Author/s:
Visser, Melissa Jane

Title:
Characterisation of the penumbra in paediatric arterial ischaemic stroke.

Date:
2023-02

Persistent Link:
https://hdl.handle.net/11343/333752

CHARACTERISATION OF THE PENUMBRA IN PAEDIATRIC ARTERIAL ISCHAEMIC STROKE

MELISSA JANE VISSER

ORCID: 0000-0002-5313-3347

FEBRUARY 2023

SUBMITTED IN TOTAL FULFILMENT OF THE REQUIREMENTS OF THE DEGREE OF MASTER OF PSYCHOLOGY (CLINICAL NEUROPSYCHOLOGY) / DOCTOR OF PHILOSOPHY

MELBOURNE SCHOOL OF PSYCHOLOGICAL SCIENCES
THE UNIVERSITY OF MELBOURNE

BRAIN AND MIND, CLINICAL SCIENCES
THE MURDOCH CHILDREN'S RESEARCH INSTITUTE

ABSTRACT

Stroke is among the top 10 causes of death in the paediatric population, with arterial ischaemic stroke (PAIS) accounting for the majority of cases. CT is the standard diagnostic imaging modality in adults presenting with focal neurological deficits, based on a high a-priori probability of stroke. In children, there are much higher rates of mimics, therefore, MRI is preferred given its higher sensitivity in detecting acute ischaemia. MR diffusion-weighted imaging (DWI), and perfusion-weighted imaging (PWI) are used to detect the presence of cerebral ischaemia and hypoperfusion in acute stroke. The mismatched volume between PWI and DWI lesions is thought to represent "*penumbra*", the tissue at risk of infarction that is potentially salvageable if reperfused within a short timeframe.

Multiple studies have shown that lesion volume is a predictor of poorer outcomes in paediatric stroke. Therefore, the aim of acute interventions is to minimise extent of brain injury. Though studies in the literature are building, reperfusion treatments, including thrombolysis and mechanical thrombectomy, are not yet used routinely in paediatric stroke due to limited safety and efficacy data and difficulty identifying children with salvageable brain, who are most likely to benefit from these therapies. Therefore, the time course of penumbra in children, and its relationship to the final infarct lesion volume, is an area of clinical importance.

The novel contributions made by my PhD are in the furthering of knowledge of penumbra characterisation and cognitive sequelae of PAIS. The first component of this thesis involved a synthesis of current penumbra definitions in PAIS. Considerable inconsistency of penumbra definitions and methods were identified. Though most used DWI/PWI or CT perfusion, there were 11 different imaging modality combinations.

Definitions largely followed adult-based literature, though there was a paucity of clearly defined, quantitative methods.

In components two and three, I applied adult-based definitions and imaging methodologies to a paediatric sample. Here, I evaluated the technical feasibility of penumbra imaging using RAPID (Rapid Processing of Perfusion and Diffusion) software, an automated segmentation tool used in adults. I found that penumbra can be promptly assessed in PAIS using RAPID. Favourable mismatch profiles persisted beyond the standard 4.5 hours window for thrombolysis, suggesting potential therapeutic benefit. I also found pilot-level indication that DWI and PWI thresholds used in adults may require modification in PAIS.

The final thesis component explored the relationship between infarct lesion volume and cognitive outcome following PAIS with a subsample of participants assessed at 12 months post-stroke. The main finding was that both larger acute and chronic lesion volumes were associated with poorer neurological and cognitive outcomes. A case study with a particularly large lesion experienced a progressive decline in IQ only detected on longer term follow-up.

In conjunction with these novel findings, this exploratory pilot research identified important methodological challenges and limitations to inform the design of prospective studies in this area. Future research will improve understanding of the penumbra in PAIS, to inform clinical treatment decision making, and to salvage viable brain tissue, and improve cognitive outcomes for children diagnosed with PAIS.

DECLARATION

i. THIS THESIS COMPRISES ONLY MY ORIGINAL WORK TOWARDS THE PHD EXCEPT WHERE INDICATED IN THE PREFACE;

ii. DUE ACKNOWLEDGEMENT HAD BEEN MADE IN THE TEXT TO ALL OTHER MATERIAL USED;

iii. THE THESIS IS FEWER THAN 100,000 WORDS IN LENGTH, EXCLUSIVE OF TABLES, FIGURES, BIBLIOGRAPHIES AND APPENDICES.

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MELISSA J. VISSER

PREFACE

Whilst the conceptualisation, design, analyses, and the writing of this thesis was my principal responsibility, I would like to acknowledge the contribution and assistance of the following collaborators. Prof Bruce Campbell, Gagan Sharma and Prof Fernando Calamante from the Melbourne Brain Centre at the Royal Melbourne Hospital, and Dr Chris Adamson and Michael Kean from the Murdoch Children's Research Institute for technical assistance, proofing, and help with analysis and interpretation of the published work in Chapter Seven. For Chapter 5, I would like to acknowledge my co-authors Dr Jesse Shapiro, Ms Vanessa Rausa for assistance with data collation and analysis. For Chapter 9, I would specifically like to thank Dr Nicholas Ryan and the other members involved with the Stroke Recovery Study at the Royal Children's Hospital for the opportunity for data collaboration. My supervisors Prof Vicki Anderson, A/Prof Mark Mackay, and Dr Joseph Yuan-Mou Yang contributed to conceptualisation, review and editing of my writing throughout all chapters in this thesis.

The following sections of this thesis contain published work, or work under review for publication.

Chapter Seven:

Visser, M., Yang, J., Calamante, F., Kean, M., Adamson, C., Sharma, G., … Mackay, M. (2021). Automated Perfusion-Diffusion Magnetic Resonance Imaging in Childhood Arterial Ischemic Stroke. *Stroke*, 52, 3296–3304

Note: Chapter Seven uses American English spelling, as published in STROKE, which is different to the other chapters in the thesis.

Chapter Five:

Visser, M., Mackay, M.T., Rausa, V., Shapiro, J., Yang, J., Anderson, V. (under review) Imaging of the ischaemic penumbra in paediatric arterial ischaemic stroke: a systematic review. *European Journal of Paediatric Neurology*

Other articles published during candidature

Bressan, S., Clarke, C.J., Anderson, V., Takagi, M., Hearps, S.J., Rausa, V., Anderson, N., **Doyle, M**., Dunne, K., Oakley, E., Davis, G.A. and Babl, F.E. (2020), Use of the sport concussion assessment tools in the emergency department to predict persistent post-concussive symptoms in children. *J Paediatr Child Health*, 56: 1249-1256. https://doi.org/10.1111/jpc.14910

Vicki Anderson, Gavin A. Davis, Michael Takagi, Kevin Dunne, Cathriona Clarke, Nicholas Anderson, Vanessa C. Rausa, **Melissa Doyle**, Georgia Parkin, Katie Truss, Emma Thompson, Silvia Bressan, Stephen Hearps, and Franz E. Babl. *Journal of Neurotrauma. Jun 2020.1392-1400*. http://doi.org/10.1089/neu.2019.6683

Michael Takagi, Stephen J. C. Hearps, Franz E. Babl, Nicholas Anderson, Silvia Bressan, Cathriona Clarke, Gavin A. Davis, **Melissa Doyle**, Kevin Dunne, Chloe Lanyon, Vanessa Rausa, Emma Thompson & Vicki Anderson (2020) Does a computerized neuropsychological test predict prolonged recovery in concussed children presenting to the ED?, *Child Neuropsychology*, 26:1, 54-68, DOI: 10.1080/09297049.2019.1639653

McCann, M.E., et al. (**GAS Consortium**), Neurodevelopmental outcome at 5 years of age after general anaesthesia or awake-regional anaesthesia in infancy (GAS): an

international, multicentre, randomised, controlled equivalence trial. *Lancet*, 2019. **393**(10172): p. 664-677.

Conference presentations

International Stroke Conference – Honolulu, Hawaii, USA – February 2019. Oral presentation:

Doyle, M. J., Yang, J. Y.-M., Adamson, C., Sharma, G., Calamante, F., Campbell, B., Anderson, V., & Mackay, M. T. (2019). Abstract 54: Utility of Automated Perfusion-diffusion Magnetic Resonance Imaging in Acute Childhood Arterial Ischaemic Stroke. *Stroke*, *50*(Suppl_1), A54–A54. https://doi.org/10.1161/str.50.suppl_1.54

Students of Brain Research, Student Symposium November 2019 – Poster presentation

Awards

2017-2020 Australian Government Research Training Program Scholarship

(Acknowledged as the source of funding for this thesis)

2019 Paul Dudley White International Scholar

Impact of COVID-19 Pandemic

This research was partly conducted during the COVID-19 pandemic. This involved complete closure to clinical research at the institute at which this research was conducted. It is worth noting the isolation and reduction of contact with supervisors and research colleagues during this time, and the impact on recruitment and ability to seek timely assistance with some analyses and other aspects of this thesis.

ACKNOWLEDGEMENTS

Firstly, I'd like to thank our study participants and their families who made this research possible. Your contribution will hopefully help other children with stroke going forward, and your strength, passion and memory has been a constant inspiration for me to continue working in this very important area of research.

I'd like to thank my supervisors for all of their guidance and support. I feel very fortunate to have had such an incredible support system throughout my PhD, and I'd like to thank you all for your time you've given me and the wisdom you've imparted.

Prof Vicki Anderson, you have been a constant shining light throughout this journey, and your never-ending advice and care has meant so much to me. As both an exceptionally accomplished researcher and neuropsychologist, you have been an inspiration to me for many, many years, since I first reached out to you nearly ten years ago.

Thank you so much A/Prof Mark Mackay, for your patience and support, and for imparting so much of your endless knowledge to me. You shared your infectious passion for this area of clinical practice and research and you have taught me so much about the research process and the translation of science to practice that I'll carry with me throughout my professional career.

Thank you to Dr Joseph Yang, who helped me learn and understand the neuroimaging component of my research, which you taught me almost from scratch. I'm grateful for your time, perseverance and understanding and particularly for your detailed and insightful feedback that you've generously provided me – you have taught me many useful and practical skills for my future research.

There are many other people who have made contributions to this thesis, including my advisory committee members Dr Richard Beare, and Prof Kim Felmingham. Thank you for lending your expertise and suggestions to me along the way. Thank you also to my collaborators and co-authors Prof Bruce Campbell, Gagan Sharma, Prof Fernando Calamante, Mike Kean, Dr Chris Adamson, Dr Jesse Shapiro, and Vanessa Rausa. This research would not have happened without you. I am so lucky to have so many amazing people with varied expertise in my professional corner.

To my colleagues, past and present, at the Murdoch Children's Research Institute. You have all helped me enormously, both personally and professionally throughout this thesis, and leading up to it. A special mention to Dr Michael Takagi, who put so much time and effort into my professional development to help me get accepted into the Neuropsychology Masters course. I cannot thank you enough.

My friends and co-PhD friends who have been on this journey with me and supported me from catch-ups and writing days, to ranting debriefs. Especially (soon to be Dr) Jane Bairnsfather, (soon to be Dr) Hana McDonald, Jessica Kennedy, Harriet Wills, Dr Georgia Parkin, Dr Caitlin Yolland, my favourite cousin Dr Matthew Mitchell, and the amazing people I got to work with in the clinical research group at the Florey Institute of Neuroscience and Mental Health.

Thank you to my family, especially my parents, my in-laws, my grandparents, my sister-in-law, and my brothers. I would not be where I am today without the understanding of the value of hard work, and the 'go for everything' attitude instilled in me from a young age (thanks Pa). Who knew that my childhood stubbornness would lead to persistence in adulthood that undoubtedly helped me through this thesis. Your support means the world to me.

This thesis is dedicated to my husband, Justin. *Thank you* does not adequately encapsulate my gratitude for everything you've done for me since we embarked on this PhD journey together. I appreciate each and every time you've helped and supported me over these years, from the morning coffees to the consolation. Your never-ending support, guidance and love means everything to me, and I would not have made it here without you. Together, anything is possible, and this thesis is a testament to that.

CONTENTS

LIST OF ABBREVIATIONS

ADC	Apparent Diffusion Coefficient
AIF	Arterial Input Function
ASL	Arterial Spin Labelling
(c)(p)(pc)ASL	(continuous)(pulsed)(pseudocontinuous)
CBF	Cerebral Blood Flow
CBV	Cerebral Blood Volume
CSVT	Cerebral Sinovenous Thrombosis
CT(P)	Computed Tomography (Perfusion)
DSC-PWI	Dynamic susceptibility contrast perfusion weighted imaging
DSC	Dice Similarity Coefficient
DWI	Diffusion Weighted imaging
EF	Executive Function
FLAIR	Fluid Attenuated Inversion Recovery
fMRI	Functional Magnetic Resonance Imaging
FSIQ	Full scale intelligence quotient
HS	Haemorrhagic Stroke
ICC	Intraclass coefficient
IQ	Intelligence quotient
LVO	Large Vessel Occlusion
MCA	Middle Cerebral Artery
MRI	Magnetic Resonance Imaging
MRP	Magnetic Resonance Perfusion
MTT	Mean Transit Time
PAIS	Paediatric Arterial Ischaemic Stroke
PR	Perceptual Reasoning
PS	Processing Speed
PSOM	Paediatric Stroke Outcome Measure
PWI	Perfusion weighted imaging
RAPID	Rapid processing of Perfusion and Diffusion
SCD	Sickle Cell Disease
SWI	Susceptibility Weighted Imaging

TIPS(TER)	Thrombolysis in Paediatric Stroke Study (Extended Results)
T_{max}	Time to Maximum residue function
tPA	Tissue Plasminogen Activator
TTP	Time to Peak
VC	Verbal Comprehension
WM	Working Memory
WTM	Watershed Transform Method

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1. INTRODUCTION

1.1. Problem statement

Many studies have documented the importance of timely treatment to salvage brain tissue in adult ischaemic stroke (Dhamija & Donnan, 2007; Rymner et al., 2010). Reperfusion therapies, including intravenous thrombolysis and mechanical thrombectomy are routinely used to treat adults with ischaemic stroke. Patients who present within a six-hour window, or who are deemed to have salvageable brain tissue on acute scan up to 24 hours following symptom onset are often eligible for these treatments. Currently, although evidence of safe implementation of reperfusion therapies is increasing, patients who present with paediatric arterial ischaemic stroke (PAIS) are not routinely treated with thrombolysis or thrombectomy. This is due to the limited research into the safety and efficacy of such treatments (Heit et al., 2021). Patients are often given secondary preventative antithrombotic treatments and are monitored acutely with supportive care

measures provided, such as seizure control, and optimising oxygenation (Ferriero et al., 2019; Rivkin et al., 2016). Case studies and multicentre cohort studies suggest that reperfusion therapies can be useful and safe in patients under 18 years. (Kossorotoff et al., 2022; Pacheco et al., 2018; Sporns et al., 2020). However, further studies are needed to increase our understanding of the vascular pathophysiology of paediatric stroke and methods to determine which patients are the most likely to benefit from such treatments.

Unique plasticity and functional specificity characteristics of the developing brain make it impracticable to translate knowledge of the mature adult brain directly to children (Kolb & Gibb, 2011). Childhood acquired brain injury is occurring within a developmental context. Contrary to some belief regarding plasticity that children have more opportunity for neural reorganisation, there is a vulnerability in very young children. In particular, literature has shown that those sustaining brain insults in the perinatal period or less than one year of age, tend to have poorer outcomes, due to disruption in the processes of rapidly developing neural networks (Anderson et al., 2011). In older children, there is less plasticity, or capacity for reorganisation, as these patients are heading towards the functionally specific organisation of the adult brain. This group can also be vulnerable to poorer outcome. Adult stroke patients may experience a loss of function, however, in children, they can experience disruption *attaining* function, rather than losing it. Lesion location and laterality are very important in predicting adult outcome and recovery following neuropathology, however, this relationship is less consistent following paediatric stroke (Allman & Scott, 2013; Levine et al., 2005; Westmacott et al., 2018). There is, however, a strong relationship across most studies between lesion size and cognitive outcome, underlining penumbral tissue as a crucial

target to improve outcome in PAIS (Abgottspon et al., 2022; Everts et al., 2023; Hajek et al., 2014; Studer et al., 2014).

Magnetic resonance perfusion-weighted imaging (PWI) and diffusion-weighted imaging (DWI) can detect and quantify the extent of hypoperfusion and ischaemia in the acute period following stroke onset (Ermine et al., 2021). Areas of perfusion abnormality on PWI are thought to represent volume of hypoperfusion, and diffusion restriction is thought to represent cytotoxic oedema, or the core of irreversibly damaged ischaemic tissue. The mismatched lesion volume between these two imaging modalities is hypothesised to be an estimate of penumbra, or salvageable brain tissue, which is the target for reperfusion therapies in adult ischaemic stroke (Ramos-Cabrer et al., 2011). This tissue has reached a level of hypoperfusion which renders it dysfunctional, but it has not yet reached the threshold of ischaemia resulting in structural or morphological damage (Jones et al., 1981). This tissue is at risk of infarction if restoration of blood flow does not occur within a timely manner, from reperfusion therapies, recanalization, and / or sufficient collateral flow from other circulations via pial collaterals (Liu et al., 2018; Rusanen et al., 2015).

Children with PAIS often present to hospital and have brain scans later than adults with ischaemic stroke (Mallick et al., 2015; Rafay et al., 2009). This is problematic due to the direct relationship between time and level of ischaemia within acutely hypoperfused brain tissue (Dhamija & Donnan, 2007; Donnan et al., 2007). The DEFUSE-3 and DAWN trials demonstrated that reperfusion treatment in adults with ischaemic stroke can be beneficial outside the standard time window of six hours from stroke onset, when using tissue-based selection of patients (Albers et al., 2018; Nogueira et al., 2018). This is important because there are also delays to presentation in the adult population (Leung &

Caplan, 2016; Takarada et al., 2021). Automated software, which utilises perfusion imaging data to estimate volume of hypoperfused tissue, and diffusion weighted imaging to estimate the volume of infarct core, was used in the DEFUSE-3 trial. Patients with a large mismatch according to specific criteria were more likely to benefit from reperfusion treatment (Albers et al., 2018; Nogueira et al., 2018).

It cannot be assumed that the pathophysiological mechanisms underpinning penumbral evolution in adults are analogous in children, as there are varying stroke aetiologies and age-related differences in cerebral perfusion in the developing brain, including differences in collateral flow. The time course of penumbral evolution in adults is well understood, however in PAIS it is poorly characterised.

1.2. Overall aim and scope of thesis

The overall aim was to improve our understanding of penumbra characterisation in children, and provide further rationale to penumbra-targeted revascularisation therapies to potentially improve outcome in PAIS. Specifically, this thesis aimed to look at the natural history of penumbral tissue evolution in PAIS, and to see whether it could be feasibly quantified using automated software for timely interpretation. It also aimed to look at the relationship between lesion volume change and penumbra definitions in our sample. This thesis also explored lesion size at varying time-points and how this might affect cognitive outcome, while identifying important limitations and methodological considerations.

1.3. Thesis overview

Chapter Two of this thesis provides important background information and an updated literature review in childhood stroke and neuropsychological outcome. This is followed

in Chapter Three by an exploration of the neuroimaging literature in stroke and acute cerebral ischaemia in both adults and children. From this literature review, the study questions and aims were elicited, which are detailed in Chapter Four.

An objective understanding of the imaging modalities and definitions used for penumbra identification in PAIS was warranted as this is a key methodological concept used in this thesis. To address this, a systematic literature review was conducted in Chapter Five. This study ascertained how different imaging modalities have been used to characterise penumbra in children and identified if there were any consistencies in these definitions. The findings from this chapter informed the methods for the subsequent studies in this thesis.

In Chapter Six, I present some important methodological groundwork which was undertaken prior to characterisation of the penumbra. In novel research, trialling methods and encountering difficulties are key learnings. I implemented a semi-automated manual segmentation technique which needed to be tested to validly address the remaining study questions of this thesis. Additionally, I examined the application of an automated software program that is validated for use in adults (RAPID) and applied it to a paediatric stroke cohort. This had to be tested prior to interpretation and analysis in the following chapter.

In Chapters Seven and Eight, I present the findings of the investigation into the feasibility of applying RAPID to this paediatric sample. I highlighted the considerations both technically and pertinent to the population (paediatric factors), with applying this type of software. This approach is clinically relevant because refining the protocol for defining penumbra in PAIS, will enable clinicians to implement automated software and potentially make decisions regarding treatment in the acute setting. I also present a case

series of mismatch profiles and explore alternate definitions. This exploration utilised lesion volume change from acute to chronic infarct as an adjunct marker of penumbra.

In Chapter Nine, I discuss the effect of infarct lesion volume on cognitive outcome in PAIS. In this chapter, I explored the impact of clinical or lesion-specific factors related to cognitive outcome in this sample. Results from a sub-sample that was analysed were investigated to see if those with larger acute and final infarct volumes had poorer cognitive outcome, and thus to determine whether prior acute brain injury literature is replicated in our sample.

Finally, in Chapter 10, I synthesise the overall findings and conclusions from all sections of this thesis, drawing attention to the novel observations and how results from this pilot work will inform future research.

As a preliminary summary, this is the first paediatric study to my knowledge to look at lesion volume with the semi-automated segmentation technique that was used, and the first study to synthesise and compare attempts to define penumbra for a clinical purpose. It is also the largest study to use an automated penumbral segmentation method in PAIS, and to delineate the problems associated with its use, particularly to identify paediatric-specific effects. Finally, the highlight of the thesis is its identification of problems, limitations, and future directions for research and clinical implications for characterising penumbra in PAIS. The novel finding of identifiable penumbra in extended windows will propel work in penumbra imaging in PAIS. If we can acutely identify penumbra, we may be able to safely salvage more brain tissue and improve neuropsychological outcomes for children with paediatric arterial ischaemic stroke.

2. LITERATURE REVIEW OF STROKE AND OUTCOME

Stroke refers to a neurological deficit which is related to acute focal injury to the central nervous system caused by a vascular abnormality (Sacco et al., 2013). This deficit can manifest in impaired motor function, cognition, speech or vision, in a manner consistent with vascular distribution, usually with accompanying neuroradiological confirmation (Li et al., 2022). As arterial ischaemic stroke tends to be a localised injury, much of what is currently known about brain-behaviour relationships stems from stroke research (Festa et al., 2007). Descriptions of clinical stroke syndromic presentations following lesions of the anterior, middle and posterior cerebral arteries were first described in the early 1900s. The neuropathology of stroke and its accompanying clinical features were continually

studied beyond this time, with advances in neuropsychology, technology, and treatments associated with cerebrovascular disease.

2.1. Adult ischaemic stroke aetiologies

An ischaemic stroke occurs when blood flow is insufficient to maintain neurological functioning, to an extent that the ischaemia reaches a critical threshold leading to irreversible structural damage (Sacco et al., 2013). The main causes of ischaemic stroke include disruption of cerebral blood flow, due to embolism, thrombus, systemic hypotension, and vessel wall pathology (eg. dissection). Thrombosis is caused by a locally formed clot which impedes blood flow through an artery. This can often proceed from atherosclerosis, when plaques form and begin to occlude the lumen of an artery. An embolism is also a clot, but one which has formed elsewhere in the vasculature and becomes lodged in a cerebral artery after travelling distally through the blood stream (Festa et al., 2007). Stroke can also be caused by a haemorrhagic mechanism, (infarcted brain tissue due to an intracranial bleed) or be caused by an occlusion in the venous system, such as Cerebral Venous Sinus Thrombosis. This thesis will focus on arterial ischaemic stroke.

2.2. Introduction to paediatric stroke: types and known aetiologies

Stroke is among the top 10 causes of death in the paediatric population, with an annual incidence of approximately 1.6/100,000 (Mallick et al., 2014). Paediatric stroke is a lesion due to a cerebrovascular accident that occurs between 30 days old, up until the age of 18. Perinatal stroke (occurring before birth and up to one month old) and paediatric stroke (one month to 18 years old) can significantly differ in clinical presentation (Fuentes et al.,

2016). There are three main types of childhood stroke: Paediatric Arterial Ischaemic Stroke (PAIS), Haemorrhagic Stroke (HS) and Cerebral Sinovenous Thrombosis (CVST). Paediatric HS, which is a lesion due to an intracranial bleed, generally presents with a sudden severe headache, and can be accompanied by seizures, alterations in alertness, nausea and/ or vomiting (Cárdenas et al., 2011; Fuentes et al., 2016). Almost half of paediatric strokes are due to non-traumatic HS and can be distinguished into sub-types: intracerebral haemorrhage, and subarachnoid haemorrhage (Jordan & Hillis, 2007). Another sub-type of paediatric stroke is CSVT, caused by a thrombus or clot in the venous system, which can present with more generalised symptoms and a more insidious onset than PAIS, which often has a sudden and focal onset (Cárdenas et al., 2011; Fuentes et al., 2016).

2.2.1. Paediatric Arterial Ischaemic Stroke

PAIS is a stroke due to arterial occlusion and constitutes the focus of this thesis. PAIS represents more than half of childhood stroke presentations, and has a mortality rate between 7% and 28% (DeVeber et al., 2000; Goeggel Simonetti et al., 2015; Lynch et al., 2002). Annually, PAIS is estimated to affect 1.6 – 2.4 per 100,000 children, amongst USA and European studies, and there is a high chance of PAIS recurrence in patients within a five-year period, with studies reporting rates of up to 50% (Agrawal et al., 2009; DeVeber et al., 2000; Fullerton et al., 2007; Laugesaar et al., 2010; Mirsky et al., 2017). Identified risk factors for PAIS include vasculopathy, infection, trauma, cardiac and haematological disorders (Greenham et al., 2016). Sickle cell disease (SCD) can cause secondary ischaemic and/ or haemorrhagic stroke in children.

PAIS in older children typically presents with focal neurological deficits, similar to adult symptomatology, but can also present with seizures or alterations in alertness. Up to 70

percent can have long-term neurological deficits (Cárdenas et al., 2011; Fuentes et al., 2016; Nasiri et al., 2016). PAIS patients from very low income families have been shown to have poorer neurological outcomes, than those from higher socio-economic backgrounds (Jordan et al., 2018). This has been found to occur even when patients have the same access to rehabilitation, though the cause of this is unknown.

Causes of PAIS are often complex and multifactorial (see Figure 2.1). Up to one third, of PAIS cases are idiopathic and many patients have greater than one risk factor (Friedman, 2009; Fuentes et al., 2016). Cerebral arteriopathy has been found to be the cause of approximately half of all cases of PAIS (MacKay et al., 2011). Arteriopathies in PAIS range from transient to progressive: migraine (spasm), extrinsic compression, post-varicella angiopathy/ transient (or focal) cerebral arteriopathy (FCA) of childhood, dissection, radiation-induced vasculopathy, vasculitis, and bilateral cerebral arteriopathy, including Moyamoya disease (DeVeber, 2003). Cervicocephalic arterial dissection, which is a type of arteriopathy which can be spontaneous or caused by mechanical forces, is the

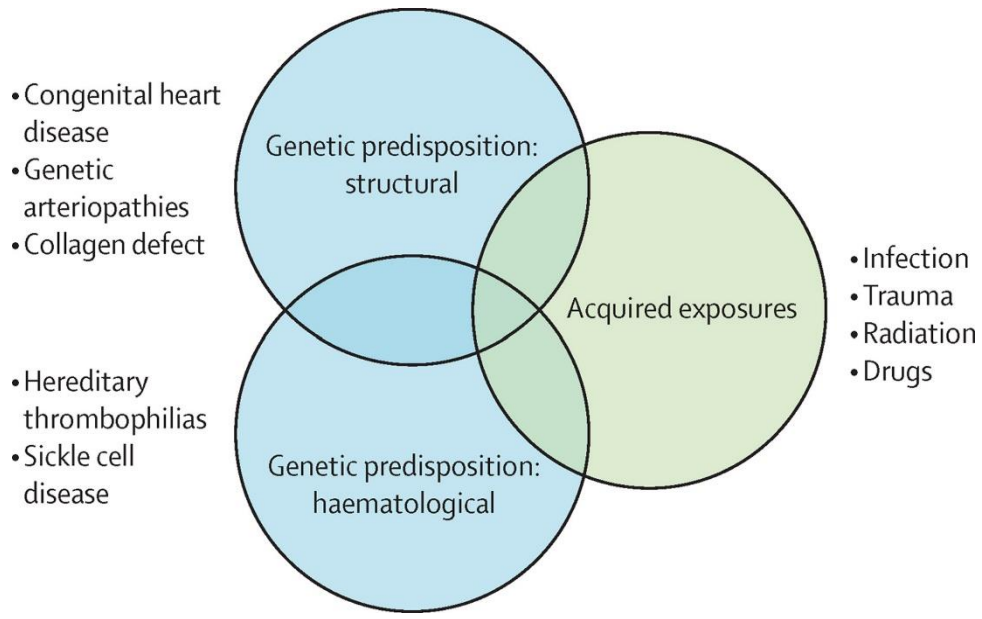

Figure 2.1 - Multifactorial causes of PAIS.
Reprinted from *The Lancet Child and Adolescent Health*, Volume 5, issue 11, Sporns, P.B, Fullerton, H.J., Lee, S., Kirton, A., Wildgruber, M. Review Current treatment for childhood arterial ischaemic stroke, 825-836, Copyright (2021), with permission from Elsevier.

cause of approximately 24% of PAIS arteriopathy cases. This is one of the three most common categories of arteriopathic stroke, alongside unilateral FCA and Moyamoya disease (Amlie-Lefond et al., 2009; Rafay et al., 2006; Wintermark et al., 2017).

FCA is typified by unilateral focal arterial stenosis, and causes a further 23% of childhood arteriopathy (Sporns et al., 2022; Wintermark et al., 2014). It can be subcategorised into inflammatory (e.g., when it occurs post-varicella virus), and dissection (with which imaging findings sometimes overlap) (Amlie-Lefond et al., 2009; Dlamini et al., 2011; Wintermark et al., 2017). PAIS typically involves the intracranial blood vessels, as opposed to those in the neck that are often affected in adult atherosclerosis. The presence of arteriopathy is also a risk factor for recurrence, and a risk factor for stroke following a transient ischaemic attack (TIA) (Fullerton et al., 2016). Up to 30% of PAIS patients have an associated cardiac disorder which leads to cardioembolism (Poisson et al., 2014). Congenital or acquired heart disease has been found to be the cause of PAIS in 25-36 percent of patients (MacKay et al., 2011; Mallick et al., 2014). The causes of cardioembolic stroke are often related to heart defects which involve the mixing of oxygenated and deoxygenated blood, such as Tetralogy of Fallot and hypoplastic left heart syndrome (Asakai et al., 2015). See Figure 2.2 for aetiological breakdown of PAIS.

Although childhood stroke patients have been thought to recover better compared to adult stroke patients due to higher plasticity in the developing brain, children do not have better outcomes (Goeggel Simonetti et al., 2015). Paediatric stroke patients must live with their stroke outcomes for longer than adults, and face difficulties in achieving, rather than losing, functional independence as there are many skills they have not yet acquired (Gordon et al., 2015; Greenham et al., 2016). This can affect the child's cognitive and

functional development, with certain factors associated with these outcomes observed in the literature (detailed further in Section 2.4 below).

2.3. Current treatments of ischaemic stroke

2.3.1. Adult Ischaemic Stroke Reperfusion Therapy: Thrombolysis

Thrombolysis is a treatment used in acute ischaemic stroke, to break down blood clots which have formed in blood vessels, with the use of an exogenous protein called tissue plasminogen activator (tPA) (Baig & Bodle, 2022). This treatment is used in pulmonary embolism, myocardial infarction, and stroke. The recommended time-window for effective tPA treatment in stroke is within 4.5 hours, however, patients who present outside this window with small infarct core and large area of hypoperfusion (penumbra, see section 3.1) may still benefit from this treatment (Paciaroni et al., 2009). The National Institute of Neurological Disorders (NINDS trial, 1995) demonstrated effectiveness of tPA for adult ischaemic stroke within a three-hour period (NINDS rt-PA Study Group,

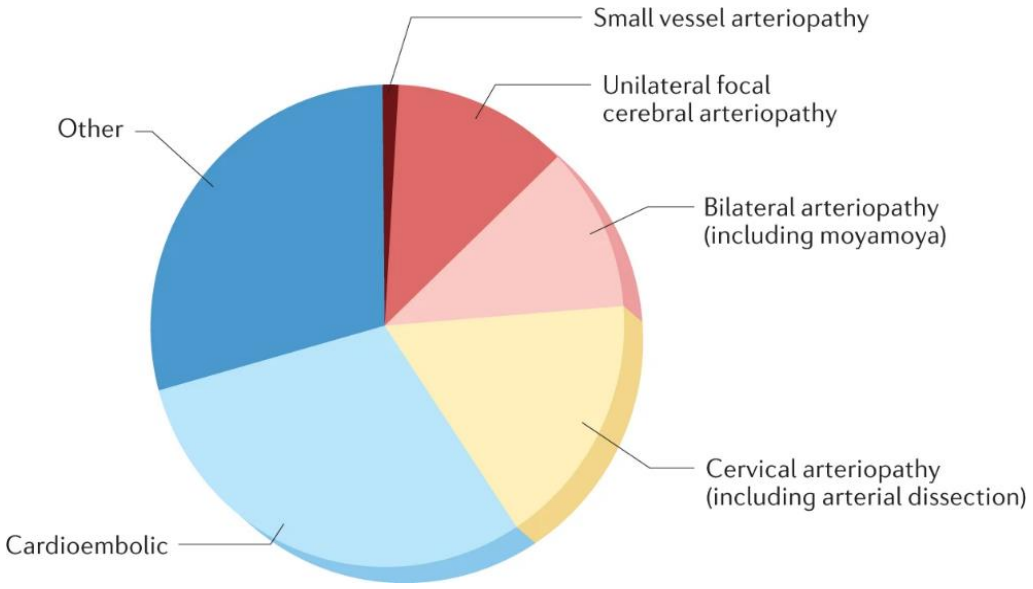

Figure 2.2 - Aetiologies of PAIS
From *Nature Reviews Disease Primers*: Sporns, P. B., Fullerton, H. J., Lee, S., Kim, H., Lo, W. D., Mackay, M. T., & Wildgruber, M. (2022). Childhood stroke. 8(1), 1–27. (using data from Wintermark et al, 2014). Reproduced with permission from Springer Nature and Wolters Kluwer Health.

1995). Subsequent studies have shown effectiveness of thrombolysis treatments when administered to adults in a 4.5 hour time-window. However, few children with ischaemic stroke receive tPA treatments within this time frame for multiple reasons (Marecos et al., 2015; Medley et al., 2019; Paciaroni et al., 2009; Rivkin et al., 2016). Barriers such as presentations outside of the recommended window, uncertainties regarding patient selection for tPA, and contraindications, contribute to this problem.

2.3.2. Adult Ischaemic Stroke Reperfusion Therapy: Mechanical Thrombectomy

Mechanical Thrombectomy (MT) involves manual intra-arterial retrieval of a clot using an endovascular device, by various methods including stent-retrieval and direct aspiration. Though tPA is considered a first line treatment, particularly when a stroke patient presents within 4.5 hours, MT is often implemented for patients with strokes caused by large vessel occlusion (LVO). A thrombus within a larger artery such as the proximal middle cerebral artery, or the distal internal carotid artery is more likely to be resistant to dissolution from intravenous tPA (Goyal et al., 2016; Saqqur et al., 2007). Meta-analysis of randomised control trials from 2015 demonstrated that the combination of MT and tPA thrombolysis in LVO resulted in improved outcomes of 20-30% compared to treatment with tPA alone. Currently, MT is used both independently and in combination with tPA treatments. It can be administered within the first six hours after symptom onset in patients undergoing standard CT or MR brain imaging. MT can also be administered up to 24 hours post stroke-onset in adults who have clinical or imaging features suggestive of salvageable brain tissue (Albers et al., 2018; Nogueira et al., 2018; Stroke Foundation, 2022).

2.3.3. Current treatments in PAIS

As noted above, the aetiologies of PAIS vary considerably compared to adult ischaemic stroke, therefore our understanding and evidence of adult treatments cannot be directly extrapolated to children. Despite advances in paediatric stroke research, discrepancies amongst disease management remain. General medical management recommendations include ICU admission and monitoring, oxygen saturation maintenance, administration of intravenous fluids, treatment of acute complications such as seizures, and antithrombotic therapy for secondary stroke prevention (Amlie-Lefond & Wainwright, 2019). For FCA, which is thought to be due to an inflammatory process, the use of corticosteroids is deemed safe, and a Delphi consensus process revealed this to be the highest trial priority amongst Australasian and European paediatric neurologists at the time of the study (Steinlin et al., 2017). A current phase III randomised control trial is underway to look for treatment differences using high-dose corticosteroids and aspirin versus standard treatment of aspirin alone.

A dearth of high-quality prospective evidence on the use of reperfusion therapies in PAIS presents a challenge, as paediatric patients are typically excluded from randomised control trials in adult ischaemic stroke. The Thrombolysis in Paediatric Stroke (TIPS) trial which was designed to assess the safety and dose-finding of tPA in PAIS was closed prematurely due to difficulties with recruitment (Rivkin et al., 2015). The TIPS Extended Results (TIPSTER) study looked at data collected from TIPS sites. The risk of symptomatic intracranial haemorrhage post-tPA treatment within 4.5 hours was found to be low, with an estimated risk of 2.1% (Amlie-Lefond et al., 2020). The efficacy and optimal dosing of tPA in PAIS patients, however, remains unknown, though off-label usage on patients

who meet TIPS criteria, using standard adult dosing is increasingly reported in the literature (Nasr et al., 2014; Rambaud et al., 2020; Vinayan et al., 2022).

For the use of MT in PAIS with LVO, the Save ChildS Study (Feasibility, Safety, and Outcome of Endovascular Recanalization in Childhood Stroke) showed multicentre retrospective evidence of safety, reperfusion rates and positive clinical outcomes which were largely comparable to adult studies (Sporns et al., 2020). This research combined data from 27 stroke centres, including 73 children, which, in the absence of higher-level prospective studies (one of which is currently underway), the authors concluded potential support for off-label usage of MT in PAIS (Sporns et al., 2020; Sporns et al., 2021). Importantly, a sub-analysis of this data found preliminary evidence of safety and beneficial outcomes of MT up to a timeframe of 24 hours post-stroke when the child presents with a mismatch between the infarct size, and clinical deficit. In addition, a recent study looking at the incidence of LVO in PAIS found that those treated conservatively had poorer outcomes than those who had MT (Bhatia et al., 2022).

Delayed recognition of PAIS has been documented with a 12-hour median time to diagnosis, and with greater than 50 percent of diagnoses confirmed more than 24 hours after presentation, even if children are brought into the emergency department soon after symptom onset (Gabis et al., 2002; Mallick et al., 2015; McGlennan & Ganesan, 2008; Ravindra et al., 2021; Srinivasan et al., 2009). Up to 75% of patients who attend the emergency department with focal neurology are found to have stroke mimics, such as migraine, and up to one quarter of them will receive a stroke diagnosis (Ladner et al., 2015; MacKay et al., 2014, 2016). Stroke mimics and poor awareness of PAIS and its symptoms, combined with difficulties accessing diagnostic neuroimaging are the main contributors to diagnostic delays (Mallick et al., 2015; Yock-Corrales et al., 2011). This

time lag to diagnosis is a particular problem in PAIS, which makes exploring treatments in extended time-windows a vital and pressing area of study. Further research is urgently needed in the use of MT in PAIS, particularly in patients with arteriopathic aetiologies. The inflamed vessels in these patients present increased susceptibility to injury with mechanical manipulation in MT with ensuant treatment complications (Sporns et al., 2021).

2.4. Cognitive outcomes following paediatric arterial ischaemic stroke

The reported cognitive outcomes following PAIS varies significantly in the literature potentially due to differing study methodologies. These include the use of cross-sectional designs, varying age ranges, time post stroke onset and sample sizes (Greenham et al., 2016). Stroke characteristics, for example, lesion size, location and hemispheric laterality (i.e. left versus right lesion), clinical observance of seizures and neurologic deficits, and developmental stage of cognitive domains have all shown to have an effect on cognitive outcome. In children, cognitive deficits may not be observable acutely, and may become evident later when developmentally appropriate, as can be seen in Figure 2.3 (Sporns et al., 2022; Westmacott et al., 2010).

The relationship between these variables is complex, and the contributing variance explained by each is dependent on other factors (Fuentes et al., 2016). Interactions between the variables is more important than any of these factors in isolation. The current literature into each of the contributing factors will be explored in this chapter. Looking at predictors and risk factors for cognitive impairment following PAIS is important, and can assist in targeting interventions, providing evidence-based knowledge and supporting health care providers and families. Early identification of children at risk of poor

outcomes may enable early targeted treatment of cognitive impairment, improving quality of life in this population (Cnossen et al., 2010; Gordon et al., 2002).

2.4.1. Neurological comorbidities and cognitive outcome

Unlike in adult ischaemic stroke, acute symptomatic seizures are common in PAIS, particularly in neonates and infants. Approximately 15-30% of PAIS patients develop epilepsy post-stroke, with larger lesions associated with higher risk (DeVeber et al., 2000). An increase in the frequency of acute seizures, along with younger age at stroke, are also significant risk factors for the development of epilepsy one year post-stroke (Fox et al., 2016). Presence of post-stroke seizures and/or epilepsy has been shown to predict poor neuropsychological outcome (Ballantyne et al., 2008; Studer et al., 2014). Persistent neurological deficits have been associated with poorer cognitive outcomes in various studies, resulting in significant deviations from normative means on Full-Scale Intelligent Quotient (FSIQ) and other indices (Allman & Scott, 2013; DeVeber et al., 2000; Gordon

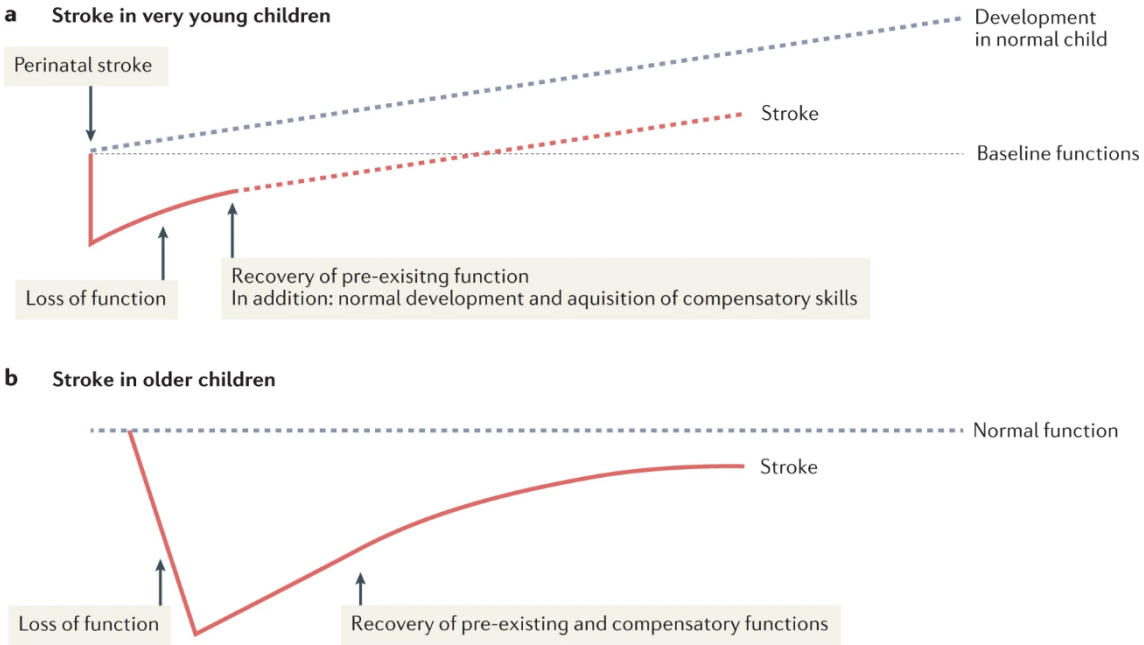

Figure 2.3 - Interruptions of cognitive function development in young children vs older children
From *Nature Reviews Disease Primers*: Sporns, P. B., Fullerton, H. J., Lee, S., Kim, H., Lo, W. D., Mackay, M. T., & Wildgruber, M. (2022). Childhood stroke. 8(1), 1–27. Reproduced with permission from Springer Nature.

et al., 2002; Studer et al., 2014). Similarly, Hajek et al., (2014) reported that greater neurological deficits as measured by the Paediatric Stroke Outcome Measure (PSOM) were related to poorer cognitive outcomes. The PSOM is a paediatric stroke specific measure of neurological recovery that provides a functional ranking across five subscales, including right and left sensorimotor, language production and comprehension, and cognitive/behavioural function (Kitchen et al., 2012). Specifically, PSOM scores were negatively correlated with FSIQ, processing speed, vocabulary and coding. A population-based study by Goeggel Simonetti et al., (2015) found that more than half of children with PAIS had long-term neurological impairments.

2.4.2. Age at stroke and plasticity – effects on cognition

The resilience of the developing brain to injury is an area of ongoing debate. On one hand, it is argued that nascent brain tissue has greater plasticity and is able to reorganise in response to injury (Chen et al., 2002). Others argue that developing brain is more vulnerable to insult, resulting in greater neuropsychological impairment (Chapman et al., 2003; Max et al., 2010). Literature indicates that both are true to some degree, with older children whose brains have less opportunity for plasticity less able to transfer function from one area to another, but also that very young children seem to have poorer post-stroke outcomes than older children. This vulnerability may stem from the disruption of brain maturation processes which interrupts ongoing development (Allman & Scott, 2013). Brain maturation does not occur in a linear fashion, but rather in a stepwise manner, with critical periods more sensitive to disruption (Anderson et al., 2009). An example of this was summarised in a review by Anderson (2002), who plotted the trajectories of different aspects of executive function (EF) development (Figure 2.4).

In support of this model of the non-linear relationship of age at insult and cognitive outcome, Allman & Scott, (2013) looked at age effects of PAIS onset and identified significant differences in FSIQ, verbal comprehension (VC), and perceptual reasoning ability (PR), with those aged one to six years performing better than those younger than one year and older than six years. This indicates a protective age effect for stroke occurring in middle childhood, compared to early and perinatal, and later childhood (Allman & Scott, 2013; Everts et al., 2008). A recent study also found a U-shape relationship between cognitive outcome and age, however, the effect was in the exact opposite direction with those who had a stroke beyond the neonatal period but younger than six years displaying poorer outcomes across domains of cognitive flexibility, processing speed (PS) and verbal learning (Abgottspon et al., 2022). These effects were evident while controlling for lesion characteristics such as location and size, providing

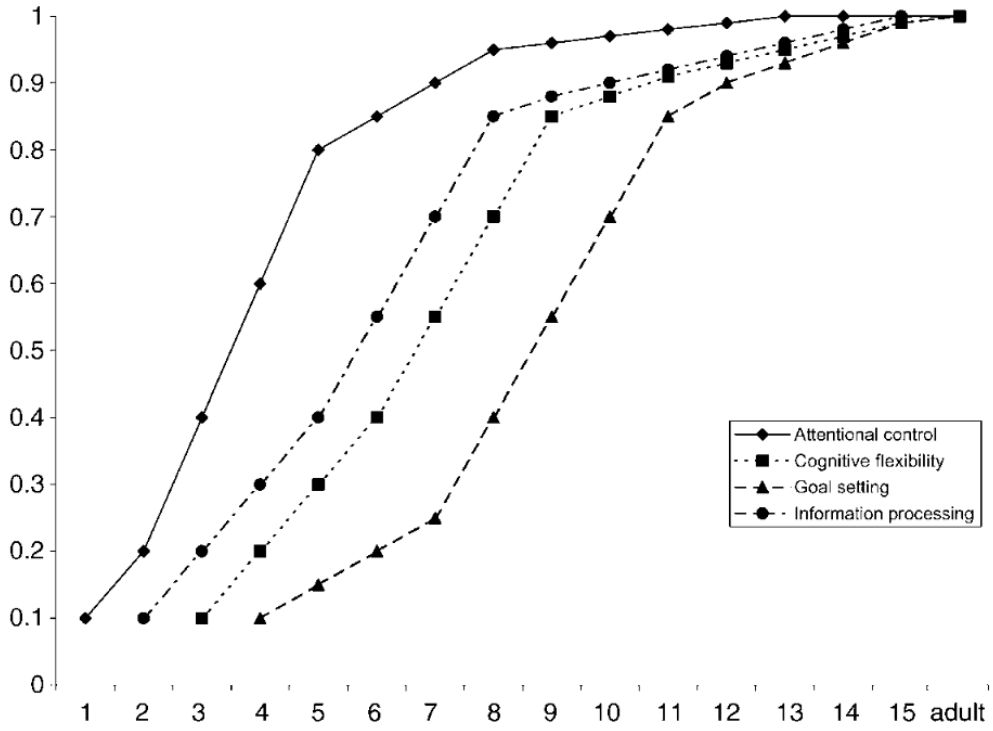

Figure 2.4 Trajectories of development of executive function – Cognitive development by Age (years)
From *Child Neuropsychology*, Anderson, P. (2002). Assessment and Development of Executive Function (EF) During Childhood. 8(2), 71–82. Reprinted with permission of Informa UK Limited, trading as Taylor & Taylor & Francis Group, http://www.tandfonline.com

stronger evidence for a unique contribution of age at stroke to cognitive outcome. Various other studies have also looked at age at stroke, specifically children less than one-year-old. PAIS in this age group indicates deficits in general intellect and visuospatial processing (Allman & Scott, 2013; Max et al., 2010; Studer et al., 2014; Westmacott et al., 2010).

Perceptual reasoning ability and fine motor control in another study was positively correlated with age, adding to our understanding that particular domains appear to be more vulnerable to disruption at earlier ages (Peterson et al., 2019). Westmacott et al. (2010) reported a specific effect on verbal IQ for children following perinatal stroke (ie. within one month of birth), compared to all other age groups, and lower FSIQ and the ability to hold information in mind while working on it (working memory, WM) compared to children older than six. Another study also found that younger age at stroke implicated poorer cognitive outcomes, in line with the hypothesis of vulnerability of the nascent brain and disruption of development (Allman & Scott, 2013; Anderson et al., 2009; Max et al., 2010; Westmacott et al., 2010).

Language may also be affected by PAIS, with language deficits and poorer discourse ability identified in children younger than two, but not in older children (Avila et al., 2010; Chapman et al., 2003). Perinatal stroke has been shown to be associated with delayed language onset in children with lesions to either hemisphere, suggesting that acquisition of language ability, as opposed to maintenance in adulthood, requires both hemispheres (Bates et al., 1997; Ilves et al., 2013; Marchman, Miller, & Bates, 1991; Stiles et al., 2010; Vicari et al., 2000). Following initial delay, however, children may recover with a normal developmental language trajectory (Marchman et al., 1991; Stiles et al., 2010). Jacola et al., (2006) looked at reorganisation of language function following left perinatal middle

cerebral artery (MCA) infarction. They found that stroke subjects showed either bilateral or right hemisphere functional magnetic resonance imaging (fMRI) activation compared to typically developing children with left hemisphere activation. These results were replicated by Ilves et al., (2013), who also used language fMRI to ascertain language dominance, with their findings showing that left hemisphere perinatal arterial ischaemic stroke was associated with bilateral or right hemisphere language predominance, but this effect was not observed in patients whose stroke occurred in childhood.

Rodrigues et al., (2011) examined the effect of early stroke (less than one year old) and academic ability later in life, finding that those who had early stroke had poorer academic outcomes in spelling, reading and arithmetic. EF appears to be a particularly sensitive domain to stroke at very early and later childhood periods, which suggest developmentally vulnerable periods for this function (Max et al., 2010). Conversely, one study reported the EF was higher in children who had stroke in early childhood (O'Keeffe et al., 2014). This study excluded perinatal stroke, and had an average age of five years at stroke onset, with the youngest child being four months old. This may indicate that this sample was not young enough to capture the EF vulnerability indicated in previous research.

2.4.3. The impact of time-post-stroke to assessment on cognitive outcome

An important consideration when interpreting results from PAIS studies is the impact of time-post-stroke on the follow-up cognitive assessment. In studies looking at cognitive outcomes following PAIS, there is high variability in time-post-stroke among protocols (Allman & Scott, 2013). Due to maturational changes in the developing brain, cognitive outcome of children with early lesions is expected to be altered over time. A longitudinal study of a sample of children who had perinatal unilateral lesions showed that IQ was

not significantly lower when tested before the age of seven, but became apparent when tested beyond this age (Levine et al., 2005). This study demonstrates that some changes in IQ may not become apparent until the brain regions responsible for development have reached a level of maturity, suggesting not a loss of skill, but an impediment of skill development (Stiles et al., 2010). This may explain some of the variance between studies in cognitive outcome due to heterogeneity in time-post-stroke and age. Similar effects were demonstrated in a longitudinal study which noted that deficits in FSIQ, WM, and PS were evident in school age children with history of PAIS, but who were cognitively normal when assessed in preschool age (Westmacott et al., 2010). Other studies have reported stable or improved outcomes longitudinally (Ballantyne et al., 2008; Everts et al., 2008). These inconsistencies may be due to sample differences or the variance in follow up intervals.

2.4.4. PAIS aetiology, sex differences and cognitive outcomes

PAIS has a different pathogenesis to adult ischaemic stroke, and is not associated with modifiable environmental factors for atherosclerosis, such as hypertension, smoking, diabetes and hyperlipidaemia (Cárdenas et al., 2011; Friedman, 2009; Greenham et al., 2016). As outlined in 2.2.1, aetiology of PAIS is largely intrinsic to the central nervous system, as opposed to exogenous causes or factors. Boni et al, (2001) examined the differences in cognitive and social outcomes in Sickle Cell Disease (SCD) children with and without stroke. They found that poorer social information processing was associated with children with SCD who had a stroke in the past, but not SCD children without stroke. In contrast, in Moyamoya disease which can cause PAIS, cognitive changes and poorer outcomes can be the direct result of the condition itself, irrespective of stroke occurrence (Williams et al., 2012). Although there is a predominance for PAIS in male

children, sex does not seem to have an effect on cognitive outcome (Fullerton et al., 2003; Golomb et al., 2003). Braun et al., (2002) looked specifically at the effects of sex on cognitive outcomes following focal cortical brain damage of various aetiologies in both children and adults, without any evidence of an overall observable distinction between males and females.

2.4.5. Lesion characteristics – volume, laterality and location

Arguably one of the most consistent factors associated with cognitive outcome in PAIS is lesion size / volume. Increased lesion volume and infarcts spanning both cortical and subcortical regions have been associated with poorer cognitive outcomes across studies. Lesion volume and PSOM scores were found to be negatively correlated with cognitive outcome in a study by Hajek et al., (2014). Specifically, lesion volume was significantly correlated with poorer IQ and the vocabulary subtest, but notably, lesion volume was not significantly correlated with EF performance. In this study, it was suggested that although larger lesions seem to be related to greater cognitive impairment, the effect was dependent on the severity of ongoing neurological deficits, an important interaction to be considered (Hajek et al., 2014).

A recent study with 52 PAIS patients found that larger lesions were associated with poorer general intellect, WM, some EF, PS and visual-motor skills (Abgottspon et al., 2022). Increased lesion size has also been shown to be predictive of poorer psychological outcomes five years post-stroke (Greenham et al., 2017). Other research has indicated that larger lesions, specifically those that span greater than 10% of total brain volume, are associated with poorer outcome (Ganesan et al., 1999; Zecavati et al., 2014). Where pathology is localised to subcortical lesions, those with larger lesions are more likely to be diagnosed with a learning disorder (Westmacott et al., 2018).

The impact of infarcts that span cortical and subcortical regions has also been associated with poorer cognitive outcome. Studer et al., (2014) found cognitive performance overall to be in the low-average range, with specific deficits found in PS, WM and visual construction skills. Subcortical-cortical lesions were related to lower FSIQ, VC, WM and PS, in keeping with previous research (Westmacott et al., 2010).

Hemispheric effects are more mixed. Better outcome after PAIS in the right hemisphere has been reported, with left hemisphere lesions associated with lower scores on working memory, verbal memory, and receptive language (Allman & Scott, 2013). These authors found that infants in the sample were the only age group to demonstrate significant laterality effects, with left hemisphere patients performing significantly worse compared with right hemispheric stroke patients.

A longitudinal study of unilateral perinatal lesions suggested that smaller lesions in early life may have a greater impact on cognitive outcome over time (Levine et al., 2005). Children were tested twice, when less than seven years old and then when older than seven. Larger lesions were associated with poorer IQ on initial testing, but smaller lesions resulted in a significantly greater decline in IQ over time. This effect was seen regardless of lesion laterality or seizure status. These results may imply that functional plasticity following early brain lesions may inadequately sustain a normal rate of development due to limitations on processing capacities. This may lead to restricted plasticity for protracted skill development as task demands increase (Levine et al., 2005; Stiles et al., 2010).

Although there is less functional specificity in the child brain, with lesions resulting in more generalised than specialised effects on function, lesion location has been shown to account for some variance in outcome Lesions to the occipito-temporal pathway following PAIS can result in similar effects as in adults, with left hemisphere damage

impairing processing of sections of spatial array, and right hemisphere damage impairing configural processing. However, there is evidence to suggest that children with perinatal stroke may be more likely to demonstrate adaptive organisation. Though similar deficits are seen in children and adults, children's deficits may be milder. A number of studies looking at visual processing following perinatal stroke show mild, but lesion location-specific difficulties (Stiles & Nass, 1991; Stiles et al., 2008; Stiles, Reilly, Paul, & Moses, 2005).

Regarding cortical location, heterogeneity exists in methodology and outcomes. Firstly, an interaction of cortical location and age has been demonstrated. Perinatal subcortical, and childhood cortical lesions have been found to be related to lower FSIQs, whilst childhood subcortical lesions have been related to deficits in attention, WM and EF (Westmacott et al., 2010, 2018). This last finding fits well with our knowledge of the importance of subcortical structures such as the basal ganglia in EF skills, which has been well documented in both adult and paediatric neuropsychological research (Elliott, 2003; Leisman et al., 2014; Takeuchi et al., 2013). Fronto-striatal pathways and other complex interconnections from the subcortices and the prefrontal cortex are consistently related to basic attention and higher-order attentional functions (Alves et al., 2022; Arnsten, 2009).

Age-related differences in ipsilateral volume loss of subcortical structures in PAIS have been noted. A recent study found that thalamic volume loss is greater in children compared to adults following stroke (Mastej et al., 2022). Furthermore, another study found that ipsilateral hippocampal volumes were relatively preserved in younger PAIS patients, compared to older children suggesting differences in plasticity (Ritchey et al.,

2019). The volume of viable tissue in subcortical structures may be a contributing factor to cognitive outcome and warrants further research.

Other studies have found that although PAIS patients overall fell within the average range on FSIQ, those with combined cortical-subcortical lesions had significantly lower performance regardless of age, again highlighting the importance of lesion size (Studer et al., 2014; Westmacott et al., 2010). A recent study looked specifically at stroke lesions isolated to the cortical region in PAIS, and found several noted interactions (Peterson et al., 2019). Similarly to Studer et al., (2014), their sample of 27 children were performing generally in the average range, though with reduced performance on processing speed and fine motor coordination. They also found that right-sided lesions with frontal involvement were related to significantly poorer performance across cognitive tasks, and as similarly identified above, medium/large lesion sizes were also related to poorer outcome. In summary, age seems to play an overarching role in overall intellectual outcomes, however the cortical location can have domain specific effects within this broader age-mediated context. Overall, the interactions of lesion characteristics identified across the PAIS populations are shaping our understanding of cognitive trajectories. However, we need larger samples with consistency in methodology for further robust clarification of these interactions.

2.4.6. Current limitations in cognitive outcomes in PAIS literature

An important consideration when looking at cognitive outcome in paediatric stroke is the interaction of the above variables, which are summarised in Table 2.1. A comprehensive review by Fuentes et al., (2014) identified these particularly integral interactions for interpretation: "(a) Age at Stroke and Lesion Location; (b) Lesion Characteristics and Neurologic Impairment; (c) Lesion Volume and Time Since Stroke;

(d) Sex and Lesion Laterality; and (e) Seizures and Time Since Stroke" (p. 34). These effects are also dependent on the cognitive domain being measured, as they do not all reflect overall cognitive ability equally. Because cognitive outcome post-stroke is highly complex, it is difficult to accurately model in small samples, which are inevitably typical in these studies. Larger studies are emerging, with pooled data from multiple sites and paediatric stroke registries.

Additionally, most studies have used either no control group, made comparison to normative data, or a healthy control group. Using a chronic illness control group, (Hajek et al., 2014) found that the chronic illness controls also demonstrated cognitive deficits, albeit not as poor and widespread as the PAIS group. Most of these studies have been cross-sectional, and therefore, more prospective longitudinal research with well validated tools, considering developmental differences, is needed to replicate and extend current findings.

Another consideration when evaluating PAIS studies is exclusion criteria, in particular, exclusion of more severe cases, who are unable to complete testing. This approach may introduce bias into the results. Furthermore, as many studies are retrospective, there are limitations in control over assessment and protocol adherence. Future research into cognitive outcomes following PAIS, should base theoretical models on previous literature. As variance explained by factors already associated with cognitive outcome is controlled for, the accuracy of estimated variance related to variables of interest will improve.

Overall, in the last few years there have been many important developments in the understanding of neuropsychological outcomes following PAIS. With advances in neuroimaging, more detailed analyses of lesion characteristics are possible, particularly

hyperacute changes in ischaemia and perfusion. Improved understanding of changes in stroke infarct volume and the neuropsychological correlates of this is needed. This is particularly important, given the findings that lesion volume is associated with poorer cognitive outcomes. Neuroimaging approaches such as DWI and PWI which can non-invasively study pathophysiological changes following stroke, can provide more advanced understanding of brain-behaviour relationships.

Stroke variable	Effect on cognition / domains	Significant interactions with other variables
Age at stroke	Non-linear – conflicting evidence for worse younger and older aspects of PAIS including perinatal (FSIQ, VCI, PRI), but other evidence of the opposite direction (cognitive flexibility, PS, Verbal learning). Perinatal stroke = worse outcomes.	Stroke location: perinatal subcortical ↓ Childhood cortical ↓
Time of assessment since stroke	Highly variable – For some cognitive domains (such as executive function) that are not fully developed, deficits may become more evident longer term. FSIQ, WM, PS, deficits observable older than 7 years old.	Age at stroke – some sensitive domains such as EF may be more vulnerable to insult in the perinatal / very young age-group.
Lesion location	Subcortical stroke ↓ attention & executive function. Cortical stroke ↓ processing speed. Cortical-subcortical stroke ↓ general intellect	Age at stroke seems to mediate the effect of lesion location.
Lesion volume	↑ lesion volume = ↓ overall cognitive function	Neurological impairment (increased likelihood of post-stroke epilepsy) and time post stroke – possible mediation of effect. Time post stroke.
Lesion laterality	Heterogenous, but some indication of left lesions = ↓ language outcome	Age – perinatal stroke more likely to show evidence of reorganisation of language dominance.
Neurological co-morbidities	Post-stroke epilepsy & other neurological impairments increases the likelihood of poorer outcome (FSIQ and other domains)	Time post stroke – ongoing neurological change can be related to increased likelihood of stroke recurrence, and poorer outcome.

Table 2.1 – Main clinical and neurological variable interactions on cognitive outcome identified in the literature. ↑ (increase), ↓ (decrease). FSIQ (Full scale intelligence quotient), WM (working memory), PAIS (paediatric arterial ischaemic stroke), VCI (verbal comprehension index), PRI (perceptual reasoning index), PS (processing speed), EF (executive function)

2.5. Cognitive outcomes in adult ischaemic stroke

Stroke in adults commonly causes neuropsychological deficits which can result in disability, and indirectly affect patients' capacity to actively engage with rehabilitation. Following adult ischaemic stroke, patients can experience a number of cognitive and motor difficulties. Approximately 30% of patients experience neuropsychological deficits six to twelve months post-stroke (Ferreira et al., 2015; Nys et al., 2005; Serrano et al., 2007). Ferreira, Moro, & Franco, (2015) looked at the neuropsychological profiles of adults after their first ischaemic stroke and found a pattern of deficits in domains of attention, visuospatial ability, and verbal memory. Though this study did not consider lesion characteristics, several relationships with cognitive deficit were identified, including being female, more severe neurological deficits at presentation, (as determined by the National Institute of Health Stroke Severity scale [NIHSS]) and higher co-morbid functional impairment. Cortical strokes in adults have been found to be more likely associated with neuropsychological deficit than sub-cortical or infratentorial stroke (Nys et al., 2007). Aphasia and hemi-spatial neglect are commonly reported post-stroke, though are more likely following cardioembolic aetiology than large-vessel or small vessel disease, as are changes in working memory and attention, executive function, visual perception and learning (Cumming et al., 2013; Gottesman & Hillis, 2010; Hoffmann, 2001). Aphasia is more often seen following a left hemisphere stroke, whereas hemi-spatial neglect is more often seen following a right-hemisphere stroke. These changes can result from either the infarct itself, or in both the acute and chronic timeframe, from the involvement of areas of hypoperfusion adjacent to the infarct (Cumming et al., 2013).

2.5.1. Changes in cognition – a marker for cerebral hypoperfusion

Chronic hypoperfusion has been identified as an important factor for cognitive outcome following both childhood and adult stroke. Adult patients with global haemodynamic compromise from heart failure show deterioration in attention, executive function and memory (Festa et al., 2011; Vogels et al., 2008). These changes may be due to ongoing reductions in cerebral blood flow, but also due to higher likelihood of cardiogenic embolism. As chronic hypoperfusion can reduce cerebral grey matter volume, the observed effects on cognitive outcome may also be related to overall brain volume (Almeida et al., 2012). Recent research in children with stroke has also observed the negative effect of hypoperfusion on cognition, continuing up to two years post-stroke (Steiner et al., 2021).

Acute cerebral perfusion, which is an important focus for the remainder of this thesis, has also been demonstrated to affect changes in cognition in adults, which can be transient in the acute phase, where perfusion fluctuates (Gottesman & Hillis, 2010).

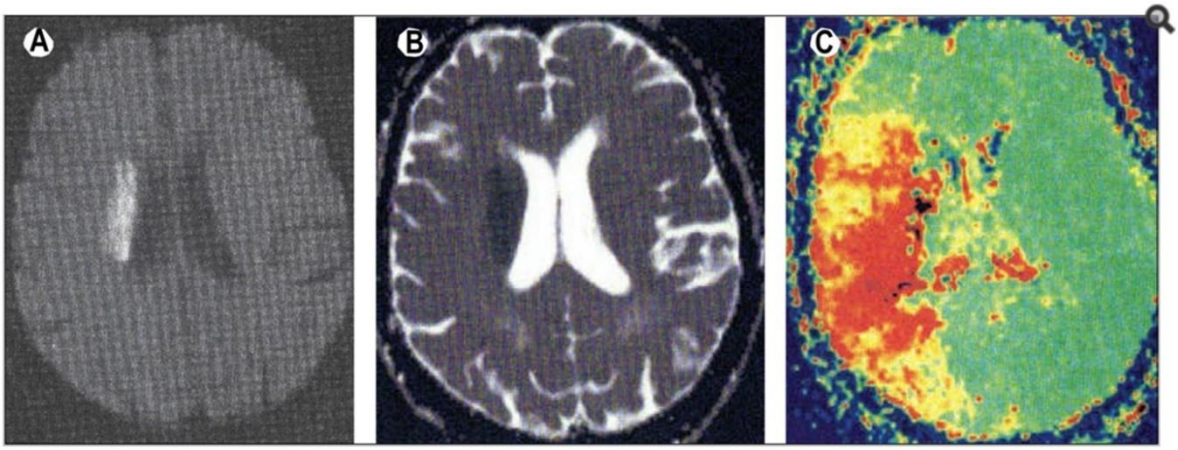

Figure 2.5 - MRI sequences from an 85-year-old woman with a right-sided infarct and a large area of hypoperfusion. The infarct is demarcated on diffusion weighted imaging (A) and apparent diffusion coefficient map (B), large area of hypoperfusion as seen on PWI imaging, demarcated by the area in red (C) Severity of ischaemia correlated with severity of cognitive deficit post-stroke.
Reprinted from *Lancet Neurology*, Vol 9, issue 9, Gottesman, R., & Hillis, A., Predictors and assessment of cognitive dysfunction resulting from ischemic stroke, 895-905., Copyright (2010), with permission from Elsevier.

Hypoperfused and dysfunctional tissue surrounding the infarct core (penumbra, see 3.1) has been shown to result in acute cognitive deficits, that can be improved with reperfusion therapies (Gottesman & Hillis, 2010). An example of this can be seen in Figure 2.5, which demonstrates the acute neuroimaging of an 85-year-old woman with a relatively small infarct as detected on DWI, but with a large area of hypoperfusion as seen on PWI. The degree of hypoperfusion correlated with the severity of cognitive deficit (Gottesman & Hillis, 2010).

Areas of hypoperfusion tend to re-perfuse or progressively be recruited into the infarct core within hours post stroke onset. Cognitive deficits beyond the acute phase are more likely to be due to infarcted tissue, rather than hypoperfused tissue. Acute changes in clinical picture that are seemingly more severe than the size of the infarct may be due to hypoperfused, dysfunctional tissue, and this "clinical mismatch" has been used as a selection criteria for reperfusion therapies in extended time windows in both adult and paediatric stroke research (Nogueira et al., 2018; Sporns et al., 2020). A further look at clinical-infarct mismatch is explored in section 8.5.

2.6. Summary

This chapter has presented a background of the important definitions and findings in stroke in general and delved more specifically into PAIS. Exploring the literature and understanding mechanisms of ischaemic stroke in adult populations is highly relevant, as much of our current approach stems from this literature. Adult stroke has a much higher prevalence and, therefore, has allowed more robust studies on the mechanisms, treatments, and outcomes. When looking at outcomes post-stroke, it was observed that there has been expanding research in paediatric cohorts, which has identified a complex picture of the multiple variables which may impact outcomes. Given that risk factors and

stroke aetiologies in PAIS are less modifiable than in adults, a search for ways to improve outcome has led me to look at adult treatments that have a direct effect on acute lesion characteristics. In the next chapter, I will review the theoretical definition of the ischaemic penumbra, which will become the primary concept going forward, and the key target of the thesis study questions and methods throughout the remaining chapters. I will also present the different neuroimaging techniques used to detect and define acute cerebral ischaemia in stroke.

3. LITERATURE REVIEW OF IMAGING IN ACUTE CEREBRAL ISCHAEMIA

The diagnosis and understanding of acute stroke have been greatly improved by the introduction of, and advances in neuroimaging techniques. This largely began with the advent of computerised tomography (CT) scanning in the 1970s, particularly in imaging acute haemorrhagic stroke (Davis et al., 2003). Conventional magnetic resonance imaging (MRI) became widely available about a decade later; however, clinical application of MRI in acute stroke has advanced significantly since this time, and enabled a noteworthy increase in research into stroke pathogenesis, treatment and lesion studies of brain-behaviour relationships (Kakkar et al., 2021; Kidwell et al., 2000; Turkeltaub, 2019). In

this chapter, I will discuss acute detection and quantification of ischaemia, imaging of the ischaemic penumbra, focussing on perfusion-diffusion mismatch, and paediatric-specific neuroimaging considerations. Neuroimaging is the key to confirming stroke diagnosis. In order to understand the applications of CT- and MRI based imaging, it is useful to first understand introduce the concept of the ischaemic penumbra.

3.1. The ischaemic penumbra

The term *ischaemic penumbra* was coined by Astrup and colleagues (1981) after a series of pioneering experiments conducted looking at the pathophysiology of cerebral infarction in baboon cortices. These researchers directly measured pathophysiological changes in brain tissue using multiple measures such as pH, potassium levels, and electrical activity, while inducing hypoxia by altering the cerebral blood flow. One of the key concepts

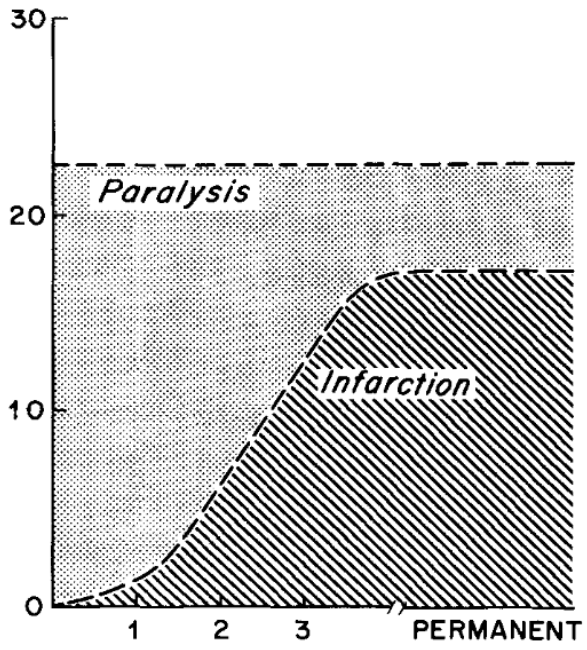

Figure 3.1 - Threshold of cerebral ischaemia, as a function of time. CBF (cc/100 gms/mins) by Time (hours of MCA occlusion)
From *Journal of Neurosurgery,* Jones, T. H., Morawetz, R. B., Crowell, R. M., Marcoux, F. W., FitzGibbon, S. J., DeGirolami, U., & Ojemann, R. G. (1981). Thresholds of focal cerebral ischemia in awake monkeys, 54(6), Reprinted with permission frm the Journal of Neurosurgery

underlying the definition of the ischaemic penumbra is that there are two ischaemic thresholds in the pathophysiology of cerebral infarction: one resulting in functional impairment of the affected tissue, and one resulting in morphological/structural damage (Astrup et al., 1981; Jones et al., 1981). These thresholds can be seen in Figure 3.1. Tissue became reversibly dysfunctional when local cerebral blood flow fell below 23mls/100gms/minute (penumbra). When the level of ischaemia increased to below 10mls/100gms/minute for 2 hours, it became irreversibly infarcted (core). However, these are not distinct tissue types or thresholds, as an extremely important factor is time. These areas of hypoperfusion are better conceptualised as an ischaemic zone consisting of penumbral tissue at different stages. Jones et al., (1981) noted that even profoundly ischaemic tissue remains reversible for a short period of time.

Conceptually, the ischaemic penumbra was described as this hypoxic brain tissue surrounding the infarct core, which is still morphologically intact, but functionally impaired. By definition, the hypoxic tissue damage is not severe enough to cause complete failure of energy metabolism, and the tissue is able to restore its physiological function if restoration of circulation and reperfusion occurs (Astrup et al., 1981; Paciaroni et al., 2009). This tissue is usually peripherally surrounding the irreversibly necrotic tissue (or infarct core), which may expand into penumbral tissue over time (see Figure 3.2). An important aspect of hypoperfusion surrounding the penumbra (and sometimes miscategorised as such) is benign oligemia, which appears as mild hypoperfusion. This is cerebral tissue which is not actually at risk of infarction, but recovering from tissue depolarisation, and is an important delineation from ischaemic penumbra (Chiu et al., 2018). As factors such as collateral blood supply, residual flow, and duration of blood flow disruption can impact the vulnerability of tissue to ischaemic damage, characterising penumbral tissue is dynamic, time-dependant and ill-defined (Paciaroni et al., 2009). The

necrotic core is also penumbral, even for a very small amount of time, before the necrosis propagates, which makes separating the hypoxic stage of the tissue difficult. As the penumbra is thought to represent "tissue at risk of infarction", imaging research has sought to differentiate this area from necrotic core to guide treatment recommendations. Reperfusion that is achieved early after stroke onset endeavours to prevent the infarct core from expanding into the penumbra, which is the target in current adult ischaemic stroke treatments, as described in section 2.3.

3.2. Acute ischaemia: stroke infarct detection

3.2.1. CT and MRI in acute ischaemic stroke in adults

Computerised tomography is often used acutely where there is clinical suspicion of cerebrovascular abnormality, due to its relatively quick acquisition time and cost-effectiveness (Tomandl et al., 2003). However, while CT has high specificity in acute cerebral haemorrhage, false-negative results are common in the context of acute ischaemic lesions, small lesions, and posterior circulation lesions (Chalela et al., 2007;

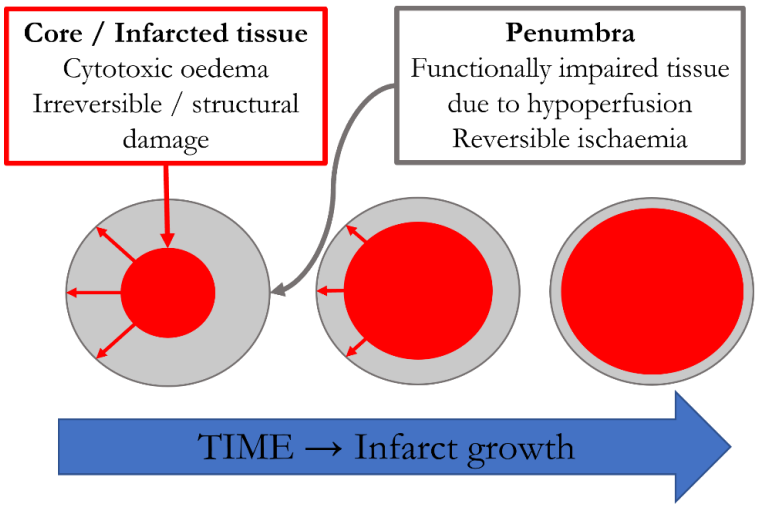

Figure 3.2 - Diagrammatic Representation of the ischaemic penumbra

Hwang et al., 2012; Potter et al., 2019). Conversely, DWI is very sensitive to acute ischaemia, and remains the most diagnostically robust modality in the acute setting (Bulut et al., 2014; Lansberg et al., 2000). This is especially important when diagnosis is uncertain to prevent erroneous diagnoses resulting in inappropriate treatment.

DWI is an MRI-based imaging method which measures amount of signal attenuation resulting from water molecule diffusion in different biological tissue medium. For example, the DWI signal contrast differs between the healthy brain grey and white matter. Diffusion of water molecules is restricted within the infarct core, resulting in less DWI signal attenuation compared to healthy brain tissue, hence, the infarct core appears hyperintense on acute DWI (Baliyan et al., 2016). DWI hyperintensities can be seen on acute images which are potentially indicative of cytotoxic oedema or due to enlargement and constriction of axons and dendrites (neurite beading) (Budde & Frank, 2010). Other imaging modalities, such as CT and MRI T2 weighted sequences are not as sensitive to early ischaemic change. Maps calculated to represent the degree of diffusivity of the water molecules in a particular location is an Apparent Diffusion Coefficient (ADC) map. A low ADC corresponds to high signal intensity (restricted diffusion) on a DWI image. Only acute infarcts are represented by low intensity on ADC maps (Fink & Caplan, 2003).

Figure 3.3 demonstrates the time-course of changes detectable on imaging following an acute adult ischaemic stroke (Baird & Warach, 1998; Gadian et al., 2000). ADC value drops early, indicating a restriction of the diffusivity of the intracellular water molecules. This changes when tissue necrosis occurs (i.e., breakdown of cellular membranes in the infarcted tissue), as the diffusivity increases. The ADC value approaches the baseline value around two-weeks post-onset in adults, which is known as pseudonormalisation. This does not indicate a truly normal state, as revealed by the increase in T2 signal

(Gadian et al., 2000). The time course of pseudonormalisation of ADC values may be slightly different in the developing brain due to differences in brain structure and metabolic maturity. One case report of two neonatal stroke patients suggested a slightly different pattern, demonstrating earlier ADC pseudonormalisation (around one week post-stroke) compared to 10-14 days in adults (Mader et al., 2002).

3.3. Acute ischaemia: stroke infarct measurement

3.3.1. Alberta Stroke Program Early CT score in PAIS

The Alberta Stroke Program Early CT score (ASPECTS) is a rating of ischaemic volume across cross-sectional CT imaging, and is often performed to both guide treatments and assess their outcomes (Barber et al., 2000; Beslow et al., 2012). This method, originally developed for use in adult stroke, uses a systematic quantitative scoring system, with a value assigned to areas throughout the middle cerebral artery territory. To have a point deducted from a normal CT rating of 10, each structure in the system must be expertly

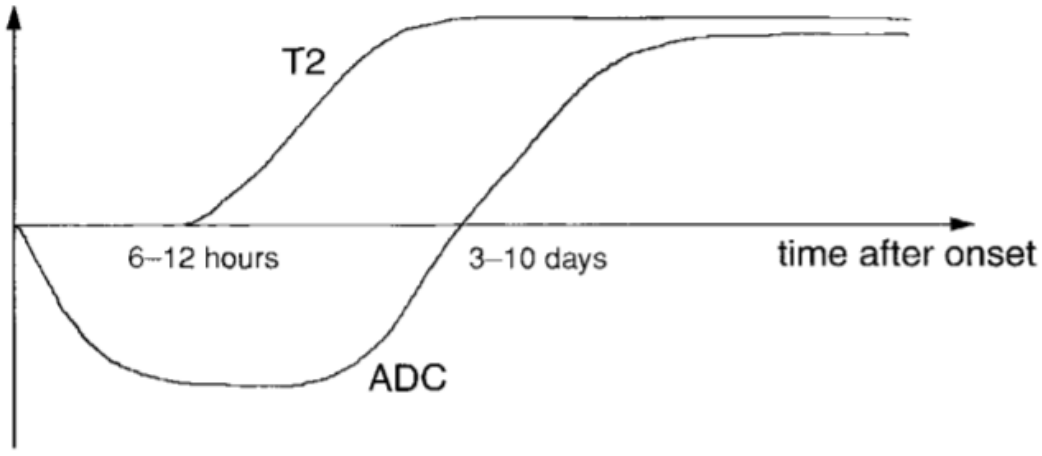

Figure 3.3 - Schematic time-course showing apparent diffusion coefficient and T2 changes following acute in adults stroke

From *Journal of Child Neurology,* Gadian, D. G., Calamante, F., Kirkham, F. J., Bynevelt, M., Johnson, C. L., Porter, D. A., Chong, W. K., Prengler, M., & Connelly, A. (2000). Diffusion and Perfusion Magnetic Resonance Imaging in Childhood Stroke 15(5), 279–283, using data from *Journal of Cerebral Blood Flow and Metabolism,* Baird, A. E., & Warach, S. (1998). Magnetic resonance imaging of acute stroke. 18(6), 583–609 Reused with permission from SAGE Publications

rated (generally by a neurologist, or neuroradiologist) as demonstrative of early ischaemic change (Barber et al., 2000). This score correlates with functional outcome and has good interrater reliability. This method was developed for CT, but has been applied to other types of imaging that detect ischaemic change, with this change defined by each method, including focal swelling or parenchymal hypoattenuation on CT, restricted diffusion on DWI, asymmetrical prominent veins on Susceptibility Weighted Imaging (SWI), hyperintensity on Fluid-attenuated Inversion Recovery Imaging (FLAIR), and prolonged mean transit time or reduced cerebral blood flow and volume on MR Perfusion (Kao et al., 2012; Tei et al., 2011).

A modified version of ASPECTS for paediatric assessment has been used (modASPECTS) (Beslow et al., 2012). The modified version was conducted using DWI and compared to manual segmentation of lesion volumes, showing a moderate correlation. This version adds a point rather than subtracting a point for each region, and also includes ACA and PCA regions for a maximum total score of 30. This study also categorised large strokes as ≥5% of supratentorial brain volume, with a paediatric ASPECTS of 5 or more correctly categorising stroke size with 80% sensitivity and 87% specificity. ModASPECTS correlates with stroke severity scores, haemorrhagic transformation, and functional outcome one year post stroke onset in PAIS patients (Beslow et al., 2021). This categorical scale, similar to other measurements in paediatric stroke such as the PSOM, are the current best measures that we have and can be calculated relatively quickly in the clinical setting, however, they are ordinal in nature, which limits the comparability and statistical analyses that are possible. Additionally, in terms of accurate volume estimation, each region in the scoring system is given equal weight, despite the varying sizes of the structures, meaning that the ASPECTS score may

underestimate or overestimate infarct volume, and there is no differentiation between a small amount of affected region and a large amount (Beslow et al., 2012).

3.3.2. Volumetric analyses of infarct core in adult ischaemic stroke

Image segmentation uses neuroimaging techniques to quantify regions of interest, by the process of assigning labels to pixels (in 2D images) or voxels (in 3D images) (Suetens et al., 1993). Segmentation methods can either be "low-level" – where imaging operators examine homogeneity amongst local visual characteristics to guide labelling, or "model-based" methods which use knowledge of object structure or shape to identify regions of interest. In medicine, image segmentation can be used in diagnosis and disease monitoring in a range of conditions, including characterisation of infarct core in stroke.

Understanding the size of an infarct core is advantageous for multiple reasons. In the acute time-frame, size of the infarct is an important variable that helps guide treatment decisions. Changes in infarct volume over time provide information about the presence of penumbral tissue, and the final infarct volume has been shown to be related to outcome (Ospel et al., 2021; Zaidi et al., 2012). Lesion studies can provide information about brain-behaviour relationships, and therefore accurate volume estimation is an important methodological consideration (Verma et al., 2022).

Lesion studies and segmentation take place in the context of time-related stroke progression and dynamic changes that become apparent in response to injury (Thiyagarajan & Murugan, 2021). These changes are visible as variation in tissue contrast, particularly between acute injury (< seven days) and chronic injury (≥ three months) (Carey et al., 2013). MRI modalities for infarct core segmentation include DWI, T1 weighted imaging, T2 weighted imaging, and FLAIR. DWI is the preferred modality for

acute stroke segmentation, whereas T2 and FLAIR images are more commonly used for chronic infarct segmentation (Carey et al., 2013; Rivers et al., 2007; Xavier et al., 2003)

The partitioning of images to segment stroke infarcts can be achieved through manual, semi-automatic, and automatic methods. Manual segmentation techniques are often used for volumetric analysis, particularly for research purposes, and are considered the gold standard for the measurement of chronic infarcts (Verma et al., 2022). They are generally executed by a trained clinician or radiologist, and commonly involve manual demarcation of the region of interest in a slice-by-slice manner (Starmans et al., 2020). The main drawback of manual segmentation techniques is that they are time-consuming to implement and can be prone to intra and interrater differences. However, a study looking at both the intra and interrater reliability of manual segmentation measurement for various methods including DWI, FLAIR, and Mean Transit Time (see section 3.4.3), showed low percentage change between raters and high intra-rater reliability. This reduction in measurement error was increased by holding sources of error constant such as differences in analysis software, MRI scanner, and pulse sequence parameters. This is an important consideration, as lesion boundaries are not often sharp in contrast, and signal intensities may be mistaken for non-pathological structures when using automatic software (Luby et al., 2006).

Semi-automated methods attempt to overcome the limitations of manual segmentation, with the added assistance of algorithms aimed at saving time or reducing the effort required by the user (Starmans et al., 2020). For example, this could be by expanding a segmented region over multiple slices to reduce the need for individual slice segmentation. This provides an element of objectivity into the analysis, however, there is still the possibility of interobserver variability due to the manual aspect of the method.

One such semi-automated method is the watershed transform method (Beare & Lehmann, 2006). This method is based on a geophysical model of rain falling on a terrain. An algorithm is used to determine boundaries of a lesion dependent on the minimum that water would travel down steepest mountains (analogous to the height of the intensity of the image). By manually demarcating areas of a lesion, regions of interest are produced, and can be manually edited if needed using imaging software.

Automatic, and semi-automatic segmentation techniques are more applicable for implementation within the acute clinical setting due to their rapidity of application. Automatic methods can be implemented with less need of user interaction, and are reliably reproducible (Paing et al., 2021). However, though they are more objectively measured, this does not preclude the need for expert interpretation, nor does it ensure accuracy or protection from systemic errors (Psychogios et al., 2021; Starmans et al., 2020). Computer-assisted software and programs which implement this technology play a major role in the treatment decisions and monitoring of disease progression within the clinical setting (Petrick et al., 2013).

A type of automated segmentation, threshold-based infarct segmentation, has been used to rapidly and automatically segment infarct volume in adult ischaemic stroke. An ADC value of $\leq 620 \times 10^{-6}$ mm^2/s has been validated in adult stroke research (Purushotham et al., 2015). Using Computed Tomography Perfusion (CTP, Section 3.4.3) a relative cerebral blood flow of less than 30 percent is often thought to represent infarct core (Yoshie et al., 2020).

An increase in the implementation of more advanced segmentation techniques which utilise machine learning are also becoming more common in the adult literature. A machine learning based method called Deep Learning uses algorithms which are

continually trained on large clinical acute stroke datasets. These are emerging to automatically segment lesions in stroke, and predict final infarct size (Bridge et al., 2022; Liu et al., 2021; Wang et al., 2022). A limitation with this type of automated segmentation is that they require large amounts of data to develop accurate models. As Deep Learning algorithms improve, these techniques may offer clinical benefits such as real-time interpretation of images, and suggestion of further studies that may be required during scanning, and could even provide further avenues for neuroanatomical atlas studies to inform prognosis (Bridge et al., 2022).

3.3.3. Infarct volume change over time

There is a change in visually inspected lesion volume over time in imaging analyses, due to swelling, reperfusion, and tissue loss (Zille et al., 2012). Infarct volume can also change over time, as hypoperfused tissue is recruited progressively into the infarct core. Studies in adults have shown that infarcts reach their peak volume approximately three days after onset, decreasing until a week post stroke, then reaching their final size between seven and 30 days post-stroke (Gaudinski et al., 2008; Lansberg et al., 2001). Gaudinski et al., (2008) compared lesion volume using fluid-attenuated inversion recovery (FLAIR) imaging 30 days after stroke onset, to 90 days after stroke. Although changes in volume did continue up to the 90-day time-point, there was no significant difference found between the two follow-up lesion volumes. This suggests that images taken 30 days following stroke are sufficient representations of final infarct volume. For chronic volumetric analyses, T2 FLAIR has been shown to be the most accurate, potentially due to the increase in spatial resolution and the reduction of oedema. (Luby et al., 2006)

3.3.4. Volumetric analysis in paediatric arterial ischaemic stroke

There are few studies that have used volumetric analyses to estimate infarct volume in paediatric stroke, with much of the earlier literature using visually based categorisations and ratings. A recent study by Jiang et al. (2021) used acute infarct volume measurement to look at the relationship between infarct size and various measures of outcome. Similar to the semi-automated watershed transform method mentioned in section 3.3, the authors manually segmented the borders of restricted diffusion on axial DWI images, while using ADC maps for visual comparison to prevent segmentation of T2 shine through. Automatic calculations of segmented infarct volume across three planes were estimated by utilising slice thickness and gaps between slices (Jiang et al., 2021).

Manual or semi-manual segmentation methods are time consuming, thus, finding an accurate and reliable way to rapidly segment infarcts is critical for clinical application, as the time scale of manual segmentation is suboptimal in acute stroke care. To do so, naturally, requires comparison of reliable manual segmentation techniques to automated estimation. Filippi et al. (2015) sought to address this with a reliability comparison of a computer assisted volumetric infarct segmentation and manual technique. They found that the volumetric results of the computer-assisted method and the manual method were highly correlated (mean infarct volumes 65.6mls vs 63.7mls respectively) and took significantly less time (<1 minute vs 7.3 minutes, respectively). They used a proprietary segmentation algorithm originally created for identifying lesions in the liver, which automatically segmented the infarct based on analysing active contours within the DWI image. The region of interest was then identified across neighbouring slices to automatically calculate an infarct volume. By also looking at outcome in relation to infarct

volumes using the PSOM, they found that higher volumes and infarct percentage were correlated with poor post-stroke outcome.

To understand infarct progression, volumetric segmentation of chronic lesions is necessary, however, methods seem to be less reliable in the literature compared to acute DWI segmentation. Chen et al., (2009) described the difficulties with chronic lesion segmentation of encephalomalacia on T2 images, as the infarct includes atrophy and transsynaptic degeneration, with the hyperintensity on these images unable to clearly differentiate. The clinical significance of final volume is also difficult to interpret as the individual differences between infarcts and brains make absolute comparison difficult. As the developing brain is constantly changing, chronic lesion volume may be underestimated due to overall brain growth, which also limits comparability. Further research is needed to improve characterisation of final infarct volume in children, in a way that is clinically meaningful.

3.4. Perfusion Imaging and Penumbra definition in adult ischaemic stroke

Perfusion imaging of the brain has been used to provide significant information about numerous neural conditions, such as CNS tumours, epilepsy, and stroke (Lee et al., 2021). Different types of perfusion imaging and their expansion over the years has been an integral tool in the identification of clinically significant salvageable brain tissue in acute stroke. Definitions of penumbra in adult ischaemia have expanded over the years, as research and technology has evolved, since the initial discoveries of morphologically intact yet dysfunctional tissue at risk of infarct progression were described first in animal studies. Clinical definitions have focused on finding non-invasive ways to image

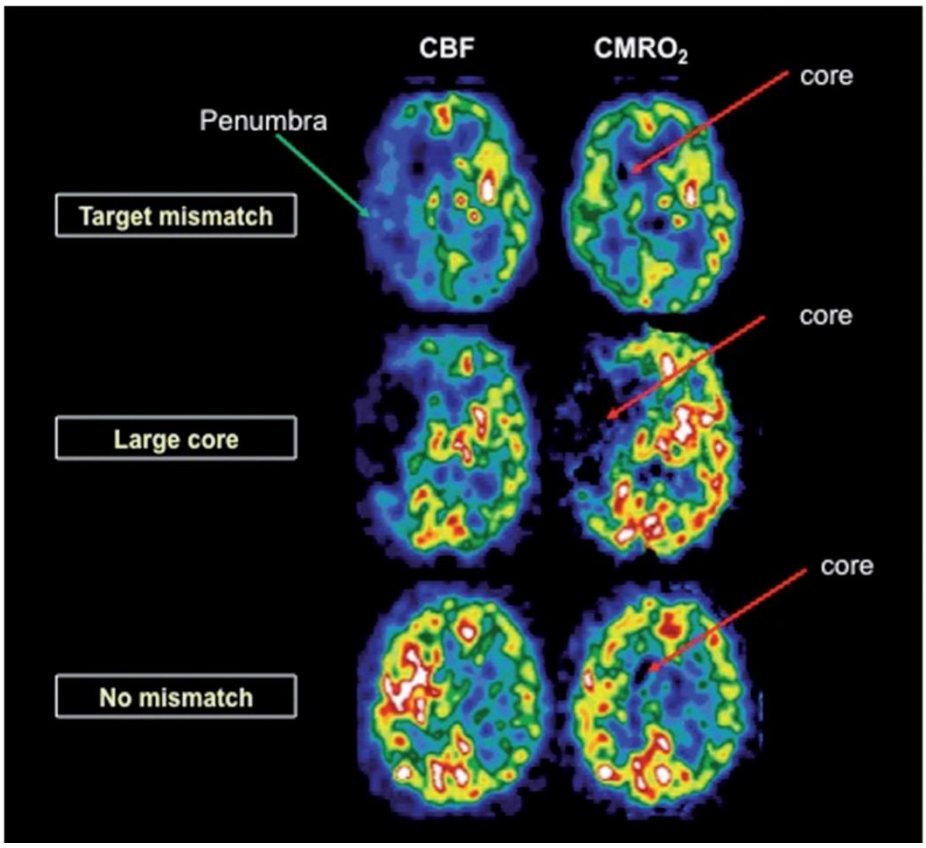

Figure 3.4 – PET images demonstrating penumbra and core.
Reprinted from Agarwal, S., Warburton, E. A. & Baron, J.-C., From Time is brain to Physiology is brain: a case for reflection in acute stroke treatment decisions, *Brain,* 2015, 138, 1768-1770, by permission of Oxford University Press, Modified/Reprinted from *The Lancet*, Vol. 341, Issue 8850, Marchal, G., Rioux, P., Petit-Tabou, M.C., Derlon, J.M., Baron, J.C., Serrati, C., Viader, F., de la Sayette, V., Le Doze, F., Lochon, P., Petit-Taboué, M.C., Orgogozo, J.M. PET imaging of cerebral perfusion and oxygen consumption in acute ischaemic stroke: relation to outcome, 925-927., Copyright (1993), with permission from Elsevier.

penumbra, for purposes of mechanistic understanding of pathological processes, and increasingly for treatment decisions, particularly following a delay to presentation. Each of these definitions have strengths and disadvantages and accordingly, some are used more often than others. The goal with each is, to distinctly define the ischaemic core and the area of hypoperfused tissue, to look for an observable mismatch.

3.4.1. Positron Emission Tomography

One such definition is using Positron Emission Tomography (PET) scans obtained with injection of ^{15}O-labeled radioactive tracer, which maps cerebral blood flow and oxygen metabolism biomarkers (Chalet et al., 2022). Research using this modality has delineated the core as very low CBF (which may be increased due to partial reperfusion that can occur in early infarction), low cerebral metabolic rate of oxygen ($CMRO_2$), and variable oxygen extraction fraction, (OEF). As can be seen in Figure 3.4, the penumbral tissue has low CBF (<20ml/100 g/min), with increased OEF and relatively preserved CMRO2 (Agarwal et al., 2015; Baron et al., 1984; Marchal et al., 1993). Although this provides an accurate prediction of penumbra due to the ability to quantitatively measure perfusion and metabolism of the tissue, the disadvantages of using ^{15}O-labeled PET include the limited availability and the expense of these scans, and the exposure to radiation (Ermine et al., 2021). The most commonly used isotope with PET scans is ^{18}F-Flurodeoxygluose (^{18}F-FDG) PET which measures glucose metabolism. The clinical utility of this isotope has not been extensively studied in acute ischaemic stroke management, though there has been consistent demonstration of reduction of ^{18}F-FDG uptake within the infarct core in animal studies (Fukumoto et al., 2011; Sobrado et al., 2011). There has been more variability in glucose metabolism in penumbra regions, with variance in temporal and spatial uptake reported (Bunevicius et al., 2013; Heiss et al., 1992; Nasu et al., 2002).

Therefore, more rigorous research is needed to determine the utility of ^{18}F-FDG PET to characterise penumbra in acute ischaemic stroke.

3.4.2. Single photon emission computer tomography

Single photon emission computer tomography (SPECT) is an imaging modality which uses a radioactive agent to evaluate perfusion and functionality of certain tissue types (Yandrapalli & Puckett, 2022). It has also been used to define penumbra, with the infarct core often defined by a cut-off > 70% reduction in tracer signal when compared to the analogous location on the contralateral hemisphere. The hypoperfusion lesion is often characterised as a slightly lower reduction in contrast signal, ranging from 40 – 70% (Heiss, 2000). SPECT scans are generally more available and lower cost than PET scans, however, the use of radioactive agents limits their use, and the analyses and estimation of penumbra is difficult to accurately determine. Figure 3.5 (a) shows what is likely the infarct core and hypoperfusion, as three days later (b) much smaller area of hypoperfusion is noted. Transient reperfusion ("luxury perfusion", see section 3.5) can be seen within two weeks (c) with the final infarct volume visible four months post stroke (d) (McArthur et al., 2011). SPECT images also tend to have coarse spatial resolution, and inaccuracies in interpretation can occur in the case of partial reperfusion (Ermine et al., 2021).

3.4.3. Computed Tomography Perfusion

Another commonly used low-cost measure of penumbra in adult ischaemic stroke is the use of CT perfusion (CTP), with penumbra defined by the area of prolonged mean transit time (MTT), decreased CBF, intact or increased cerebral blood volume (CBV), while infarct core is indicated by the area of increased MTT, and markedly decreased CBF and CBV (Lin et al., 2013; Wintermark et al., 2002, 2004) (see Figure 3.6 for an example of CTP to characterise penumbra, and Table 3.1 on pg. 53 for perfusion parameter definitions). A generally accepted definition of infarct core is similar to PET, with a very low relative CBF < 30%. CTP involves an injection of an iodinated contrast media, which helps assist the estimation of perfusion lesion, with a T_{max} >6s, or a delay time of >3s (Chen et al., 2021; Lin et al., 2016; Vagal et al., 2019). The disadvantages with this method

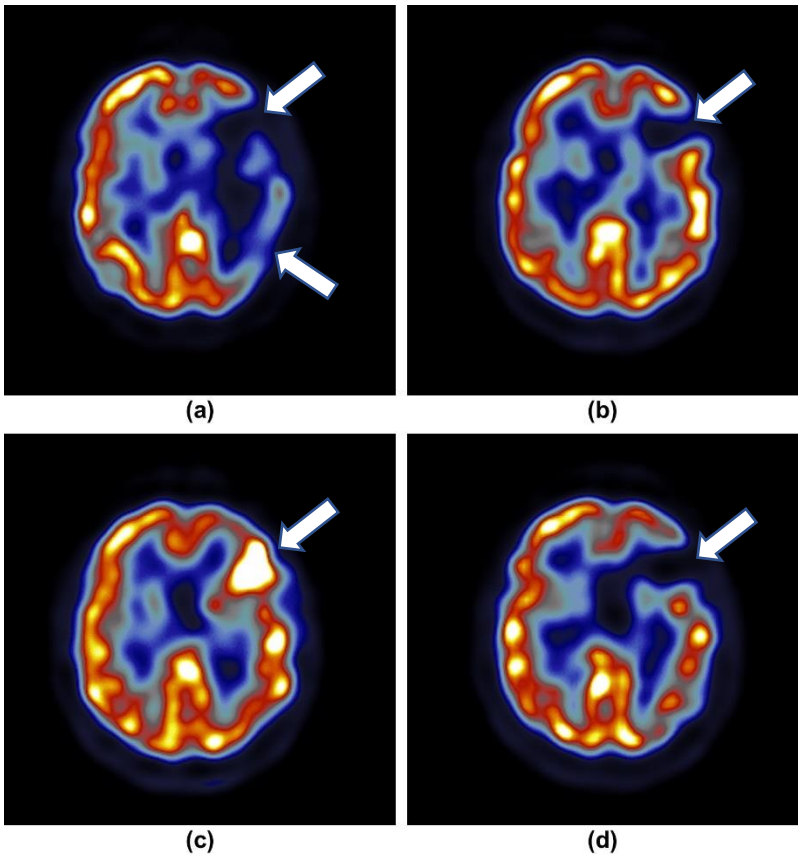

Figure 3.5 - SPECT scan acute and subacute images post-stroke. (a) acute infarct core and hypoperfusion (b) likely infarct core (c) luxury perfusion two weeks post stroke (d) final infarct volume four months post-stroke. Reprinted with modification from *Clinical Radiology,* Volume 66, McArthur, C., Jampana, R., Patterson, J., & Hadley, D. Applications of cerebral SPECT, pages 651-661, Copyright (2011) with permission from Elsevier

include the exposure to radiation, and the reduced reliability when compared with MR methods. The contrast used in CTP can also be contraindicated in patients with history of severe renal impairment or anaphylaxis to contrast media (Munich et al., 2016).

3.4.4. Dynamic Susceptibility Contrast Perfusion

Alongside CTP, the generally accepted "gold-standard" of penumbra estimation is the "Perfusion-diffusion mismatch". Dynamic susceptibility contrast (DSC) perfusion imaging, or bolus-tracking MRI uses an exogenous contrast agent (gadolinium-based) to monitor the first bolus of agent through brain tissue with a run of T2 and T2* weighted

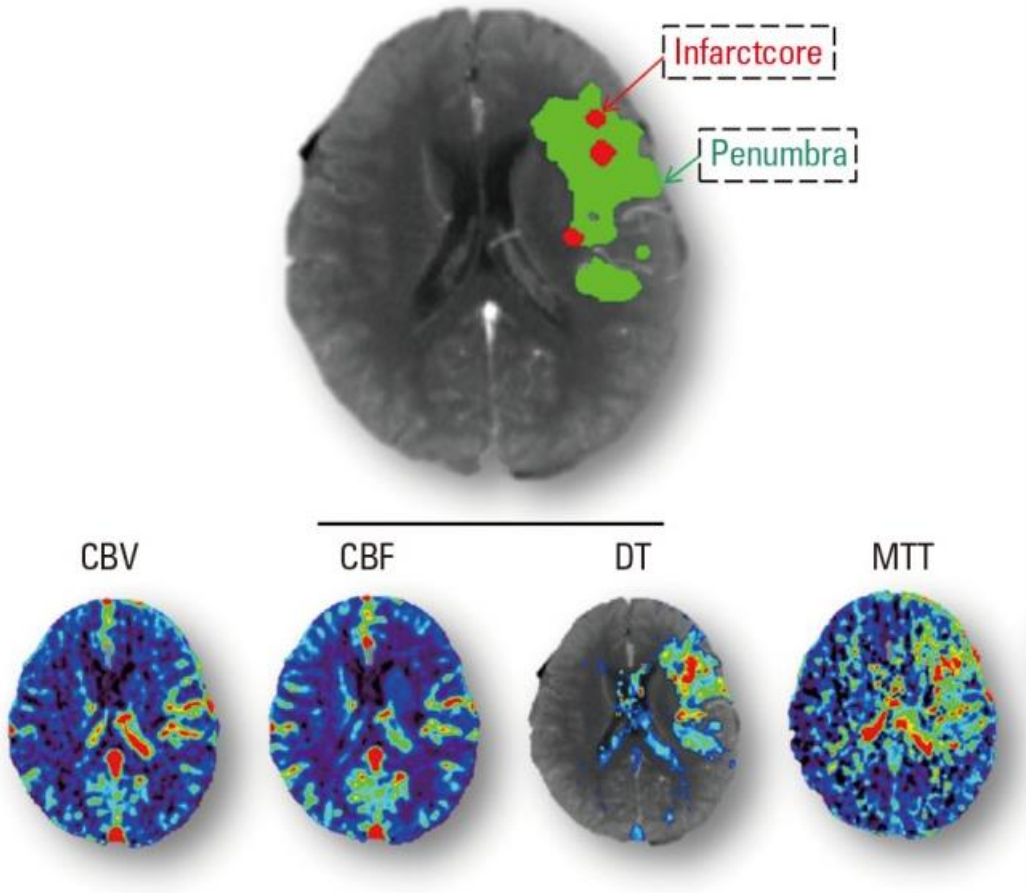

Figure 3.6 - Computed Tomography Perfusion Parameters for characterising penumbra. For each perfusion parameter map: CBV (Cerebral Blood Volume), CBF (Cerebral Blood Flow), DT (Delay Time) and MTT (Mean Transit Time), red/orange represents higher values, and blue/purple represents lower values.
Reproduced with permission under the creative commons licence from Lin L, Bivard A, Parsons MW. Perfusion patterns of ischemic stroke on computed tomography perfusion. *Journal of Stroke*. 2013 Sep;15(3):164-73.

images, to determine areas of cerebral hypoperfusion. From this CBF and CBV can be quantified, as can additional parameters MTT and T_{max} (see Table 3.1 for definitions, and Figure 3.7 for diagrammatic representation).

By subtracting the DWI lesion, which is the area of restricted diffusion, from the area of hypoperfusion, often defined by a T_{max} threshold of >6s, the remaining area is thought to represent at-risk and salvageable tissue. Studies suggest this to be the most accurate way to characterise the ischaemic penumbra within the clinical setting, however, MRI scanners may not always be readily available in a timely manner within emergency departments. There are also contraindications for some patients, such as those with implanted devices or claustrophobia (Ermine et al., 2021; Ghadimi & Sapra, 2022).

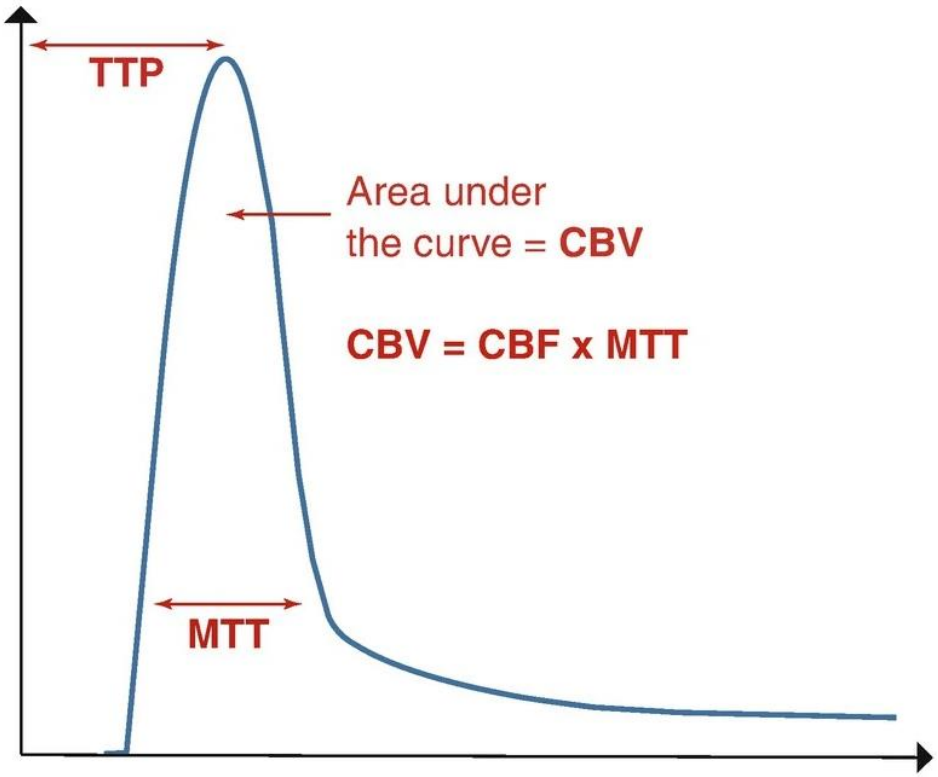

Figure 3.7 - Dynamic Susceptibility Contrast Imaging Perfusion Parameter Curve. Tracer Coneentration by Time (secomds)
From Advanced Physiologic Imaging: Perfusion – *Theory and Applications BT - Glioma Imaging: Physiologic, Metabolic, and Molecular Approaches* Kickingereder, P., Park, J. E., & Boxerman, J. L. (2020). *(W. B. Pope (ed.); pp. 61–91).* Springer International Publishing. Reproduced with permission

The use of DWI to detect infarct core has shown to have a sensitivity of 95% and a specificity of nearly 100% (Lövblad et al., 2001). As discussed in Section 3.1, DWI looks at the relative diffusivity of water molecules in cerebral tissue. Areas of restricted diffusion appear bright (high intensity) on DWI, potentially indicating areas of pathology, cytotoxic oedema, or structural damage.

Perfusion Imaging Term	Definition
Cerebral blood volume (CBV)	"The fraction of tissue volume occupied by blood" (Calamante, 2012, p. 293).
Cerebral blood flow (CBF)	"The rate of blood delivery to tissue" (Calamante, 2012, p. 283)
Mean transit time (MTT)	"The time that has elapsed between arterial inflow and venous output" (Sivaswamy et al., 2010, p. 268)
Time to peak (TTP)	"The time between injection and detection of maximal concentration of contrast in the region of interest" (Sivaswamy et al., 2010, p. 268)
Time to Maximum (T_{max})	"The time to maximum of the residue function obtained by deconvolution" (Calamante et al., 2010, p. 1167).

Table 3.1 - Perfusion Imaging terms and definitions

3.4.5. Non-contrast alternatives to penumbra estimation

Arterial Spin Labelling

Later sections in this chapter will explore imaging in paediatric cerebrovascular disease in more detail, but an important bridge between imaging in PAIS and adult ischaemia is the consideration of non-contrast perfusion estimation. DSC MR perfusion requires an injection of contrast agent, which can be a contraindication in pregnant patients and those with severe renal issues or previous reaction to contrast agents (Beckett et al., 2015). In PAIS, the question about the use of contrast is also related to concerns around cumulative gadolinium deposition in the brain, which is a consideration with the need of repeat imaging (particularly in clinical populations such as paediatric cancer). The risk in paediatric patients therefore, in the context of lifetime exposure risk, is higher than in

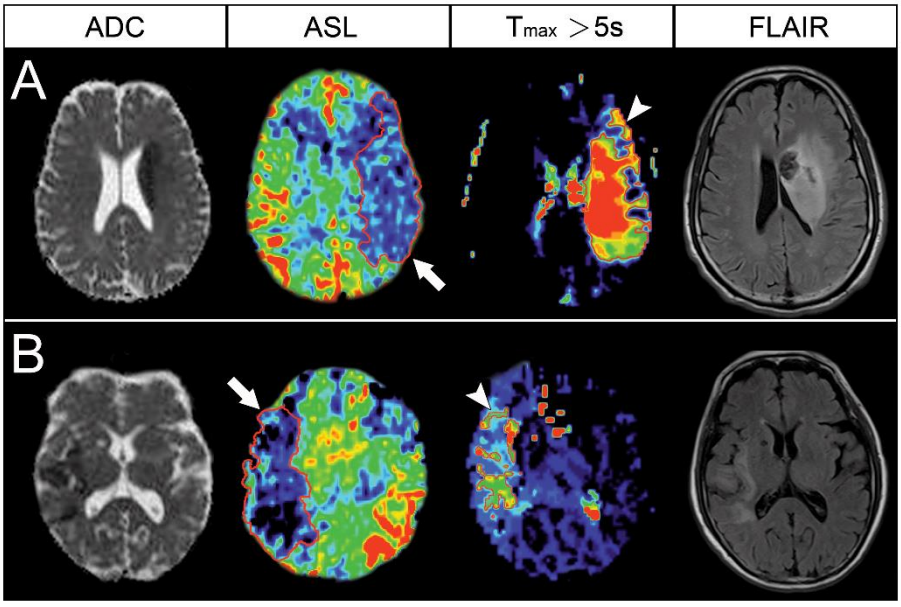

Figure 3.8 - Comparison of ASL to DSC perfusion (T_{max} >5s), showing an overestimation of hypoperfusion, with DSC closer to final infarct volume on follow-up FLAIR
From Huang, Y. C., Liu, H. L., Lee, J. D., Yang, J. T., Weng, H. H., Lee, M., et al. (2013) Comparison of Arterial Spin Labeling and Dynamic Susceptibility Contrast Perfusion MRI in Patients with Acute Stroke. *PLoS ONE* 8(7): e69085. Reproduced under Creative Commons Attribution Licence.

adults, though no clear clinical manifestations of such have yet been definitively identified (Guo et al., 2018; Zaki et al., 2020)

A non-contrast option for MR perfusion imaging is Arterial Spin Labelling (ASL), which uses an endogenous tracer consisting of magnetically labelled protons in arterial blood water by radiofrequency pulses, to measure cerebral blood flow. With this method, the core is estimated in the same way, however, the perfusion lesion is often defined as a CBF threshold, minus the core. Similarly, this is a rapidly obtained method which is quantitative, and allows for repeat scanning without contrast. Research has shown that ASL correlates with degree of stenosis, diffusion restriction and follow up T2 infarct volumes (Mastrangelo et al, 2022; Chen et al., 2009).

Importantly, ASL research has mostly been in adult stroke and therefore cannot be simply extrapolated to paediatric stroke. This is due to differences in site of intracranial arteriopathy (intracranial vs extracranial), the number of vessels that can be involved, and the developmental differences in CBF both across childhood, and between childhood and adulthood (Alloush et al, 2022; Jain et al, 2011; Parkes et al, 2004). ASL can also have poor spatial resolution, with Continuous ASL (cASL) being more likely to produce magnetisation transfer effects, and pulsed ASL (pASL) leading to a lower signal to noise ratio. The pulse pattern of cASL approaches a continuous inversion of flowing spins, whereas pASL seeks to label a fixed amount of blood, by a short inversion pulse (Chen et al., 2010). A third type of ASL, pseudo-continuous ASL or pcASL uses a similar continuous pulse sequence strategy like cASL, though with lower power deposition similar to pASL (Dai et al., 2008). On ASL CBF maps there can be artifacts detected due to slow collateral flow, and comparative research has indicated the possibility of ASL overestimating the perfusion deficit in adult ischaemia (Ermine et al., 2021; Huang et al.,

2013). Currently, ASL is not used widely in clinical practice, and is largely limited to research purposes (Meoded et al., 2014).

Susceptibility Weighted Imaging

Looking for other non-invasive ways to estimate perfusion changes in paediatric patients has been a focus across the literature in recent years. There have been some studies trialling these modalities in adult samples. One such estimation is to use susceptibility weighted images (SWI) to look at changes in the venous system as a proxy measure of arterial hypoperfusion. SWI is a high-resolution MR modality that is sensitive to paramagnetic substances, such as iron, calcifications, and blood products (Darwish et al., 2020). Some studies have used an overlay of acute SWI, and follow-up images such as T2 or CT, to look for areas of gliosis or encephalomalacia that exceed the area of acute SWI hypo-intensity, to define lesion growth or expansion (Meoded et al., 2014; Polan et al., 2015). More recently, SWI has been looked at as having a role to identify hypoperfused tissue in ischaemic stroke. Asymmetrically prominent veins, as detected on SWI are considered to represent hypoperfusion, and as such, have thought to be a promising non-contrast marker of penumbra, when compared with DWI infarct volume. Studies have compared a SWI-DWI mismatch to PWI-DWI mismatch (using MTT) in adult ischaemic stroke (Darwish et al., 2020; Kao et al., 2012). The authors used a categorical rating to define infarct growth, using DWI ASPECTS on acute and follow-up images, and mismatch was defined using ASPECTS rating for both MTT maps and SWI images. Their results showed a comparable estimation of perfusion deficit and a prognosticator of lesion growth, showing that SWI may be another important non-contrast alternative for stroke evolution prediction, particularly when standard PWI measures are contraindicated.

3.4.6. Selection for reperfusion treatment in adult ischaemic stroke

Adult trials have shown benefit with patient selection and outcome, with the assistance of contrast perfusion imaging and estimation of penumbra in patients with anterior circulation large vessel occlusion. A meta-analysis identified that the adjunct use of perfusion imaging with standard radiological assessment for patient selection for reperfusion treatment was related to independent functional status three months post-stroke (Ryu et al., 2018). This is a critical finding, as often patients who are selected for reperfusion treatment based on their perfusion imaging results, are those that do not meet standard time-limited criteria. A target mismatch profile was defined in a large adult stroke trial as MRI or CT-assessed ischemic core lesion volume ≤ 50ml, T_{max} >10s lesion ≤100ml, mismatch volume ≥ 15ml, and mismatch ratio > 1.8 (Albers et al., 2006; Lansberg et al., 2012). They also identified mismatch profiles that showed less or little

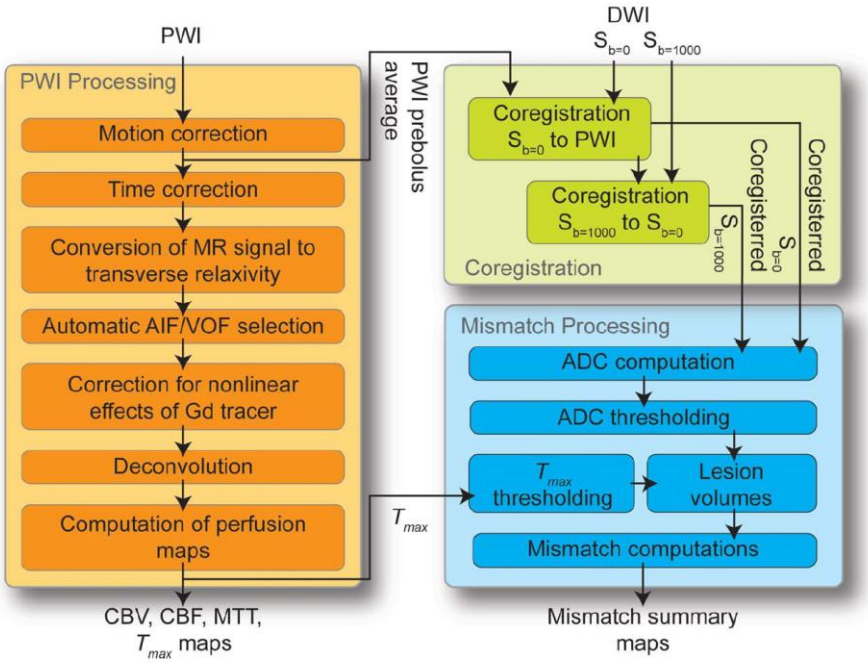

Figure 3.9 -Details of the RAPID process for automatic mismatch map calculations
Straka, M., Albers., G. W., Bammer, R. Real-time diffusion-perfusion mismatch analysis in acute stroke. Journal of Magenetic Resonance Imaging. (2010); 32: 1024-1037. Reproduced with permission by John Wiley and Sons.

benefit, or that were more likely to have adverse outcomes (such as a malignant profile). These findings were from studies that looked at the role of perfusion imaging and outcome, but did not necessarily use mismatch profiles to prospectively select patients. Further prospective research in adults identified that patients with a 'target mismatch' profile, as previously defined, showed more benefit to outcome, even in extended time-windows out to 24 hours post stroke-onset (Albers et al., 2018).

The aim of diffusion-perfusion (DWI/PWI) mismatch imaging is to acutely select patients who may benefit from reperfusion therapies with certain mismatch profiles (Straka et al., 2010). Providing reperfusion therapy to patients with very little mismatch or very large ischaemic lesion may not be beneficial and can even cause harm (such as complications from treatment, including haemorrhage). Generating DWI-PWI mismatch maps manually can rely heavily on user input and training, thus can be time-consuming in an area that is as time-dependent as acute-stroke treatment selection. A validated method designed to automatically quantify the mismatch to facilitate timely assessment and treatment in a clinical setting is RAPID "RApid processing of PerfusIon and Diffusion) (Straka et al., 2010). Using deconvolution of arterial and tissue signals, the RAPID system computes CBV, CBF, MTT and time until residue function reaches its peak (T_{max}) to produce perfusion maps. Diffusion-perfusion mismatch is then automatically quantified by infarct core segmentation (based on thresholded ADC maps) in comparison to hypoperfusion tissue volume based on T_{max} maps (Figure 3.9). This technique was validated for use in routine clinical assessment and clinical trials in adult stroke patients. RAPID showed very high correlations with manual quantification for both DWI and PWI, with 100% sensitivity and 91% specificity for mismatch identification (Straka et al., 2010). Other commonly used automated software programs, such as MIStar, OLEA and Syngo.Via, have shown varying degrees of core and

hypoperfusion estimation concurrence with RAPID (Psychogios et al., 2021; Suomalainen et al., 2022). However, so far only RAPID has been implemented in large adult stroke trials and has shown to be predictive of final infarct volume (Albers et al., 2016, 2018; Campbell et al., 2019; Nogueira et al., 2018).

By using this tissue-based patient selection, as opposed to previous time-based criteria, unnecessary endovascular intervention with risk may be avoided. Patients may have little benefit due to little or no salvageable brain tissue, or may have vessels which have spontaneously recanalised (Fink & Caplan, 2003; NINDS rt-PA Study Group, 1995). Spontaneous reperfusion following stroke in adults has been shown to be common, occurring in more than half of patients, and is associated with an improved outcome (Baird et al., 1994; Jorgensen et al., 1994). There are also patients who would benefit from tPA treatment, but are outside of the 4.5 hour window, who may be denied valuable treatment, without determining individual pathophysiological features, such as DWI-PWI mismatch. By using imaging techniques to estimate presence and extent of penumbral tissue, appropriate selection of patients most likely to have improved outcome can be achieved.

Recanalization versus reperfusion are both target outcomes during acute treatment of ischaemic stroke, therefore, patient selection requires consideration of those most likely to achieve these outcomes. The former relates to the reopening of an occluded vessel, while the latter relates to the restoration of cerebral blood flow to ischaemic brain tissue, including by collateral circulation. They are often used interchangeably, however, reperfusion can be achieved without recanalization, and is more highly correlated with favourable outcomes (Angermaier & Langner, 2016; Cho et al., 2015).

3.5. Perfusion imaging in PAIS – paediatric specific considerations

An important area for consideration in PAIS is collateral grading which has been explored in reference to lesion volume change and clinical outcome. Lee et al., (2022) found that collateral supply surrounding the infarct that was rated as good, defined by >50 % distal filling using either Magnetic Resonance Angiography (MRA) or Computed Tomography Angiography (CTA), was related to smaller final stroke burden and slower early infarct growth rate in childhood stroke. This was however, not related with better clinical outcome, as defined by the modified ranking scale (mRS) (which is a global outcome rating of post-stroke disability) and the PSOM. An important observation from this work, was that approximately half of their sample were rated as having poor collateral supply, and most of these participants were under five years of age. This was interesting, as often paediatric patients are thought to be likely to have good collateral flow compared to adults. It is not yet known from this study however, whether overall cognitive and behavioural outcome may have been affected by the reduction in lesion growth, as the mRS and PSOM do not capture more in-depth levels of outcome such as cognition, quality of life, mental health or behaviour. This robustness of collateral circulation, however, is a crucial contributing factor to our understanding of what constitutes a favourable mismatch profile in PAIS, which could attenuate growth of the ischemic core for a longer period by providing indirect perfusion (Lee et al., 2022).

As mentioned in Section 3.4.5, Arterial Spin Labelling has gained considerable attention for perfusion imaging in paediatric patients, due to not requiring contrast or radiation exposure. Despite this, there are noted limitations for the use of ASL in PAIS mentioned in Chapter 3.4.5, particularly limited paediatric studies and inability to simply extrapolate

from adult research. In small neonatal arterial ischaemic stroke samples, ASL has been used to explore perfusion abnormalities (De Vis et al., 2013). Wintermark and Warfield (2012) observed that within a small sample of four neonates with AIS, cerebral blood flow was increased within the stroke lesion in one patient and decreased in the core of the other. However, the periphery of the core demonstrated an increase in CBF. This is thought to represent the concept of "luxury perfusion", which describes transient reperfusion, or collateral flow and recanalization. This may indicate a protective mechanism against haemorrhagic transformation and can also be an indication of favourable tissue outcome (Wintermark & Warfield, 2012). In PAIS, one of the disadvantages of ASL mentioned earlier (the reduced signal to noise ratio), may be offset in children due to increases in water content and higher CBF. The changes in perfusion, however, caused by the need for general anaesthesia for image acquisition in some paediatric patients, may preclude this benefit (Lee et al., 2021; Makki et al., 2019; Proisy et al., 2016).

There is a paucity of longitudinal studies in healthy paediatric samples using DSC-MR perfusion. Contrast perfusion has been used in clinical samples, particularly those with brain tumours. One such study aiming to look at the feasibility, safety and quality of DSC MR images in children with brain cancer, identified higher quality perfusion maps comparable to adults, and no local or systemic adverse effects (Gaudino et al., 2019). There is limited data in the use of DSC MR perfusion in PAIS patients, which have been largely case studies. Additionally, it is not yet currently known whether adult definitions of infarct core and hypoperfusion are applicable in children, particularly to identify candidates for reperfusion therapies (Avital et al., 2022; Gaudino et al., 2019; Kulhari et al., 2017).

3.5.1. T_{max} thresholds for hypoperfusion in PAIS

T_{max} thresholds for the delineation of hypoperfusion levels in children may be different than in adult ischaemic stroke. It is feasible that the age of the patient, and history of chronic illness that effect the heart and/or lungs may influence change in cerebral perfusion needed to meet metabolic demand (Dehaes et al., 2015; Heit et al., 2021). Measures of cerebral perfusion, including CBV, CBF and MTT, have been found to change substantially as infants grow into toddlers (Wintermark et al., 2004). A longitudinal study of 96 children scanned initially between ages two to four, and followed up every six months, demonstrated that CBF across multiple cortical regions increases significantly over childhood in a linear fashion, peaking around the age of seven (Paniukov et al., 2020). Lee et al, (2019) noted that a T_{max} value of > 4s seemed to correlate better with clinical stroke severity (as measured by the PedNIHSS, see Section 8.5) than T_{max} >6s, and they also indicated a suggestion of influence of cardiac status. One patient with heart failure correlated with T_{max} >6s, and two without heart failure correlated with T_{max} >4s. This relatively longer hypoperfusion in those with impaired cardiac function may be explained by the impact of cardiac output on brain perfusion (Heit et al., 2021). Further research into the hypoperfusion thresholds across childhood in PAIS is needed. With these considerations in mind, no clear definition of penumbra in PAIS was identified in this literature review, with evidence so far of a mixture of methods and usage of adult parameters.

3.6. Resulting research questions from literature review

Throughout this chapter, I have explored the expanding role of neuroimaging techniques for identification and treatment of acute ischaemic stroke. I have encountered important definitions to inform my own methods going forward, and to highlight inconsistencies

and gaps in understanding particularly between the adult and the paediatric stroke literature. Initial observations from the paediatric stroke literature have highlighted issues with consistency and methodology. No clear consistent definitions are initially evident, as they are in adults. The heterogeneity encountered makes it difficult to clearly synthesise and present an understanding of penumbra in PAIS. A lack of comprehensiveness alongside a risk of selection bias in data collation delivered the first study aim for this thesis. As noted above, penumbral tissue remains an integral target in adult ischaemic stroke, and a central concept to the understanding of differences between the pathophysiology of ischemia between children and adults. As identification of the penumbra in PAIS is a key concept for this thesis, a solid, evidenced-based understanding of the current methods used in the literature was needed to enable methodological design. Therefore, a systematic review is warranted to more objectively understand the scope of published penumbra definitions in PAIS.

4. AIMS & HYPOTHESES

4.1. Study rationale

A comprehensive literature review is required to investigate how penumbral tissue has been defined in the paediatric literature. Findings from this literature review were used to inform study methodology for the thesis. Despite the differences in stroke aetiology and cerebrovascular development between children and adults, much remains unknown about the presence of, and the characteristics of penumbral tissue following PAIS. There is an urgent need to address this knowledge gap given the lack of clinical consensus and guideline about tissue perfusion-based candidate selection for acute reperfusion therapy in children. There is also little research into the evolution of penumbra and infarct core and their relationship to neurocognitive outcome. As existing evidence suggests that larger stroke lesions are associated with poorer cognitive outcome, this logically leads to

the question of whether presence of penumbra, which may lead to lesion growth, may also lead to poorer cognitive outcome post-stroke in children.

4.2. Aims, Study Questions, and Hypotheses

4.2.1. Study 1

In children with PAIS:

Question 1: How has the penumbra been defined in research studies?

- **Aim 1(a):** To summarise how different imaging modalities have been used to identify the ischaemic penumbra in the PAIS literature
    - **Hypothesis 1(a):** There will be heterogeneity in imaging modalities used to measure penumbra.
- **Aim 1(b):** To determine if there is consistency in definitions of penumbra in the PAIS literature
    - **Hypothesis 1(b):** Penumbra definitions will largely follow those used in adult ischaemic stroke.

As only one participant in the study sample of children with PAIS was treated with tPA, I aimed to answer the following study questions, by looking at the natural history of penumbra in the paediatric population.

4.2.2. Study 2

In children with PAIS:

Question 2: Is it feasible to identify the ischaemic penumbra, as defined by PWI-DWI mismatch, in PAIS patients, using automated software?

- **Aim 2(a):** To explore the feasibility of using automated perfusion-diffusion imaging software to characterise penumbra in PAIS.
    - **Hypothesis 2(a):** Penumbral tissue can be detected using automated perfusion weighted (PWI)/diffusion weighted (DWI) magnetic resonance imaging (MRI).

- **Aim 2(b):** To explore the relationship between time-to-imaging and ADC values to aid understanding of the utility of stroke lesion segmentation thresholds.
    - **Hypothesis 2(b):** That ADC values in children will follow a similar pattern to adults, which will affect the automated segmentation of infarcts in later time-windows.

4.2.3. Study 3

In children with PAIS:

Question 3: Is there evidence to suggest the favourable mismatch definition used (target mismatch in adults) represents "meaningful" penumbra, and is there any suggestion of changes needed?

- **Aim 3(a):** to investigate lesion volume change from acute to final infarct, and the relationship with penumbral definitions
    - **Hypothesis 3(a):** That chronic lesions will generally appear smaller than acute lesions, but the ratio of lesion volume change will be higher

(potentially representing lesion growth) in those demonstrating a favourable mismatch profile than those who did not.

- **Aim 3(b):** To explore the impact of clinical factors such as time from symptom onset to imaging, and aetiology, on lesion characteristics.
    - **Hypothesis 3(b1):** The acute lesion volume will correlate with time from symptom onset to imaging.
    - **Hypothesis 3(b2):** Penumbral tissue will be seen more often in children with cardio embolic mechanism than with other aetiologies.

4.2.4. Study 4

In children with PAIS:

Question 4: Are there clinical and lesion-specific imaging biomarkers which predict cognitive outcomes in these children**?**

- **Aim 4(a):** To investigate the relationship between imaging characteristics, such as acute and chronic lesion volume and location, and cognitive outcomes.
    - **Hypothesis 4(a):** Larger acute lesions, larger final infarct volume, those with penumbral tissue acutely, and those involving cortical and subcortical regions, will be associated with poorer cognitive outcomes.
- **Aim 4(b):** To explore the relationship between clinical factors including age, stroke severity, and aetiology, and cognitive outcomes.
    - **Hypothesis 4(b):** Age at stroke and stroke severity will be associated with more cognitive and neurological deficits, but the relationship with age will be non-linear.

5. STUDY 1: IMAGING OF THE ISCHAEMIC PENUMBRA IN PAEDIATRIC ARTERIAL ISCHAEMIC STROKE – A SYSTEMATIC REVIEW

As identified in Chapter Three, a delve into previous paediatric literature highlighted inconsistencies in the definition and understanding of penumbra. As outlined in Chapter 3, penumbral tissue is an important treatment target, and is inextricably linked to time-post-stroke onset. Therefore, having a clear definition and understanding of salvageable tissue has important ramifications for treatment and clinical decision making. The paediatric stroke literature in this area has lagged behind the adult counterpart for many reasons. It is, however, no less pertinent, and requires a nuanced understanding particularly as the developing brain changes so much from birth to early adulthood. A

systematic approach ensures that the aims and study design for further research both within the scope of this thesis and beyond can progress towards the goal of rapid patient selection of those most likely to benefit from reperfusion therapies in PAIS.

5.1. Study 1 – Manuscript under review (European Journal of Paediatric Neurology)

IMAGING OF THE ISCHAEMIC PENUMBRA IN PAEDIATRIC ARTERIAL ISCHAEMIC STROKE: A SYSTEMATIC REVIEW

Melissa J. Visser BA[1,2]

Mark T. Mackay, MBBS PhD[4,6,7,8]

Vanessa C. Rausa MPsych[1,6]

Jesse Shapiro PhD[1,2]

Joseph Yuan-Mou Yang*, MBChB PhD[3, 4, 5, 6]

Vicki Anderson*, PhD[1,2,6]

1. *Brain and Mind, Murdoch Children's Research Institute, Parkville, Australia.*

2. *Melbourne School of Psychological Sciences, University of Melbourne, Parkville, Australia.*

3. *Neuroscience Advanced Clinical Imaging Service (NACIS), Department of Neurosurgery, Royal Children's Hospital, Parkville, Australia.*

4. *Neuroscience Research, Murdoch Children's Research Institute, Parkville, Australia.*

5. *Developmental Imaging, Murdoch Children's Research Institute, Parkville, Australia.*

6. *Department of Paediatrics, University of Melbourne, Parkville, Australia.*

7. *Florey Institute of Neurosciences and Mental Health, Parkville, Australia.*

8. Department of Neurology, Royal Children's Hospital, Parkville, Australia.

*Authors J. Yang and V. Anderson are to be considered joint senior authors

Correspondence to: Ms Melissa Jane Visser, Brain and Mind, Murdoch Children's Research Institute, 50 Flemington Road, Parkville, Victoria, Australia. 3052.

Email: melissa.doyle@mcri.edu.au. Phone: 61-3-9345 5661. Fax: 61-3-9345 5977.

Cover title: Defining Penumbra in Childhood AIS

Number of Tables: 4

Number of Figures: 2

Search terms: Stroke, Ischemia, Paediatrics, Penumbra, Neuroimaging.

Subject terms: Paediatrics, Magnetic Resonance Imaging (MRI), Ischemic Stroke.

Manuscript word count of paper: 4495

Declarations of interest: None

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Highlights:

- No clear consistent penumbra definition in PAIS
- Penumbra definitions often follow adult models
- Most studies identified used visually-based criteria
- Significant heterogeneity exists in the current literature

Abstract

Background: The area of salvageable brain tissue surrounding an infarct (penumbra) in ischaemic stroke is the target for acute recanalization therapies in adults. Less is known about the pathophysiology of paediatric arterial ischaemic stroke (PAIS), although it is recognised that the cerebrovascular system and stroke aetiology are different in the developing brain. We aimed to determine if there are consistencies within the literature in both imaging modalities used, and definitions used, to characterise penumbra in PAIS.

Methods: Relevant databases were selected: Scopus, PubMed, Medline, and PsycInfo. Studies must have been original research and have used a neuroimaging modality to identify possible penumbral tissue in PAIS. A systematic review was performed from literature published up until December 2022. The primary outcomes were type of neuroimaging performed and the definition of identifying penumbral tissue in PAIS.

Results: Twenty-two papers met eligibility criteria. Penumbra definition across studies were highly variable with 14-visually based, 4 semi-quantitatively and 4 quantitatively defined, using 11 differing imaging modality combinations, the most common using DWI and MR contrast perfusion. Seven studies used non-contrast MR estimates of hypoperfusion. Only 3 quantitative studies used some overlapping definition criteria, which were based on adult studies.

Conclusions: There is little consensus regarding definition of the ischaemic penumbra in children, and imaging modalities used vary widely. Included studies were mostly low evidence retrospective case series with heterogenous study design, indicating the need for higher quality prospective studies with larger samples to provide more rigorous understanding of penumbra estimation in PAIS.

Key words: Stroke, Ischemia, Paediatrics, Penumbra, Perfusion, Neuroimaging.

5.2. Introduction[1]

Paediatric Arterial Ischaemic Stroke (PAIS) is a debilitating childhood cerebrovascular condition which results from a blockage or disruption to the blood flow in the arteries of the brain, leading to focal infarction. PAIS is a leading cause of morbidity and mortality in the paediatric population. It has a wide-ranging estimated incidence across studies, with a recent systematic review reporting an overall incidence of 5.6 per 100,000, with the highest rates in neonates (24.6 per 100,000 live births) (Oleske et al., 2021). Children with stroke, on average, live longer with the prolonged effects of their condition than adults. More than half of the survivors of paediatric stroke have ongoing neurological sequelae that significantly affect their development and quality of life. These children are also at an increased risk of recurrent strokes. In contrast to adults who can experience a loss of function, children with stroke can also experience an inability to achieve function, and often grow into their deficits (Anderson, Spencer-Smith, & Wood, 2011).

Treatment recommendations for paediatric stroke are extrapolated from the adult stroke literature, where the aetiology is largely thromboembolic. In contrast, PAIS aetiology is varied, and includes nonatherosclerotic arteriopathies, complications from certain infections and cardiac, neoplastic, haematologic, vascular and toxic conditions (Bernard et al., 2012; Gumer et al., 2014). Differences have also been documented in developmental haemostasis, clot composition, and function which may influence response to thrombolytics including tissue plasminogen activator (tPA), which are standard of care in adults (Ignjatovic et al., 2015). Thus, extrapolating data and treatment

[1] *Non-standard abbreviations: PAIS – Paediatric Arterial Ischaemic Stroke. MRP – Magnetic Resonance Perfusion*

recommendations directly from adult stroke literature may not be appropriate. Ischaemic stroke treatments focus on dissolution of clots (tPA) or physical retrieval of the thrombus through mechanical thrombectomy (MT). For PAIS, these treatments while allowable, based on consensus-based guidelines, are not approved by regulatory authorities due to a lack of randomised control trials which include paediatric patients (Ferriero et al., 2019; Medley et al., 2019; Royal College of Paediatrics and Child Health (RCPCH), 2017). While evidence of safety and efficacy of reperfusion therapies such as thrombolysis and MT is building from small prospective studies and case studies, application to PAIS populations still requires further research (Sporns et al., 2022).

Stroke symptoms in children are sometimes non-specific, which can result in delays to hospital presentations, accurate investigation, and diagnosis (Mackay et al., 2016; Rafay et al., 2009). The recent shift in adult practice, from time-based to tissue-based patient selection for reperfusion therapies, may have advantages for children. Research has shown treatment benefit to outcome, when adult patients have penumbral tissue identified on imaging, even if they present outside the standard time-window for thrombolysis and endovascular clot retrieval (Albers et al., 2018). The ischaemic penumbra in stroke refers to tissue surrounding the infarct core which is functionally impaired and at risk of infarction if blood flow is not restored in sufficient time. Left unsalvaged, this tissue is progressively recruited into the infarct core until it reaches the maximum volume originally at risk (Donnan et al., 2007). The advancement of imaging techniques in recent decades has led to exponential growth in non-invasive characterisation of the penumbra. One of the most common current definitions using magnetic resonance imaging (MRI) is the estimation of the mismatch between hypoperfusion indicated on perfusion MRI, and the infarct core, estimated by reduced apparent diffusion coefficient (ADC) of water molecules, identified by the area of

hyperintensity on diffusion-weighted images (DWI) (Donnan et al., 2007). In adult ischemic stroke, there have been attempts to quantify and stratify penumbral characteristics to identify patients most likely to benefit from reperfusion therapy, such as the DEFUSE/EPITHET criteria with perfusion diffusion mismatch defined as PWI lesion 120% greater than DWI lesion, with a target mismatch of DWI core <70mls, mismatch volume ≥15mls and ratio ≥1.8 (Albers et al., 2006). This study also identified mismatch profiles that might be less likely to benefit from reperfusion therapies, such as lesions <10mls, or even those at risk of adverse outcomes, such as a malignant profile with 100ml DWI lesion, 100ml PWI lesion on T_{max} >8s. The target mismatch profiles showed significant clinical benefit, with a later trial showing that benefit with endovascular thrombectomy extends up to 16 hours post-stroke onset (Albers et al., 2018).

Automated processing of multimodal imaging to estimate salvageable penumbral tissue, has been used in adult research to rapidly identify patients most likely to benefit from reperfusion therapies (Albers et al., 2018). This may be advantageous in the acute clinical setting without the need to rely solely on expert visual estimation, which is dependent on clinical judgement and timely availability within the emergency department. However, significant differences in automated core and penumbra segmentation have been identified across software packages, as well as artifactual hypoperfusion, therefore caution and expert oversight in interpretation has been recommended (Psychogios et al., 2021; Siegler et al., 2019).

Purushotham et al., (2015) suggested an ADC threshold for automated delineation of the infarct core from non-core voxels as an ADC value of $<620 \times 10^{-6}$ mm^2/s. Perfusion thresholds for both CT perfusion and dynamic susceptibility contrast-enhanced

perfusion MRI can be measured by the T_{max} value, which is the time it takes for the contrast bolus to reach the brain parenchyma from the proximal arterial circulation (Calamante et al., 2010). Adult studies have indicated that a T_{max} threshold of >6 seconds is the most applicable to adequately capture hypoperfusion deficit for automated penumbra estimation (Straka et al., 2010). From a large-scale research perspective, correlations between quantitative definitions of penumbral volume and treatment outcomes have been established in adults. No such specific thresholds have yet been determined in PAIS.

Lesion size on neuroimaging has been associated with poorer neuropsychological outcome in paediatric acquired brain injury (ABI), and paediatric stroke specifically (Ganesan et al., 1999; Greenham et al., 2016; Zecavati et al., 2014). In stroke, the presence of penumbral tissue may influence infarct size through treatment opportunities that reduces lesion growth. Infarct expansion into penumbral tissue could potentially impact the outcomes of the patient, which provides a strong rationale for the targeting of penumbra in paediatric AIS. An increased understanding of penumbral time-course and evolution unique to the child brain is warranted.

In this review, we sought to summarise how different imaging modalities have been used to identify the ischaemic penumbra in the paediatric stroke literature. We aimed to look at: (1) consistency in imaging modalities, and (2) consistency in definitions used in the literature to estimate penumbra in PAIS.

5.3. Methods

5.3.1. Search strategy

A systematic review was conducted with reference to the PRISMA guidelines (Moher, Liberati, Tetzlaff, & Altman, 2009). A literature search was performed on electronic databases Scopus, MEDLINE, CINAHL and EMBASE searching all available papers until December 2022. Search terms included the following themes and their related word-variants and MeSH terms: penumbra or mismatch, Arterial Ischaemic Stroke, childhood or paediatric, and neuroimaging, CT or MRI. Full search terms for each database can be found in Supplementary Material 1.

5.3.2. Eligibility Criteria

Inclusion criteria for this review included studies which met the following (a) English language (b) original research articles (conference abstracts excluded due to level of detail in methodology required), (c) human research with sample consisting of neonates or children with PAIS, aged between the perinatal period and 18 years (d) confirmed infarction on neuroimaging (e) the authors used neuroimaging to estimate or to quantify the presence and / or size of penumbra.

All studies from searches were imported into Covidence review software (Veritas Health Innovation, Melbourne, Australia. Available at www.covidence.org). Titles and abstracts were screened to identify papers which possibly met inclusion criteria. Full text review was performed by two independent authors to reduce selection bias (M.V. and V.R or J.S). Discrepancies between reviewer categorisations were resolved by consensus agreement. If papers met inclusion criteria and selection agreement, data was extracted.

5.3.3. Data Analysis

Age, sex and sample size, study aims and outcomes were collected. Neuroimaging modality used to identify penumbra, and definition of penumbra, if specified, was also retrieved. Frequency data from imaging modalities used for each study were obtained, and in-text definitions of penumbra or salvageable tissue were collated. Studies were rated as *quantitative* if a purely numerical definition was used, *visual* if a purely visual definition was used, or semi-*quantitative* if there was a quantitate component to a visual definition.

5.3.4. Quality of evidence

The overall risk of bias of the included studies (regarding their generalisability, causality, and validity of their study outcomes and / or interventions) was not particularly informative for the purposes of our systematic review, as we were aiming to synthesise description of penumbra, rather than their impact on interventions or outcomes. As much of this area of literature is case reports and for which there is no widely validated tool for the assessment of risk of bias, we used an amended version of a tool proposed by Murad, Sultan, Haffar, & Bazerbachi, (2018), based on convergent domains from previously reported criteria, combined with GRADE criteria (quality of evidence and strength of recommendations) (Atkins et al., 2004). Considerations for quality of evidence for the purposes of this review are detailed below:

1. Type of evidence (study design):
    a. case study (very low), observational study (low) and randomised control trial (high).
    b. retrospective or prospective.
2. Study quality (methodology):

a. Was penumbra clearly defined / replicable? Quantitative Penumbra (High), Clear Visual Penumbra Definition (Moderate).

b. Were all important data cited in the report / study?

3. Consistency

a. Was the penumbra definition used in other research studies?

b. Patient ages (clear and comparable)

c. Time to imaging (clear and comparable)

4. Clinical applicability

a. Consider quantitative vs visual definition

b. Potential feasibility of implementation in clinical setting (considering need for processing & expertise) (if applicable)

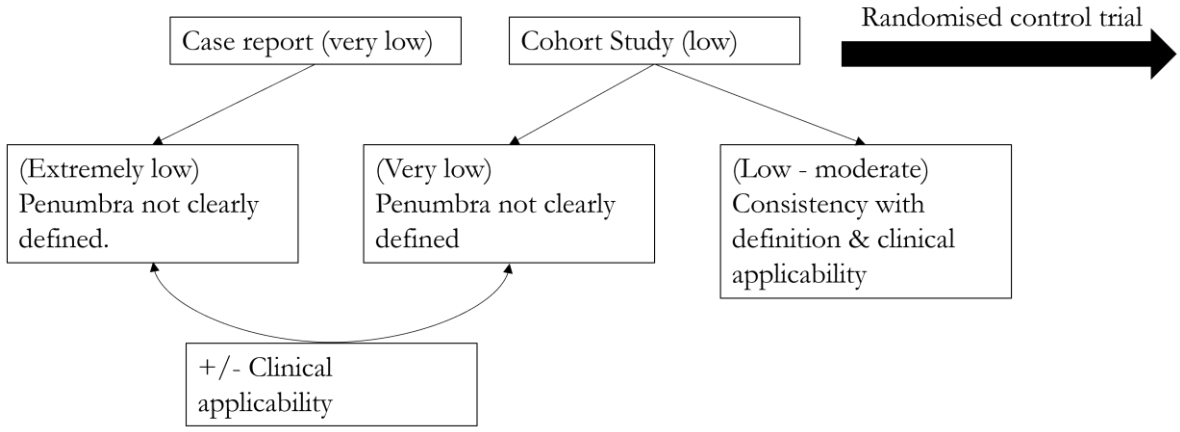

Figure 5.1 - Quality of evidence reference chart

5.4. Results

The initial search of the literature produced 1833 studies, following removal of duplicates (Figure 5.2). Full text evaluation on 274 studies detected during an abstract screen identified 22 studies which met inclusion criteria. A tabled summary of the extracted data

from included studies is presented in Table 1. For the purposes of our review, we grouped eligible studies according to their age group (neonates, children) and design (case study, retrospective and prospective cohort study).

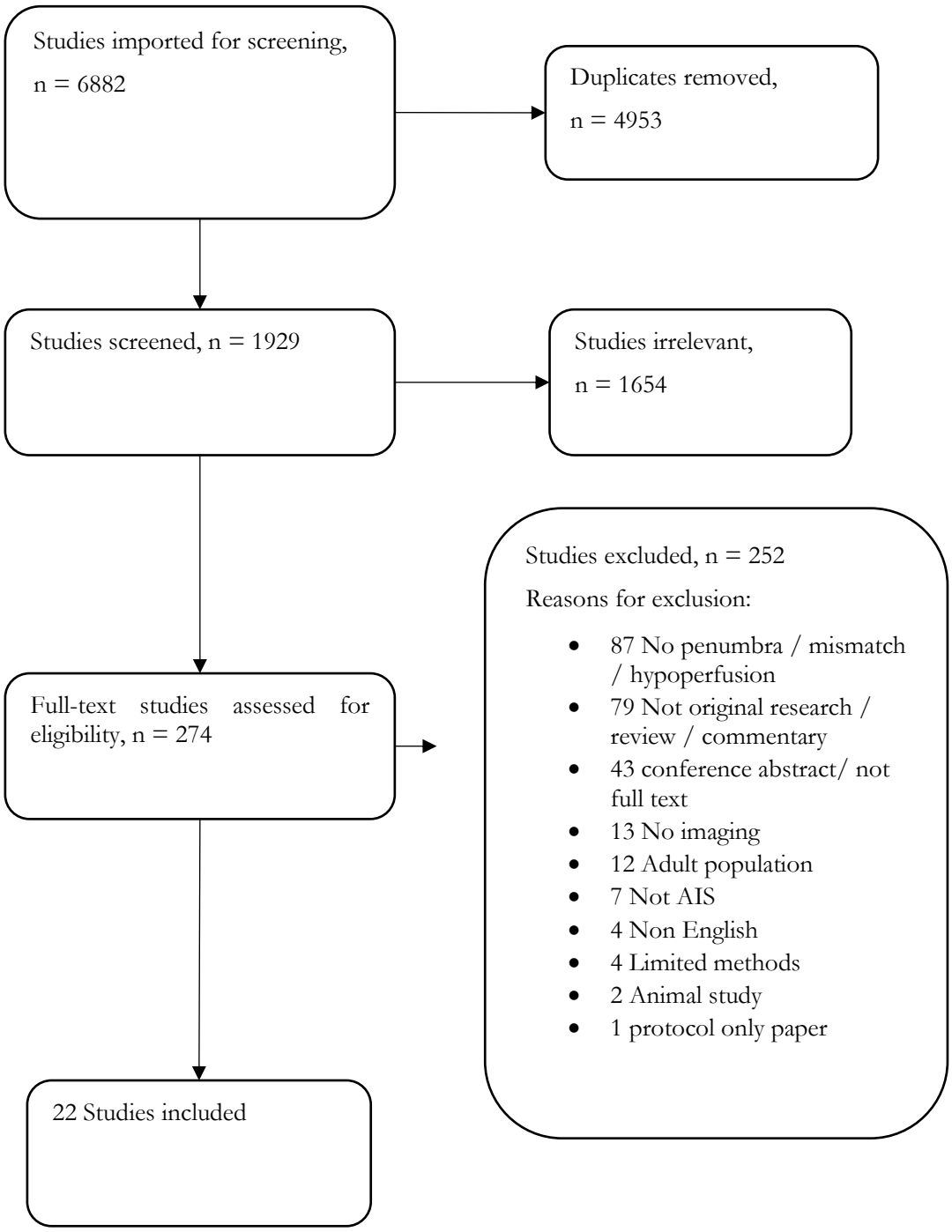

Figure 5.2 - PRISMA flow diagram

Authors	Study Design (N)	Neonatal / Perinatal or children	Time from symptom onset to imaging (if known)	Imaging modalities for Penumbra	Infarct core & Hypoperfusion definitions	Penumbra definition method
Watson, C.G., et al (2016)	Retrospective cohort study (N=25)	Neonatal	Median 2 days (range 0-8 days)	DWI, MRP (ASL), (or SWI if ASL poor)	C: ADC hypointensity regions H: Decreased perfusion on CBF map compared with the control region	Visual
Meoded, A., et al (2014)	Retrospective cohort study (N=8)	Combined	N = 1 (<6 hours), N = 6 (<24 hours), N = 1 (<72 hours)	DWI, SWI	C: Area of restricted diffusion on DWI H: Venous signal intensity & caliber (SWI) visually categorised compared to contralateral hemisphere (grading system)	Semi-quantitative
Polan, R.M., et al (2015)	Retrospective cohort study (N=24)	Combined	Mean 2 days (range 0 - 7 days)	DWI, SWI, MRP (ASL)	C: Restriction diffusion on ADC maps (visually graded for each arterial territory) H: Hypointense venous signal (SWI) (and CBF perfusion ASL in a subsample) visually graded for each territory.	Semiquantitative
Avital et al., (2022)	Case report (N=1)	Child	Unclear	DWI, MRP (contrast)	C: Restricted diffusion on DWI H: Mean transit time map (visually larger than core)	Visual
Bolognese, M., et al (2011)	Case report (N=1)	Child	Acute <5 hours (treatment time, imaging preceded treatment).	DWI, MRP (unspecified).	C: Restricted diffusion on DWI H: Perfusion deficit on MRP (unspecified)	Visual
Chalian, M.,et al (2011)	Case report of two cases (N=2)	Child	Case #1 - MRI 13 hours post, Case #2 - MRI 16 hours post	DWI, SWI.	C: Restricted diffusion on DWI. SWI hypointense intramedullary veins in area of acute ischaemia matched with DWI. H: Prominent hypo-intense veins on SWI, draining tissue greater than the area of restricted diffusion.	Visual
Kulhari et al. (2017)	Case report (N=1)	Child	7 hours	DWI, MRP (contrast)	C: Restricted diffusion on DWI (volume rapidly estimated by ABC/2 method) H: Increased MTT on PWI map	Visual
Fujimoto, M., et al (2013)	Case report (N=1)	Child	CTP done acutely (no specific time given).	CTP.	C: Decreased CBV on CTP H: Diminished CBF and prolonged MTT (extended beyond area of decreased CBV) on CTP	Visual
Gerstl, L., et al (2016)	Case report (N=1)	Child	CT approximately <1 hour post onset, followed by MR	DWI, MRA	C: Restricted diffusion on DWI H: Absence of diffusion restriction in territories occluded as detected by MRA	Visual

Pabst, L, et al (2022)	Case report (N=1)	Child	6 hours	DWI, MRP (ASL)	C: Restricted diffusion on DWI H: "Decreased perfusion" on ASL CBF	Visual
Sainz de la Maza, S., et al., (2014)	Case report (N=1)	Child	6 hours	CT, CTP.	C: Unclear, likely CT showing "early signs of right" MCA infarction. H: Perfusion CT revealed mismatch area – nil other details provided.	Semi-quantitative
Mittal, O.S., et al (2015)	Case report (N=1)	Child	Unknown	DWI, MRP (contrast)	C: Restricted diffusion on DWI H: Seen with mean transit time on MRP	Visual
Orman, G., et al, (2020)	Case report (N=1)	Child	First CT at 1-2 hours post. Acute MRI 8-9 hours post-onset	DWI, SWI, MRP (contrast)	C: Restricted diffusion on DWI H: Hypointensity of veins on SWI compared to contralateral area, matched with the area of altered perfusion on PWI	Visual
Tai, W.A., et al (2012)	Case report (N=1)	Child	3 hours 15 minutes	DWI, MRP (contrast).	C: Restricted diffusion on DWI H: Decreased CBV in corresponding core area, with elevated CBV surrounding infarct in the periphery of the MCA territory.	Visual
Van den Wijngaard, I., et al (2014)	Case report (N=1)	Child	Unknown. tPA within 4 hours of onset. MT five hours post-onset	CTP	C: Decreased CBV and CBF on CTP H: Increased MTT and TTP on CTP	Visual
Xavier, A., et al (2012)	Case report (N=1)	Child	3 days	CT & DWI, CTP.	C: Change on CT and restricted diffusion on DWI H: Increased MTT and TTP on CTP, with subsequent decrease in CBF also, with preserved CBV.	Visual
Lee, S., et al (2019)	Retrospective cohort study (N=12)	Child	Unknown.	DWI, MRP (contrast) or CTP	C: Using RAPID software. Infarct volume on DWI (ADC value <620 x 10^{-6} mm/s) or CBF <30% on CTP (not explicitly stated, standard for RAPID) H: MRP or CTP T_{max} >6 seconds, but also looked at T_{max} >4s.	Quantitative
Sporns, P.B., et al (2019)	Retrospective cohort study (N=12)	Child	Symptom onset to thrombectomy: 2 hours (range:1-4)	CTP	C: CBV maps on CTP (visual) H: CBF approximately 30 mL/(100 gms/minute) defined as hypoperfusion.	Semi-quantitative
Sporns, et al., (2021)	Retrospective cohort study (N=20)	Child	Median 5.6 hours, IQR 7 – 14.6	DWI or CTP (core)	C: ADC <620 $\times 10^{-6}$ mm^2/s (if MRI was performed) or CBF <30% (if CTP was performed). H: Inferred by presence of clinical deficit defined by PedNIHSS score (as per DAWN study).	Quantitative

Zebedin, D., et al (2013)	Retrospective cohort study (N=10)	Child	N = 1 (3 hours), N = 4 (3 - 6 hours), N = 4 (6 - 9 hours), N = 1 (9 hours).	CTP	C: Decreased CBF, CBV and increased MTT on CTP H: Decreased CBF and normal or elevated CBV	Visual
Visser, M.J. et al, 2021	Retrospective cohort study (N=29)	Child	Median 13.7 hours	DWI, MRP (contrast)	C: Infarct volume on DWI (ADC value <620 x 10^{-6} mm^2/s) H: MRP or T_{max} >6 seconds	Quantitative
Chen, J., et al., (2009)	Prospective cohort study (N=10)	Child	Range of 4 to 125 hours post symptom onset (Median 24.2 hours).	DWI, MRP (ASL)	C: Restricted diffusion on DWI H: CBF values<60% (2 SDs) of the mean CBF value in the contralateral ROI.	Quantitative

Table 5.1 - Data extraction results. ADC (Apparent diffusion coefficient), DWI (diffusion-weighted imaging), MRP (Magnetic Resonance Perfusion), MTT (Mean Transit Time), TTP (Time to Peak), CBF (Cerebral blood flow), CBV (Cerebral Blood Volume), CT (Computed tomography), CTA (computed tomography angiography) CTP (Computed Tomography Perfusion), SWI (susceptibility-weighted imaging), ASL (arterial spin-labelling). C: Core, H: Hypoperfusion, SD (Standard Deviation), ROI (Region of Interest).

Perfusion Modality Combinations (core/hypoperfusion)		Study design		Penumbra definition	
DWI/MRP	6	Case report	13	Visually-based	14
CTP (core & hypoperf)	4	Retrospective cohort	8	Semi-quantitative	4
DWI/ASL	3	Prospective cohort	1	Quantitative	4
DWI/SWI	2				
DWI/MRP or CTP	1				
DWI or CTP (core only)	1				
DWI/SWI (& ASL)	1				
DWI/SWI & MRP	1				
CT/CTP	1				
DWI/MRA	1				
CT & DWI/CTP	1				

Table 5.2 - Number of studies identified in each category. DWI (diffusion-weighted imaging), MRP (Magnetic Resonance Perfusion with contrast/unspecified), CT (Computed tomography), CTA (computed tomography angiography) CTP (Computed Tomography Perfusion), SWI (susceptibility-weighted imaging), ASL (arterial spin-labelling).

5.4.1. Study demographics

Sample sizes across included studies ranged from one to 29 participants. Age at stroke was also highly variable, ranging from neonates to 18 years. Reasons for imaging were to: describe perfusion or penumbra pattern without intervention (in 3 studies), to identify penumbra non-invasively (in 5 studies), and to characterise perfusion patterns to guide endovascular treatment or describe their outcomes (in 14 studies). Most eligible studies were case reports (59%). Penumbra definitions were implemented in the acute clinical setting in 64% of studies. Further information about acute feasibility is in Appendix A.1

5.4.2. Study Quality

As demonstrated in Appendix A.2, overall quality of evidence was rated as Very Low-Low (1), Low (5), Low-moderate (11), Moderate (2), Moderate-High (3). Acute clinical applicability of each definition was estimated in combination with level of methodological detail provided for penumbra estimation across studies. Level of expertise and time for implementation in the acute system was considered, with overall results to be mostly rated in the Low-Moderate level (64%). There were three studies rated as Moderate, two of which had a degree of visually-based quantitative estimation required and were implemented acutely. One study was purely visually estimated, though with clear criteria which progressed within the acute clinical period. Only three studies were rated as High in this category as they utilised automated segmentation software, or quantitative criteria which can be rapidly implemented to estimate penumbra.

5.4.3. Consistency of imaging modalities

There was wide variation in the imaging modalities used to approximate/characterise penumbra, with 11 different combinations identified in this review. The most common techniques were aligning with adult studies, using DWI/PWI mismatch (with contrast) (27%), or changes in CT perfusion parameters (18%) to delineate core and hypoperfusion. An additional study used a combination of these techniques, while another two used CT (and/or DWI) to estimate core alongside CTP for hypoperfusion. For core segmentation, most studies (64%) used only DWI to segment or visualise the infarct core, but five studies used CT and/or CTP, with three studies using a combination of DWI and/or CT/CTP. One of these studies used imaging to assess the core infarct alone.

A further seven of the 22 studies utilised non-contrast perfusion imaging techniques to estimate hypoperfusion, all using DWI to estimate core. SWI was used as an alternate measure of hypoperfusion in four studies, two of which had an additional perfusion modality used. ASL was used in three studies.

5.4.4. Consistency of penumbra definitions

Most studies used qualitative, visually based definitions, where the area of hypoperfusion was rated as larger than the infarct core (68%). Full definitions taken from the text of each paper can be seen in Appendix 1.

Four studies used a semi-quantitative approach, which combined visual estimation with a degree of quantitative assessment. One used CT perfusion, visually defining hypoperfusion as areas with ≤ CBF 30 mL/(100g brain tissue min) (Sporns et al., 2019). One study defined the penumbra as demonstrating hypoperfusion as greater than two thirds of the infarct core (Sainz de la Maza et al., 2014). Two studies using SWI involved expert categorisation using visually graded criteria to define areas of hypoperfusion and mismatch with infarct core (Meoded et al., 2014; Polan et al., 2015).

Only four studies used a purely quantitative method to define penumbra with clear criteria, though there was only overlap in criteria within three studies. Three of these studies implemented the DEFUSE-3 or DAWN trial criteria using perfusion/diffusion mismatch using contrast MRP or CTP, and/or an ADC value of <620 x 10-6 mm^2/s for the infarct core (Lee et al., 2019; Sporns et al, 2021; Visser et al., 2021). Another study used ASL, with hypoperfusion defined by comparison to perfusion in the unaffected hemisphere, and a mismatch > 20% of the infarct core defined on DWI (Chen et al, 2009).

5.5. Discussion

Neuroimaging techniques used in adults to measure penumbra are feasible in children at the acute phase of stroke, including neonates. However, there is no current consensus or definition for the imaging approximation of the ischaemic penumbra in PAIS. We aimed to examine the imaging modalities used to characterise penumbra within the paediatric literature, and overall, there were 47 children identified as having suggested salvageable tissue or mismatch across studies, using more than 11 different combinations of imaging modalities, and ages ranging from neonates to 18 years old. Though two studies in this review identified that RAPID software may be used in PAIS, more research is needed to validate appropriate penumbra parameters in children, while taking into account clinical feasibility of imaging acquisition and processing.

The second aim of this review was to examine consistencies within the definitions of penumbra in the PAIS literature, with variance across definitions identified. Almost all studies consistently defined penumbra by a larger looking area of hypoperfusion when compared to the infarct core. This was mostly accomplished from a visually larger appearance of hypoperfusion when compared to the infarct core, with quantitative definitions explored further below. Consensus for the representation of what is meaningful or favourable penumbra was not identified in this review and requires further research. This concept is largely represented by penumbra characterisation that leads to patient selection for reperfusion therapies, with some studies defining favourable mismatch, or penumbra within the context of patient selection criteria.

5.5.1. Study quality

The quality of studies identified in this review were largely of low quality in terms of sample size, study design, and information provided in penumbra definition. Due to the rarity of paediatric stroke presentations, the sample sizes were frequently small, with the majority being case studies. Therefore, in terms of type of evidence, most studies were rated Very Low to Low. When considering the definition of penumbra, availability of relevant information, and potential clinical applicability, there was more spread in the quality of evidence rating, with five studies that had an overall rating of Moderate, to Moderate-High.

5.5.2. Non-contrast hypoperfusion methods

The use of a contrast injection, such as gadolinium, for MR perfusion is not universally routine in paediatrics, due to concerns around gadolinium deposition in the brain and nephrogenic fibrosis in individuals with renal impairment (Miller et al., 2015; Nardone et al., 2014). This accounts for the attempts to identify non-invasive techniques to measure hypoperfusion in children.

Although contrast MRP or CTP was one of the most common modalities in the estimation of penumbra in over one-third of cases, some paediatric studies explored non-contrast alternatives to characterise hypoperfused brain. Several studies used susceptibility weighted imaging (SWI), a non-contrast neuroimaging modality, which looks at the magnetic susceptibility from blood and other tissues such as hemosiderin, calcifications, or air (Chalian et al., 2011; Orman et al., 2020; Polan et al., 2015). No exogenous tracer is required, instead the magnetic signal from deoxygenated blood in the venous system is used to identify areas of perfusion abnormality (Thomas et al., 2008).

These studies aimed to detect a 'venous ischaemic penumbra' by superimposing DWI images onto SWI images. Mismatch was defined as visually larger areas of prominent hypointense draining veins on SWI, which have been noted as a marker of ischaemic brain tissue, that exceed the area of restricted diffusion, or infarct core, on DWI. This modality has been used in adult stroke research, demonstrating lesion growth in those with a higher number of affected vascular territories on SWI compared with DWI (Darwish et al., 2020). As SWI data are often collected as part of standard stroke MRI protocols, this technique may be clinically useful, however more feasibility data is needed, particularly as this is a qualitative technique which requires expert visual estimation.

Arterial Spin Labelling (ASL) measures tissue perfusion, similar to standard contrast MR perfusion. It uses magnetically labelled arterial blood water protons as an endogenous tracer, as opposed to gadolinium, which is advantageous for use in paediatric settings due to the above-mentioned risks. A benefit of ASL imaging is that results are more readily quantifiable, compared to standard MR perfusion with contrast (Petcharunpaisan, Ramalho, & Castillo, 2010). Four studies identified in this review used ASL as either a primary or secondary method to measure hypoperfusion (Chen et al., 2009; Lee et al., 2019; Pabst et al., 2022; Polan et al., 2015; Watson et al., 2016) The main drawback with ASL is the very low signal to noise ratio and poor temporal resolution, compared to contrast perfusion imaging. There is also a need for further studies to understand the utility of ASL in paediatric stroke, as studies to date have largely been in adult stroke samples, and do not take into account developmental differences in CBF and stroke aetiology (Alloush et al, 2022; Jain et al, 2011; Parkes et al, 2004).

5.5.3. Quantitative definitions of penumbra

There were four studies identified in this review that used a semi-quantitative definition to describe the penumbra. Sporns et al., (2019) looked at five paediatric patients with AIS secondary to cardiac aetiology, for the purposes of describing outcomes retrospectively following thrombectomy. The authors defined at-risk tissue using CTP, with hypoperfusion defined as areas of tissue with CBF of less than 30 mL/(100g min). This approach with clear definition of hypoperfusion, alongside visual estimation of penumbra. This combined approach did not seek to define an amount of mismatch that was appropriate or ideal for treatment. A visually defined mismatch or penumbra has had a clear role in clinically-based penumbra estimation, and can be seen throughout the adult literature and through many of the smaller case studies within this review. Overall, Sporns et al, (2019) indicated a step towards quantitative or automated selection, using visual penumbra estimation on a quantitatively defined hypoperfusion map, using a well-used method within a clinical setting.

Taking this a step further, Lee et al., (2019) and Visser et al, (2021) retrospectively sought to define penumbral tissue in children with AIS using completely automated quantification, analogous to adult studies. Lee et al, (2019) used both CTP and MRP, along with DWI, processed through RAPID software (iSchemaView, Menlo Park CA), a validated automated software used in adult stroke. The authors investigated the perfusion imaging characteristics in five children with large vessel occlusions, and identified two children that had penumbral tissue as per the target mismatch definition used in the adult stroke-based DEFUSE studies (Albers et al., 2006, 2018). Interestingly, they identified two more children that had penumbra using T_{max} >4 seconds instead of T_{max} >6 seconds, which correlated with acute clinical deficit. Similarly, Visser et al, (2021) used contrast

MRP and DWI, through RAPID, and identified three children with perfusion-diffusion mismatch, using the same adult definition, and demonstrated potential feasibility of this technique for use in PAIS.

Another quantitative definition identified in this review was in alignment with the DAWN trials conducted in adults (Nogueira et al., 2018; Sporns et al., 2021). These studies have suggested that a purely imaging-based 'mismatch' to select patients for mechanical thrombectomy treatment may not be the only or best way to estimate penumbra. They used a mismatch between quantitative scores of clinical symptom severity and infarct volume in both late presenting adults and children with ischaemic stroke (Nogueira et al., 2018; Sporns et al., 2021). This retrospective study had the largest number of PAIS patients rated as having mismatch in this review, and a very clear criteria which can be implemented acutely.

The only prospective study identified by our search, utilised ASL to quantitatively define at-risk tissue using objective criteria (Chen et al., 2009). The authors clearly defined hypoperfusion (the PWI-based lesion) using interhemispheric comparisons and normative data, with an operationalised 'mismatch' as the PWI lesion being at least 20% greater than the diffusion lesion. Alongside Lee et al (2019), and Visser et al., (2021), these are the only studies to propose criteria for mismatch ratio as an approximation of the penumbra, from a quantitative approach. As previously mentioned, using ASL to detect the perfusion lesion may have greater applicability than contrast-based MRP or CTP for use in paediatric patients. Using very different criteria and modalities, these five studies are currently the most scientifically robust identified in our review for the objective definition of penumbra in PAIS, though the extent of their methodological differences does highlight the need for further research in this area.

5.5.4. Review limitations

This review has several limitations. Due to the inhomogeneity of the literature, inclusion criteria and definitions identified in the studies were varied. This is because we not only looked at studies that were using a definition of penumbra for a clinical purpose, but those that mentioned 'mismatch' or 'penumbra' without a clear operationalised definition, limiting comparability. Most of these papers identified in our study were case studies, with varying aims, which precluded our ability to conduct comprehensive comparative analyses. As is often the case within the paediatric stroke literature, most sample sizes were very small, with vastly different ages and time from symptom onset to imaging. The modalities differed not only amongst type of imaging used, but also the parameters used, and the aims of identifying penumbra. Due to the variance in our studies, we were unable to determine clear commonality between studies in PAIS, which is in itself an important finding and rationale for further research.

5.5.5. Recommendations for future studies

Large prospective studies are required to answer the question of what constitutes a "favourable" penumbra, and the best imaging modality and quantification method, which encompasses fast and objectively processed results, such as an automated software. It is also important to look at sequence acquisition time, to be within an acceptable timeframe in the acute stroke setting. These are essential steps towards reaching consensus on the best way to image penumbra in PAIS. Detection of a 'mismatch' between an area of hypoperfusion and an area of estimated infarction, does not tell us how much of that tissue will proceed to infarction, and how much is benign oligemia which will resolve without intervention.

The role of spontaneous reperfusion and collateral circulation is an important consideration. Research has indicated that children with strong collateral circulation surrounding the area of infarction tend to experience less lesion growth than those that do not, with many small infarcts occurring in subcortical structures such as the basal ganglia (Lee et al., 2022; Vagal et al., 2018). It might be that subcortical strokes are less likely to demonstrate salvageable tissue primarily, and therefore may require a different treatment approach.

Many ischaemic strokes are cryptogenic or secondary to non-atherosclerotic arteriopathies, which question the utility of characterising the penumbra as a treatment target in children, as some treatments may not be appropriate for certain aetiologies. Ongoing research in this area looking at not only penumbra identification, but the wider clinical picture, will help define what a favourable profile for reperfusion therapies looks like in PAIS.

5.5.6. Conclusion

The penumbra remains an important target for potential stroke intervention in the paediatric population. However, it is not currently possible to provide recommendations on the types of brain imaging modalities used to define penumbra in PAIS. Up to 11 different combinations of imaging techniques were used to define PAIS penumbra in the included studies. They are mostly low evidence retrospective case series with heterogenous study design, and analytic bias, many of which are inherent to the non-specific nature of PAIS presentation, and our lack of understanding of PAIS pathophysiology and optimal treatment paradigms.

Higher quality prospective studies, with more carefully selected cases with similar clinical profiles would provide more rigorous understanding of penumbra estimation in PAIS. This future research with larger samples, potentially through inter-institutional data pooling, is needed to identify not only the most accurate measurement of penumbra in children, but also a protocol which is feasible to quantify salvageable tissue within an acute timeframe.

Authors	Aim	Age (N), sex	Stroke / CVA type	Penumbra Definition in text	N = mismatch / penumbra	Acute feasibility
Watson, C.G., et al (2016)	To describe the perfusion pattern in perinatal stroke	Neonates < 28 days old (25)	11 PAIS, 5 PAIS & Venous infarction, and 9 Venous infarction.	Core was defined as dark areas on ADC. Perfusion assessed by ASL (or SWI where ASL was poor), defined as decreased perfusion signal compared with control region.	0 (no neonatal PAIS had hypoperfusion)	Expert visual rating required (retrospectively analysed)
Meoded, A., et al (2014)	To identify if a mismatch can be detected with changes in venous drainage on SWI and diffusion; a 'venous ischaemic penumbra' as an alternative to DWI/PWI mismatch.	Median 0.2 years, Range 1 month - 16 years, (8), 6 F	Acute PAIS (3 cardioembolic)	SWI images were overlaid onto DWI to obtain a single image, to look for mismatch - defined by the area of restricted diffusion being smaller than the area with hypointense venous signals. Visually-graded categorical criteria (smaller, matched, larger).	1	Expert visual rating required with specific criteria (retrospectively analysed)
Polan, R.M., et al (2015)	To use SWI venous signal patterns to predict stroke evolution and the development of malignant oedema in PAIS.	Med 4.7, range 2 days to 17 years (24), 14M	PAIS of varying aetiologies	Mismatch determined by the "the number of vascular territories showing restricted diffusion" and the number showing SWI-hypointense venous signal. "The number of territories with SWI-hypointense venous signal" was greater than, equal to, or less than those with restricted diffusion. This was also compared with "the number of territories with abnormal (increased or decreased) perfusion."	6	Expert visual rating required with specific criteria (retrospectively analysed)
Avital, D, et al (2022)	To describe the course and improved clinical outcome of FCA likely induced by SARS-COV-2 infection in a toddler.	1 (1) F	R MCA infarct, FCA, possibly due to recent COVID-19 infection	"MRI of the brain in axial views on DWI sequence, demonstrating an area of restricted diffusion... indicative of irreversible ischemic infarction. MRP MTT maps demonstrating a large ischemic salvageable penumbra in the right MCA territory."	1	Expert visual rating (completed acutely/ clinically)
Bolognese, M., et al (2011)	To present case details of a patient with AIS due to fibromuscular dysplasia who was successfully treated with tPA	12 (1), F	Unilateral PAIS due to fibromuscular dyplasia	Perfusion MR "an extended perfusion deficit beyond the territory of the diffusion-weighted image lesions in the cortical areas of the complete MCA territory, characteristic of a significant mismatch between the hypoperfused area and the diffusion impairment."	1	Expert visual rating (completed acutely / clinically)
Chalian, M.,et al (2011)	To present two cases of acute AIS that had SWI imaging to examine critically perfused brain tissue.	12, 6 (2), M	Case #1 - unilateral ACA infarct secondary to SCD and MMD. Case #2 - Bilateral infarction.	Area of SWI-hypointense veins drain area "significantly larger" than area of restricted diffusion. Hypointense intramedullary veins matching restricted diffusion, and dilated hypointense sulcal veins matching the area of infarct progression on follow-up imaging, suggesting critically hypoperfused tissue beyond infarcted tissue.	1	Expert visual rating required (retrospectively analysed)

Kulhari et al. (2017)	To present a case report of a child who underwent mechanical thrombectomy at 8 hours post-onset, who had a large penumbra, and a positive outcome.	9 (1) M	PAIS due to primary restrictive cardiomyopathy	Penumbra defined by large area of hypoperfusion on PWI (MTT), vs small infarct volume.	1	Expert image analysis required for core volume (though can rapidly calculated) and expert visual rating for PWI (completed acutely/clinically)
Fujimoto, M., et al (2013)	To describe a case of paediatric AIS (cervicocephalic arterial dissection) that was treated using thrombus aspiration	15 (1) F	PAIS (spontaneous intracranial (cervicocephalic) arterial dissection with distal MCA occlusion)	Perfusion abnormality on CTP: "CT perfusion demonstrates decreased cerebral blood volume in the anterior right middle cerebral artery (MCA) territory, with diminished cerebral blood flow and prolonged mean transit time through right MCA territory." Potentially salvageable tissue stated, based on hypoperfusion in posterior part of MCA territory.	1	Expert visual rating (completed acutely / clinically)
Gerstl, L., et al (2016)	To report a case of paediatric AIS of cardioembolic origin, successfully treated with thombolysis and thrombectomy.	3 (1) M	PAIS (cardioembolic intracranial occlusion due to congenital heart disease(hypoplastic left ventricle)).	Describes infarct in left MCA; "no diffusion restriction in posterior circulation, and complete occlusion of BA and MCA (MRA)". Mentions 'salvageable tissue in both vascular territories' in discussion. Salvageable tissue inferred from lack of diffusion restriction in brain stem and posterior circulation, with complete occlusion of BA and MCA.	1	Expert visual rating (completed acutely / clinically)
Pabst, L, et al (2022)	To report the successful mechanical thrombectomy procedure of a child with multisystem inflammatory syndrome (MIS-C) due to COVID-19, resulting in an ischaemic stroke.	"School-aged child" (1) M	MIS-C, large vessel occlusion	"Perfusion imaging was suggestive of a large ischemic penumbra." "DWI sequence on initial MRI demonstrating acute ischemic infarct... ASL Perfusion demonstrating decreased perfusion within right MCA territory."	1	Expert visual rating (completed acutely / clinically)
Sainz de la Maza et al., (2014)	To report the successful treatment of PAIS in a previously-healthy 12 year-old girl with mechanical thrombectomy	12 (1) F	Transient Cerebral Arteriopathy	"Perfusion CT revealed a mismatch area greater than two-thirds of right middle cerebral artery vascular territory, suggesting a large area of penumbra."	1	Expert visual rating (completed acutely / clinically)
Mittal, O.S., et al (2015)	Brief case report as part of an illustrative teaching case.	17 (1) M	Acute PAIS	"a perfusion scan showed a large perfusion/diffusion mismatch" "MRI shows a small area of diffusion restriction with a large perfusion deficit seen with mean transit time suggestive of acute ischemia with a large penumbra"	1	Expert visual rating (completed acutely / clinically)

Orman, G., et al, (2020)	To report the acute and follow-up findings and neuroimaging characteristics of acute ischaemic stroke caused by large, thrombosed aneurysm. To discuss the value of using SWI in combination with DWI to identify the ischaemic penumbra.	17 (1) M	Acute PAIS caused by aneurysm.	Penumbra: impaired perfusion (Using PWI & SWI) which is substantially larger than the area of restricted diffusion (DWI). (Visually defined)	1	Expert visual rating (completed acutely / clinically)
Tai, W.A., et al (2012)	To describe a case of a patient with paediatric AIS who underwent thrombolytic therapy following multimodal imaging with perfusion, outside the standard time window	13 (1) M	Acute PAIS	DWI "evidence of acute ischemia", CBV "decrease in the corresponding region with surrounding elevation in the periphery. "This pattern of circumscribed ischemic core with a larger area of mismatch in the remainder of the middle cerebral artery territory indicated potential regions at-risk of evolving infarction."	1	Expert visual rating (completed acutely / clinically)
Van Den Wijngaard, I., et al (2014)	To present a case report of a paediatric patient treated with mechanical thrombectomy for a large vessel occlusion caused by atrial myxoma	14 (1) M	Acute embolic PAIS (MCA occlusion) cause by atrial myxoma	Hypoperfusion: Increased MTT and TTP. Core: decreased CBV and CBF "indicating a favourable penumbral pattern."	1	Expert visual rating (completed acutely / clinically)
Xavier, A., et al (2012)	To report the case of a successful recanalisation following delayed thrombectomy	16 (1) M	PAIS due to ICA occlusion	CT & DWI "showed an area of core infarct". Penumbra described with two definitions, progressively "Preserved CBV & CBF, and increased MTT and TTP", with subsequent imaging showing penumbra with "preserved CBV, but decreased CBF and increased MTT & TTP".	1	Expert visual rating (completed acutely / clinically)
Lee, S., et al (2019)	To report the demographic and neuroimaging considerations and clinical course of a retrospective cohort of PAIS patients with large vessel occlusion, to help inform potential thrombectomy eligibility.	Mean 9.7 (SD 5) (12) 8F	PAIS due to large vessel occlusion	Penumbra defined using subset of patients who had perfusion imaging, which was processed with RAPID automated segmentation software. Other criteria specifically for thrombectomy consideration, not explicitly penumbra was small infarct core <70mls (DWI) or ASPECTS =>7 (non-contrast CT), large vessel occlusion evidence on MRA or CTA, salvageable penumbra: (MR or CT perfusion) using T_{max} (>6s or >4s), stroke severity on NIHSS => 6.	4 (2 using T_{max} >4s)	Expert interpretation of automated quantitative maps with clinical information (completed acutely / clinically, compiled retrospectively)

Sporns, P.B., et al (2019)	To retrospectively look at the outcomes of thrombectomy performed in childhood AIS across three hospitals.	Med 14 , 7.8-16 (12) 6 M	PAIS. (5 Cardiac aetiology)	"Tissue at risk and early infarct in CTP were estimated visually using quantitative cerebral blood flow and cerebral blood volume perfusion maps, respectively. Any cerebral blood-flow lesion of 30 mL/(100gmin) or less was defined."	Unclear	Expert visual rating (completed acutely / clinically, compiled retrospectively)
Sporns P.B et al (2021)	To determine whether thrombectomy is safe in children up to 24 hours after onset of symptoms when selected by mismatch between clinical deficit and infarct.	Median 10.5, IQR 7-14.6 (20) 7M	PAIS, large vessel occlusion	Mismatch : (1) a score of ≥10 on the Pediatric NIH Stroke Scale (PedNIHSS) and an infarct volume of ≤50 mL or (2) a score of ≥20 on the PedNIHSS and an infarct volume of 51 to 70 mL. Core defined as ADC <620 ×10−6 mm^2/s (MRI) or CBF <30% (CTP).	17	Expert image analysis for core estimation, and clinical assessment (analysed retrospectively)
Zebedin, D., et al (2013)	To report the results of contrast-enhanced perfusion multi detector CT for identifying perfusion abnormalities in PAIS	Mean age = 13, range 8 - 17 years (10) 7F	2 with PAIS, 8 with migraine	Decreased CBF, CBV and a long region MTT as infarct core and a CBF/CBV mismatch with decreased CBF and a normal or elevated CBV as penumbra.	1	Expert visual rating required (analysed retrospectively)
Visser, M.J. et al, 2021	To investigate the feasibility of assessing automated perfusion-diffusion mismatch, commonly used for adult studies, in childhood AIS.	Median 8 (29) 52% male	Acute PAIS (varying aetiologies)	Ischemic core was defined as apparent diffusion coefficient <620×10−6mm2/s and hypoperfusion as T_{max} >6 seconds. Favorable mismatch profile was defined as core volume <70 mL, mismatch volume ≥15 mL, and a mismatch ratio ≥ 1.8	3	Expert interpretation of automated quantitative maps (analysed retrospectively)
Chen, J., et al., (2009)	To look at the utility of ASL in measuring changes in cerebral blood flow in paediatric AIS.	1.2-16 (median 7.5) (10), 7 M (70%)	PAIS of varying aetiologies/ stroke risk factors.	Hypoperfusion: Interhemispheric perfusion difference (unaffected-affected)/unaffected x 100%. IHPD =>20% = significant hypoperfusion, mean +2 SDs from a control sample. "PWI/DWI mismatch was then defined as the volume difference between PWI and DWI greater than 20% of diffusion lesion volume."	2	Expert non-automated manual segmentation of core and hypoperfusion required (prospective data collection, though analyses not for acute decision making)

Table 5.3 - Appendix A.1 – Additional Study Information

Authors	Overall QoE rating	Type of evidence	Methodology	Consistency	Clinical Applicability
Watson, C.G., et al (2016)	Low-moderate	Low-moderate	Moderate	Moderate	Low-moderate
Meoded, A., et al (2014)	Low-moderate	Low	Moderate	Low	Low-moderate
Polan, R.M., et al (2015)	Moderate	Low-moderate	Moderate-high	Low-moderate	Low-moderate
Avital, D, et al (2022)	Low-moderate	Very Low	Low-moderate	Low-moderate	Low-moderate
Bolognese, M., et al (2011)	Low	Very Low	Low-moderate	Low-Moderate	Low-moderate
Chalian, M.,et al (2011)	Low-moderate	Very Low	Moderate	Low-moderate	Low-moderate
Kulhari et al. (2017)	Low-moderate	Very Low	Moderate	Low-moderate	Low-moderate
Fujimoto, M., et al (2013)	Very Low-low	Very Low	Low	Low-moderate	Low-moderate
Gerstl, L., et al (2016)	Low	Very Low	Very Low-low	Very Low-low	Low-moderate
Pabst, L, et al (2022)	Low	Very Low	Very Low-low	Very Low-low	Low-moderate
Sainz de la Maza et al., (2014)	Low	Very Low	Low	Low	Moderate
Mittal, O.S., et al (2015)	Low	Very Low	Low	Low	Low
Orman, G., et al, (2020)	Low-moderate	Very Low	Moderate	Low-moderate	Low-moderate
Tai, W.A., et al (2012)	Low-moderate	Very Low	Moderate	Moderate	Low-moderate
Van Den Wyngaard, I., et al (2014)	Low-moderate	Very Low	Low-moderate	Low-moderate	Low-moderate
Xavier, A., et al (2012)	Low-moderate	Very Low	Moderate	Moderate	Moderate
Lee, S., et al (2019)	Moderate-high	Low	High	Moderate	High
Sporns, P.B., et al (2019)	Moderate	Low	Moderate	Moderate	Moderate
Sporns, P.B., et al (2021)	Moderate-high	Low	High	Moderate-High	High
Zebedin, D., et al (2013)	Low-moderate	Low	Low-moderate	Moderate	Low-moderate
Visser, M.J. et al, 2021	Moderate-high	Low	High	Moderate	High
Chen, J., et al., (2009)	Low-moderate	Low-moderate	High	Low-moderate	Low-moderate

Table 5.4 - Appendix A.2 - Quality of evidence ratings

Supplementary material 1: Search terms

CINAHL

(arterial isch#emic stroke or stroke or AIS or isch#em*) AND (p#ediatric*or child* or infan* or newborn* or neonat*) AND (neuro#imaging or MRI or magnetic resonance or computed tomography or CT or imaging or thrombect* or thrombolys*) AND (hypoperfus* or perfus* or mis#match or penumbra or thrombect* or thrombolys*)

Scopus

(TITLE-ABS-KEY ((*arterial AND isch*emic AND stroke) OR stroke OR ais OR ischemic OR ischaemic) AND TITLE-ABS-KEY (paediatric OR pediatric OR child* OR infan* OR newborn OR neonat*) AND TITLE-ABS-KEY (neuroimaging OR mri OR (magnetic AND resonance) OR (computed AND tomography) OR ct OR imaging) AND TITLE-ABS-KEY (hypoperfus* OR perfus* OR mismatch OR penumbra OR thrombect* OR thrombolys*))

Ovid (Medline, Embase):

(arterial isch*emic stroke or stroke or AIS or ischemic or ischaemic).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

AND

(paediatric or pediatric or child or childhood or children or infan* or newborn or neonat*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

AND

(neuroimaging or MRI or magnetic resonance or computed tomorgraphy or ct or imaging).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

AND

(hypoperfus* or perfus* or mismatch or penumbra or thrombect* or thrombolys*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

Limit "all child (0 to 18 years)" and humans and English (child unspecified in Embase)

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6. STROKE LESION SEGMENTATION METHOD DEVELOPMENT - IMAGING METHOD CHALLENGES ENCOUNTERED

Upon highlighting the inconsistencies within the paediatric stroke literature in terms of methods of penumbra segmentation, it is important to carefully consider methodology for the subsequent studies. As is common in understudied areas, the imaging methods used for stroke lesion segmentation are not always well-established, and not necessarily robust or appropriate across samples. As part of the initial evaluation of using RAPID software in the PAIS sample, it is important to assess the accuracy of infarct segmentation

volume based on a thresholded ADC value. I developed a methodological framework to test this. To address the overall study questions (two, three and four), it was also necessary to be able to measure lesion size to estimate lesion growth or change over time.

In this chapter I aimed to:

1. To define study-specific reference standard of the stroke lesions segmented, based on a novel semi-automated method.
2. Evaluate the initial run of RAPID data outputs using the clinical data and provide methods to resolute problematic scenarios relating to data types and data processing.
3. Compare these reference stroke lesion segmentations against those automatically segmented in RAPID.

No validated measures for the specific use of determining penumbra in children were identified in Study One. To assess the applicability and accuracy of automated software within the study sample, manual segmentation was also needed as reference standard for method validation. There are various techniques to segment stroke lesions, however, no "gold-standard" was identified for use within the sample. I decided to trial a semi-automated technique to compare to automatic results, and to also answer the research questions of lesion growth and change over time, as automated software is only used acutely.

As highlighted in section 3.3.4, volumetric segmentation has been used in paediatric stroke, with varying methodology. As detailed below, volumetric segmentation was deemed to be preferable for the study sample, rather than the categorical size definition used by some other studies, to perform comparisons using continuous variables.

At the time of writing, only one additional study external to this thesis had used RAPID automated software to characterise penumbra in a PAIS sample. With no published preferable method in a similar sample to ours, the technical feasibility was yet to be tested, to learn about any particular methodological considerations required for successful implementation and to validly answer our study questions. In this chapter, the results obtained from piloting the chosen methods are documented, as are some of the challenges encountered.

6.1. Study-specific manual segmentation method testing & validation

6.1.1. Segmentation aims:

1. To accurately and reproducibly segment acute and chronic lesions to determine infarct volume. This is to be able to answer research questions regarding:
    - The feasibility of using RAPID software for automated infarct segmentation
    - Volumetric change over time, in reference to presence or absence of penumbra
    - Effect of lesion volume on outcome
2. To evaluate the intra-rater reliability of using the semi-automated watershed transform method to segment lesions overtime.
3. To have a reference standard to compare the automated acute DWI segmentation method to evaluate reliability.

6.1.2. Participants

Twenty-nine participants were identified retrospectively from The Royal Children's Hospital between 2007-2013 with a unilateral arterial ischaemic stroke. These participants also underwent DSC contrast perfusion imaging. Age range was 1-16 years and 57% were male. The inclusion criteria were: term neonates and children who presented to the RCH with provisional PAIS who underwent imaging within 24 hours of symptom onset and with radiological diagnosis of unilateral, single vascular territory PAIS.

The exclusion criteria were neonates and children with focal deficits or encephalopathy not due to PAIS, MR imaging showing cerebral venous sinus thrombosis, intracranial haemorrhage, periventricular leukomalacia or hypoxic ischaemic changes.

6.1.3. Segmentation methods

The decision to implement DWI as the acute infarct method and T2 FLAIR for the chronic infarct segmentation was informed by literature review in Chapter Three. All images were pre-processed prior to segmentation. For acute lesion volume, DWIs obtained within 24 hours of stroke onset were used. Data pre-processing performed included thermal noise correction, Gibbs ringing correction, DWI geometric distortion correction to remove DWI noise and artifact. FSL EDDY was applied to correct for eddy-current and motion. Scalar maps were then derived from the DWI data (generation of TRACE and ADC images). Both the TRACE and ADC images were upsampled to structure scan resolution to aid with lesion segmentation.

A watershed transform method (WTM) based segmentation technique was used (Beare & Lehmann, 2006). This WTM was performed for segmentation of the acute and chronic infarcts for all eligible participants. The image segmentations were performed by the same

rater twice, with three weeks interval between attempts. At the second session, the rater was blinded to the participant ID and previous round of segmentation results (i.e. sorted the data in a randomised order with participant IDs removed), to look at intra-rater reliability of this method.

Statistical tests implemented to measure the intra-rater reliability of the segmentation protocol included both descriptive and inferential analyses. Descriptive statistics were the intraclass coefficient (ICC) score to look at the strength of the fixed degree of relatedness between the two timepoints, and the Dice Similarity Coefficient (DSC) to observe the degree of spatial overlap between images. Good intra-rater agreement using the ICC is generally thought to be ≥ 0.75, with scores above 0.90 rated as excellent (Koo & Li, 2016). DSC, which is the most common method to look at the degree of spatial overlap and agreement in image segmentation can be interpreted by scores >0.70 as good, and ≥ 0.75 as excellent agreement (Zou et al., 2004). In addition to standard correlations, both parametric (paired t-test) and non-parametric comparisons (Wilcoxon signed rank paired test) were done to look for statistically significant differences between the results.

6.1.4. Expert Review of Manual Segmentation

Prior to the completion of the first segmentation, an expert paediatric stroke neurologist, blind to clinical information of the stroke patients, systematically examined each case following initial segmentation of acute and chronic lesions, to determine any areas that were deemed incongruently segmented. A collaborative list of segmentation criteria was established, and this was then used as a guide. The criteria were applied to adjust and complete the first segmentation, and then referred to during the second randomised segmentation.

Segmentation Analyses Criteria:

- Diffusion weighted images
    - Hyperintensity clearly resulting from artefact, particularly susceptibility artefact near the skull base – not included in the analysis
    - Hyperintensity lining the ventricles – also not included in the analysis
    - Refer to ADC map for lesion comparison
- T2 FLAIR images and/or diffusion weighted images:
    - Tissue that looks abnormal, but is symmetrical – not included in the analysis
    - Circumscribed holes in tissue included – clear post-stroke atrophy.
    - Comparison of intensity value to contralateral side to help determine if brighter or not for areas that are difficult to delineate.

Following both segmentation attempts, the cases were expert reviewed again, and segmentation delineation was deemed appropriate.

6.2. Segmentation Results

6.2.1. Acute segmentation

Statistic	N	Mean	Standard Deviation	Minimum	Maximum
Acute Volume Time 1 (mls)	29	14.95	19.05	0	79.81
Acute Volume Time 2 (mls)	29	15.02	19.28	0	80.57
Acute ADC Time 1 ($x10^{-6}$ mm^2/s)	29	440.39	98.80	0	557.61
Acute ADC Time 2 ($x10^{-6}$ mm^2/s)	29	440.07	99.39	0	561.55
Acute DSC	28	0.96	0.05	0.82	1

Table 6.1 - Descriptive statistics from Acute Segmentation.

No significant differences were found between first segmentation and second segmentation, using both the mean (paired t-test) or the median (Wilcoxon signed rank sum test, due to non-parametric nature of the distribution). Very high correlations were found between both volumetric segmentations (r_s = 0.994, ICC = 0.99). Overall DSC for acute volumes = 0.96 (Table 6.1) These are thresholded values, hence participant #2 which was a very small not easily differentiated lesion was not included (N = 28). Bland-Altman plot of the acute segmentation, which looks for systematic biases in measurements, found that 93% of segmentations were within one standard deviation of the mean volume difference between time-points (Figure 6.1).

6.2.2. Chronic Segmentation

Similar to the acute segmentation, both segmentation timepoints indicate very high correlations (r_s = 0.99 & ICC = 0.99), and no significant differences were found between the volumes between times, with both the paired t-test and Wilcoxon signed rank sum >.05. Dice Similarity Coefficient analyses revealed the overall DSC for chronic lesion volume comparison was 0.851 (Table 6.2). Only one patient's chronic lesion segmentation was rated below the 0.75 cut-off for a *very good* overlap, which had a very small volume (0.07mls vs .27mls). Bland-Altman plot (Figure 6.2) for the chronic segmentation shows that most values are clustered around zero, not indicating problematic systemic biases.

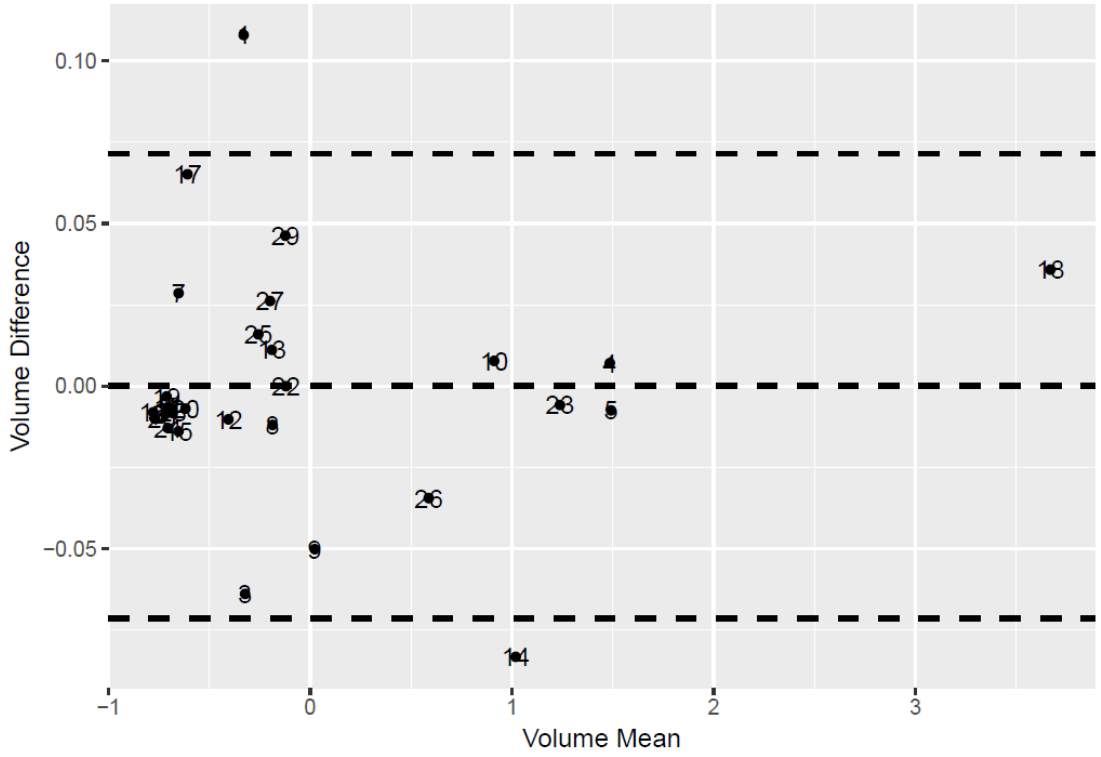

Figure 6.1 – Bland-Altman plot for acute segmentation attempts which shows most values clustered close to zero.

Statistic	N	Mean	Standard Deviation	Minimum	Maximum
Chronic Volume Time 1 (mls)	29	12.16	19.21	.06	98.74
Chronic Volume Time 2 (mls)	29	12.47	21.43	.05	111.49
Chronic DSC	29	0.85	0.08	0.56	0.98

Table 6.2 - Descriptive statistics from Chronic Segmentation.

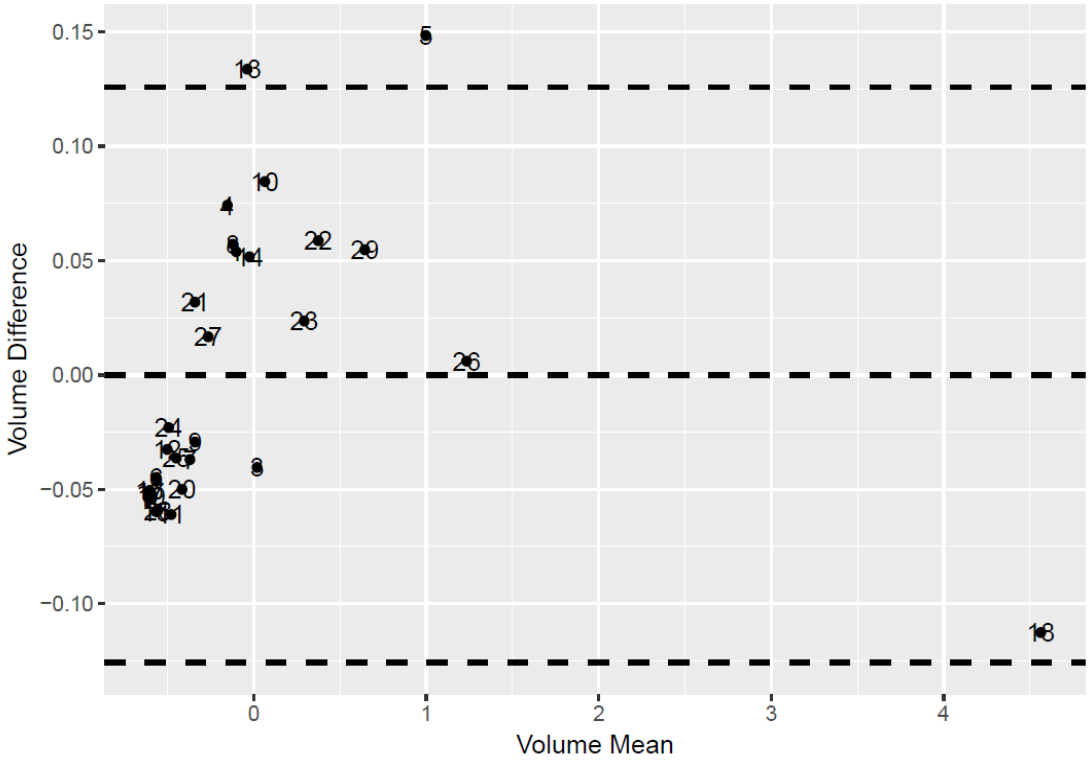

Figure 6.2 - Bland-Altman plot for chronic segmentation attempts showing most values falling close to 0.

6.3. Discussion of segmentation results

In the acute segmentations, once participant two had been removed due to the threshold, all cases DSC were at or above 0.75, suggesting very good spatial overlap and agreement between time one and time two. No profound systemic bias was indicated, and results were highly correlated for both acute and chronic segmentations. For the chronic cases, there were two cases just below the suggested very good DSC score. Taking into consideration that the chronic lesions are more difficult to visually segment, to have 90% above the 'very good' cut-off is reasonable. The chronic segmentation for participant two was still below expectations because the lesion was extremely small. However, given that the acute segmentation was not included, the inclusion of this case is due to the observational study design. As the novel semi-automated manual segmentation results appeared to be reliable, I was then able to look at the comparison with RAPID acute segmentation in the following section, the ADC thresholds and lesion volume change in Chapter 8, and to explore the effect of lesion volume on outcome in Chapter 9.

6.4. RAPID software initial run and errors encountered

6.4.1. Issues with site data

The results of the first attempt at running the pre-processed DICOM DWI and PWI images through RAPID had several invalid features prompting further investigation. There appeared to be left to right flip in 12 cases, identifying an issue with orientation of some of the data. At the standard T_{max} >6s for perfusion, 11 cases indicated no perfusion deficit at all, and most showed bilateral perfusion deficits (n = 15), that were not always near the infarct itself and did not seem to be valid. 27/29 indicated no penumbra, with the majority of these showing negative mismatch (i.e., volume of thresholded ADC mask

was greater than the hypoperfusion volume). Others showed bilateral hypoperfusion, or no ADC detected. Only two cases in total met penumbra criteria, with both an acute infarct detected and a unilateral perfusion deficit.

These issues were realised to be due to site-specific difficulties with our retrospective clinical data. These were related to the way that the DWI and PWI images were processed prior to submitting to the RAPID software. These included differences in slice thickness, orientation issues, retrospective analyses of our research design which meant that scans were collected over a large span of years, and on different MR machines.

Specific to our retrospective sample, another issue was the differences in the DWI sequences acquired. Some patients had standard DWI and ADC maps, in whom the upload of DICOM data to RAPID was not problematic. In others who had diffusion tensor imaging (DTI), and high angular direction, high b-value HARDI acquisitions, additional non-trivial data processing steps were first required to enable the diffusion data in DICOM format to be imported into RAPID for processing. This involved first reconstructing diffusion tensor from the DTI and HARDI data, then computing the TRACE-DWI map by summing the diagonal component of the diffusion tensor. The combined b = 0 s/mm^2 and the TRACE-DWI map were then saved in DICOM format with the same world coordinate frames as the acquired data.

A more in-depth discussion of the specific technical difficulties is beyond the scope of this thesis, however, the importance of having expert assistance when setting up a program using a software so that the data is in the correct format was noted. This issue will be prevented when designing a prospective study, as would implementing a standard MRI protocol on the same machine. Having amended these issues, this enabled valid analyses and interpretation of the RAPID data for the subsequent studies.

6.4.2. Bolus properties & Arterial Input Function

Bolus properties were rated as adequate in 23/29 cases, with 6 rated as "poor" with notching of peaks and some temporal dispersion. 10 percent of children had moderate to severe head motion. These numbers changed slightly after the amendment of data format following this initial run through RAPID, and are detailed in Chapter Seven. Those with unamendable poor bolus properties following the improvement of our data processing, though excluded from penumbra analyses, were included descriptively due to the observational nature of this work.

Automated Arterial Input Function (AIF) selection for some cases were sub-optimal. The AIF is a deconvolution of the contrast agent input to the tissue of interest (Calamante, 2013). Research has indicated that differences in AIF location can affect the size of the T_{max} hypoperfusion lesion (Thijs et al, 2004). AIF locations in the contralateral MCA region have been identified to be associated with follow up lesion volumes. 11 cases in the present sample had automated AIF located in the same arterial field and hemisphere as the lesion, when it is preferable to be in the contralateral hemisphere / arterial field. There were a couple where it was difficult to determine the neuroanatomical location of the AIF. At least one case had an unclustered AIF. To rectify, the AIF locations that were initially in suboptimal locations were manually re-localised to the contralateral MCA in the RAPID program, for analyses used in Study Two. This extra manual step did not add significantly to the processing time.

The technical issues identified in this initial run of the RAPID software were rectified and ready for data analysis in the following chapter. Using the amended RAPID data, infarct volume segmentation was able to be validated compared to manual segmentation.

6.5. Stroke lesion volumes: RAPID vs reference standard

RAPID Infarct segmentation Threshold ADC <620x10^-6 mm^2/s	Manual Segmentation (Mean ADC volume Time 1 & Time 2)
13	12.44
64	62.51
107	52.07
14	13.20
15	17.30
2	46.69
3	2.13
18	16.32
47	49.65
4	3.55
108	103.32
4	4.51
22	18.24
52	55.70
7	2.08
12	14.46
77	37.75
39	16.07
28	18.15

Table 6.3 - RAPID automated segmentation infarct volumes and Manual segmentation infarct volumes

Within the amended data, RAPID did not detect an infarct core in 10/29 cases, compared to 11/29 from the first run. Of those which RAPID did detect the infarct core, there was no significant difference in lesion volumes between manual segmentation and RAPID segmentation (Table 6.3). Median ischemic core was 17.3mls IQR 12.8-48.2 (manual) versus 18mls IQR 9.5-49.5 (RAPID) which was not significantly different ($W = 58$, $Z = -1.49$, $p = 0.14$).

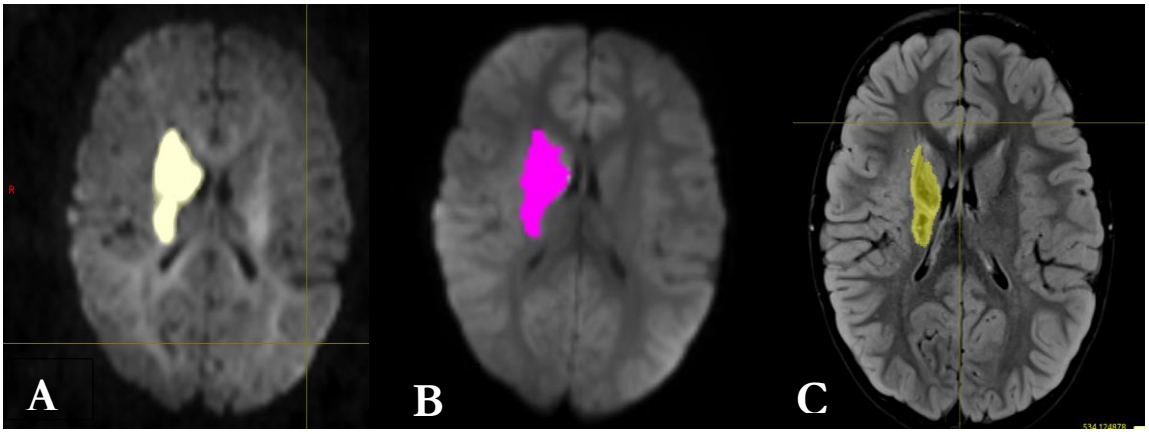

Figure 6.3 – An example of segmentation methods used (A) Manual segmentation of acute infarct volume using DWI (B) Automated segmentation of infarct volume using RAPID (C) Manual segmentation of chronic infarct volume using T2 FLAIR

6.6. Summary

In this chapter, I have answered some necessary methodological considerations to enable valid analyses in subsequent chapters. Overall, I tested the novel semi-automated manual segmentation protocol and found that it had strong intra-rater reliability across timepoints (acute and chronic) and modalities (DWI and FLAIR). The initial RAPID data analysis demonstrated some unexpected challenges which were largely due to the nature of our retrospective data, requiring time-consuming post-processing and expert input. This very important challenge that was encountered should be noted for future research, as the objective of automated penumbra segmentation is rapid implementation and interpretation in a clinical context. While the amended RAPID data still did not segment almost one third of acute infarcts, this is believed to be an interpretable finding which I was able to complete further analyses on in the following study.

7. STUDY 2 – AUTOMATED PENUMBRA SEGMENTATION IN PAEDIATRIC ARTERIAL ISCHAEMIC STROKE

After exploring definitions of penumbra, and overcoming the challenges in methodology, the central question of this thesis was able to be explored. This chapter sought to address the second Study Question and accompanying aims by looking at the feasibility of applying automated software to characterise penumbral tissue in PAIS, and to assess ADC values over time. In the following chapter, I will look at this second aim in more detail, and also explore whether 'mismatch' identified in this chapter may have progressed to infarction. Both of these elements provide vital information about penumbra in children and key directions for future research. This chapter is presented as the author-

accepted manuscript of a peer-reviewed article published in *Stroke* (2021; 52: 3296-3304); please note that the spelling in this chapter is intentionally US English, as published.

7.1. Study two published paper STROKE

AUTOMATED PERFUSION-DIFFUSION MRI IN CHILDHOOD ARTERIAL ISCHEMIC STROKE

Melissa J. Visser, BA[1,2]

Joseph Yuan-Mou Yang, MBChB PhD[3, 4, 5, 8]

Fernando Calamante, PhD[6]

Michael Kean, BSc[4]

Christopher L. Adamson, PhD[4]

Gagan Sharma, MCA[7]

Vicki Anderson, PhD[1,2,8]

Bruce C.V. Campbell, PhD[7,9]

Mark T. Mackay, MBBS PhD[4,8,9,10]

1. *Brain and Mind, Murdoch Children's Research Institute, Parkville, Australia.*
2. *Melbourne School of Psychological Sciences, University of Melbourne, Parkville, Australia.*
3. *Neuroscience Advanced Clinical Imaging Suite (NACIS), Department of Neurosurgery, Royal Children's Hospital, Parkville, Australia.*
4. *Neuroscience Research, Murdoch Children's Research Institute, Parkville, Australia.*
5. *Developmental Imaging, Murdoch Children's Research Institute, Parkville, Australia.*
6. *The University of Sydney, Sydney Imaging and School of Biomedical Engineering, Sydney, Australia.*

7. *Department of Medicine and Neurology, Melbourne Brain Centre at the Royal Melbourne Hospital, University of Melbourne, Parkville, Australia.*

8. *Department of Pediatrics, University of Melbourne, Parkville, Australia.*

9. *Florey Institute of Neurosciences and Mental Health, Parkville, Australia.*

10. *Department of Neurology, Royal Children's Hospital, Parkville, Australia.*

Correspondence to: Associate Professor. Mark T. Mackay, Department of Neurology, Royal Children's Hospital Melbourne, 50 Flemington Road, Parkville, Victoria, Australia. 3052.

Email: mark.mackay@rch.org.au Phone: 61-3-9345 5661. Fax: 61-3-9345 5977.

Twitter handle: @MelissaJVisser

Cover title: Automated Perfusion-diffusion MRI in Childhood AIS

Number of Tables: 2

Number of Figures: 3

Search terms: Stroke, Ischemia, Pediatrics, Neuroimaging, Perfusion Imaging.

Subject terms: Pediatrics, Magnetic Resonance Imaging (MRI), Ischemic Stroke.

Manuscript word count of paper: 5906

Abstract

Background and Purpose: Recent studies using automated perfusion imaging software have identified adults most likely to benefit from reperfusion therapies in extended time windows. The time course of penumbral tissue is poorly characterized in paediatric arterial ischemic stroke (PAIS). We explore the feasibility of using automated perfusion-diffusion imaging software to characterize penumbra in childhood PAIS.

Methods: An observational cohort study of children with acute unilateral PAIS presenting to our institution. Diffusion-weighted imaging and dynamic susceptibility

contrast perfusion MRI performed within 72 hours of symptom onset were necessary for inclusion. Perfusion-diffusion mismatch was estimated using RAPID software. Ischemic core was defined as ADC <620 $\times 10^{-6}$ mm^2/s and hypoperfusion as T_{max} >6 seconds. Favorable mismatch profile was defined as core volume <70mls, mismatch volume ≥15mls and a mismatch ratio ≥1.8.

Results: Twenty-nine children (median 8-years-old, IQR=4.4-14.6) were included (26 unilateral MCA and 3 unilateral cerebellar infarcts). Median PedNIHSS was 4 (IQR=3–11). Most cases had cryptogenic (n=11) or focal cerebral arteriopathy (n=9) etiologies. Median time-to-imaging=13.7 hours (IQR=7.5-25.3). RAPID detected an ischemic core in 19 (66%) patients. In the remaining cases, the mean ADC values were mostly higher than the threshold as the majority of these presentations were delayed (median >21 hours), and/or infarct volumes were small (<3.5mls). Overall, three children, imaged at 3.75, 11, and 23.5 hours had favorable mismatch profiles.

Conclusion: This study demonstrates it is feasible to rapidly assess perfusion-diffusion mismatch in childhood PAIS using automated software. Favorable mismatch profiles, using adult-based parameters, persisted beyond the standard 4.5 hours window for thrombolysis, suggesting potential therapeutic benefit of RAPID use. Further work is required to determine the utility of perfusion-based imaging to guide clinical decision making, whether adult thresholds require modification in childhood PAIS, and to investigate the effect of time-delay and etiology on mismatch characteristics.

ABBREVIATIONS

ADC: Apparent Diffusion Coefficient

AIF: Arterial Input Function

PAIS: Paediatric Arterial Ischemic Stroke

DSC: Dynamic Susceptibility Contrast

DWI: Diffusion Weighted Imaging

MRP: Magnetic Resonance Perfusion

PWI: Perfusion Weighted Imaging

7.2. Introduction

Adults with ischemic stroke are routinely treated with reperfusion therapies, including intravenous thrombolysis within 4.5 hours of stroke onset and endovascular thrombectomy within 6 hours of symptom onset.[1] In contrast, children with acute arterial ischemic stroke (PAIS) rarely access these treatments, largely due to diagnostic delays extending beyond recommended time-windows, and limited safety and efficacy data for reperfusion therapies.[2–4] In adults, recent trials have led to extended time windows for thrombolysis and thrombectomy, based on advanced imaging with perfusion-diffusion MRI or CT perfusion, processed using automated software with standardized thresholds.[5–8] This imaging identifies patients with potentially salvageable ischemic penumbra, and these trials have demonstrated benefit from reperfusion, independent of the time elapsed from stroke onset.

Adopting a similar tissue-based approach to select children most likely to benefit from reperfusion therapies may allow increased use of these treatments, in view of the logistical difficulties in achieving rapid diagnosis of stroke in children. Given the difference in stroke etiologies, pathogenesis and frequent diagnostic delays, the time-course of ischemic penumbra in children cannot be assumed to be the same as adults.[9–11] There is a need to better understand the relationship between time-to-imaging, penumbra characteristics, and the optimal imaging parameters for administering reperfusion therapies in PAIS.

The aims of this retrospective pilot study were: (i) to investigate the feasibility of assessing automated perfusion-diffusion mismatch, commonly used for adult studies, in PAIS, and (ii) to explore the relationship between time-to-imaging and ADC values to aid understanding of the utility of segmentation thresholds.

7.3. Methods

7.3.1. Data availability

Anonymized raw data are available and can be shared upon appropriate request.

7.3.2. Participants

This was a retrospective observational clinical cohort study. To be eligible for inclusion, children had to be aged one month to 18-years-old, have presented to The Royal Children's Hospital (RCH), Melbourne, Australia, between 2005 and 2014 with radiologically confirmed unilateral acute PAIS. Additionally, they had to have undergone diffusion weighted imaging (DWI) and dynamic susceptibility contrast (DSC) magnetic resonance perfusion (MRP) within 72 hours of symptom onset. The number of cases meeting inclusion criteria during the study period determined the sample size.

The study was approved by The RCH Human Research Ethics Committee (HREC 28093). Informed consent was obtained from the patients and/or their guardians/parents.

7.3.3. Clinical data

Clinical data were collected acutely and on routine clinical follow-up (approximately three months post-stroke). Variables collected included age at stroke, sex, time-to-imaging (i.e. from symptom onset to MRI being acquired), and stroke severity using the Pediatric NIH

Stroke Scale (PedNIHSS).[12] Neurological impairment at presentation was classified using the Pediatric Stroke Outcome Measure (PSOM) score.[13] Stroke etiology was determined by reviewing clinical risk factors and the results of standardized investigations, which included MRI, intracranial and neck vascular imaging (MR angiogram and/or digital subtraction angiography), echocardiogram, and prothrombotic studies. The etiologies were categorized based on the Childhood AIS Standardized Classification and Diagnostic Evaluation (CASCADE) classification system.[14]

7.3.4. Neuroimaging data

MRI was performed on 3.0-Tesla Siemens scanners (MAGNETOM Trio, Erlangen, Germany). Sequences included Sagittal 3D T1-weighted, T2-weighted, fluid-attenuated inversion recovery (FLAIR), DWI, and dynamic susceptibility contrast MRP.

DSC-MRP: This was acquired using a gradient-echo echo-planar imaging (EPI) sequence with 32 channels head coil (TE = 32ms; TR = 1400ms; flip angle = 90; degrees; acquisition matrix = 128 x 128 mm; FOV = 230 x 230 mm^2; slice thickness = 4.50 mm; in-plane voxel resolution = 1.8mm). A bolus of 0.1 mmol/kg body weight contrast agent (Magnevist [2005-2012], Gadavist [2012-2014] Scherring [Bayer]) was injected intravenously at 2ml/second, followed by a 10ml saline flush using power injector (Medrad Spectris Solaris).

DWI: There were three different DWI sequences over the course of the study. In eight cases, the DWI was acquired using the readout-segmented echo-planar imaging (RESOLVE) sequence,[15] which included one $b=0s/mm^2$ volume and a computed TRACE-DWI map, derived from the three $b=1000s/mm^2$ DW volumes obtained from three orthogonal DW gradient directions. Other sequence parameters were: TR4700ms;

TE72ms; 9 Segments acquisition matrix192x192mm; FOV220x220mm; slice thickness3.5mm.

In the remaining 21 cases, the Diffusion Tensor Imaging (DTI; n = 14 cases), and the High Angular Resolution Diffusion Imaging (HARDI; n = 7 cases) data were acquired using the twice-refocused pulsed-gradient spin echo (PGSE) EPI sequence along multiple non-collinear DW directions. The DTI acquisition parameters were: 20 non-collinear DW directions; $b=1000s/mm^2$; 2 $b=0s/mm^2$; 2.3mm isotropic voxels; TE80ms; TR5600ms, flip angle90 degrees. The HARDI acquisition parameters were: 60 non-collinear DW directions; $b=3000s/mm^2$; 7 $b=0s/mm^2$; 2.3 mm isotropic voxels; TE110ms; TR7600ms; flip angle90 degrees.

7.3.5. Data processing in RAPID

Both the MRP data and, in cases with the DWI-RESOLVE acquisition, the acquired data in DICOM format were imported directly into RAPID (iSchemaView, Menlo Park CA) for processing. In cases with the DTI and HARDI acquisitions, the DWI data were first converted to NIfTI format, and denoised, and corrected for Gibbs-ringing artifacts, and motion and eddy current distortions using the MRtrix3 software (version 3.0_RC3; http://www.mrtrix.org/).[16–19] Diffusion tensor was reconstructed from the DTI or HARDI dataset. The TRACE-DWI map was then computed by summing of the diagonal component of the diffusion tensor. The $b=0s/mm^2$ volume and the TRACE-DWI map were saved in DICOM format with the same world coordinate frames as the acquired data, which was then imported into RAPID for processing. RAPID internally calculated the ADC map.

The RAPID software performs motion registration, automated the arterial input function (AIF) selection, deconvolution and generates the following parametric perfusion maps:

relative cerebral blood flow (rCBF); relative cerebral blood volume (rCBV); mean transit time (MTT); and time until residue function reaches its maximum (T_{max}) based on three different thresholds (i.e. >4s, >6s and >10s).[20] Using the apparent diffusion coefficient (ADC) map, RAPID segments and computes the ischemic core volume based on an ADC threshold of $<620 \times 10^{-6}$ mm^2/s.[21] Perfusion-diffusion mismatch volume was calculated automatically by RAPID as the difference between the ischemic core and the T_{max}>6s hypoperfusion lesion volumes. The mismatch ratio was calculated as the ratio between the two (i.e. hypoperfusion to ischemic core) volumes.

7.3.6. RAPID Analyses

RAPID output was analyzed to determine the proportion of children with perfusion-diffusion mismatch at presentation, defined as a core volume of <70mls, mismatch volume of ≥15mls; and a mismatch ratio ≥1.8 (ratio MRP/DWI).[22] If mismatch was present, it was described in terms of the topographical distribution of the ischemic core, and the perfusion deficit.

To better understand the MRP data quality and the quality of RAPID-derived results within our sample, we conducted a post-processing review of RAPID output maps, AIF locations, bolus properties and motion degradation artefact. An adult stroke neurologist (B.C.), blinded to the patients' clinical history, independently inspected all output perfusion parametric maps to check for motion artefacts and that the contrast bolus curve and AIF location were appropriate. Manual AIF selection was performed if the automated AIF was ipsilateral or non-arterial.

7.3.7. Effect of time-to-imaging on ADC values

To address our second aim, mean ADC values for the entire sample were calculated from manual segmentations of the ischemic core. For these segmentations, the ADC map was converted to NIfTI format, and upsampled to 0.8 mm^3 isotropic resolution. We used the scanner-derived ADC map from cases with RESOLVE acquisition, and a derived ADC map from the diffusion tensor for cases with DTI and HARDI acquisitions. A manually-initiated, semi-automated segmentation procedure, based on the Watershed Transformation method,[23] and a segmentation protocol were adopted from previous stroke studies.[24,25]

The ischemic core was manually segmented based on diffusion-restricted areas shown on the TRACE-DWI map. All image segmentations were performed by the primary author (M.V.), reviewed and approved by the senior pediatric neurologist (M.M.) as an expert rater. A repeat of all segmentations was performed by M.V., (eight-week interval between attempts), which demonstrated excellent intra-rater test-retest reliability (mean Intra-class coefficient (ICC) 0.99). Mean ADC values were calculated from the analogous DWI map. Cases were categorized into <6 hours, 6 to 12 hours, 12 to 24 hours and >24 hours post-symptom onset to imaging. The mean ADC values from the manually segmented ischemic cores were categorized, and the spread of the data were summarized as median and associated interquartile ranges (IQR).

7.4. Results

During the study period, 187 children presented to The Royal Children's Hospital with confirmed PAIS (Figure 7.1). Of these, 29 children (median age 8, IQR=4.4–14.6) met eligibility criteria. Patient demographics are shown in Table 7.1**.** Median time post-symptom onset to imaging was 13.7 hours (IQR=7.5-25.3). Four children were imaged

within 6 hours, and a further 10 within 12 hours of symptom onset**.** Median PedNIHSS was 4 (IQR=3–11). The majority of children had unilateral subcortical middle cerebral

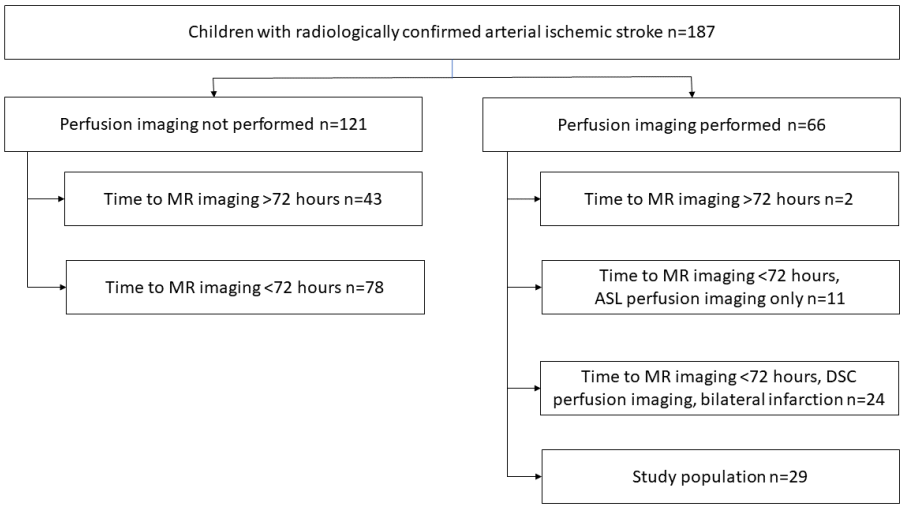

Figure 7.1 - Flow diagram of patient selection

artery (MCA) infarcts. Twelve children had vascular occlusion on MR angiography, which included five with large anterior vessel occlusion (ICA or M1), five children with M2 occlusions, and two children with posterior circulation occlusions. All three children who had mismatch had a large vessel occlusion, one of whom received intravenous alteplase. A further nine children had vascular stenoses and eight had normal vascular imaging. Focal cerebral arteriopathy was the most common identifiable etiology (31%). The cause of stroke was undetermined in 41%. The median stroke volume for the entire sample was 12.6mL [IQR=2.7- 28.0]. Eighteen participants required general anesthetic prior to their imaging scans. Those requiring GA were significantly younger (median age 4.4, IQR=2.8-7.3) than those who did not (median age 14.8, IQR=10.7-15.7) (Mann-Whitney $U = 6$, $Z = -4.15757$, $p < .001$).

7.4.1. RAPID outputs

Table 7.2 presents the MRP bolus properties. Five cases were rated as demonstrating poor bolus properties. Three children had moderate to severe head-motion on imaging. There was no relationship between age and bolus properties or head motion. The automatically selected AIF locations were deemed appropriate in 21 out of the 29 children, generally within the contralateral M1 or M2 segment of the MCA. In six, the AIF were within the ipsilateral arterial field, and in two others, the selected AIF locations were extra-arterial (venous locations). Those with suboptimal AIF selections were manually selected and reprocessed.

Based on the RAPID outputs with adequate bolus properties, penumbra, as defined by the presence of perfusion-diffusion mismatch, was identified in three children - all with a subcortical/cortical lesion, imaged at 3.75 hours (cardioembolic stroke), 11 hours (cryptogenic stroke), and 23.5 hours post-symptom onset (focal cerebral arteriopathy) (Figure 7.2). No patient with an isolated subcortical lesion had favorable mismatch.

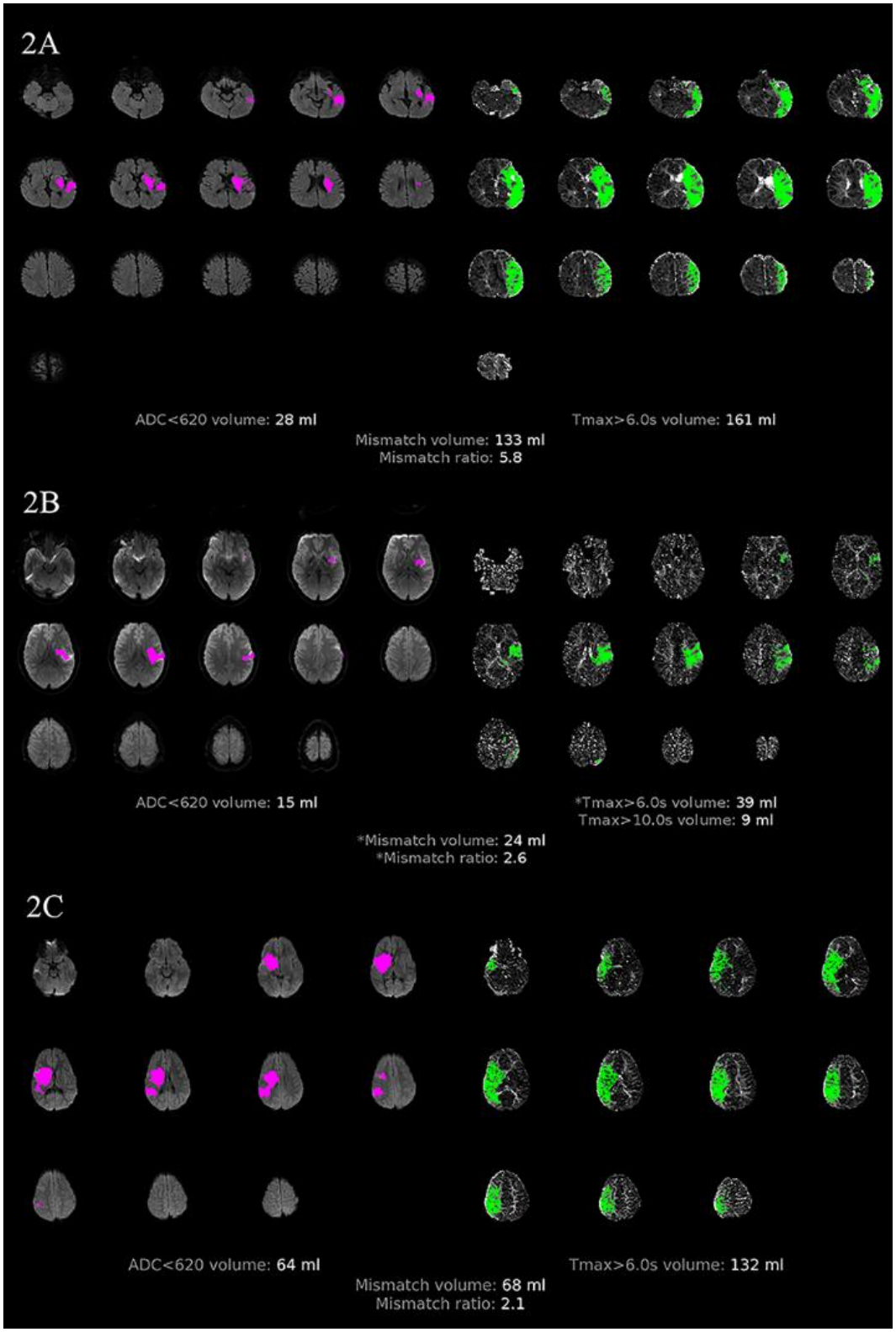

Figure 7.2 - RAPID imaging for children with favorable mismatch profiles. Figure 2A is a 22-month child with a cardioembolic left MCA stroke, in the setting of hypoplastic left heart syndrome. MRA showed a left M1 occlusion. Diffusion (left) and T_{max} perfusion (right) MRI at 3.75 hours post-onset. Figure 2B is a 15-year-old child with a cryptogenic left MCA territory stroke. MRA and echocardiography were normal. Diffusion (left) and T_{max} perfusion (right) MRI at 11 hours post. Figure 2C is a three-year-old child with a focal cerebral arteriopathy resulting in a left MCA territory stroke. Diffusion (left) and T_{max} perfusion (right) MRI at 23.5 hours post-onset. Note: varying slice numbers due to different slice thicknesses for output view.

7.4.2. Time-to-imaging & ischemic core characteristics

Using manual segmentation, the mean ADC values increased as time-to-imaging increased (Figure 7.3). 79% of 14 children imaged within 12 hours post-symptom onset had mean ADC values below $<620x10^{-6}mm^2/s$, whereas 33% of 15 children imaged beyond this time had mean ADC values below this threshold. There was no correlation found between age and mean ADC values ($r = -0.05$).

Using RAPID, an ischemic core was detected (using the automated ADC threshold $<620x10^{-6}mm^2/s$) in 19 (66%) patients at a median post-symptom onset time-to-imaging of 11 hours (IQR=7.2-23.5). For cases where no automated RAPID core was detected, the mean ADC values, time-to-imaging, and the overall size of the ischemic core lesion were compared with those that RAPID did segment an ischemic core, using non-parametric comparisons (Mann-Whitney *U*). In these remaining ten patients, the mean ADC values from manual segmentation were above the ADC threshold of $<620 \times 10^{-6}$ mm^2/s in six cases, and the remaining four had lesions that were small, only 3.5mls or less in volume. Overall, the median post-symptom onset time-to-imaging was 21.8 hours (IQR=14.3-27) for these ten cases, and the manually segmented volumes were significantly smaller (median 2.7mls, IQR=1.1–6.2) than those cases which RAPID automatically detected an ischemic core (median 17.3mls, IQR=12.4–49.6) (Mann-Whitney $U = 20$, $Z = 3.41829$, $p < .001$).

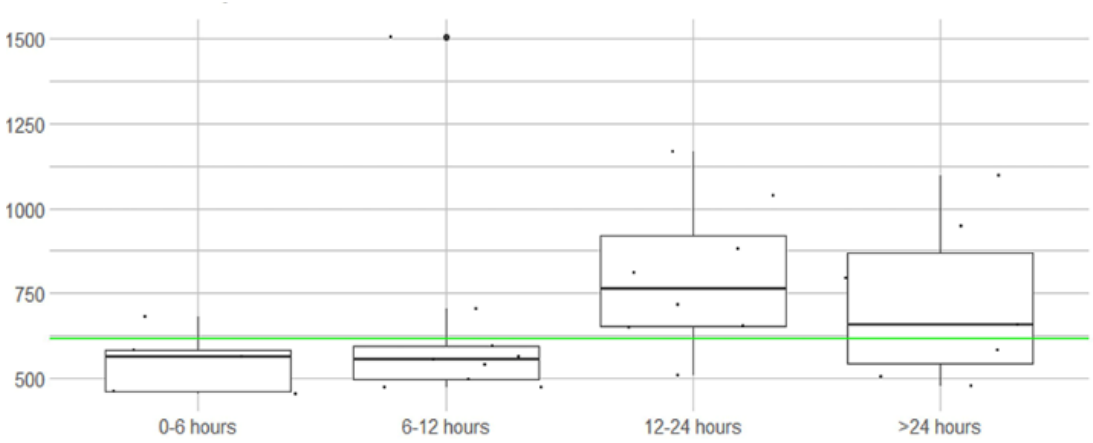

Figure 7.3 - ADC values ($mm^2/s \times 10^{-6}$) by time to presentation. X axis indicates time-to-imaging from symptom onset. The green line represents the $<620 \times 10^{-6}$ mm^2/s threshold used by RAPID. Y axis indicates spread of ADC values calculated from manual segmentation.

7.5. Discussion

This study investigated the feasibility of assessing automated perfusion-diffusion mismatch, commonly used for adult studies, in children with PAIS. We examined the characteristics of the ischemic penumbra, as defined by perfusion-diffusion mismatch profile, and the relationship between core ADC values and time-to-imaging. Three children had favorable perfusion-diffusion mismatch profile according to the defined criteria[25], including one child imaged as late as 23.5 hours post-symptom onset. ADC values appeared to follow a similar pattern to what was expected, given adult stroke time-frames, however, a larger sample is needed to determine if there is a more optimal threshold for automated segmentation in childhood PAIS. The late imaging window meant that the ADC<620 x 10^{-6} mm^2/s threshold typically used to define ischemic core using RAPID under-segmented the visually apparent lesion in some cases. The MRP contrast bolus properties were mostly adequate, suggesting that MRP can be reliably carried out in PAIS.

Currently, there is substantial gap between adults and children to access reperfusion therapies. Factors which have led to such treatment discrepancy include limited safety

and efficacy data for reperfusion therapies in PAIS, and diagnostic delays, extending beyond the standard recommended time windows for treatment.[2–4] Contributors to these delays include poor recognition of stroke symptoms, which may differ in childhood stroke (e.g. non-focal presentations with seizures), and difficulty in rapidly accessing confirmatory brain imaging.[26] Recent studies in adults have demonstrated the utility of perfusion mismatch selection to extend the treatment window to 9 hours and wake-up onset stroke for intravenous thrombolysis[5,6] and up to 24 hours for endovascular thrombectomy.[7] Adopting a similar approach should, in principle, benefit children, although more studies need to be done to better understand the penumbral characteristics in children compared to adults, underpinned by differences in stroke pathogenesis, clot composition and the immature state of the cerebral circulation.[9–11]

The earliest pediatric studies describing use of MRP were in children with sickle cell and with Moyamoya disease.[27,28] These studies were performed to investigate the hemodymanic effects of chronic ischemia and for pre-surgical planning prior to the development of automated perfusion software, such as RAPID, and therefore, are directly relevant to our current study. The data post-processing required to generate the perfusion maps was time-consuming, reducing utility for rapid clinical decisions. Another used pulsed Arterial Spin Labelling (ASL) perfusion imaging to investigate cerebral blood flow in ten PAIS children.[29] ASL is an alternate perfusion imaging modality that uses arterial blood water as an endogenous tracer to examine CBF changes in the brain. For ASL there is no need for gadolinium injection, which is a positive for safety of serial imaging.[29] In the current study, however, DSC perfusion imaging was used to avoid the reduced signal to noise ratio of ASL, and to replicate the current adult model of penumbra estimation. Further prospective research is needed to evaluate the clinical benefits of both modalities in acute PAIS. Previous studies have reported the use of CT

perfusion imaging software to characterize perfusion-diffusion mismatch in pediatric populations.[30, 31] In one study, mismatch was defined by visually-rated criteria using CBF, CBV and MTT parameters. As this technique also relied on expert review, finding a fully automated mismatch software may also be useful in an acute clinical setting. Additionally, performing CT imaging exposes the child to radiation, making it a less desirable imaging modality compared with using the MR perfusion imaging techniques. To the best of our knowledge, only one study has reported use of RAPID software in a paediatric population.[31] This retrospective study assessed the feasibility of selecting children for thrombectomy using various neuroimaging modalities, which included five patients who had RAPID imaging. This study suggested that a T_{max} >4 seconds might be more representative of critical hypoperfusion for patients scanned within 6 hours. One patient within our study, scanned at seven hours, met criteria for mismatch with T_{max} >4s, but not T_{max} >6s. Relatively high ADC values in some children may relate to the imaging occurring late and after a degree of reperfusion. Similarly, to optimize ADC thresholds, further research is needed to explore this potential MRP parameter change within a larger sample of children.

As contrast injection in pediatric patients is sometimes administered without a power-injector (which provides a more controlled pass of bolus) because of small veins, this can have an impact on the quality of the resulting perfusion maps. However, our sample demonstrated adequate bolus properties for the majority of children, with only a few curves indicating notching and temporal dispersion. Head motion was also a relatively minor factor, affecting 10% of our sample.

RAPID identified the infarct core in 19 (66%). In the remaining 10 children, this subgroup tended to have delayed imaging, in turn leading to higher ADC values, and/or

had smaller lesions. The limited ability to detect smaller lesions may have been due to the minimum lesion cluster size filter which RAPID uses to reduce artefacts.[20]

7.5.1. Penumbral profiles

Favorable mismatch profiles were identified in three children, including two who were imaged beyond the standard 4.5 hours window for thrombolysis, indicating that tissue-based selection may be beneficial in certain patients. The majority of children (21 out of the 24 cases with adequate bolus properties), however, had no detectable mismatch. The likely explanations for this observation included that expansion of the lesion core or spontaneous reperfusion had already occurred in children with delayed imaging; or that there was no penumbral tissue to begin with. No patients with pure subcortical lesions had a favorable mismatch profile. Subgroup analyses by stroke location and etiology was not possible due to the small number of children recruited to the study. Focal cerebral arteriopathy, which is one of the most important causes of pediatric stroke, is characteristically associated with subcortical infarction, centered on the basal ganglia. Although lacunar strokes do not typically meet perfusion-diffusion mismatch criteria for thrombolysis treatment, research in adults has indicated that a mismatch between the visible diffusion lesion and an absence of FLAIR hyperintensity in the region of ischemia may indicate a treatment-responsive group.[32] In contrast, stroke as a result of congenital heart disease, often affects both the cortex and subcortical structures, and typically involves an embolic mechanism, similar to the adult ischemic stroke due to atherosclerosis or cardiac disease. It is possible that children with large vessel occlusions due to cardioembolism may have similar penumbral characteristics to adults.

Limitations of our study include its retrospective design, and a small sample of childhood PAIS with different etiologies. Perfusion imaging was not performed in almost two-thirds

of children. The reason for this could not be determined in most cases because of the retrospective nature of case ascertainment. In some children, particularly in delayed time windows, this was because contrast imaging of the neck vessels was required to investigate for possible dissection, which precluded the acquisition of contrast perfusion imaging due to gadolinium dosage limits. In other cases, there was nonadherence to imaging protocols by the MRI technologists. The accrual of cases over a decade, across different practice eras, differences in MRI hardware, DWI data and sequence types employed over time affect the MRI data quality and comparability of diffusion data, making our pooled analysis less valid. The 72-hour cut-off for inclusion in the study was arbitrary, because this was an exploratory study conducted in an era where there was no data available about the time course of the penumbra in the childhood stroke population, and prior to implementation of standardized institutional pediatric stroke imaging protocols.

Our sample included children with a wide variation in time to imaging from symptom onset, a common limitation with delayed stroke presentations to the emergency department. It is important to note that while our sample did not have many children with mismatch, the included cases were not collected prospectively or consecutively. This is a limitation of our study design with a convenience sample of those who had DSC-MRI, and therefore, proportions of mismatch or their etiologies cannot be generalized. The study cohort consisted of children recruited over 10 years, during a period when reperfusion therapies were not offered to children with stroke, with the exception of one child with perfusion-diffusion mismatch (Figure 2C) who received tPA in 2014, following approval of off label treatment by our institutional clinical ethics committee.

The use of gadolinium contrast is given in most Australian pediatric stroke centers, and the perfusion imaging in this sample was obtained opportunistically in children with

confirmed stroke undergoing MRI. However, due to practice variation across the world, the use of gadolinium contrast may not be appliable as part of standard clinical care for other centers, because of concerns related to nephrogenic systemic fibrosis and gadolinium deposition in the brain.[33,34] Therefore, MRI stroke protocols should be tailored to minimize the need for gadolinium administration. Currently at our institution, contrast perfusion imaging is only performed in children with suspected stroke who present within the time window for reperfusion therapies, following diffusion imaging to confirm infarction, susceptibility weighted imaging to exclude hemorrhage, and time of flight MR angiography to confirm a vessel abnormality.

The utility of our definition of "favorable mismatch", which is based on adult stroke practice, is a potential limitation. It has not been validated in children and it is unknown whether this definition is therapeutically helpful for the pediatric population. Selection of the ADC and T_{max} threshold for RAPID processing were also chosen from evidence-based adult parameters, the limitations of which are not currently known for children, especially given the prevalence of delayed time to diagnosis in children. In this study, we were unable to obtain follow-up imaging at day seven to determine final DWI volume, though in future research this could be investigated to characterize final stroke burden compared to acute perfusion deficit. The methodological issues encountered and clinical relevance require further investigation in larger prospective studies.

7.6. Conclusion

In conclusion, this study demonstrates that it is feasible to rapidly assess perfusion-diffusion mismatch in childhood PAIS using automated software. Favorable mismatch profiles, using adult-based parameters, were noted as late as 23.5 hours post-symptom onset, well beyond the standard 4.5-hour time-window for thrombolysis. However, the

utility of perfusion-based imaging to guide clinical decision making requires further investigation in prospective studies. The effects of etiology on mismatch characteristics and the clinical relevance of acute perfusion-diffusion mismatch with regard to long-term outcomes need to be determined. Improved understanding of penumbral characteristics and optimization of automated DWI/PWI thresholds and mismatch criteria in childhood PAIS, may facilitate tissue-based patient selection for reperfusion therapies.

Tables

	N or Median	Interquartile range or percentage
Sex (male), N %	15 male	52%
Age (years), median (IQR)	8	4.4 - 14.6
PedNIHSS, median, (IQR)	4	3 – 11
Time-to-imaging (hours), median (IQR) 0-6 hours (N) 6-12 hours (N) 12 – 24 hours (N) >24 hours (N)	13.7 4 10 8 7	7.5 – 25.3 14% 35% 28% 24%
Infarct location (N) MCA Subcortical MCA Mixed cortical-subcortical PCA	 17 9 3	 59% 31% 10%
Median ischemic core volume, cm^3	12.6	2.7 - 28.0
ADC value (mm^2/s)*, median (IQR)	595.3	506.7 – 801.7
Vascular territory N MCA PCA	 26 3	 90% 10%
Etiology (N) Small vessel arteriopathy	 1	 3.4%

Unilateral (focal) cerebral arteriopathy	9	31%
Bilateral cerebral arteriopathy	1	3.4%
Aortic/cervical arteriopathy	1	3.4%
Cardioembolic	5	17.2%
Other: Undetermined	12	41.4%

Table 7.1 - Sample demographics *Mean ADC values calculated for each patient and Median used to describe spread of data.

	N
Movement artefact	
Minimal	26
Moderate	2
Severe	1
Contrast injection bolus	
Adequate	24
Notched peak	2
Temporal dispersion	2
Notched peak and temporal dispersion	1
AIF automatic location selection	
Contralateral hemisphere or ACA, acceptable	21
Ipsilateral arterial field, suboptimal	6
Non-arterial	2
Perfusion-diffusion mismatch characteristics	
(Poor bolus properties)	(5)
No perfusion or diffusion lesion detected	8
Negative diffusion*	2
Negative perfusion†	8
Matched	3
Favourable mismatch	3

Table 7.2 - Bolus properties and RAPID outputs *DWI (core) not detected but perfusion lesion detected, or DWI < PWI but favourable mismatch criteria not met. †Perfusion lesion detected, less than core volume. PWI T_{max} >6s, DWI <620 $\times 10^{-6}$ mm^2/s.

Source of Funding

Study funding: M. Mackay received grant funding from Murdoch Children's Research Institute. M. Visser receives funding from an Australian Government Research Training Program (RTP) Scholarship. J. Y.-M. Yang received positional funding from the Royal Children's Hospital Foundation (RCH 1000). The research was also supported by the Victorian Government Operational Infrastructure Fund

Conflict(s)-of-Interests/Disclosures

Disclosures: None.

Supplemental Materials

Online Figure I

7.7. References

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8. STUDY 3 – MEANINGFUL PENUMBRA? CLINICAL FACTORS, & LESION VOLUME CHANGE EXPLORED

In this chapter, I attempt to answer the question "does our mismatch definition represent "meaningful" penumbra and are changes needed?" To do this, I first expand on the findings from RAPID in the previous chapter, by examining the ADC values in more depth (to further address Aim2b). Secondly, I look at how infarct volume changes over time, and how this relates to clinical factors such as time-to-imaging and aetiology. Finally, this chapter takes a more in-depth look at the definitions of the penumbra and the relationship with lesion volume change, with an exploratory look at mismatch profiles and comparisons.

8.1. Apparent Diffusion Coefficient thresholds

In Study Two, RAPID was implemented to provide an estimate of the infarct volume that was quickly obtained by using a pre-selected ADC threshold. As detailed in Chapter Three, however, sub-acute ADC values pseudonormalise within 3-7 days following PAIS (van der Aa et al., 2013). This adds difficulty in the context of delayed presentation and diagnosis in children with PAIS, as the infarct ADC value may have pseudonormalised by the time children are imaged. To the best of our knowledge there are no studies investigating whether infarcted tissue in non-neonatal children follows a similar ADC value pattern. The relevance of the threshold identified in adult ischemic stroke for children is also unclear. As children often present outside the standard time-windows for stroke treatment in adults, it is important to look at the accuracy of automated segmentation using adult thresholds. If there is an opportunity to accurately define different ADC thresholds based on timing of presentation, this could allow RAPID infarct segmentation for PAIS patients with delayed imaging. In this chapter, the aims are to examine the mean ADC values for infarcted tissue in the study sample of PAIS, and to look specifically at the trend over time.

8.1.1. Segmentation and estimation methods

Methods for the estimation of the ADC value of infarcted tissue are detailed in Chapter Six. The mean ADC values used in this estimation were derived from the manually segmented lesions using the watershed transform method. The ADC values collected per identified region of interest were then averaged to get an overall mean value. The mean ADC values were plotted by time-to-scan to observe the differences and explore the relationship between these variables. As can be seen in Figure 8.1, which is a clearer presentation of the results than in Figure 7.3, the mean ADC value for patients who

present beyond 12 hours are higher than the ADC threshold that is used by RAPID. Although the average and most values are above 620 x10^{-6} mm^2/s, the spread is much wider in both groups beyond twelve hours, which indicates that adjusting the threshold for later presentations may not be simple. Equally, making the threshold too high to encapsulate the majority of values may result in the false positive inclusion of healthy tissue.

Overall, the trend appears to be similar to that in adults, with most ADC values above the threshold in later time brackets. However, a much larger sample size may identify the most appropriate threshold in PAIS, in a similar way to the work done in adult stroke to find the current RAPID threshold. In the sample, as detailed in the previous chapter, RAPID software not detecting the infarct in those with delayed imaging was a problem

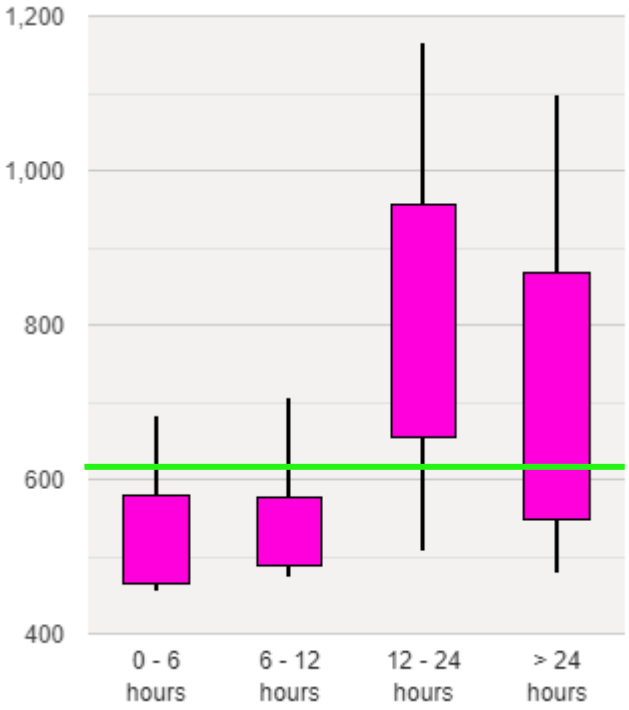

Figure 8.1 – Box and whisker plot of Mean ADC values by time to presentation. X axis indicates ADC values (mm2/s x 10 -6), Y axis indicates hours from stroke onset to imaging. Green line indicates automated threshold used by RAPID (ADC <620x10-6 mm2/s).

that was not unexpected, due to ADC pseudo-normalisation in the subacute period after infarction.

Despite the visual identification of a higher and more varied ADC value range in later scan times, I sampled some cases to see if a more appropriate ADC threshold could be identified. I piloted using the mean ADC value from our manual segmentation, and altered the threshold in RAPID, based on the time-to scan presentation.

Time-to-scan (TTS) group results:

1. 0-6 hours overall mean ADC value = 571.17.
2. >6 – 12 hours overall mean ADC value = 637.01.
3. 12 – 24 hours overall mean ADC value = 802.65.
4. >24 hours overall mean ADC = 723.66.

One case from TTS group 3 and TTS group 4 are presented here.

Case #1: As can be seen in Figure 8.2, this was initially segmented missing a clear amount of the lesion (automated segmentation (<620 $x10^{-6}$ mm^2/s): 2mls). Manual segmentation was 46mls. Mean ADC value for this region-of-interest (ROI) defined by manual segmentation was 717.25 (SD 210.38). Time to scan was 24 hours, placing this participant in TTS group 3 (mean ADC value = 802.65). This adjustment only segmented 8ml, significantly under-segmenting the lesion. To achieve a comparable result to the manual segmentation, an ADC threshold was greater than doubled, to an ADC value of <1300 $x10^{-6}$ mm^2/s. This particular result was well above the suggested mean ADC for this time bracket.

Case #2: Initially, no diffusion lesion was segmented using the standard RAPID threshold (automated segmentation (<620 $x10^{-6}$ mm^2/s): 0mls (Figure 8.3)). Manual

segmentation was 5mls. Mean ADC value for ROI: 794.42 (SD 218.38). Time to scan was 32 hours, placing this participant in the TTS group 4 (mean ADC = 723.66). This figure demonstrates that by adjusting the ADC threshold to more closely match the mean

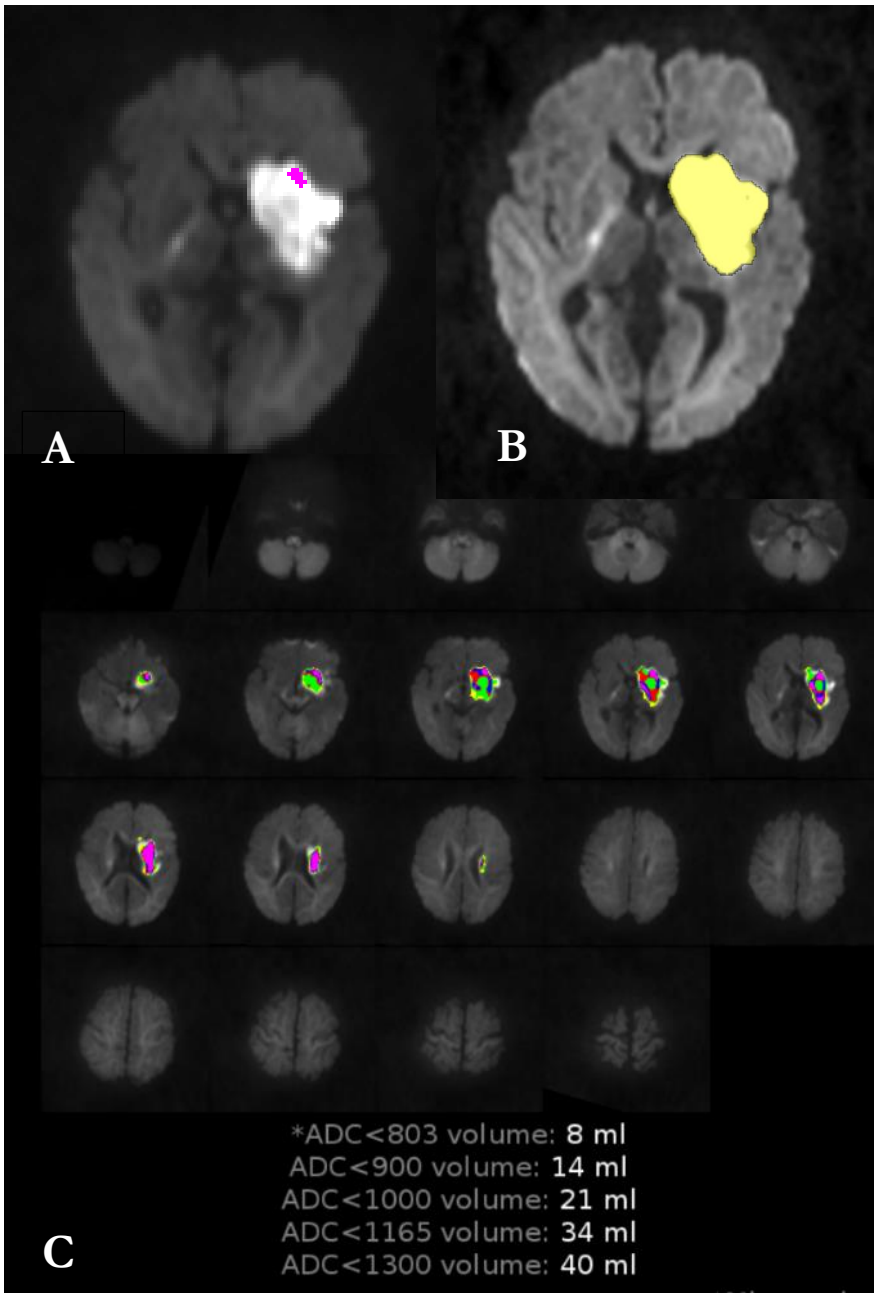

Figure 8.2 - ADC threshold adjustment case 1 RAPID output. (A) RAPID automated segmentation using pre-defined threshold (ADC <620 x10-6 mm2/s) volume 2mls (B) Manual segmentation DWI volume 46mls (C) Time Group 3 Mean ADC < 803 x10-6 mm2/s only segmented 8mls. An ADC value closer to double the original value (<1300) was needed to get closer to the manual segmentation.

ADC value from the small sample with reference to scan time, there are still some infarcted sections not included, and there is also some clear inclusion of healthy tissue.

With these two significantly varied examples, it is unlikely to be as simple as adjusting the ADC threshold to accurately segment these infarcts from later presentations. It may be that much larger studies may be able to determine if there are any mediating variables that have an effect on establishing an individualised ADC threshold for those that present

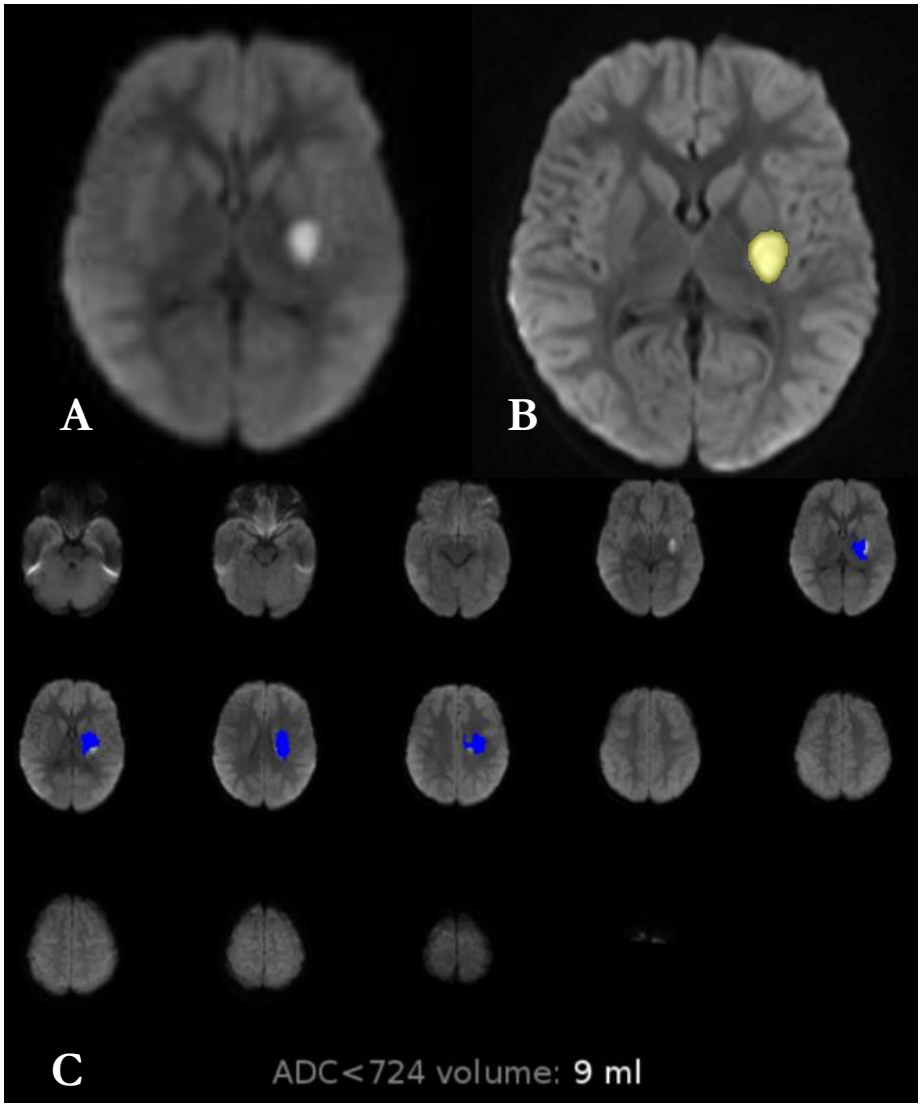

Figure 8.3 - ADC threshold adjustment case 2 RAPID output. (A) RAPID automated segmentation using pre-defined threshold (ADC <620 x10^{-6} mm^2/s) volume 0mls (B) Manual segmentation DWI volume 5mls (C) Time Group 4 Mean ADC < 724 x10^{-6} mm^2/segmented 9mls. This ADC value over segmented and included some healthy tissue.

in extended time-windows. It is likely, however, that due to the acute changes in diffusivity, a threshold-based ADC map may be suboptimal for cases who are imaged late. For these cases, future research could trial a different automated segmentation algorithm, perhaps using machine learning or deep learning.

Overall, more than half of the sample were scanned later than 12 hours post-onset, and one-third did not have an infarct detected by RAPID using the standard threshold which was not amended by adjustment of mean ADC value by time to scan. Therefore, continued work to determine if it is possible to accurately segment infarct core from delayed imaging with either a tweak in software parameters, or an alternate method remains an important area for future research.

8.2. Infarct volume change in paediatric arterial ischaemic stroke

Accurate determination of infarct volume and penumbral changes is challenging when imaging is not conducted in the subacute period (i.e., within days post-stroke), particularly as children often require general anaesthesia for MRI scanning. In my study, final infarct volume was estimated by using the same manual volumetric analyses as used to determine acute infarct volume. T2 FLAIR weighted images were determined to be the most useful in this aspect, as per previous research (Federau et al., 2016; Gaudinski et al., 2008). By assessing scans approximately 90 days post-stroke, the final infarct volume should be reached without expecting significant change beyond this time.

Final infarct volume is visually smaller due to the reduction in oedema, and widening of sulci due to atrophy. It is difficult to compare acute to chronic volumes, as the rate of which the lesion would appear smaller is unknown, which makes 'lesion growth' difficult to define. This difficulty has been identified in paediatric stroke literature (Chen et al., 2009). As mentioned in Section 3.5, one recent study highlighted the importance of

collateral blood supply in PAIS, demonstrating that distal filling of >50% resulted in smaller final stroke burden and slower early infarct growth rate in children who underwent MT (Lee et al., 2022). Though collateral flow was not measured in the present study, this is an important factor for future research.

Lesion growth or change was defined as a ratio of the chronic lesion (mls), divided by the acute lesion (mls). To increase the comparability of the lesion change ratios by reducing the effect of time, I used chronic scans as close to 90 days post-stroke as possible. Chronic scan time-post stroke onset (days) median = 99, IQR 64.5-116. Multiple factors appear to explain some variance in this lesion change ratio, and I aimed to look at the effects of both acute time to imaging and age at stroke.

Overall, the average proportion of the chronic lesion size to the acute lesion size for our sample was 0.72. Similar to the overall acute and chronic infarct volumes identified in Chapter 6, this rate of change also supports the original hypothesis (Hyp3a) that chronic

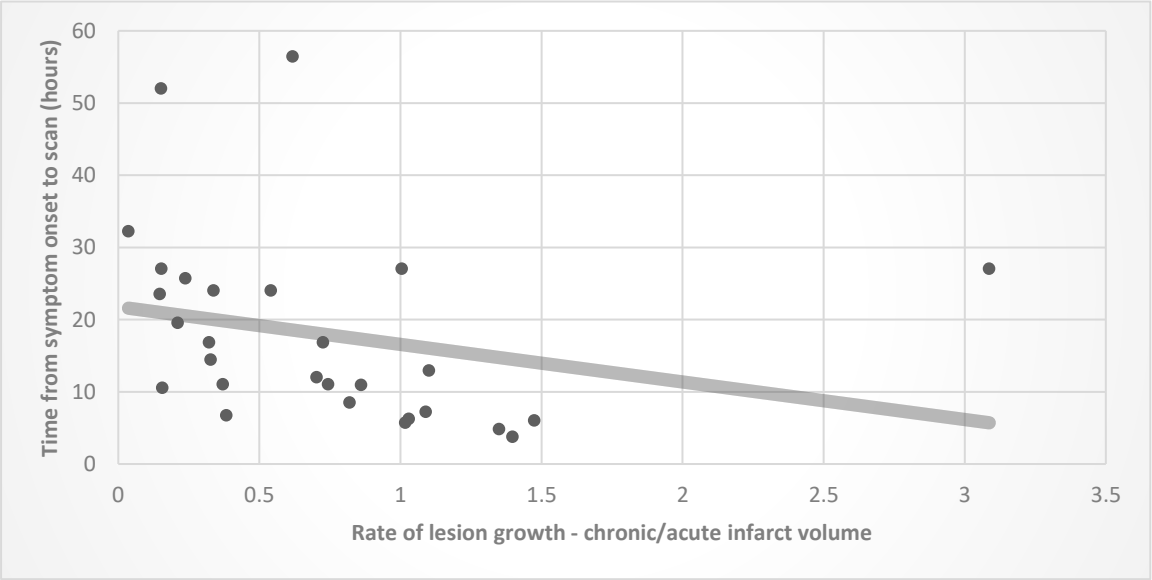

Figure 8.4 - Lesion growth by time from symptom onset to MRI scan

lesion volumes would be smaller than the acute lesion volumes following atrophic processes.

As can be seen in Figure 8.4, there is a trend indicating that PAIS patients who were scanned earlier were more likely to experience a higher proportion of chronic lesion volume compared to the acute lesion volume (r_s = -0.56, p > 0.01). Though there are some data points that do not fit the overall trend, particularly after 12 hours, those scanned within 12 hours post-onset seem to suggest an increase in lesion change amongst the most acute infarcts within our sample.

Figure 8.5 illustrates the rate of change (chronic lesion (mls)/ acute lesion (mls)) by age at stroke. No significant relationship was identified between age at stroke onset and lesion volume change (r_s = -0.3956, p = 0.18).

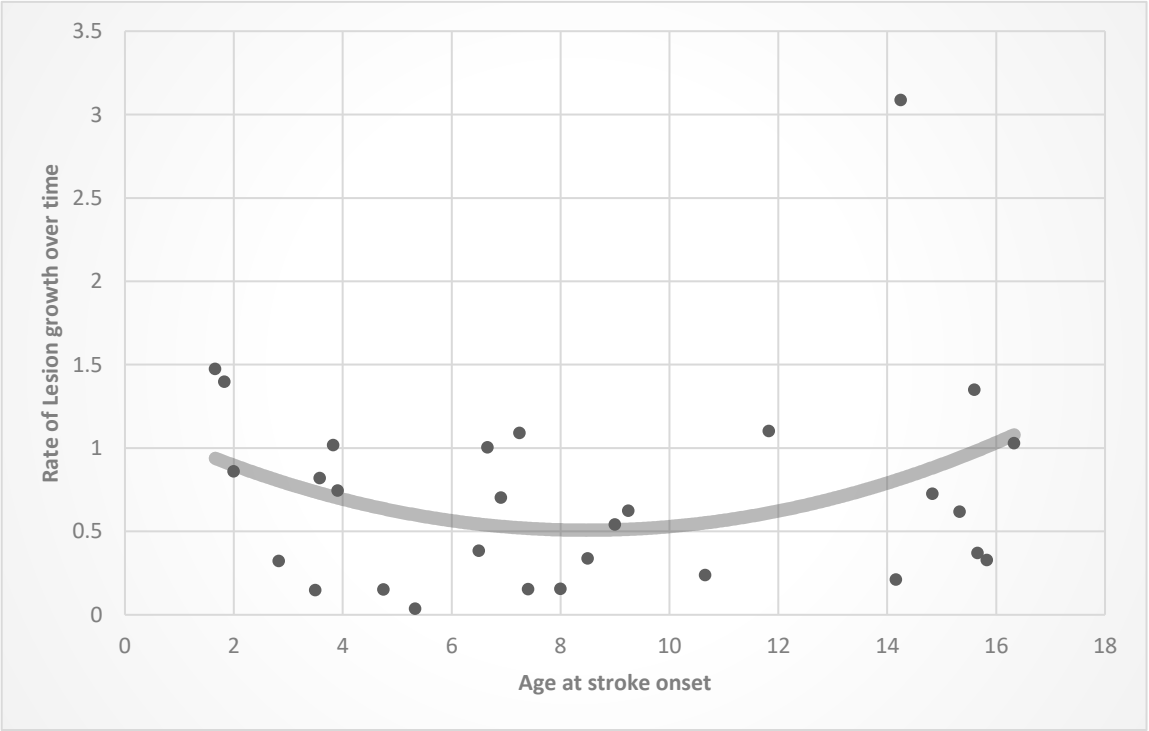

Figure 8.5 - Relationship between age and rate of lesion growth

8.3. Aetiology

The subsample of patients that had definable mismatch on imaging was low (n = 3), therefore analyses looking at the relationship between aetiology and likelihood of having penumbral tissue was not possible. These three participants had different aetiologies (cardioembolic, focal cerebral arteriopathy, cryptogenic). I hypothesised (Hyp3b2) that those children whose stroke mechanism was similar to adult stroke (i.e., cardioembolism) would be more likely to have penumbra than those with a more cerebrally intrinsic aetiology (ie. arteriopathy); however, a much larger sample of participants with mismatch will be needed to answer that question. Another difficulty inherent in looking at the relationship between aetiology and outcomes is that between 10 – 25 percent of strokes in children are cryptogenic (Mittal et al., 2015). In our sample, 41% were of an undetermined aetiology.

With our limited data, to attempt to answer Study Question 3, Aim 3(b), I looked for differences in lesion volume and the relationship to aetiology, of which frequency data is presented in Table 8.1. Kruskal-Wallis analysis of the three aetiologies with greater than one datapoint in the study sample (I.e. FCA, cardioembolic and cryptogenic aetiology) revealed no significant differences in acute lesion size ($H = 0.33$, $p = .85$), or rate of lesion change ($H = 0.87$, $p = .65$). Non-parametric comparison of the acute lesion volume and lesion volume change for the two known aetiologies with greater than one datapoint (FCA and cardioembolic) was also not significant ($U = 19$, $p = 0.34$), ($U = 21$, $p = 0.45$, respectively).

Aetiology	Frequency	Acute volume (mls)	Lesion volume change (Chronic / Acute)
Focal Cerebral Arteriopathy	9	Median 12.66, IQR 13.45	Median 0.82, IQR 0.49
Cardioembolic	5	Median 14.46, IQR 50.36	Median 1.01, IQR 1.10
Cryptogenic	11	Median 13.20, IQR 29.61	Median 0.38, IQR 0.34
Single Vessel Arteriopathy	1	1.73	3.09
Moyamoya Disease	1	49.65	0.24
Dissection	1	3.55	0.16
Anaemia	1	4.51	0.86

Table 8.1 – Aetiologies and lesion volumes

8.4. Case studies – Penumbra and Infarct Volume Change

In this section, I explore the individual case studies of those children who met criteria for penumbra based on adult thresholds used by RAPID. In addition, some extended criteria are included, using T_{max} >4s, which was thought to correlate more accurately with stroke severity in previous research (Lee et al., 2019). This brings the case study total to six participants; though there are very small numbers in this exploratory pilot study, I explored indicators of differences in lesion growth between those defined as having favourable mismatch profiles and those that didn't. The aim is to see if there are any

preliminary indications that my definition may represent truly salvageable brain tissue, using a higher ratio of chronic to acute lesion volume as a marker.

8.4.1. Case study #1

Case #1 (Figure 8.6) was a three-year-old female with a cortical-subcortical focal cerebral arteriopathy. She met standard imaging criteria for penumbra using RAPID. She was scanned 23.4 hours after symptoms onset and had a PedNIHSS of 4. Acute DWI lesion volume using RAPID was 64 mls, and using manual segmentation was 62.5 mls. Hypoperfusion lesion was 197 mls at T_{max} >4s, and 132 mls at T_{max} >6s. Mismatch ratio was 2.1. Chronic proportion of acute lesion identified was only 15% (chronic volume = 9.2mls). Both acute and follow-up PSOM (12 years post-stroke) were 2.

8.4.2. Case study #2

Case #2 (Figure 8.7) was a 15-year-old male with a cortical-subcortical cryptogenic stroke, who was scanned at 11 hours post-symptom onset. This participant also met standard imaging criteria for penumbra using RAPID. He had a PedNIHSS of 10. Acute DWI lesion volume using RAPID was 15 mls, and using manual segmentation was 17.3 mls. Hypoperfusion lesion was 84 mls at T_{max} >4s, and 39 mls at T_{max} >6s. Mismatch ratio was 2.6. Chronic proportion of acute lesion identified was 37% of the acute size (chronic volume = 6.4 mls). Acute PSOM score was 0 (nil follow-up PSOM data available for this patient) .

8.4.3. Case study #3

Case #3 (Figure 8.8) was a 15-year-old female with a subcortical focal cerebral arteriopathy scanned at 4.8 hours post-symptom onset. She had a PedNIHSS of 3. She

had penumbral mismatch using T_{max} >4 seconds, scanned within 6 hours post-symptom onset. This patient's DWI lesion was not detected by RAPID, likely due to the small size of the lesion, and therefore was not included in Chapter 7. Using the DWI manual segmentation (3.6mls), this patient met criteria for favourable mismatch at T_{max} >4 seconds (41mls). T_{max} >6 seconds demonstrated a relatively matched perfusion deficit (3 mls). Manual segmentation did suggest lesion growth, with a chronic lesion size estimated to be 130% of the acute lesion (4.8mls). Acute and follow-up PSOM scores (nine years post-stroke) were both 0.5.

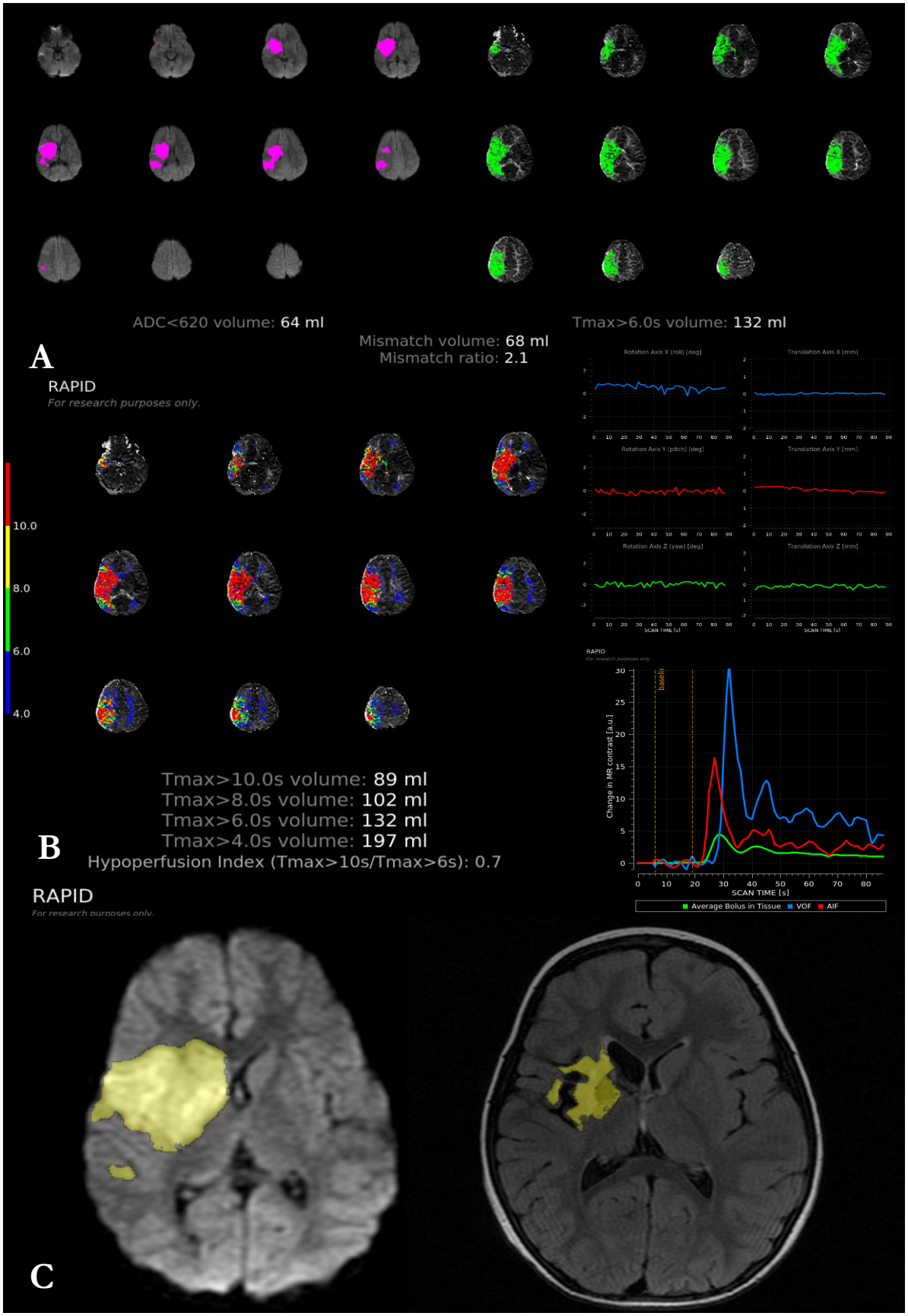

Figure 8.6 - RAPID output and manual segmentation Case study 1. (A) Automated mismatch segmentation core (left) hypoperfusion (right) using standard RAPID thresholds (B) Alternate T_{max} thresholds, head motion, and AIF curves. (C) Manual segmentation of acute DWI lesion 62.5mls (left) and chronic T2 FLAIR lesion 9.2mls (right)

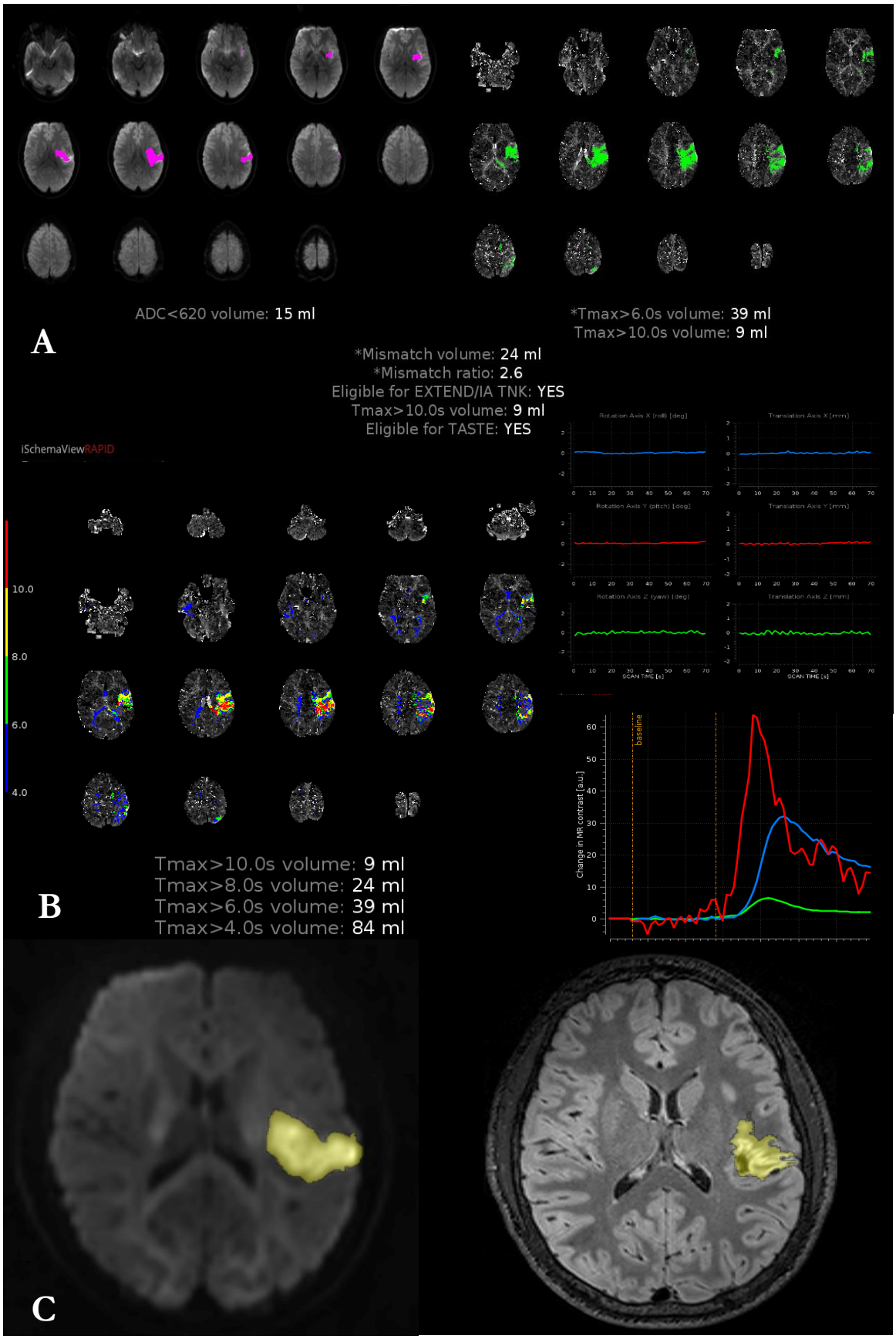

Figure 8.7 - RAPID output and manual segmentation Case study 2 - A) Automated mismatch segmentation core (left) hypoperfusion (right) using standard RAPID thresholds (B) Alternate T_{max} thresholds, head motion, and AIF curves. (C) Manual segmentation of acute DWI lesion 17.3mls (left) and chronic T2 FLAIR lesion 6.4mls (right)

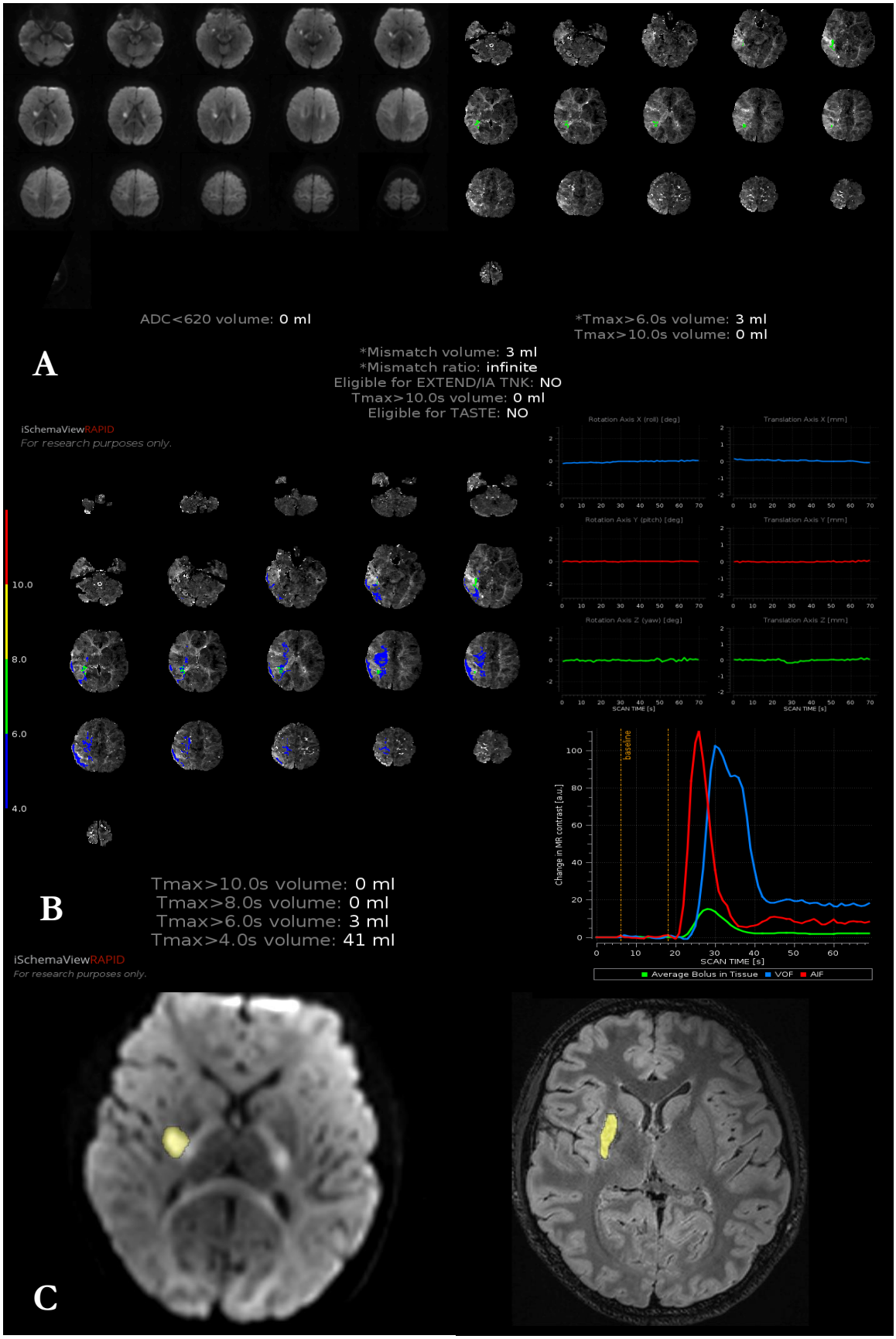

Figure 8.8 - RAPID output and manual segmentation Case study 3 - A) Automated mismatch segmentation core (left) hypoperfusion (right) using standard RAPID thresholds (B) Alternate T_{max} thresholds, head motion, and AIF curves. (C) Manual segmentation of acute DWI lesion 3.6mls (left) and chronic T2 FLAIR lesion 4.8mls (right)

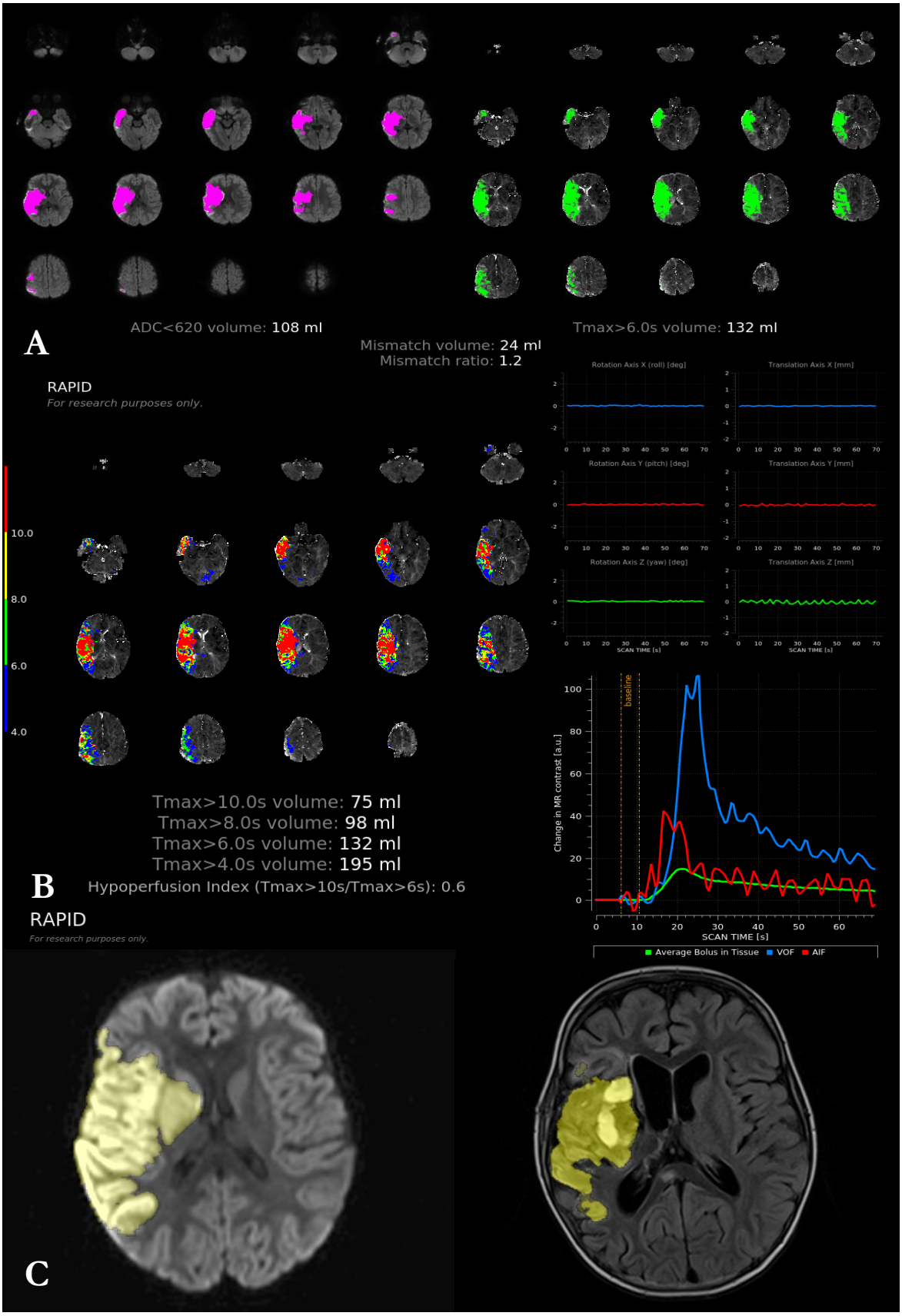

Figure 8.9 - RAPID output and manual segmentation Case study 4 (A) Automated mismatch segmentation core (left) hypoperfusion (right) using standard RAPID thresholds (B) Alternate T_{max} thresholds, head motion, and AIF curves. (C) Manual segmentation of acute DWI lesion 103.3mls (left) and chronic T2 FLAIR lesion 105.1mls (right)

8.4.4. Case Study #4

Case #4 (Figure 8.9) was a three-year-old boy with a cortical-subcortical cardioembolic stroke, with a PedNIHSS of 11. He was scanned at 5.7 hours post stroke onset. As in Case #3, this participant had suggestion of mismatch using T_{max} >4s. Acute volume using RAPID was 108 mls, using manual segmentation was 103.3 mls), T_{max} >4 = 195 mls (Ratio 1.8), T_{max} > 6 = 132 mls (Ratio 1.22). This participant did not have a favourable mismatch profile, due to an infarct segmented of greater than 70mls. The ratio of chronic lesion (105.1mls) over acute (103.3mls) was 102%, suggesting the possibility of infarct progression. Acute PSOM was 6 (nil follow-up PSOM data available for this patient).

8.4.5. Case study #5

Case #5 (Figure 8.10) This case was a seven-year-old female with a subcortical focal cerebral arteriopathy. She was scanned at 7.2 hours post-symptom onset, and had a PedNIHSS stroke severity score of 19. This participant was mentioned briefly in the discussion section of Chapter Seven, as meeting all criteria for favourable mismatch profile, except using T_{max} >4s (88 mls) instead of 6s (23 mls). The acute DWI lesion was 22 mls using RAPID, and 18.2 mls using manual segmentation. Mismatch volume using T_{max} >4s was 66 mls, and a mismatch ratio of 4.0. Chronic lesion volume was 19.9 mls. Chronic lesion proportion of acute lesion was greater than 1 (109 %), suggesting infarct growth. Acute PSOM was 2 and follow-up (five years post-stroke) was 1.

8.4.6. Case study #6

Case #6 (Figure 8.11) was a one-year-old boy with a cortical-subcortical cardioembolic stroke, who was scanned at 3.7 hours after symptom-onset. This participant met standard imaging criteria for penumbra using RAPID. This patient was the only one in this sample

that received reperfusion treatment (treated with tPA acutely). Acute lesion volume using RAPID was 28 mls, and manual segmentation was 18.1 mls. Hypoperfusion lesion was 209 mls at T_{max} >4s, and 161 mls at T_{max} >6s (I.e. mismatch ratios of 7.5 and 5.8, respectively). Chronic infarct volume was 25.4 mls. Chronic proportion of acute lesion identified was 140% of the acute size suggesting infarct growth. Acute PSOM was 2 and follow-up (two years post-stroke) was 1.

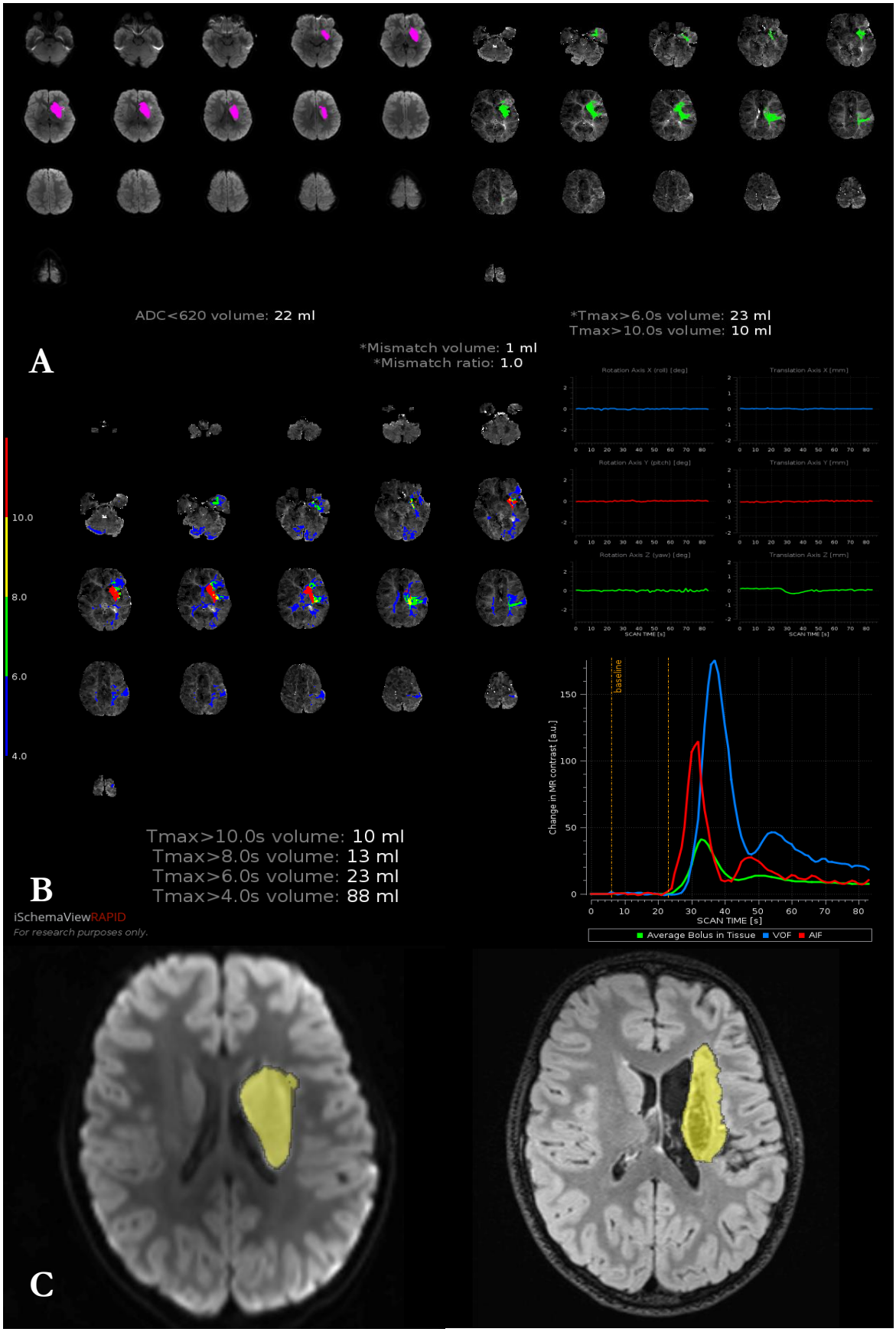

Figure 8.10 - RAPID output and manual segmentation Case study 5 (A) Automated mismatch segmentation core (left) hypoperfusion (right) using standard RAPID thresholds (B) Alternate T_{max} thresholds, head motion, and AIF curves. (C) Manual segmentation of acute DWI lesion 18.2mls (left) and chronic T2 FLAIR lesion 19.9mls (right)

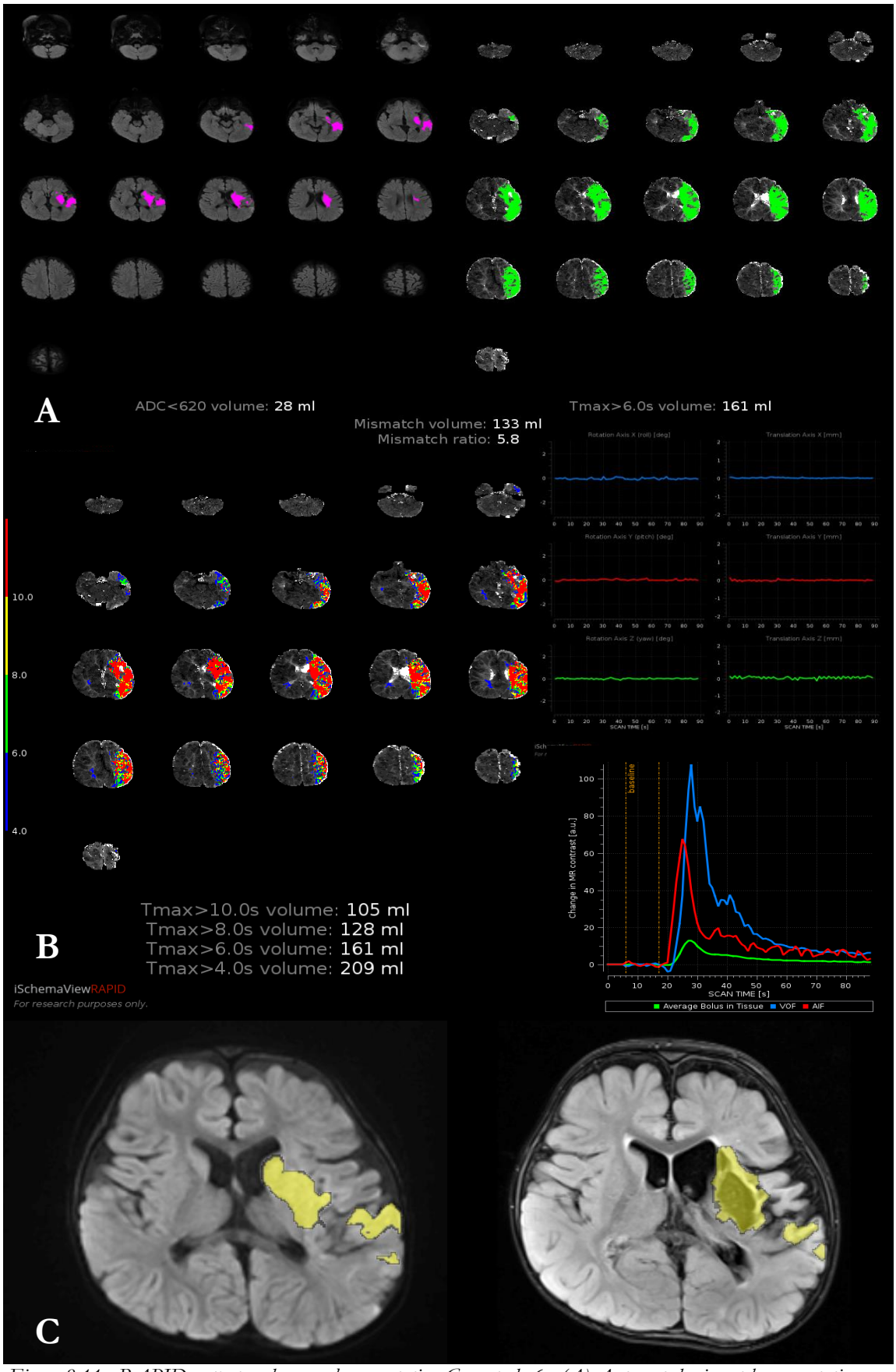

Figure 8.11 - RAPID output and manual segmentation Case study 6 - (A) Automated mismatch segmentation core (left) hypoperfusion (right) using standard RAPID thresholds (B) Alternate T_{max} thresholds, head motion, and AIF curves. (C) Manual segmentation of acute DWI lesion 18.1mls (left) and chronic T2 FLAIR lesion 25.4mls (right)

All six participants in these case studies had good / adequate bolus properties, with minimal head motion. Four out of six cases (66%) had chronic lesion proportions greater than 100% of the acute lesion volume. Only four other cases in the remainder of the sample (17%) also met this proportion, with the average chronic lesion of the entire sample only 72% of the acute lesion. Non-parametric comparison between case studies with suggested mismatch (N=6) and the remainder of the sample (N=22) showed no significant difference in lesion volume change ($U = 46$, $p = 0.11$) or acute stroke severity rating ($U = 50.5$, $p = 0.11$). Non-significance was not entirely unexpected due to the vastly different sample sizes between groups. Though not significant, the average lesion change was 89% (Median = 106%) in the suggested penumbra group, and 68% (Median = 58%) in the remainder of the sample, demonstrating a trend towards larger lesion growth in those with penumbra using both T_{max} >6s and T_{max} >4s. This is further demonstrated in Table 8.2 and Figure 8.14.

8.5. Clinical Mismatch – an alternate measure of penumbra

Definitions of penumbra to identify patients most likely to benefit from reperfusion therapies have largely attempted to do so via multimodal imaging techniques, as previously explored. The DAWN trial in adults, and more recently a sub-analysis of the Save ChildS study in PAIS used a selection of patients by assessing their clinical deficit (as per their NIHSS or PedNIHSS respectively), compared to their infarct volume on DWI. The PedNIHSS calculates stroke severity using a paediatric-specific version of the National Institute of Health Stroke Score (NIHSS), with 11 neurological domains screened (Tam, 2019). Scores range from 0-42, categorised by Mild (1-5), Mild to Moderately-Severe (5-14), Severe (15–24), and Very Severe (>25). Favourable mismatch was defined by either:

1. (Ped)NIHSS ≥ 10, and an infarct volume of ≤50mls

or

2. (Ped)NIHSS ≥ 20 and an infarct volume of 51-70mls

Those not meeting either of these criteria did not have a clinical mismatch. Seven participants in our sample met these criteria, using both RAPID DWI segmentation and manual segmentation. Two patients met the above criteria, using the manual segmentation performed in Chapter 6 but not with RAPID segmentation. These were two cases where RAPID either appeared to include healthy tissue and/or missed infarcted tissue (Figure 8.12). From these nine total participants, only two clinical mismatch patients overlapped with those identified in the above penumbra case series. Case #2 (page 164), and Case #5, (page 169).

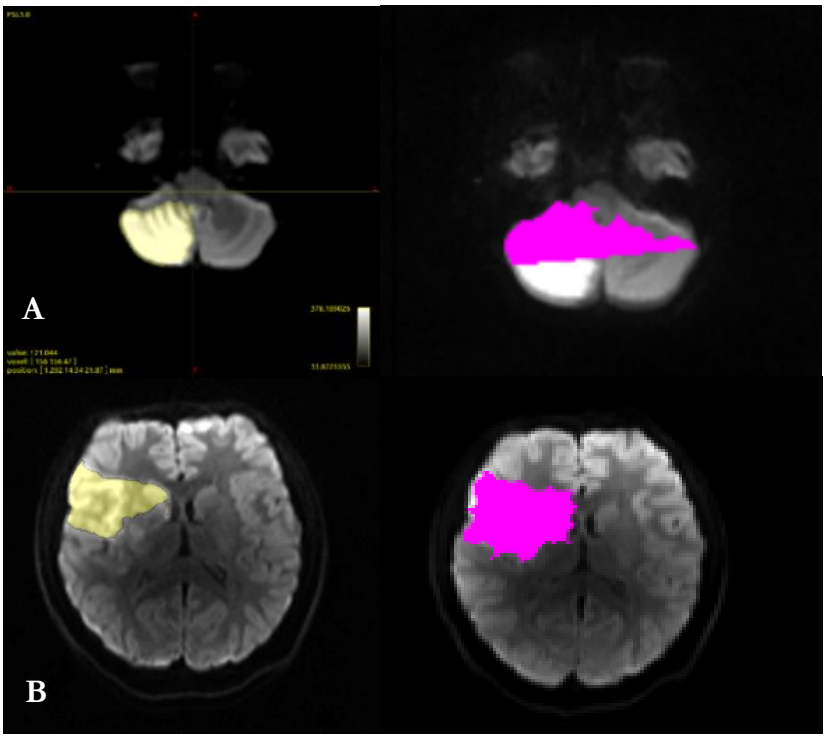

Figure 8.12 - Two cases with suboptimal RAPID DWI segmentation. A – Cerebellar stroke, missing some and over-including other infarcted tissue. B – Overinclusion of infarcted tissue

A comparison between lesion volume change in participants identified as having penumbra using imaging (using expanded RAPID criteria), and those using clinical mismatch criteria shows overlap, though with a visually different spread of scores (Figure 8.13). Interquartile range between imaging mismatch is more vastly spread than clinical mismatch in our sample (imaging mismatch IQR = 0.98, clinical mismatch IQR = 0.39, see Table 8.2). Note: Tukey test identified one statistical outlier in the non-mismatch sample groups, therefore overall N = 28, with outlier of 3.09 as can be seen at the bottom of the table.

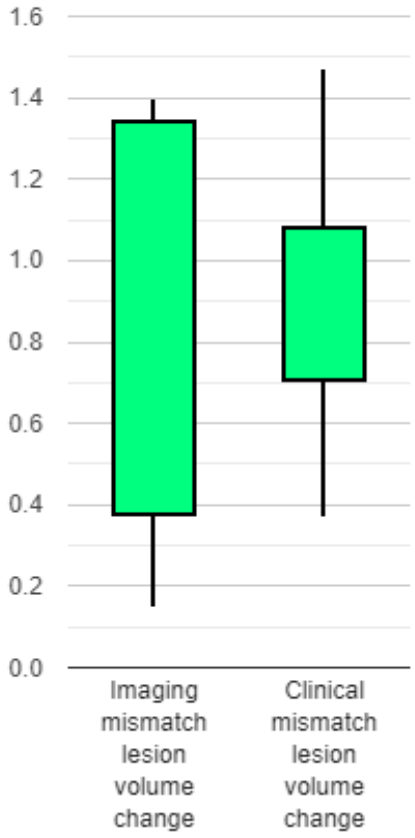

Figure 8.13 - Box and whisker spread of lesion volume change (chronic lesion (mls)/ acute lesion (mls)) between imaging defined mismatch, and clinical mismatch.

Groups:	Non-clinical mismatch	Clinical mismatch	Non-imaging mismatch	Imaging mismatch
Sample size (n):	19	9	22	6
Minimum:	0.04	0.37	0.04	0.15
Q1:	0.185	0.7	0.24	0.37
Median:	0.34	0.86	0.58	1.06
Q3:	0.77	1.09	0.82	1.35
Maximum:	1.4	1.47	1.47	1.4
Mean ($\bar{x}$):	0.52	0.89	0.57	0.90
Skewness:	0.93	0.25	0.60	-0.71
Excess kurtosis:	-0.23	0.21	-0.18	-1.46
Outliers:	3.09		3.09	

Table 8.2 - Descriptive statistics of lesion volume change amongst mismatch comparison groups

Participants with favourable clinical mismatch profiles demonstrated significantly greater lesion growth compared to those with a non-favourable clinical mismatch profile. (U = 45.5, p = 0.019). The distribution of these values can be seen in Figure 8.14.

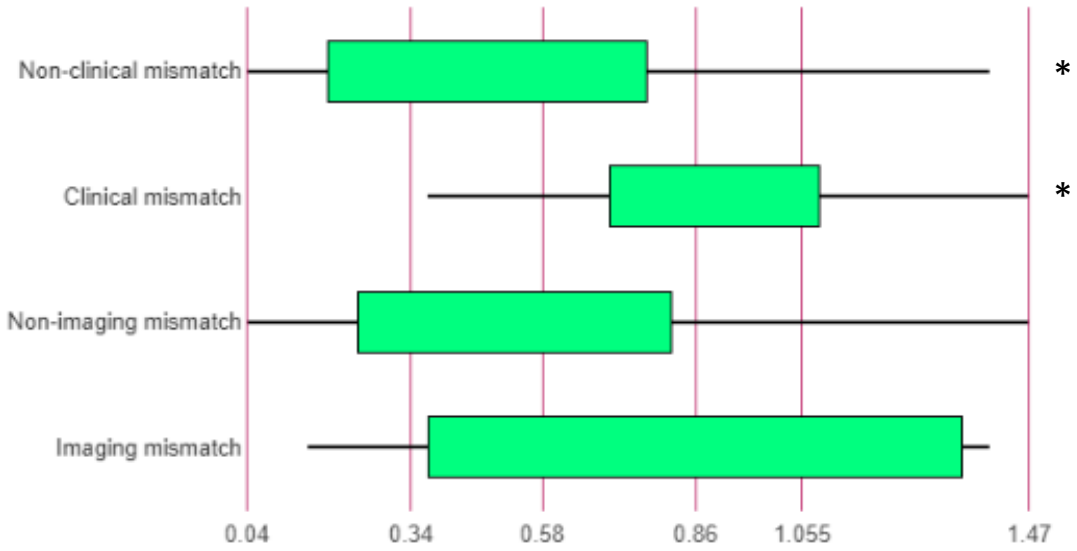

*Figure 8.14 -Box and whisker spread of lesion volume change (chronic (mls)/ acute (mls)) between clinical and imaging mismatch and non-clinical or imaging mismatch (*significantly different non-parametric*

8.6. Discussion

The goals of this study were to: I) identify whether the adult-based definition of mismatch was indicative of true (meaningful) penumbra in this paediatric sample; ii) explore alternate definitions as informed by literature review; and iii) to look more deeply at the adult ADC threshold used to segment infarcts in this PAIS sample. Due to the small number of participants meeting adult-based mismatch criteria, this was done by investigating lesion volume change as an adjunct marker of penumbra, and by exploring case studies.

The penumbra case studies highlighted several important trends. Lee et al (2019) noted a suggestion of T_{max} >4s being a more robust measure of hypoperfusion deficit in children for penumbra segmentation, finding that it related more to stroke severity than the adult-based designation of T_{max} >6s. Three study participants did not meet criteria for favourable penumbra when using the adult-based T_{max} >6s designation, but had suggestion of mismatch tissue when using the T_{max} >4s segmentation for perfusion deficit. They also all had a documented chronic / acute lesion volume ratio greater than one, which was only observed in 31% of the total sample, with the average ratio of 0.72. Though this is not definitively representative of lesion growth, it was hypothesised (Hyp3a) that the chronic lesion would appear smaller than the acute lesion, though the rate of reduction would be less in those with suggested growth. By this hypothesis, it is therefore possible that the cases using T_{max} >4s may have represented meaningful penumbra as they trended towards relatively larger final infarct volumes.

Two participants with suggested mismatch had smaller chronic lesion proportions. Case #1 had a very small chronic lesion of 9.1mls, which was only 14% of the acute lesion volume. It is important to note however that this case very likely included some lesion

progression given the time-to-scan was just shy of 24 hours. Case study participant #2, who had a chronic lesion proportion of 37% was on the older end of the spectrum and was scanned at 11 hours post onset. This acute segmentation volume may also have included lesion progression. Overall, the sample indicated a trend of higher chronic proportion of acute lesion volume with those scanned earlier, which supports this as a potential factor. However, the theoretical understanding of ischaemic progression lends validity to the suggestion that later scans would more closely reflect final infarct.

Overall, the six participants in the case series with expanded criteria (T_{max} >4s) may have had penumbra discernible on imaging. Supporting the inclusion of participants with larger infarcts and by using T_{max} >4s, was the appearance of larger chronic lesions than the rest of the sample, with four out of six cases demonstrating likely lesion growth. All four were scanned within eight hours of symptom onset, with the other two scanned greater than 11 hours post onset.

Despite the small samples and limitations with inferential statistics, comparison of lesion growth between penumbra and no penumbra groups using clinical mismatch criteria was noted to be statistically significant. Interestingly, there was only an overlap of two participants that also met imaging criteria, one using standard adult parameters, and one using T_{max} >4s. This may indicate that clinical mismatch approach to patient selection for reperfusion therapies may be more beneficial in preventing lesion growth, but much higher-powered studies with larger samples sizes are needed for further investigation.

Aim 3(b) sought to determine the effect of clinical factors such as aetiology on presence of penumbra, however, this was not feasible given the rarity of favourable mismatch in our sample. Using rate of lesion change as a proxy, no significant difference was found

as a result of aetiology. This is very likely affected by sample size, but also by the proportion of cryptogenic stroke in PAIS.

The overarching limitation to addressing our third Study Question and accompanying aims was the inability to robustly compare groups, or look at the predictive value of variables of interest to presence of penumbra, due to the very small sub-sample with favourable mismatch. As noted previously, the study sample may have included lesion locations and aetiologies less likely to have penumbral tissue. However, potential trends were identified that warrant further investigation.

8.7. Conclusion

In this chapter, I explored secondary analyses in more detail to answer Study Question 3 and accompanying aims, which included three main areas: acute ADC values and the effect of time, penumbra and stroke aetiology of our sample, and penumbra and infarct volume change – case observations of imaging-defined mismatch and clinical mismatch. I found that the ADC values in the sample followed a similar pattern to acute adult stroke, which provided pilot-level evidence that acute imaging parameters may be very similar when considering automated threshold-based core segmentation. I highlighted difficulties in definitively answering both aims in Study Question 3, which sought to analyse clinical factors and lesion volume change related to acute mismatch characteristics. Given the unexpectedly small number of participants with mismatch identified, these hypotheses can only be addressed with future larger scale research.

Two overarching definitions of penumbra were looked at in this chapter. Imaging-based definitions, (including the standard adult "target" mismatch, and the expanded criteria to include Tmax >4s for hypoperfusion), and clinical mismatch including those with smaller infarcts relative to higher stroke severity ratings. A non-significant but clear trend

suggested that the six cases identified with imaging-based penumbra, particularly those using Tmax >4s, were more likely to have a higher chronic lesion volume proportion compared to the acute lesion. Those meeting clinical mismatch criteria for penumbra also trended in the same direction, and this was found to be statistically significant. Though non-definitive, this pilot-level data does indicate that this is an important area for future research. As more information is gathered across future studies, preferably with research designed which allows sub-acute imaging, there will be improved understanding of the time-course of penumbra in paediatric stroke.

9. STUDY 4 - EXPLORING COGNITIVE OUTCOMES FOLLOWING PAEDIATRIC ARTERIAL ISCHAEMIC STROKE

Targeting penumbra is not only important to prevent brain tissue loss. As documented in 2.4.5, previous research has demonstrated that the lesion size (or volume) is a lesion characteristic related to neuropsychological outcome following PAIS. The literature also suggests that penumbral tissue is directly related to lesion growth, providing reason to suspect that targeting penumbra may increase the likelihood of positive cognitive outcome. Previous chapters have indicated that the size of a lesion changes from the acute period to the chronic period when it reaches final infarct volume. Past studies

looking at the effect of lesion size on outcome have often used final infarct volumes from years after the initial stroke, or they have not reported on the time since stroke for segmentation or categorisation (Everts et al., 2023; Long et al., 2011; Westmacott et al., 2018). One study used acute DWI scans to describe lesion volume and the effect on outcome, though time to imaging was not clear (Jiang et al., 2021). Although favourable penumbra mismatch profiles were rare in our sample, reducing final infarct volume is an adjacent goal of reperfusion therapies. Therefore, in this chapter I aim to look at the effect of both acute and chronic lesion volumes, as a related outcome of potential penumbra, on cognitive outcome.

9.1. Introduction

Factors relating to the cognitive outcome of a paediatric stroke patient are heterogenous, but lesion size has been consistently linked to outcome in children with acquired brain injury, with larger lesions relating to poorer outcome. Some studies have shown an overall deficit in general intellect, while others show more focussed effects particularly working memory, behavioural aspects of executive function, and the diagnosis of a learning disability (Ballantyne et al., 2008; Greenham, Anderson, & Mackay, 2017; Hajek et al., 2014; Long, Spencer-Smith, et al., 2011; Westmacott et al., 2018). However, methods used to measure lesion size or volume in PAIS are remarkably variable. A study by Westmacott and colleagues (2009) used a categorical rating scale for lesion sizes in neonatal brains, using the number of slices indicating atrophy, porencephaly or ventricular dilation to quantify the size of the lesion. Whilst appropriate for the neonatal sample, in this study, more than the number of slices affected is needed when comparing paediatric stroke lesions more generally. This is because some lesions may affect a larger proportion of a smaller number of imaging slices, or the reverse may also be true.

Similarly, another study found that larger lesions in children with acquired brain injury were related to poorer outcomes, also measured categorically (Long et al., 2011). In this study, lesion size was categorised using a coding protocol which included assessment of laterality, extent of injury, and number of brain regions affected, leading to characterisation of stroke burden into small (10% or less of parenchymal tissues), medium (more than 10% to 24 %), and large (greater than 25%).

Several studies have combined categorical criteria and manual region of interest (ROI) drawing to define lesion size, often expressed as a percentage of total brain volume. Lopez-Espejo and colleagues, (2017) used volumetric manual segmentation of lesions within 36 hours after symptom onset, using acute DWI images, expressed as a percentage of brain volume. These percentages were then classified into large (>4%), and small (<4%). They found that larger lesions were more strongly related to functional impairment (mRS score) on follow-up assessment. Ganesan et al., (1999) used manual segmentation of abnormal tissue, which was defined as abnormal hyperintensity on T2 images. The study found that children who had infarcts that were equal to or greater than 10 percent of total brain volume were more likely to have functional impairments (as assessed by a parent questionnaire), with medium to high effect. Neither of these studies looked specifically at neuropsychological outcome and both used a manual approach.

The major advantages of volumetric analyses over expert categorisation are that they enable quantitative analysis. Additionally, as there is less human judgement, particularly in some automated or semi-automated techniques, they are less susceptible to intra and inter-rater variables. To the best of my knowledge, no study to date has used semi-automated volumetric analyses to examine the relationship between cognitive outcome and lesion size in paediatric stroke. In addition, the use of dichotomised (acute & chronic)

lesion volumes - to see if there is an effect of time on this relationship - has also yet to have been completed. There is a need to replicate findings from other acquired brain injury studies in children, particularly in paediatric stroke, as it may be possible given current research avenues, to target penumbra and impact lesion size.

The current work is in conjunction with the Stroke Recovery Study (SRS), which aimed to describe lesion characteristics, clinical and neurological sequelae of patients presenting to our tertiary paediatric hospital (Gordon et al., 2015). The current work aimed to build on these findings by examining the links between brain lesion volume and cognitive outcome at 12 months, and four to six years post-stroke. I hypothesised (Hyp4a) that children with larger lesions (both the acute infarct and 90-day chronic infarct volume) would have poorer cognitive and neurological outcomes at both 12 months and four to six years post-stroke. However, the cognitive impairment of these individuals would likely still fall within the average range as per replicated findings from the PAIS cognitive literature. I also aimed to compare categorical ratings of acute lesion size with volumetric data obtained from both the acute infarct segmentations.

9.2. Study methods

This study was a mixed-methods observational study with retrospective analysis, consisting of a sample of children aged 1-18 who presented to The Royal Children's Hospital in Melbourne between 2008 and 2014. Participants had a unilateral arterial ischaemic stroke and had undergone acute perfusion imaging. The current study combined two paediatric stroke cohorts: i) the Stroke Recovery Study (SRS), and Penumbra in Paediatric Stroke Study (PPS study). The SRS study is a prospective, longitudinal cohort study which investigated clinical and functional outcomes and their predictors, over at least six-months post-stroke onset. This study classified infarct lesion

size based on vascular territory effected, divided into 'large - major vessel', 'medium - branch', or 'small – perforator artery'. The PPS study is an observational study with retrospective data collection which aimed to look at characterising penumbra in PAIS, using DWI and PWI. The PPS study also implemented a semi-automated volumetric segmentation technique, to observe changes in lesion size from acute presentation to estimated final infarct volume, while looking at the effect of time-to-imaging.

9.2.1. Outcome measures

The Kaufman Brief Intelligence Test (K-BIT) is a relatively quick measure used to screen IQ in people aged between 4 and 90 years-old (Gray, 2013). The K-BIT composite score has good reliability and concurrent validity with more robust measures of IQ, though with a higher standard error (Canivez, 1996; Webber & McGillivray, 1998). Periods of testing were six months and 12 months post-stroke, with some patients having follow-up assessments as late as four to six years post-stroke.

Clinical variables were collected, including stroke severity score, Paediatric Stroke Outcome Measure (PSOM) data and aetiology. The PSOM is a paediatric stroke-specific measure of neurological recovery (Kitchen et al, 2012). The total PSOM score was used, which is the sum of the five sub-scale scores across cognition and behaviour, right and left sensorimotor, and language. This score ranges from 0-10, with higher scores indicating more deficit.

To compare the volumetric data with the categorical data estimating lesion size, the information was graphically presented for visual comparison, as sample size precluded any inferential analyses. Volumetric analyses were completed as previously reported in Chapter 6. Overall study methods for the collection of cognitive outcome data have also been previously reported (Gordon et al., 2015). Parametric correlation analyses using

Pearson's *r* were implemented to observe trends in data for both the effect of acute and final infarct volume on estimated IQ at 12 months post-stroke, and on a smaller sub-sample at four to six years post-stroke. Non-parametric correlation analyses using Spearman's Rho (r_s) were conducted to observe the relationship between PSOM scores and lesion volumes. IQ change over time was detailed in a case-study analysis as a result of initial findings.

9.3. Study results

9.3.1. Participant demographics

Twelve children were identified as eligible for inclusion, 11 of which had follow-up outcome data at 12 months post-stroke. (Median age at stroke was 8.9 (IQR 6.2-12.5), 50% male). A smaller subset of seven participants also had outcome data at four to six years post-stroke. Aetiologies included *cryptogenic* = 6, *focal cerebral arteriopathy* = 3, *moyamoya disease* = 1, *dissection* = 1, *cardioembolism* = 1. Median PedNIHSS (stroke severity) = 9. For lesion size, only one participant was rated as "*major vessel*", and only two were "*small perforator*" lesions, visually represented in Figure 9.1. Due to the uneven distribution of numbers per category, no inferential comparison of continuous and categorical lesion size, nor lesion location and cognitive outcome, were completed. For lesion location, 7/11 (64%) were purely subcortical, with two cerebellar, and two combined cortical-subcortical lesions.

9.3.2. Lesion volumes and cognitive outcome

Figure 9.2 demonstrates a strong trend for larger lesions to be associated with poorer cognitive outcome (r = -0.66). Acute lesion volume using the semi-automated

segmentation protocol was a significant predictor of IQ at 12 months as per our subsample of 11 ($R^2 = 0.44$, $F(1, 9) = 6.91$, $p = .03$). Figure 9.3 demonstrates a moderate correlation between final infarct volume (chronic) and cognitive outcome at 12 months post-stroke. ($N = 11$, $r = -0.567$).

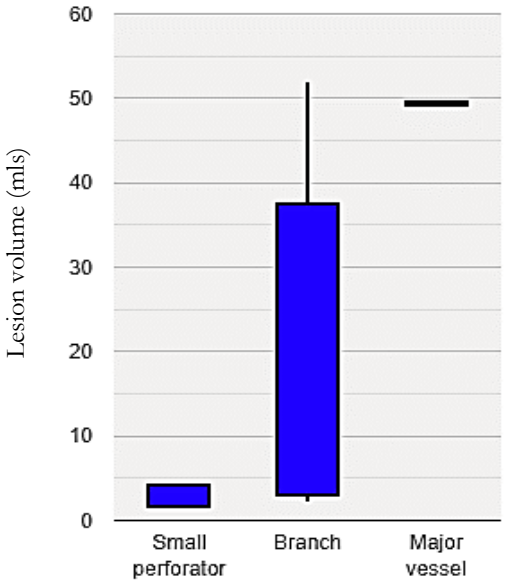

Figure 9.1 - Plot of categorical lesion size vs continuous lesion volume (mls).

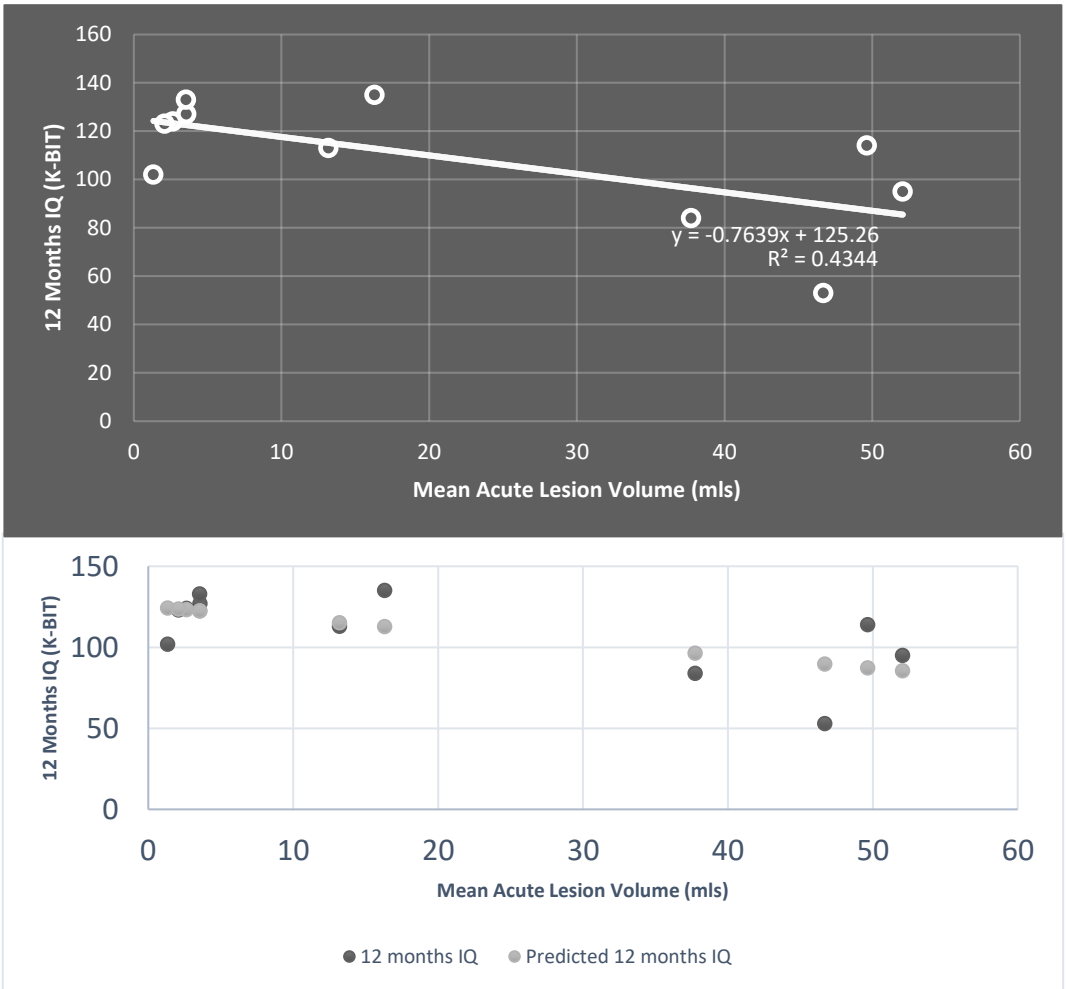

Figure 9.2 - Acute lesion volume by IQ (K-BIT) at 12 months post-stroke (Top image) Mean acute volume Line Fit plot (Bottom image)

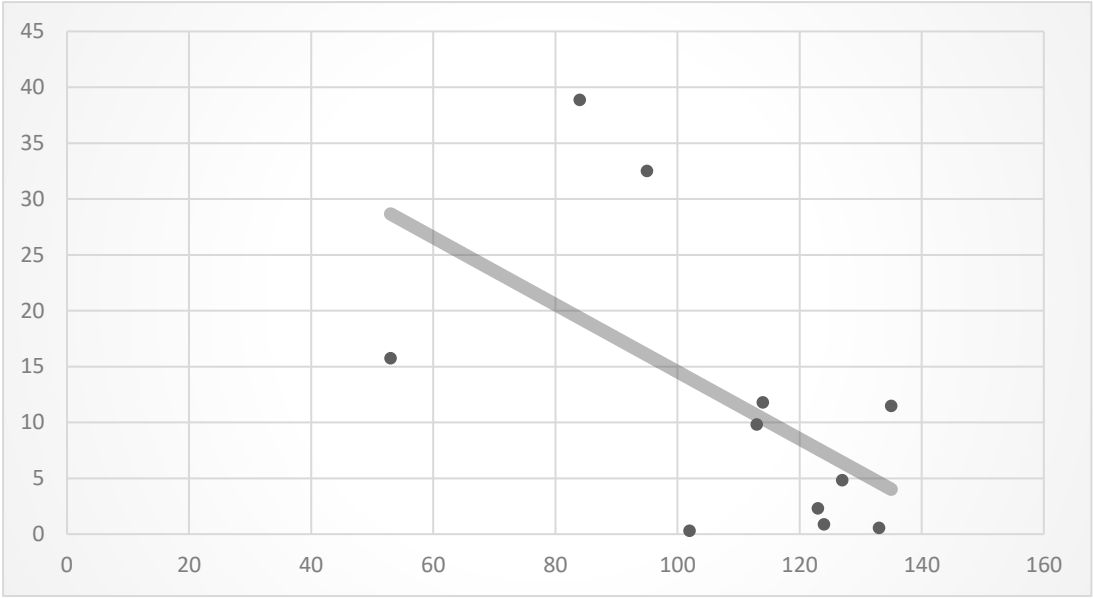

Figure 9.3 - Chronic lesion volume (mls) and 12 months IQ (K-BIT)

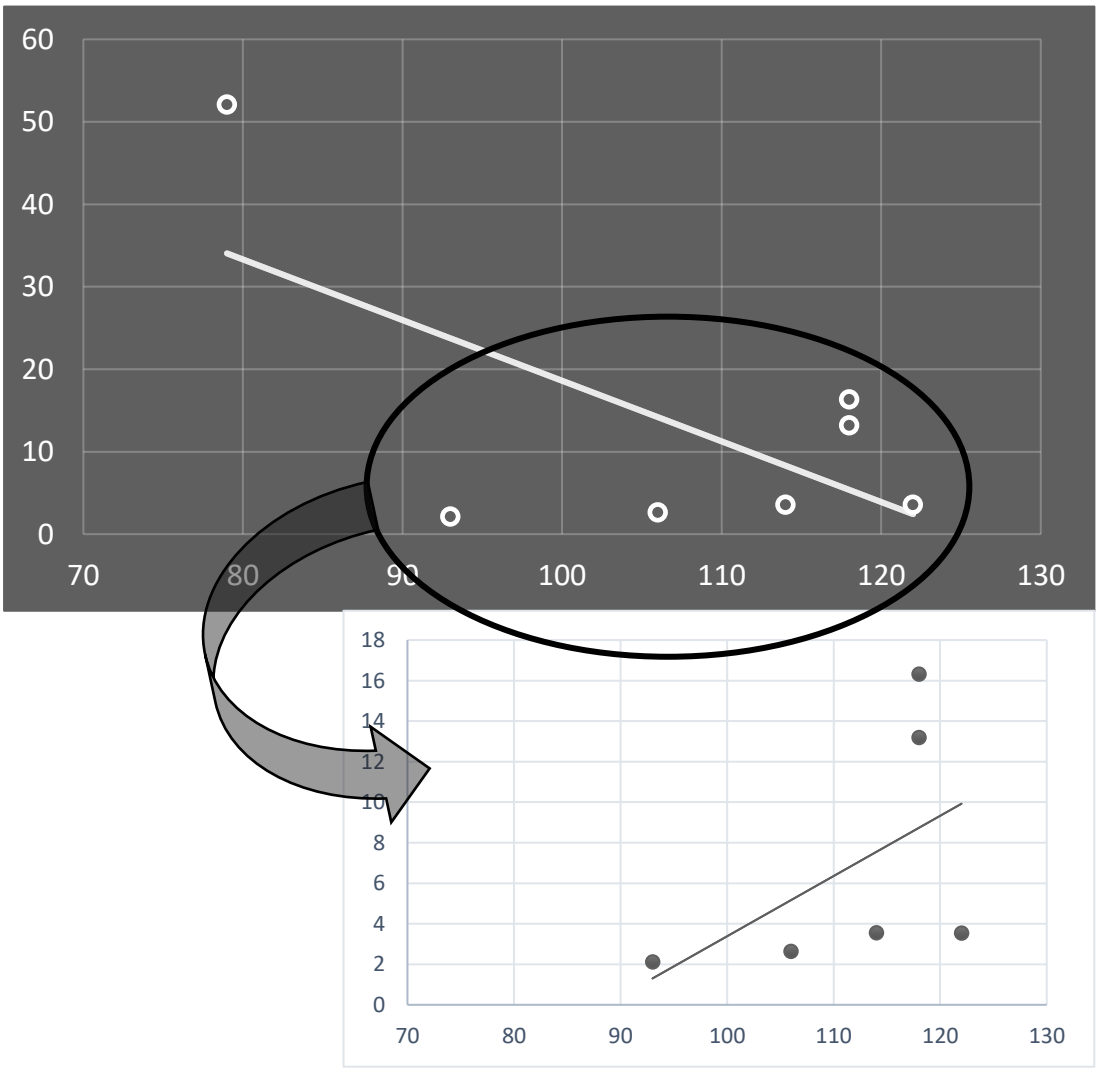

Figure 9.4 - Acute lesion volume and IQ 4 - 6 years post-stroke. Inset of participants following removal of one patient driving the initial trend.

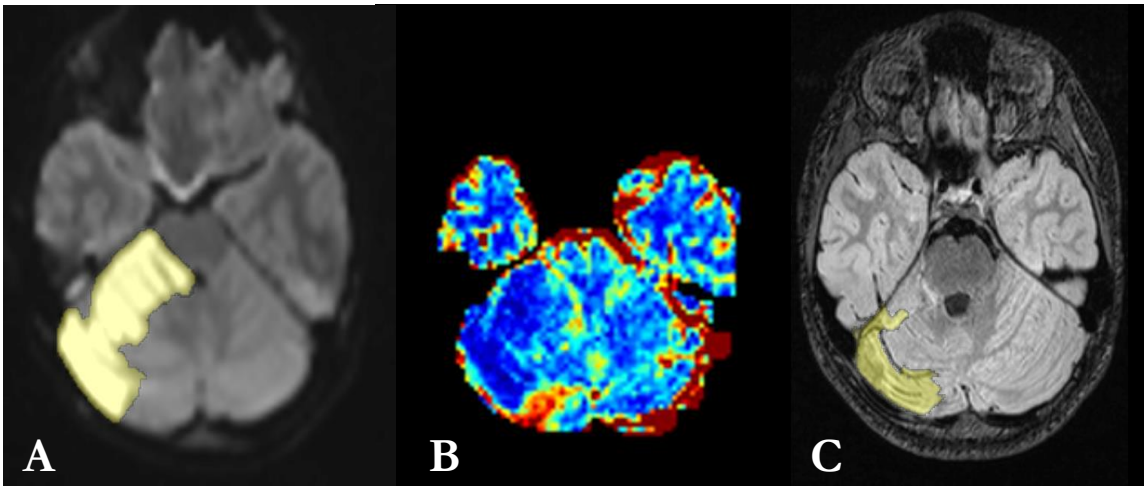

Figure 9.5 – Case study of a 9-year-old boy with a cryptogenic cerebellar stroke associated with poor cognitive outcome 4 - 6 years post-stroke (A) DWI showing manually segmented infarct (B) RAPID relative CBF map showing area of hypoperfusion (C) T2 FLAIR showing chronic infarct.

A smaller subset of participants were followed up four to six years post-stroke. A weak relationship between lesion volume and IQ estimate can be observed which seems to be largely due to one value. When removing the participant with significantly larger lesion volume, the trend disappears (Figure 9.4)

9.4. Case study:

This participant was a 9-year-old boy with a cryptogenic cerebellar stroke, who was scanned 11.5 hours post-symptom onset. At presentation, he had a PedNIHSS of 23, and an acute PSOM score of 3. He was categorised as having a branch artery infarction, with volumetric segmentation of 52.07mls (acute), and 32.51mls (final). Infarct volume was likely heading towards progression at the time of scanning. This participant met clinical mismatch criteria as described in 8.5, in the previous chapter. The participant's IQ change can be seen in Figure 9.7. Estimated IQ within the first year after stroke is within the lower end of the *average* range. However, extended follow-up revealed an IQ decrease to the *well-below average* range. Follow-up 9 years-post stroke PSOM = 1.

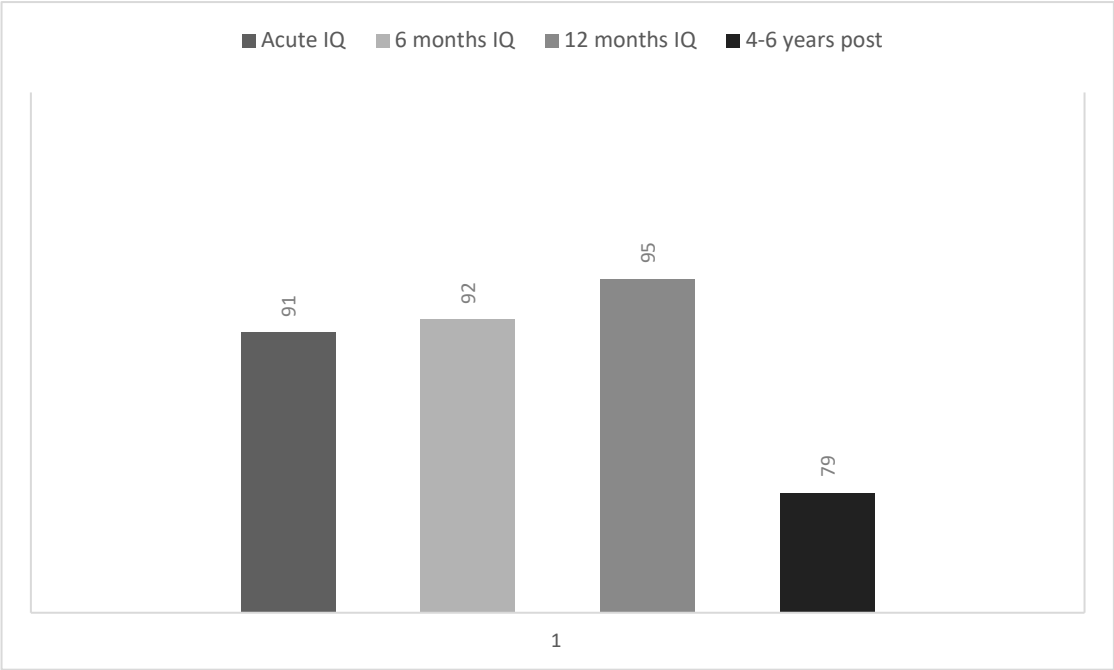

Figure 9.7 - Case study IQ composite change over time

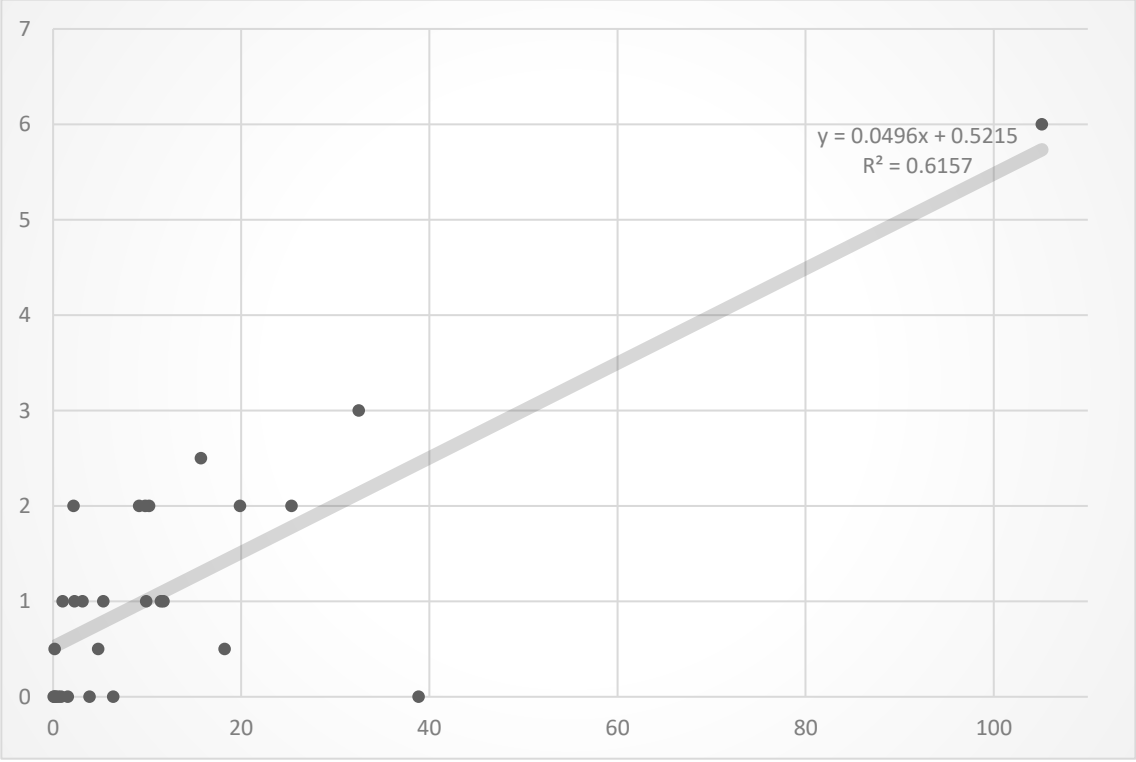

Figure 9.6 - Acute lesion volume by PSOM scores

For neurological outcome, non-parametric correlation using our larger perfusion imaging sample size (N=28, one patient had passed away), lesion volume was significantly

correlated with total PSOM scores (r_s = 0.61, p = <0.01, Figure 9.6). No such correlation was found between total PSOM scores and cognitive outcome (r_s = 0.02, p = 0.95).

9.5. Discussion

Multiple previous studies have demonstrated that children with PAIS generally fall within the average range in terms of their general intellectual ability (Allman & Scott, 2013; Everts et al., 2023; Max et al., 2010; Peterson et al., 2019; Studer et al., 2014; Westmacott et al., 2010, 2018). However, differences across cognitive domains have been noted, and variables such as age, aetiology, and time post stroke make predicting outcomes very difficult. Prior studies have demonstrated the importance of lesion size for neuropsychological outcome in paediatric acquired brain injury (Abgottspon et al., 2022; Anderson et al., 2014; Everts et al., 2023; Long, Anderson, et al., 2011; Steinlin et al., 2005).

This study provides preliminary results to support these previous studies. I found that cases with larger lesions on acute scans were associated with poorer cognitive outcome at 12 months post-stroke. Persistent cerebrovascular damage, in the form of hyperintensities on chronic follow up imaging representing atrophy, gliosis and encephalomalacia, remnants of previous infarction, were also related to poorer outcome. The correlations may suggest that the severity of the lesion volume in general is detrimental to outcome. However, these patients had a varied time-course in their scan time from symptom onset, and not enough is known about the acute changes in lesion size in PAIS.

Though this effect was not as strong when looking four to six years post-stroke, as most participants had *average* to *high average* IQ estimates, there was a case study level suggestion that a particularly large acute lesion volume may have an effect on cognitive outcome

well into the future. It was noted that the case study participant had the largest lesion volume in the subsample of participants, which was more than double the other lesion volumes. Though this participant may not have experienced a significant loss of their current functioning in the sub-acute period, this may have become more evident as cognitive development and demands continue to increase from late primary school into early adolescence. Maturation of cognitive processes continues well beyond this participant's age at stroke onset, with adolescents beginning to develop skills in abstract thinking and figurative language, and experiencing a growth in their ability to think logically (Anderson et al., 2001; De Luca et al., 2003; Luna et al., 2004).

The significance of understanding the relationship between lesion size and outcome in stroke patients is that it adds weight to the importance of implementing interventions that restore blood flow to penumbral tissue to limit stroke lesion growth. This knowledge also provides important prognostic information for patients and their families. In this preliminary observational study, I noted the relationships between both acute and chronic lesion volumes and outcome. The slightly weaker correlation using chronic volumes was not unexpected, as acute segmentation can be clearer to visually discern areas of hyperintensity on DWI, with high concurrence between evaluators (Gaudinski et al., 2008; Luby et al., 2006). This weaker correlation was also likely related to the small sample size. With chronic volumetric segmentation, there are areas that have been affected by atrophy, leading to widening of sulci, visible holes in the tissue, and less distinguishable or confluent areas of hyperintensity on T2 FLAIR imaging. Other studies have also expressed chronic lesion volume as a percentage of brain volume to account for differences in the size of paediatric brains, which is a limitation for the current study (Jiang et al., 2021; Lee et al., 2022). For chronic lesions, further research is needed to

determine the more appropriate imaging metrics to use, such as sequential imaging to include tissue loss in the equation.

Automated lesion segmentation tools are efficient and a sensible choice to be implemented as part of the acute stroke imaging workflow. They also have potential to reduce measurement errors and eliminate examiner biases. However, fully automated lesion segmentation of chronic lesions may not be accurate due to the lack of sharp contrast boundaries, and similarities in intensity with non-pathological areas (Luby et al., 2006). This study aimed to look at using a combined, semi-automated segmentation protocol to address this, and to identify similarities between approaches. It was also found that categorically assigning infarct volume as according to specific arterial type (perforator, branch, and major vessel) was well aligned with our volumetric segmentation, as expected. This is important, as expressing lesion size using volumetric analyses (i.e., as a continuous variable) is more quantitatively comparable than qualitative, categorical based methods.

9.5.1. Limitations and future directions

This exploratory pilot study is limited by its sample size, with only 11 participants at twelve months, and only seven participants at four to six years post-stroke. This lowers the power and interpretability of results. I was unable to examine relationships between other important variables, such as age. Previous research indicates that age may have a curvilinear effect on outcome, therefore, it is difficult to interpret these results without this consideration. In the sub-sample that had follow-up four years post-stroke, there was only one participant that had a relatively large infarct, with a significantly lower IQ at follow-up assessment. Further research is needed to examine the long-term effects on children with particularly large lesions, to see if this trend is replicated. This study was

limited by the fact that the other three participants with larger acute lesions (>20mls) were lost to cognitive follow-up.

There is some evidence that acute stroke severity may be a predictor of outcome. The participant in our case study had a stroke severity score, as per the PedNIHSS of 23, which was by far the highest in this sample (median stroke severity score =9). It may also be that small perforator or branch lesions (or those with a smaller volume in general) may be less likely to have an ongoing impact long-term, however more research is needed to determine this.

Another limitation with the understanding of our case study, and interpretation of the overall study results is lesion location. Most of the sample had purely subcortical lesions, which were mostly small, with two located in the cerebellum, and two spanning cortical-subcortical regions. Previous research tells us that combined lesions are more likely to be associated with overall lower intelligence on cognitive testing, and deficits in various other domains (Studer et al., 2014; Westmacott et al., 2010). Research is lacking into the cognitive effects of focal cerebellar lesions, with the closest clinical research in children with tumour resection. Neuropsychological impairment was found in various areas such as executive function, visual spatial ability, and verbal memory (Levisohn et al., 2000). Further research is needed to ascertain if similar outcomes follow cerebellar infarction in PAIS.

When estimating IQ in clinical samples, it is difficult to detect change from premorbid levels, without baseline data. In adult samples, this is often estimated based on an individual's education and employment history, and their performance on neuropsychological tasks that are designed to estimate baseline IQ range. This is difficult in children. With the developing brain, cognitive skills are emerging and growing across

childhood, with acquired brain injury having the capacity to hinder the acquisition of some skills. Although our study identified a trend towards larger stroke lesions being related to poorer cognitive outcomes at one-year-post stroke, what this means from an individual perspective is unclear. An important variable not measured in the present study is the socioeconomic status of the child's family, which has been shown to have a significant impact on cognitive outcome after PAIS, controlling up to 42% of variance (Bartha-Doering et al., 2021).

Another limitation is the use of the K-BIT as a measure of IQ in the sample. Whilst the K-BIT is a validated measure for use in estimating childhood IQ, other measures have more robust psychometric properties, for detecting changes particularly amongst cognitive domains (Webber & McGillivray, 1998). With 79% of participants in the overall PAIS sample having lesions involving subcortical structures, validated measures of attention and executive function would be important to implement in future research. The associations identified in Chapter 2.4 between the basal ganglia and attentional abilities highlight the significance of this. These cognitive domains, though correlated with overall FSIQ, would need focussed assessment to look for impacts.

In the current study, I focussed on the volume of infarcted tissue, rather than the relationship between cerebral perfusion and cognitive outcome. This relationship is two-fold; taking into account individual and age-related changes in CBF, and chronic perfusion levels, both of which can have longitudinal effects on cognition. Increased CBF is associated linearly with age, and peaks around the age of seven, with increases specifically noted in areas related to neurocognitive domains such as attention, language development, and abstract thinking and decision making (Lee et al., 2021; Paniukov et al., 2020). The variance from these changes was not taken into account. The latter variable

of interest for future research would be to examine chronic perfusion levels. A study by Steiner et al., (2021) found that even two years post-stroke, PAIS patients had significantly lower CBF in the ipsilesional MCA and PCA when compared with healthy controls, and that higher interhemispheric perfusion imbalances in the MCA were associated with poorer cognitive performance. It is important to note that study participants largely fell within the normal range across cognitive domains, highlighting that PAIS patients may have subtle interference impacting their ability to reach their potential. Further research into cognitive outcomes in PAIS should consider variations in chronic perfusion levels which may explain some of the variance observed in long-term outcome (Steiner et al., 2021).

The current study provides a rationale for continued research, as both acute and chronic lesion sizes were correlated with poorer cognitive outcome. The true change in lesion size, however, is difficult to quantify in this population. This will require either large volumetric studies to provide a reference of expected final infarct shrinkage, or sub-acute follow-up imaging. To discern a notable change in lesion volume, many cases would need to be included in a model, taking into account time post-stroke and aetiology amongst other pertinent factors.

9.6. Conclusion

This pilot study lends support to previously documented observations that lesion size is related to cognitive outcomes. I provided a case-study example of long-term post-stroke cognitive deficit that developed over-time. Though this was a pilot study, it lends preliminary support to the recommendation that implementation of cognitive follow-up across childhood may be beneficial to monitor cognitive difficulties that may not have been apparent during acute and sub-acute recovery. This study uniquely highlighted the

inconsistencies in the literature of operationalising lesion size within this clinical population, and demonstrated that using a semi-automated segmentation technique may be considered as a useful method for further research.

This study took a novel look at the effect of time and infarct volume on outcome and highlighted important areas for future research. Although the acute or diagnostic infarct volume cannot be altered, the potentially modifiable final infarct volume was also related to cognitive outcome. This relationship was observed, despite chronic infarcts being more difficult to reliably segment than acute infarcts. This is particularly critical given the relationship between final infarct and neurological outcome observed up to 9 years post-stroke. This adds to the rationale for interventions that target lesion size to also improve cognitive and neurological outcome potentially after PAIS.

10. SUMMARY AND CONCLUSION

10.1. Summary of thesis

The overarching goal of treatment in childhood stroke is to reduce lifelong disability. The literature indicates that volume of brain damage is a strong predictor of long-term outcome. Therefore, the focus of acute interventions is to minimise the extent of brain injury. The ischaemic penumbra is the biological target of these therapies, which highlights the critical clinical implications underpinning this research area. In adult stroke, the catch cry is "time is brain". This is equally relevant to children, which means rapid imaging to confirm diagnosis is the key to improving access to reperfusion therapies which will save viable brain by restoring blood flow. In the past, eligibility for these off-label treatments were time-based, but imaging selection of cases is more scientifically

valid, because this has the potential to more precisely identify children with salvageable brain, rather than a "one size fits all" approach.

The main strengths of this thesis are as follows. This study was conducted in the pre-reperfusion treatment era (with the exception of one case) and provides important natural history data of the time course of the penumbra in children. Increasing understanding of the pathophysiology of salvageable brain in children is critical for clinical translation, further research design, and optimisation of child outcomes. This research is also the first and largest study to test utility and accuracy of automated software in PAIS, and to look at ADC segmentation, and the relationship with lesion growth and imaging thresholds / definitions.

My thesis had four main aims. (1) To review the paediatric literature to compile the imaging methods and definitions used to characterise penumbra in PAIS. (2) To test the feasibility and utility of automated perfusion-diffusion mismatch software in a paediatric population. (3) To look further at the thresholds and definitions used in adults, and look for suggestion of lesion growth as an adjunct marker of penumbra. (4) To explore the relationship between lesion size at different timepoints and the effect on long term outcome.

This thesis began with a detailed review into the definitions and previous research in the areas of stroke in adults and children, neuropsychological outcomes of paediatric stroke, and neuroimaging of acute cerebral ischaemia. This exploration of the literature led to generation of specific research questions and hypotheses and informed the study design and methods, particularly neuroimaging modalities for penumbral estimation and acute and chronic infarct segmentation.

10.1.1. Study One summary

Study 1 generated a detailed systematic review and critical evaluation into the ways in which penumbra, or perfusion-diffusion mismatch, have been defined and applied to the childhood stroke population. My first aim was to summarise how different imaging modalities have been used to characterise the ischaemic penumbra in the PAIS literature, and I found that most used DWI and PWI, or CTP, though at least 11 different combinations of imaging modalities were used. There was insufficient evidence that any one imaging modality is more appropriate or more accurate than the others to estimate penumbra in the developing brain. I also noted that non-contrast perfusion methods were used in a third of the sample. Additionally, I aimed to determine if there were consistencies in definitions of penumbra in the PAIS literature. Considerable heterogeneity was also identified amongst definitions, only two studies had a clear definition that overlapped with each other; many with no clear definition. Most studies used visually based qualitative methodologies, with only four with a clear quantitative technique. Most studies were of low scientific quality.

The key finding from this work was the considerable inconsistency in how the penumbra has been defined and operationalised in children, due not only to the lack of research, but also to paediatric-specific challenges. These included smaller case numbers, reduced access to urgent brain imaging, particularly in young children requiring anaesthesia or sedation, and high rates of stroke-mimics. Whilst DWI/PWI and CTP - the adult-based "gold standard" techniques - were most frequently used, I noted a divergence in the paediatric literature for penumbra identification using less-researched, non-invasive methods.

10.1.2. Study Two summary

Study 2 explored the feasibility of using an automated penumbra imaging tool validated in adults, and its application to a childhood stroke population. The use of automated perfusion imaging software was deemed feasible in PAIS, with favourable mismatch profiles persisting beyond the standard 4.5 hours window for thrombolysis in two children. Through this study I also aimed to explore the relationship between time-to-imaging and ADC values to aid understanding of the utility of paediatric specific segmentation thresholds. I found that in cases with delayed imaging or small infarct, the ischaemic core was not segmented. ADC values were above the threshold in most cases beyond 12 hours, therefore automated infarct segmentation in delayed presentations may not be accurate.

Novel findings demonstrate the feasibility to rapidly assess perfusion-diffusion mismatch in childhood PAIS using automated software. Favourable mismatch profiles, using adult-based parameters, were noted as late as 23.5 hours post-symptom onset. Further, only patients with lesions that spanned cortical and subcortical regions had favourable mismatch profiles. There were only three more participants in the whole sample who also had cortical-subcortical lesions, as the sample mostly consisted of purely subcortical lesions. It is unknown at this stage whether this represents an effect of lesion location on presence of penumbral tissue, and therefore requires future research. To the best of my knowledge at the time of writing, this is also the largest study of automated perfusion software applied to a PAIS sample. Overall, the effects of aetiology and lesion location on mismatch characteristics, and the clinical relevance of acute perfusion-diffusion mismatch with regard to long-term outcomes need to be determined.

10.1.3. Study three summary

In study three, I investigated lesion volume change from acute diagnosis to final infarct at follow up, and the relationship with penumbra definitions. I identified a novel trend towards lesion growth in an expanded PWI-DWI mismatch sample through adjustment of standard adult parameters, including participants who met penumbra criteria using a T_{max} >4s instead of T_{max} >6s threshold, though this was not statistically significant. It's also important to note that this imaging mismatch group only had six participants, so is likely to be underpowered. Four out of six cases (66%) had chronic lesion proportions greater than 100% of the acute lesion volume, indicating infarct growth, including all three of the additional T_{max} >4s cases. Only four other cases without mismatch (17%) in the remainder of the sample had chronic lesions that were larger than acute lesions, with the average chronic lesion of the entire sample representing only 72% of the acute lesion volume. Significant difference in rate of lesion growth between those meeting clinical-mismatch criteria (mismatch between higher clinical stroke severity and small acute infarct size) and those who didn't was identified. This may indicate that this is a more robust measurement of penumbra in PAIS than imaging-based criteria, though this warrants further study.

I also explored the impact of clinical factors such as age, time from symptom onset to imaging, and aetiology, on lesion volume change. No significant relationships with age, or aetiology was identified in the small study sample. However, time from symptom onset to scan was significantly related to lesion volume change. This supports the hypothesis that infarcts scanned early are more likely to continue to grow into the surrounding penumbra than those scanned in later time windows which may be closer to their final infarct volume.

10.1.4. Study four summary

To my knowledge, this is the first study to use a semi-automated stroke lesion volumetric analysis to relate acute and chronic lesion volume to cognitive and neurological outcome in a paediatric stroke sample. The main novel findings were that larger acute and chronic stroke volumes were associated with poorer cognitive outcomes at 12 months. The volume of the chronic infarct was also related to poorer cognitive outcome, and neurological outcome, up to nine years post-stroke in the sample, supporting the rationale for use of hyperacute reperfusion therapies to attenuate lesion growth. Larger chronic volumes were also associated with poorer neurological outcomes (PSOM) five to nine years post stroke. These results replicate previous reports of larger lesions leading to poorer outcome, adding further support for targeting penumbra to improve outcome.

Detailed exploration of the study cases appeared to suggest a large acute lesion volume may have an effect on cognitive outcome well into the future. It was noted that the case study participant had the largest lesion volume in the subsample of participants, which was more than double the other lesion volumes. This case study experienced a decline in IQ on long-term follow up, and is a potential example of growing into deficits. I also sought to explore the relationship between clinical factors including age, stroke severity, and aetiology, and cognitive outcomes, however, I was unable to explore this relationship due to the small sample size. Overall, the results from study four successfully replicated the work done in previous studies, and provided further emphasis on the importance of minimising lesion growth if possible (Abgottspon et al., 2022; Everts et al., 2023; Hajek et al., 2014; Long, Anderson, et al., 2011).

10.2. Key considerations & limitations

In each study in this thesis, I have detailed multiple limitations, the main points of which are summarised here. One of many difficulties with studying children with stroke, is that the research often has small samples. This was found in the systematic review, where the majority of studies mentioning penumbra were case studies. The small sub-sample of participants with a favourable mismatch profile in this study also precluded the answering of certain hypotheses. Another key difficulty is the delayed presentation of children with stroke symptoms to the Emergency Department, the further delay to scanning, and high rates of stroke mimics (MacKay et al., 2016; Mallick et al., 2015). This makes it vital to increase understanding of the time-course of penumbra in children, and how this knowledge may help clinicians move towards a tissue-based selection of children for intervention, alongside other clinical factors.

Limitations in studying the time-course of penumbra included the use of a pre-defined ADC threshold for automatic segmentation. As this pilot study was affected by site-specific issues such as varied DWI acquisition types, small numbers, and varying case accrual time, it is still possible that a better ADC threshold exists for paediatric patients. The findings from this paediatric ADC study were inconclusive, and should be looked at further in future research, particularly as there was still some accurate DWI lesion segmentation observed in this sample beyond 12 hours. To limit the technical and between-subject variability across scanners and sites there is ongoing work into methods of neuroimaging data harmonisation which could be explored for future studies (Torbati et al., 2021).

Additionally, looking at the time-course of penumbra was impeded by the lack of sub-acute imaging. This is a limitation of retrospective observational research. My research

highlighted varying degrees of chronic infarction, as measured at 90 days post-stroke, though it is difficult to ascertain how much the lesion potentially grew in the sub-acute period before neuronal recovery mechanisms and atrophy.

Implementation of neuropsychological follow-up was challenging because of the ethical considerations of contacting patients who have not previously consented to follow-up studies and who were not actively engaged with my institution's stroke clinic. This was attempted using tracing letters, where the onus is on the potential participant to make contact prior to receiving study information. Research fatigue is often reported by members of small clinical populations, and it occurs when people tire of undertaking research activities (Patel et al., 2020). Preventing these effects in relatively rare conditions like PAIS, by prospective research optionally embedded into clinical practice to reduce participant burden, is an important consideration for study design (Ashley, 2021). Although follow-up cognitive data which has been collected for clinical purposes can be released to a research team with ethical approval and participant consent, this can introduce bias into an already underpowered sample. If cognitive assessment is not routine, or prospective, it can be those who have severe ongoing cognitive impairment that are most likely to present for evaluation.

10.3. Future directions

Penumbral imaging is important in the paediatric population to enable acute reperfusion therapies to salvage viable brain tissue, and we can improve knowledge by implementing insights gained from this preliminary work. If I were to design a prospective study in the era of reperfusion therapies, there are a number of crucial considerations I would make. In the previous section, I have discussed the limitations that could be reduced or eliminated primarily with a prospective study design. However, there are many other

important aspects of methodological design to overcome these, and further progress penumbra research in children.

10.3.1. Leveraging international research networks and clinical registries and imaging databases

To overcome the limitations in sample size encountered in this field there is the need for data pooling from major paediatric stroke centres around the world. As demonstrated in the current pilot, case accrual over many years brings forth unnecessary limitations. Collaboration is needed to increase study power and strength of evidence, provided by both retrospective clinical cohort-based studies and prospectively designed research studies. Prospective registries such as the International Paediatric Stroke Study, and the Save ChildS Pro study are promising initiatives for developing consensual understanding of penumbra estimation, patient selection for treatment and cognitive outcomes (MacKay et al., 2011; Sporns et al., 2021). Designing studies with clear methodological consensus and protocols across multiple sites could help overcome the challenges experienced by paediatric stroke researchers. On designing a new study, I would implement procedures to ensure data collection methods are comparable, particularly aiming to reduce limitations of multisite imaging data acquisition.

10.3.2. Agreement on perfusion acquisition protocols

Imaging protocols require multi-site consensus for reliable and comparable imaging interpretation. Arguably the most important aspect of this is the perfusion modality for use. In Chapter Seven, I demonstrated that DSC MR perfusion resulted in adequate bolus properties across my age-varied paediatric sample, despite the need for power injection. The hesitancy for the use of contrast imaging, as identified in the literature review, stems

from concerns around gadolinium deposition in the brain, and nephrogenic fibrosis in children with existing renal impairment (Miller et al., 2015; Nardone et al., 2014). As with any investigation that contains an element of risk, the risk-benefit clinical decision must be made. In a time when a rapidly acquired and interpreted perfusion scan may lead to more precise diagnoses and selection for reperfusion therapies, applying contrast perfusion imaging could result in reduced brain damage.

In this hypothetical prospective study, DSC-MR perfusion would be acquired as part of a standardised stroke protocol, unless contraindicated. As identified within the systematic review in Chapter Five, a non-contrast perfusion option like ASL may have a complementary role, particularly for patients with renal issues or history of repeat contrast imaging for comorbidities. However, it's utility in rapid penumbra identification requires further research.

10.3.3. Determining whether the target mismatch applies to children

Determining the applicability of the favourable, or "target" mismatch in PAIS is challenging. In adults, this was done by multiple large studies, by initially observing relationships between perfusion characteristics, treatment, and outcomes, and then by applying this knowledge prospectively. At this stage, this is not possible in children with the rarity of stroke and the restrictions on standardised application of reperfusion treatments. However, going forward, there are two key research focus areas that I would examine to move towards addressing this gap.

The first relates to the automated segmentation of stroke infarcts using an ADC threshold. On a positive note, in Chapter Seven the infarcts which were detected and segmented by RAPID were not found to be significantly different than manual

segmentation. This indicates that within 12 hours, standard adult ADC threshold (<620 x 10^{-6} mm^2/s) using RAPID does an adequate job at detecting the infarct core in PAIS. In Chapter Eight, however, there was pilot indication that ADC thresholds in extended time windows may not be a viable automated segmentation method. It is possible that with more data we may be able to determine a more appropriate time-based segmentation threshold, dependent on time-to-imaging from stroke onset and other factors.

Due to the prevalence of delayed presentations in PAIS, an adjacent or preliminary study must revolve around solving this problem. Literature suggests that finding a solution may be promising in the Deep Learning model space. The difficulty with refining algorithms is that they require large datasets, highlighting the importance of multi-centred data pooling. Both threshold approaches and machine learning algorithms have this challenging requirement. As this is a growing area in adult stroke research, with some promise indicated with MR images greater than 12 hours post-onset, application to PAIS samples is a worthy area for future research.

In a new study, I would still use the adult ADC threshold acutely, as RAPID was able to accurately segment some infarcts beyond 12 hours. However, this would be with the knowledge that segmentation will more likely require secondary interpretation or expert rating of lesion size compared to hypoperfusion deficit in the acute timeframe. Target mismatch identification in these cases would be iteratively identified with post-processing and manual or semi-automated segmentation of acute infarcts.

The second component of this is determining the most appropriate threshold for hypoperfusion in PAIS. This study identified an important trend towards those with T_{max} >4s being more likely to demonstrate lesion growth than those without. Though with smaller numbers, this observation in conjunction with previously reported research,

highlights this as a potentially more appropriate imaging parameter for penumbra delineation in PAIS (Lee et al., 2019). In a new study, I would be recommending that Tmax >4s be considered as a potential indication of penumbra, that requires confirmation with larger numbers. Improved understanding of penumbral characteristics and optimization of automated DWI/PWI thresholds and mismatch criteria in childhood PAIS, may facilitate tissue-based patient selection for reperfusion therapies.

The definition of a 'favourable' profile may only apply to children with certain aetiologies, or may change throughout childhood and into adulthood as the brain matures and develops. Whether the exploration of favourable penumbra in PAIS patients with arteriopathic aetiologies is clinically useful is an open question, as mechanical thrombectomy may not be a viable treatment option due to vessel fragility and ensuant risk of bleeding (Fragata et al., 2021; Kossorotoff et al., 2022; Sporns et al., 2020). However, this is still an important area to explore, as our knowledge is still limited, and reperfusion therapies may have a treatment role for these patients.

10.3.4. Assessing the time course of infarction and change in volume over time, with where possible, sub-acute scanning

Directly related to our knowledge of whether the penumbra definition is valid in a PAIS population, is the ability to observe and increase understanding of time-course of infarct volume change. As an identified limitation, sub-acute scanning (ie. 4-7 days post stroke) is not routinely acquired. Sub-acute scanning can be acquired as part of a carefully designed prospective research study, with strict inclusion and exclusion criteria, to answer this important research question and at the same time be ethically implemented without compromising patient's clinical care. This will likely limit the case recruitment, but hopefully this can be addressed by multi-site data pooling.

Implementing a research scan with a standard protocol at the thirty days post-stroke time would also be recommended. Chronic scan segmentation would be completed using T2 FLAIR images, implementing the criteria from Chapter Six and the semi-automated method. I would also add a whole brain segmentation to account for developmental changes in brain size. To account for atrophic processes and widening of sulci in the visibly smaller chronic lesions, I would pilot the feasibility of whole brain acute segmentation (including the infarct, prior to atrophy), and comparing it with the remainder of whole brain chronic segmentation minus visible lesion. This may be a more accurate way to measure chronic infarction, which were found to be more difficult to reliably segment than acute lesions.

Two additional important perfusion markers that I would add include the acute measurement of collateral status and flow, by implementing recently published methods and potential collaboration with international stroke researchers (Lee et al., 2022). As collateral flow can sustain salvageable tissue, it is an important piece of the penumbra puzzle. The second would be a perfusion MR measurement at an agreed upon follow-up time point, to account for the variance in cognitive outcome identified in the literature explained by chronic perfusion levels (Steiner et al., 2021).

10.3.5. Assessing penumbral characteristics as a function of age, time to diagnosis and aetiology

With larger, prospective research, the unanswered questions from this thesis would likely be feasibly addressed. It is an aim for research going forward to have larger numbers, and therefore, more patients with a favourable profile. This would enable the investigation of the relationship between age, aetiology, and mismatch characteristics. As mentioned above this also has implications for treatment, as some stroke causes would not likely

benefit from certain interventions. In Chapter Four, I hypothesised that strokes of cardioembolic origin would be more likely to present with penumbral tissue acutely due to the mechanistic similarities between this stroke type, and adult ischaemic stroke, which is typically embolic in nature. This may, or may not be the case however, in light of the fact that I detected penumbra in one patient with an arteriopathy. Perhaps the question should be refocussed to the treatment options for differing aetiologies (such as that those causing large vessel occlusions which are more amenable to endovascular clot retrieval); this is an area for ongoing study. A clear protocol across sites for the collection of this data would be implemented in a new prospective study.

10.3.6. Follow-up data collection embedded into clinical practice

The use of validated age-appropriate outcome measures at agreed upon time points to investigate the relationship between infarct volume and cognitive outcomes is another critical aspect of future study design. The challenges I faced in implementing neuropsychological evaluation many years post-stroke, and the attempts to retrospectively receive consent from patients to be followed up, could be addressed by future prospective research. Going forward, I would design a study where during the acute admission, patients and their families are invited to consent to ongoing neuropsychological follow-up as part of their clinical care, but that their deidentified data is also collected for research purposes. This would be at a standardised interval across multiple sites. The consent procedure would also have an option to agree to be contacted about participation in future studies, to simplify the process of recruitment if new findings result in the development of new studies in PAIS.

To be able to understand whether treatments are meaningful and beneficial, requires not only the long-term neurological evaluation, but cognitive and psychological evaluations

as well. In a new study, I would use the neuropsychological test battery or similar as originally created for this study (detailed in Appendix 12.6). This could hopefully also serve a clinical purpose: providing recommendations or referrals for children and adolescents as they progress through their education to help maximise their potential and help with career planning and goal attainment. Important questionnaire data to collect is around socioeconomic status and parental mental health, as these have both shown to be significantly explain variance in long-term outcome following paediatric brain injury.

The current work has provided both a snapshot of challenges faced clinically in managing PAIS, and an initial feasibility study showing an automated penumbra imaging software like RAPID can be implemented in PAIS setting, although with many practical and technical caveats. Arguably the most important outcome achieved, this pilot level research aided in the methodological design of an Australian national collaborative pre- and post-implementation study called the Paediatric Acute Code Stroke study (PACS). This is national initiative in paediatric stroke, aiming to investigate the utility of decision support tools and advanced neuroimaging in shortening the time to diagnosis and increasing access to reperfusion therapies.

10.4. Conclusion

As the largest study to date to apply automated perfusion imaging software to a paediatric sample for the feasible identification of penumbra, this thesis has presented original findings including the identification of favourable mismatch profiles in extended time-windows. By detecting novel trends in lesion volume changes across time and different definitions of penumbra, this has generated additional questions and avenues to explore with further research. Building on this research will help clinicians treating children with stroke to find the most accurate, meaningful, and rapidly obtained method to identifying

salvageable brain tissue in PAIS and will take us closer to providing more patients with access to reperfusion therapies. Notably, the novel finding that larger chronic lesions seem to lead to poorer long-term outcomes directly supports the rationale for identifying penumbra and saving brain tissue in PAIS. This initial exploration has identified a range of important methodological challenges and considerations, the knowledge of which will inform and streamline further research in this pivotally important area. This is in the hope that this ongoing work will not only improve knowledge and definition of penumbra, but also serve to increase implementation of treatments which help to salvage brain tissue, minimise lifetime sequelae and improve the quality of life for children who suffer from PAIS.

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12. APPENDICES

12.1. Ethics

Approval for imaging component granted by the RCH HREC in April, 2017. Amendment to include follow-up component was approved in July 2017. Currently approved protocol (Version 4), study number: HREC 28093 E.

12.2. International Stroke Conference - abstract for oral presentation - 2019

Background and objectives: Recent studies have used automated perfusion imaging software to identify adults most likely to benefit from reperfusion therapies in extended time windows. The time course of penumbral evolution in childhood arterial ischemic stroke (AIS) is poorly characterised. We investigated the utility of automated perfusion imaging software in childhood AIS and explored the relationship between time to imaging and perfusion-diffusion mismatch. **Methods:** Convenience population of children with acute AIS, presenting to the Royal Children's Hospital Melbourne from 2005-2014, where diffusion and dynamic susceptibility contrast MRI were performed <48 hours of symptom onset. Perfusion-diffusion mismatch was estimated using RAPID (iSchemaView). Core was defined as ADC<620×10-6 mm2/s and hypoperfusion as T_{max}>6s. Favourable mismatch profile was defined as core <70mls, mismatch volume ≥15mls and ratio ≥1.8. **Results:** Twenty-nine children (median age 8, IQR 4.4-14.6) met eligibility criteria (26 unilateral MCA and 3 unilateral cerebellar infarcts). Median PedNIHSS was 4.5. Etiologies included focal cerebral arteriopathy (n=9), cryptogenic

(n=12), cardioembolic (n=5), other (n=3). Median time from onset to imaging was 13.7 hours (IQR 7.5-25.3). The visible diffusion lesion was not below ADC<620 threshold in 19 (34% of cases) (median time to imaging 21 hours). Two children with subcortical/cortical lesions, imaged at 3.75 and 11 hours had favourable mismatch profile. **Conclusions:** RAPID failed to segment the ischaemic core in cases with delayed imaging. Favourable mismatch profiles persisted beyond the standard 4.5 hours window for thrombolysis. Further work is required to investigate the effect of time-delay and aetiology on mismatch characteristics in childhood AIS.

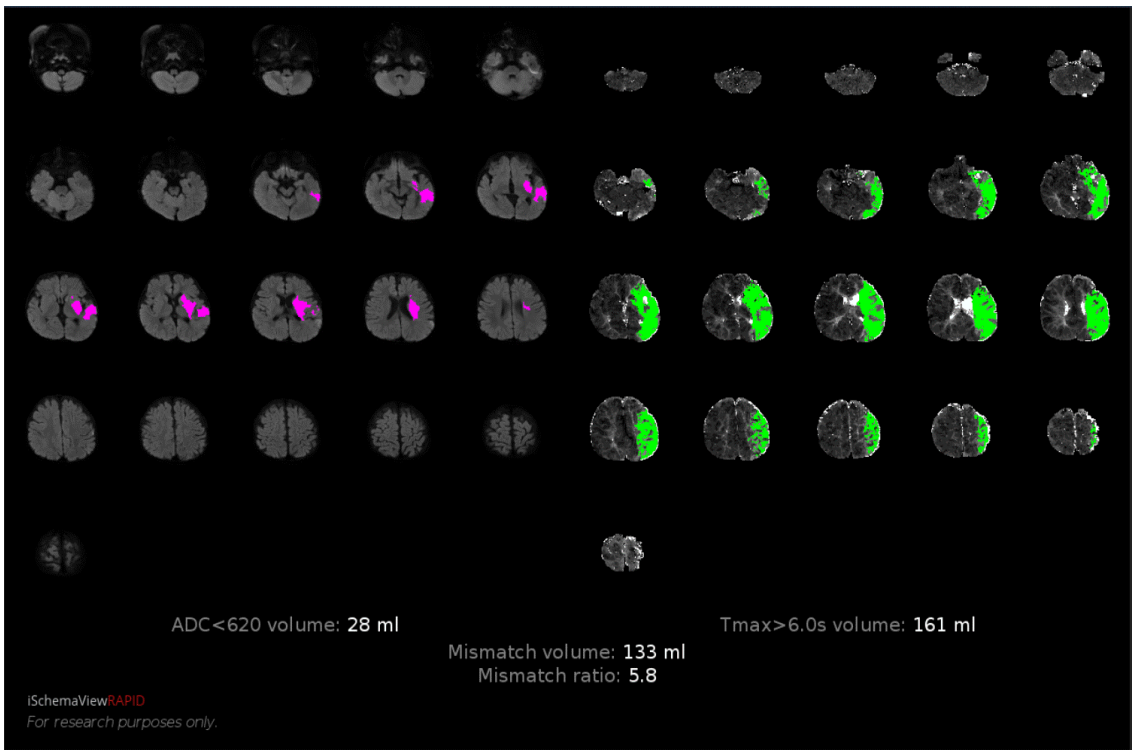
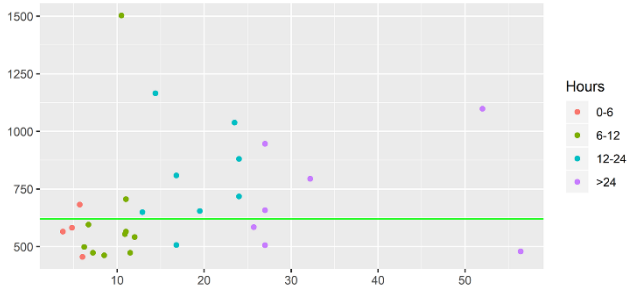


Figure: 22-month child with acute left MCA occlusion. Diffusion (left) and T_{max} perfusion (right) MRI at 3.75 hours post-onset demonstrating a favourable mismatch profile.

12.3. Supplemental material

Study 2 – Published in STROKE

Supplemental Figure I - ADC values (mm2/s x 10^{-6}) by time to presentation.



X axis indicates time-to-imaging from symptom onset. The green line represents the <620 x 10^{-6} mm^2/s threshold used by RAPID. Y axis indicates spread of ADC values calculated from manual segmentation.

12.4. Co-author contributions Study 1

Author name	Location	Contribution
Melissa J. Visser, BA	University of Melbourne, Melbourne	Design or conceptualization of the study; analysis or interpretation of the data; drafting or revising the manuscript for intellectual content
Joseph Yuan-Mou Yang, PhD	Murdoch Children's Research Institute, Melbourne	Design or conceptualization of the study; analysis or interpretation of the data; drafting or revising the manuscript for intellectual content
Fernando Calamante, PhD	The University of Sydney, Sydney	Analysis or interpretation of the data; drafting or revising the manuscript for intellectual content

Michael Kean, BSc	The Royal Children's Hospital, Melbourne	Drafting or revising the manuscript for intellectual content
Christopher L. Adamson, PhD	Murdoch Children's Research Institute, Melbourne	Analysis or interpretation of the data
Gagan Sharma, MCA	The Royal Melbourne Hospital, Melbourne	Analysis or interpretation of the data
Vicki Anderson, PhD	Murdoch Children's Research Institute, Melbourne	Drafting or revising the manuscript for intellectual content
Bruce C.V. Campbell, PhD	The Royal Melbourne Hospital, Melbourne	Analysis or interpretation of the data; drafting or revising the manuscript for intellectual content
Mark T. Mackay MBBS PhD	The Royal Children's Hospital, Melbourne	Design or conceptualization of the study; major role in the acquisition of data; analysis or interpretation of the data; drafting or revising the manuscript for intellectual content

12.5. Initial study design with follow-up component

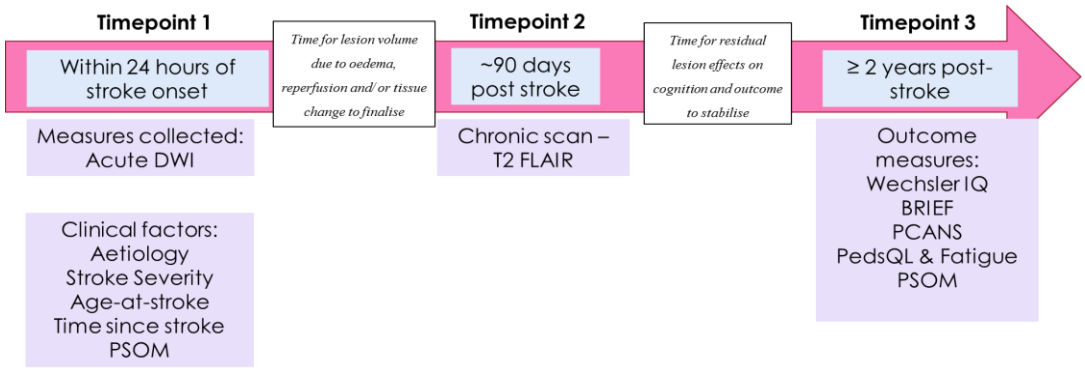


Figure 12.1 - Study design

Measure	Acute presentation	Study timepoint		**Time Estimates (mins)
		Follow-up Imaging ≈ 90 days post-stroke	Follow- up Assessment ≥ 3 years post-stroke	
Clinical history	X			
PSOM	X		X	5 – 10
PedNIHSS	X			5
MRI Stroke Protocol	X	X		30-40
BRIEF			X	5

Wechsler Intelligence Scale			X	≈ 60
Care and Needs Scale			X	5 -10
PedsQL & Fatigue			X	5 - 10

Table 12.1 - List of measures

12.6. Proposed Follow-up assessment

Patients and families previously involved in the imaging component of the study will be contacted and asked for consent to participate in a follow-up assessment at the Murdoch Children's Research Institute. This assessment will include the outcome measures indicated in Table 12.1. Age-appropriate versions of each measure will be applied and compared to appropriate age normative data. The areas that will be examined during the assessment will be full scale IQ, and domain scores (Verbal Comprehension, Perceptual Reasoning, Working Memory, and Processing Speed), Executive Function, levels of support needs, quality of life and levels of fatigue.

Participants will receive an individual summary report following the assessment, which will provide a brief overview of findings, characterising areas of strengths and weaknesses. Where any significant findings are indicated, referrals to appropriate services will be organised.

The Royal Children's Hospital Melbourne
50 Flemington Road
Parkville Victoria 3052 Australia
TELEPHONE +61 3 9345 5522
www.rch.org.au

The Royal Children's Hospital Melbourne

12.7. PLS & Consent form

INFORMATION STATEMENT AND CONSENT FORM

HREC Project Number:	28093		
Research Project Title:	Cognitive Outcome After Stroke study		
Principal Researcher:	Dr. Mark Mackay, Paediatric Neurologist		
Version Number:	8	**Version Date:**	04/04/2018

Thank you for taking the time to read this **Parent/Guardian Information Statement and Consent Form**. We would like to invite your child to participate in a research project that is explained below.

This document is 5 pages long. Please make sure you have all the pages.

If you speak a language other than English and would like this Information Statement and Consent Form in your language, please ask the person explaining this research project to you.

What is an Information Statement?

These pages tell you about the research project. It explains to you clearly and openly all the steps and procedures of the project. The information is to help you decide whether or not you would like your child to take part in the research. Please read this Information Statement carefully.

Before you decide if you want your child to take part or not, you can ask us any questions you have about the project. You may want to talk about the project with your family, friends or health care worker.

Important things you need to know

- It is your choice whether or not your child can take part in the research. You do not have to agree if you do not want to
- If you decide you do not want your child to take part, it will not affect the treatment and care your child gets at The Royal Children's Hospital

If you would like your child to take part in the research project, please sign the consent form at the end of this information statement. By signing the consent form you are telling us that you:

- understand what you have read
- had a chance to ask questions and received satisfactory answers
- consent to your child taking part in the project

We will give you a copy of this information and consent form to keep.

1. What is this research about?

A stroke is due to interruption of blood-flow to the brain. When this happens, some parts of the brain may be swollen and damaged but other parts may recover if blood-flow is restored.

We can look at how different parts of the brain are affected using MRI. MRI stands for magnetic resonance imaging. A MRI scanner is a machine that uses electromagnetic radiation (radio waves) in a strong magnetic field to take clear pictures of the inside of the body. Electromagnetic radiation is not the same as ionising radiation used, for example, in X-rays. The pictures taken by the machine are called MRI scans.

This project aims to use previously acquired MRI scans in babies and children affected by strokes in the past to see how much of the brain is affected by the stroke and whether some parts of the brain recover after blood- flow is restored. We will look at MRI scans taken at the time the stroke was diagnosed and compare them to MRI scans taken 3 months and 12 months later.

We will also look at long-term outcomes of people who had strokes as children, by looking at their cognitive, neurological and functional outcomes using standard assessment tools.

Our aim is to get the most information we can from the MRI scans and outcome measures. This information may help us determine whether it is possible in future to give treatments to dissolve clots at the time the stroke is diagnosed. This type of treatment has been shown to be effective in adults. Our long-term aims are to see whether the same treatment can be applied to babies and children affected by stroke, and to be able to give patients and families more information about how stroke can affect thinking, learning and functioning.

We hope to have 30 people who were babies and children aged from birth to 18 years old at the time of their stroke in our research project.

2. Who is funding this research project?

The Murdoch Children's Research Institute-Critical Care and Neurosciences Theme.

3. Why is my child being asked to take part?

We are asking your child to participate in this project because he/she had a stroke in the past.

4. What does my child need to do in this research project?

When your child was in the hospital, they had MRI scans done around the time of their stroke, and for follow-up assessment. They do not need to have any extra MRI scans for this research project.

This follow-up part of the study involves attending a clinical assessment, conducted at the Royal Children's Hospital. The assessment involves a standard cognitive assessment battery, including tasks which measure different aspects of your child's thinking, memory and learning. This will also include a standardised interview about functional and daily living skills, and a brief neurological screening measure designed specifically for use in paediatric stroke. We will also

ask you to complete some questionnaires about your child's background, mental health and quality of life. This assessment is estimated to take approximately two to three hours.

Optional Consent

We would like you to consider letting us send you information about new research projects that may be suitable for your child. The information we send will give you the full details about the project. It is your choice whether you agree to let your child take part in any future project or not. You are not obliged to take part in any future research you are sent information about.

5. Can my child withdraw from the project?

If you give your consent and change your mind, your child can withdraw from the project. You do not need to tell us the reason why you or your child want to stop being in the project. If your child leaves the project we will use any information already collected unless you tell us not to.

6. What are the possible benefits for my child and other people in the future?

There is no direct benefit to your child. Your child and you may feel a sense of satisfaction from taking part in medical research. This information may help us better understand how much brain tissue is affected by the stroke and how much of the brain tissue recovers. In the future, it may help us determine whether it is possible to give treatments to dissolve clots to restore blood-flow to parts of the brain that are at risk of being damaged. It may also help us understand how stroke affects children long-term. This may improve the outcome for children affected by stroke in the future.

7. What are the possible risks, side-effects, discomforts and/or inconveniences?

We do not expect the interview and/or cognitive assessment to cause you or your child any anxiety or distress. However, if your child is worried by any of the questions they do not need to answer them.

The only foreseen inconvenience is the time given by you and your child to be involved in the assessment. A suitable appointment time will be arranged with you to attend the hospital. A parking ticket for the hospital carpark will be provided so that you do not have to pay for parking.

There may be unforeseen or unknown risks. In the unlikely event that your child suffers an injury because of participating in this project, the public health service will provide hospital care and treatment at no cost to you.

During the research project, we may learn new information about the risks and benefits of this project. If this happens, we will tell you about this new information. This new information may mean that you can no longer participate in this research. If this occurs, the person(s) supervising the research will stop your participation. In all cases, we will offer you all available care to suit your needs and medical condition.

8. What will be done to make sure my child's information is confidential?

In this study, we will collect and use personal and health information about your child for research purposes. Any information we collect from your child, or their medical records, will remain confidential. We will use your child's information only for this research project. Only the researchers involved with this project and The Royal Children's Hospital Ethics Committee can have access to this information. We can disclose the information only with your permission, except as required by law.

You have the right to look at, and ask correction of, your child's information in accordance with the Freedom of Information Act 1982 (Vic). Please contact us if you would like to access this information.
The information will be re-identifiable. This means that we will remove your child's name and give the information a special code number. Only the research team will be able to break the code to match your child's name to his/her code number.

All information will be stored securely in a locked filing cabinet in the Neurology Department at the Royal Children's Hospital.

As some of the participants in this project are over 18 years old, information will be kept for at least 7 years. The research information may be destroyed or kept indefinitely in secure storage after this time.
When we write or talk about the results of this project, we will report information about the whole group of participants. This means that no one will be able to identify your child or your family.

The results of this research will be used by Ms. Melissa Doyle, to obtain a Master of Psychology (Clinical Neuropsychology) / Doctor of Philosophy degree.

9. Will we be informed of the results when the research project is finished?

An individual neurodevelopmental report will be written for your child, interpreting their results from the assessment. This will give an overview of your child's cognitive and functional profile. If you do not wish to receive individual report of your child, you can let us know at the time of the assessment. We will also send you a summary of the overall project results at the conclusion of the study. The summary will be of the whole group of research study participants, not your child's individual results.

10. Who should I contact for more information?

If you would like more information about the project or if you need to speak to a member of the research team in an emergency, please contact:

Name:	Dr. Mark Mackay, Paediatric Neurologist
Contact telephone:	03 9345 5661
Email:	mark.mackay@rch.org.au

If you have any concerns and/or complaints about the project, the way it is being conducted or your child's rights as a research participant, and would like to speak to someone independent of the project, please contact: Director, Research Ethics & Governance, The Royal Children's Hospital Melbourne on telephone: (03) 9345 5044.

CONSENT FORM

HREC Project Number: 28093

Research Project Title: Cognitive Outcome After Stroke study

Version Number: 8 **Version Date:** 04/04/2018

- I have read, or someone has read to me in a language that I understand, the information statement version listed above and I understand its contents.
- I believe I understand the purpose, extent and possible risks of my child's involvement in this project.
- I voluntarily consent for my child to take part in this research project.
- I have had an opportunity to ask questions and I am satisfied with the answers I have received.
- I understand that this project has been approved by The Royal Children's Hospital Melbourne Human Research Ethics Committee and will be carried out in line with the National Statement on Ethical Conduct in Human Research (2007) – including all updates.
- I understand I will receive a copy of this Information Statement and Consent Form.

OPTIONAL CONSENT

☐ I do	☐ I do not	consent to be contacted about future research projects

Child's Name

Parent/Guardian Name	Parent/Guardian Signature	Date
Name of Witness to Parent/Guardian's Signature	Witness Signature	Date

Declaration by researcher: I have explained the project to the parent/guardian who has signed above, and believe that they understand the purpose, extent and possible risks of their child's involvement in this project.

Research Team Member Name	Research Team Member Signature	Date

Note: All parties signing the Consent Form must date their own signature.