The Use of Hypercapnic Challenge Blood Oxygen Level Dependent (BOLD) MRI for the Investigation of Childhood Steno-occlusive Arteriopathy

PhD thesis submitted to UCL

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I, Nomazulu Dlamini, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

Background In childhood, cerebral arteriopathy causes cerebral ischaemia and infarction via two related mechanisms. The first, thrombotic vaso-occlusive stroke is the more typical mechanism of stroke in childhood. The second mechanism of infarction related to arteriopathy and not seen in other forms of ischaemic stroke is that of chronic hypoperfusion of the brain. The infarcts in this case are typically located in watershed zones and can accumulate gradually over time. Moyamoya is the prototypic arteriopathy representing the hypoperfusion injury. It is characterized by chronic progressive narrowing of the distal internal carotid, proximal middle cerebral and anterior cerebral arteries; chronic low flow infarction with accumulating 'string of pearls' in the white matter (Fig 1). It is this chronic hypoperfusion of the brain that is the subject of my PhD thesis. Cerebrovascular reactivity is a marker of cerebrovascular reserve and has been shown to be a biomarker of ischaemic risk in adults.

Objectives The primary objectives were to, in a group of children with moyamoya:

1) Validate the use of a qualitative measure of cerebrovascular reactivity as a biomarker of ischaemic risk, namely, hypercapnic challenge BOLD MRI CVR (hBOLD CVR) via two methods: a) breath-holding and b) induced hypercapnia during general anaesthesia as reliable and repeatable for use in the paediatric population

2) Assess the utility of qualitative assessment of cerebrovascular reactivity using hBOLD CVR as a tool for the identification of the risk of ischaemia in children with arteriopathy

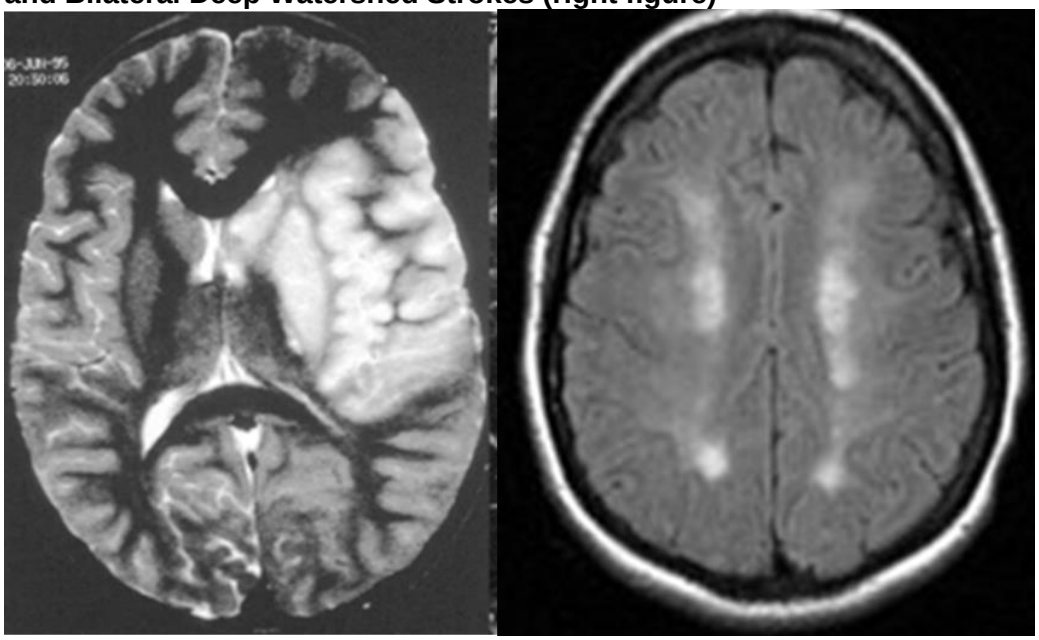
Method Hypercapnic challenge hBOLD CVR studies were obtained in children with steno-occlusive arteriopathy prospectively enrolled in The Hospital for Sick Children Stroke Registry. Semi-quantitative methods of measuring CVR were devised and used for the purpose of analysis. hBOLD CVR studies were analysed for reliability and reproducibility of the method of analysis. Clinical and radiologic data were collected and hBOLD CVR findings described for all children enrolled. Exploratory analysis of hBOLD CVR as a potential biomarker of ischaemic risk in the paediatric population were conducted. In particular association of hBOLD CVR with clinical symptomatology; parenchymal and vascular indicators of arteriopathy; neuropsychological outcome and cortical thickness were examined.

Results Forty seven children (37 Bilateral or unilateral Moyamoya arteriopathy, 6 Unilateral Non-moyamoya arteriopathy [Transient Cerebral Arteriopathy] and 4 Bilateral Non-moyamoya arteriopathy [2 PHACE(S), 1 Takayasu arteritis, 1 Sickle Cell Disease]) were enrolled and had hBOLD CVR studies. The mean age of diagnosis of arteriopathy across all groups was 8.1 years (SD 4.2) (range 7 months - 18 years). Clinical and radiographic features differed across arteriopathy groups. Most presented with acute stroke, however, among children with NF1-MM most (almost 50%) were asymptomatic and diagnosed on screening MRIs. Infarction patterns differed, with deep watershed infarction being the typical pattern in the moyamoya group in contrast to thrombotic vaso-

occlusive infarction pattern in the Non-moyamoya groups. Qualitative hBOLD CVR abnormalities were concordant with moyamoya laterality, and in unilateral moyamoya demonstrated tissue level microvascular dysfunction in the contralateral unaffected hemisphere. Qualitative hBOLD CVR abnormalities demonstrated concordance with clinically important manifestations of ischaemia including stroke, transient ischaemic attacks, cortical thinning and IQ. There was a lack of concordance with indices of executive function. In addition the moderate to severe steno-occlusive arteriopathy seen in the children with Transient Cerebral Arteriopathy was not associated with abnormality of hBOLD CVR.

Conclusion The thesis studies demonstrated that qualitative assessment of hBOLD CVR using breath-hold or general anaesthetic is feasible, reproducible and reliable in paediatric population. The utility of hBOLD CVR as a measure of tissue level microvascular dysfunction and thus a biomarker of ischaemic risk was demonstrated. However, larger longitudinal studies are required to characterize this further.

Figure 1 MRIs Showing Left Middle Cerebral Artery Ischaemic Stroke (left figure) and Bilateral Deep Watershed Strokes (right figure)



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In 1992 Dr William Logan went to Boston Children's Hospital for a two year sabbatical where he learnt about BOLD MRI. In 1996 he started applying the method to the study of cerebrovascular reactivity in adults. The first study conducted in a child was in 1998. The first study conducted in a child with moyamoya was in 2000. This work would not have been possible without the knowledge, support and expertise of this giant in medicine. I am grateful for the shoulders he has allowed me to stand on. Dr Logan thank you.

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Manuscripts

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Published Abstracts

Dlamini N, deVeber G, Kirkham F, Dirks P, Poublanc J, Logan W. Cortical Thickness and Cerebrovascular Reactivity: Potential Biomarkers of Hyporperfusion Injury in Moyamoya. Poster presentation at the Child Neurology Society Meeting, October 2016

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Dlamini N, Armstrong D, Dirks P, Moharir M, Askalan R, Kirkham F, deVeber G, Logan W. Quantitative Assessment of Cerebrovascular Reactivity in Paediatric Moyamoya. Poster presentation at the International Stroke Conference Annual Meeting, Feb 2016

Dlamini N, Dirks P, Logan W, Mikulis D, Armstrong D, Yau I, Abou-Hamden A, deVeber G. Hypercapnic blood oxygen level dependent MRI, cerebrovascular reactivity and the assessment of stroke risk in children with moyamoya.

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Poster Presentation, International Stroke Conference, California, February 2011

Williams T, Westmacott R, Logan W, deVeber G, Granite L, Dirks P, Askalan R, MacGregor D, Moharir M, **Dlamini N**. Cerebrovascular reactivity and neuropsychological functioning in pediatric moya-moya disease. Poster presented at the Annual Meeting of the International Neuropsychological Society, February 2011, Boston, MA

Dlamini N, Williams S, Kirby M, Yau I, Macgregor D, deVeber G, Logan W. Cerebrovascular reactivity in children with hematological disorders including sickle cell disease. Presented at the American Academy of Neurology 62nd annual meeting, April 10-17 2010, Toronto, ON

Dedication

This work is dedicated to:

my mother the most inspiring, loving and formidable woman I know. If I could have an ounce of your courage, and a measure of your spirit then maybe I could make a difference to the life of some in my lifetime. I am trying. And to my dad, your gentle kindness is always with me.

my husband Peter, my partner and best friend, to Delia and to our 'little dinosaur' Bukhosi. You have been my rock and my resting place. Your boundless love and encouragement to fly is more than I knew to hope for.

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the children and your families, my efforts are an attempt to match your courage

and to Him….

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Abbreviations

ACA: anterior cerebral artery

ACT: arterial circulation time

AI: asymmetry indices

AIS: arterial ischaemic stroke

ASL: arterial spin labelling

BH: breath-hold

BOLD: blood oxygen level dependent MRI

BMT: bone marrow transplant

BP: blood pressure

BRIEF: Behavior Rating Inventory of Executive Function

BRIS: Behavior Regulation／Inhibitory Self-Control Index

CA: conventional catheter angiogram

CBF: cerebral blood flow

CBV: cerebral blood volume

CMRglu: cerebral metabolic rate for glucose

CMRO2: cerebral metabolic rate of oxygen

CO2: carbon dioxide

COW: circle of Willis

CPP: cerebral perfusion pressure

CT: computed tomography

CVR: cerebrovascular reactivity

CVRE: cerebrovascular resistance

dHb: deoxyhaemoglobin

dICA: distal internal carotid

DWI: diffusion weighted imaging

ECA: external carotid arteries

ECG: electrocardiogram

EC-IC: external carotid – internal carotid

EDAS: encephaloduroarteriosynangiosis

EMS: encephalomyosynangiosis

EtCO2: end tidal CO2

FASI: foci of abnormal signal intenisity

FLAIR: Fluid attenuation inversion recovery

FSIQ: full scale intelligence quotient

FMD: fibromuscular dysplasia

fMRI: functional magnetic resonance

FSIQ: full scale intelligence quotient

GA: general anaesthetic

GEC: Global Executive Composite

hBOLD CVR: hypercapnic challenge BOLD CVR

HMPO: hexamethylpropyleneamine oxime

ICD: International Classification of Diseases

ICP: intracranial pressure

IQ: Intelligence quotient

MABP: mean arterial blood pressure

MCA: middle cerebral artery

MET: Metacognitive Index

MM : moyamoya

MMD: moyamoya disease

MMS: moyamoya syndrome

MRA: magnetic resonance angiogram

MRI: magnetic resonance imaging

NF1: neurofibromatosis type 1

NINDS: National Institute of Neurological Disorders and Stroke

NR: normal respiration

NTNP: negative pixels

OEF: oxygen extraction fraction

OxHb: oxyhaemoglobin

pACA: proximal anterior cerebral artery

PACS: picture archiving and communication system

PACNS: primary angiitis of the central nervous system

PaCO2: partial pressure of CO2

PaO2: partial pressure of O2

PCA: posterior cerebral artery

PCO2: partial pressure of CO2

PCOM: posterior communicating artery

PSOM: pediatric stroke outcome measure

PET: positron emission tomography

PetCO2: end tidal CO2

PetO2: end tidal O2

PIQ: performance IQ

pMCA: proximal middle cerebral artery

PRI: perceptual reasoning

PS: performance speed

PSI: processing speed index

PTPP: positive threshold positive pixels

PTNP: positive threshold negative pixel

rCBF: regional cerebral blood flow

REB: research ethics board

rOEF: regional oxygen extraction fraction

rCBV: regional cerebral blood volume

rCMRO2: regional cerebral metabolic rate of oxygen consumption

RM: re-breathe Mask

RNF: ring finger protein

ROI: region of interest

SCA: sickle cell anaemia

SCD: sickle cell disease

SPECT: single photon emission computed tomography

SpO2: haemoglobin oxygen saturations

TBI: traumatic brain injury

TCA: transient cerebral arteriopathy

TCD: Transcranial Doppler

TEF: Tracheoesophageal Fistula

TIA: transient ischaemic attack

TOF MRA: time of flight magnetic resonance angiogram

TR: repetition time

VIQ: verbal IQ

VCI: verbal comprehension index

WAIS-III: Wechsler Adult Intelligence Scale third edition

WISC-III: Wechsler Intelligence Scale for Children third edition

WISC-IV: Wechsler Intelligence Scale for Children fourth edition

WMI: working memory index

WMS: Wechsler memory scales

WPPSI-III: Wechsler Preschool and Primary Scale of Intelligence third edition

Xe/CT: xenon-enhanced computer tomography

1.Introduction

1.1. Overview

The current World Health Organisation definition of stroke is “rapidly developing clinical signs of focal (or global) disturbance of cerebral function, lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin” (WHO 1971). This classic definition is mainly clinical. Updated definitions have sought to incorporate tissue criteria of cell death or infarction within the central nervous system, attributable to ischemia, which *in vivo* requires neuroimaging confirmation. (Sacco *et al.* 2013). Broadly, clinical definitions of stroke include ischaemic (arterial or venous) stroke, intracerebral haemorrhage and sub-arachnoid haemorrhage. Ischaemic stroke occurs when there is critical i) compromise of the vessel lumen secondary to: i) arterial wall pathology such as dissection or stenosis ii) thrombo-embolic occlusion of the artery or vein, or critical hypoperfusion.

The incidence of paediatric arterial stroke (Figure 1) ranges from 2 – 13 per 100 000 person years in developed countries (Giroud *et al.* 1997, Lynch *et al.* 2002, Fullerton *et al.* 2003, Chung and Wong 2004). It is one of the top ten causes of death in childhood. Morbidity is high, as is the cost to families and society (Lo *et al.* 2008, Goldenberg *et al.* 2009). Unlike in adults who mostly have atheromatous disease, childhood risk factors are multiple, often occur concurrently and include cardiac disease, sickle cell disease, arteriopathy and infection to name a few (Ganesan *et al.* 2003, Fullerton *et al.* 2007, Mackay *et al.* 2011). Figure 2 Around two thirds of previously healthy children presenting with their first arterial ischaemic stroke (AIS) have a stenosing arteriopathy the

presence of which is one of the strongest predictors of recurrence and outcome (Dobson *et al.* 2002, Strater *et al.* 2002, Danchaivijitr *et al.* 2006, Ganesan *et al.* 2006, Braun *et al.* 2009) and determinate of secondary prevention (Group 2004). There are three main specific sub-types of arteriopathy in paediatrics of which transient cerebral arteriopathy (25%), dissection (20%) and moyamoya (22%) are the commonest (Ganesan *et al.* 2003, Amlie-Lefond *et al.* 2009, Tolani *et al.* 2015).

Figure 2 Stroke Types

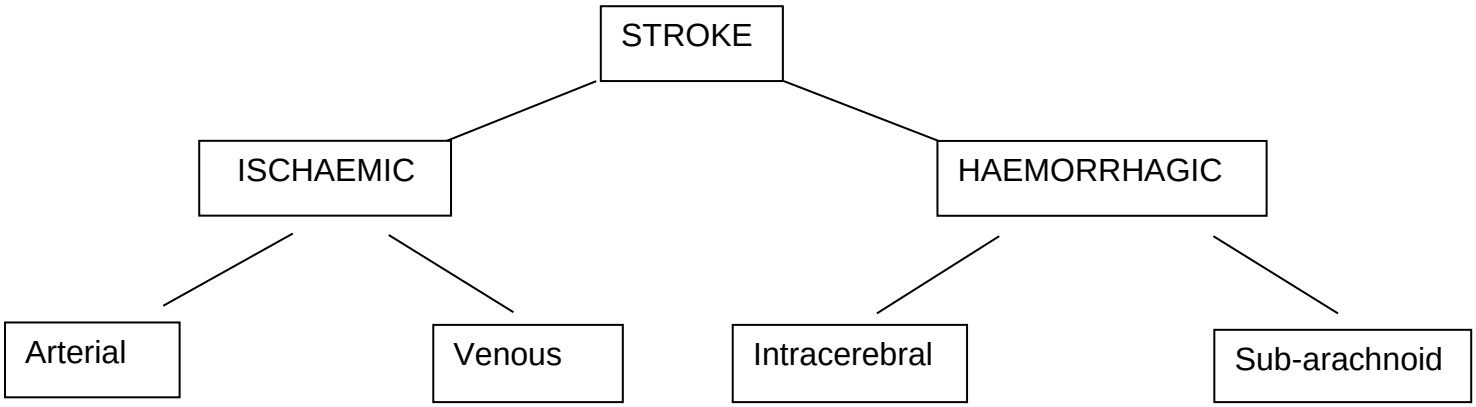


Table 1 Aetiologic Mechanisms of Arterial Ischaemic Stroke

Adults	Children
Vessel wall Arterial atherosclerosis dissection inflammatory moyamoya	Vessel wall Arterial inflammatory eg. TCA*, Vasculitis* dissection* moyamoya*
Luminal Cardioembolic eg. atrial fibrillation	Luminal Cardioembolic* eg. congential heart disease Prothrombotic states eg. primary prothrombotic conditions such as anti-thrombin III deficiency, or

	acquired prothrombotic conditions such as in dehydration
Hypoperfusion	Hypoperfusion
Other	Other Sickle cell disease* Mitochondrial disease eg. MELAS

TCA=transient cerebral arteriopathy; MELAS=mitochondrial myopsthy, encephalopathy, lactic acidosis and stroke-like episodes * common causes in childhood

Moyamoya arteriopathy is one of the most devastating conditions in paediatric cerebrovascular disease. It is common, poorly understood and associated with early death in childhood due to chronic hypoperfusion of the brain rather than thrombotic vaso-occlusion. The pathology of the primary arteriopathy is poorly understood and there are no current treatments that halt it's progression. Anti-thrombotic agents – the mainstay of treatment in thrombotic stroke – are not adequate for prevention since occlusion is not the principal pathological mechanism. Moyamoya disease is therefore a good model for the investigation of the effects of chronic hypoperfusion, and the natural history of progressive cerebral infarction over years compels us to increase our understanding of the mechanism of injury and explore opportunities for early intervention. If we are able to identify tissue level poor perfusion and quantify ischaemic risk primary preventative strategies could be employed to prevent stroke and ischaemic demise. Hypercapnic challenge blood oxygen level dependent cerebrovascular reactivity may be a useful tool for the evaluation of ischaemic risk in children with moyamoya arteriopathy. I set out to explore this in my thesis.

My primary objectives were to:

1) Validate the use of a qualitative measure of cerebrovascular reactivity, namely, hypercapnic challenge blood oxygen level dependent magnetic resonance imaging cerebrovascular reactivity (hBOLD CVR) via two methods: a) breath-holding and b) induced hypercapnia during general anaesthesia as reliable and repeatable for use in the paediatric population

2) Assess the clinical utility of qualitative assessment of cerebrovascular reactivity using hBOLD CVR as a tool for the identification of the risk of ischaemia in children with arteriopathy

In this chapter I will briefly describe our current knowledge of the cerebral arteriopathies and more specifically moyamoya, its natural history and its pathophysiological mechanisms of injury. I will discuss the rationale for current therapeutic approaches and the use of techniques – historical and new – available for the assessment of ischaemic risk within this population.

1.2. Childhood arteriopathy – the non-progressive and progressive arteriopathies

Diagnostic criteria for the arteriopathies have been proposed by Sebire et al (Sebire *et al.* 2004). Another approach to classification is by dividing the arteriopathies into non-progressive and progressive groups. However, at presentation determination of likelihood of future progression is difficult, even in unilateral arteriopathy. Hence a further revision to classification of the

arteriopathies was proposed by the International Pediatric Stroke Society (Amlie-Lefond *et al.* 2009, Braun *et al.* 2009). Focal cerebral arteriopathy may be defined as cerebral arterial stenosis not attributed to the specific sub-types. This term allows descriptive labeling at the onset of the illness (unlike transient cerebral arteriopathy (TCA)) and will include all patients including those with TCA in whom a focal arterial cerebral stenosing arteriopathy is documented on vascular imaging.

Transient cerebral arteriopathy, intracranial dissection and fibromuscular dysplasia are considered to represent the non-progressive arteriopathies. Diffuse cerebral vasculitis, moyamoya disease and some of the genetic arteriopathies form the progressive arteriopathy group. Classification between and within groups may still be challenging which is not unexpected given the commonality of underlying pathological mechanisms in some eg inflammation in TCA, primary angiitis of the central nervous system (PACNS) and moyamoya; reliance on imaging; the temporal nature of the arteriopathies, and the co-existence of arteriopathy sub-types (Chabrier *et al.* 1998, Aviv *et al.* 2006, Benseler *et al.* 2006, Sebire 2006, Braun *et al.* 2009, Kraemer and Berlit 2010, Kirton *et al.* 2013, Mineyko and Kirton 2013, Skeik *et al.* 2013, Wintermark *et al.* 2014). However, disease course, treatment strategies and prognosis differ between the groups (Fung *et al.* 2005, Benseler *et al.* 2006, Roach *et al.* 2008, Amlie-Lefond *et al.* 2009). Hence I describe the arteriopathies in the context of these two broad categories.

1.3. NON-PROGRESSIVE ARTERIOPATHIES

The non-progressive group of arteriopathies is typically unilateral (Sebire *et al.* 2004, Amlie-Lefond *et al.* 2009, Braun *et al.* 2009, Yeon *et al.* 2014).

1.3.1. *Transient cerebral arteriopathy*

Transient cerebral arteriopathy (TCA) was described by the French group at the end of the last century (Chabrier *et al.* 1998) and is one of the commonest types of arteriopathy. This aetiologically monophasic arteriopathy is characterized by non-progressive unilateral disease, affecting the supraclinoid internal carotid artery and its proximal branches, resulting typically in lenticulostriate infarction. TCA probably involves an inflammatory response to infections such as Varicella (Sebire *et al.* 1999, Askalan *et al.* 2001, Antachopoulos *et al.* 2002, Klingebiel *et al.* 2002). Cerebral imaging typically shows small subcortical infarcts located in the basal ganglia and internal capsule and on conventional or magnetic resonance arteriography, there are multifocal lesions of the arterial wall, with narrowing in the distal internal carotid and the proximal anterior, middle or posterior cerebral arteries. Similar angiographic findings exist in TCA associated with sickle cell disease (SCD) (Kirkham *et al.* 2001), although the pathophysiology might be different as histology provides evidence of endothelial proliferation and fibroblast infiltration (Rothman *et al.* 1986). Although the inciting mechanism of injury to the arterial wall is transient the arterial injury and consequences threreof are not. Recurrence occurs, particularly in the first year after stroke (Chabrier *et al.* 1998), and it is difficult to predict those at risk (Braun *et al.* 2009).

1.3.2. *Carotid arterial dissection*

In children arterial dissection in the anterior circulation is more frequent the posterior circulation (Fullerton *et al.* 2001, Rafay *et al.* 2006). Dissections (which may be multiple) occur as a result of blood penetrating and splitting the wall of an artery supplying the brain, most frequently the internal carotid (ICA) artery (Fullerton *et al.* 2001). Risk factors include trauma and infection. The diagnosis of dissection still requires a high index of suspicion in the absence of a suggestive history or neurological examination, as there remains a significant risk of misclassification (Tan *et al.* 2009). In addition intracranial dissections may be difficult to distinguish from TCA (Dlamini *et al.* 2011). Conventional angiography may still be required to confirm the diagnosis (Ganesan *et al.* 1999, Chamoun *et al.* 2008). Awareness of possible late complications is important for follow-up (Fullerton *et al.* 2001, Tan *et al.* 2009). Dissection causes local pathology through direct pressure effects and disruption of nutrient blood supply. Thrombo-embolism is the most important mechanism in dissection-related stroke. Moyamoya may occasionally develop in children who have had a dissection.

1.3.3. *Vertebrobasilar stenosis and dissection*

Vertebral artery dissection should be considered in children with posterior circulation stroke and cervico-vertebral abnormalities (Cushing *et al.* 2001, Halevy *et al.* 2008), particularly in boys (Ganesan *et al.* 2002) and if there is a history of trauma. Eighty percent of posterior circulation dissections are

extracranial. Stenosis and occlusion of uncertain aetiology also occur in the vertebrobasilar circulation but moyamoya is very rarely associated with posterior circulation vasculopathy.

1.3.4. *Fibromuscular dysplasia*

The classification of fibromuscular dysplasia (FMD) denotes a group of idiopathic, non-inflammatory arteriopathies with distinct pathological and angiographic findings (Slovut and Olin 2004). The most commonly described form of fibromuscular dysplasia is medial fibroplasia/dysplasia, typically affecting young adult females with renal and cerebral vessel involvement, distinct 'string of beads' angiography, refractory hypertension, and stroke (Slovut and Olin 2004, Touze *et al.* 2010). Pathologically this differs from the finding of intimal fibroplasia in the majority of cases of fibromuscular dysplasia in childhood stroke proven at autopsy (Harrison and McCormack 1971, Slovut and Olin 2004, Touze *et al.* 2010, Kirton *et al.* 2013). Diagnosis in childhood is difficult and requires pathological confirmation (Kirton *et al.* 2013). Clinical and radiological differences exist between the pathologically proven and not proven groups. The majority present early (under 12 months) with ischaemic (thrombotic vaso-occlusive) stroke, although some are haemorrhagic. Systemic arteriopathy, hypertension and moyamoya may co-exist. Treatment strategies are mixed and in one series included anti-thrombotic (35%) and anti-inflammatory (30%) approaches, with revascularisation surgery in 4%. Understanding of the underlying pathology and natural history remains limited

and whether FMD represents a congenital arteriopathy with or without a genetic predisposition is worth further exploration (Kirton *et al.* 2013).

1.4. PROGRESSIVE ARTERIOPATHIES

The progressive group of arteriopathies is typically bilateral although cerebral vasculitis and moyamoya may present as unilateral disease and progress to bilateral disease (Kawano *et al.* 1994, Houkin *et al.* 1996, Hirotsune *et al.* 1997, Lanthier *et al.* 2001, Benseler *et al.* 2006, Kelly *et al.* 2006, Elbers and Benseler 2008).

1.4.1. *Cerebral Vasculitis*

Multiple approaches to the classification of cerebral vasculitis in childhood exist and may be according to whether the vasculitis is primary or secondary, whether there is large or small vessel involvement or whether the temporal course is mono-phasic or progressive, hence the need for diagnostic algorithms. In 1959 Craviato and Feigin described “Noninfectious granulomatous angiitis with a predilection for the central nervous system” as the single entity now known as primary angiitis of the central nervous system (PACNS). (Cravioto and Feigin 1959) . In 1988 Calabrese and Mallek described 8 cases and proposed diagnostic criteria for PACNS in adults which have been adapted to childhood central nervous system vasculitis (Calabrese and Mallek 1988, Benseler *et al.* 2006). PACNS is characterized by evidence of cerebral vessel abnormality affecting proximal and distal segments on initial angiography

with evidence of neuroradiologial disease progression beyond 3 months when untreated (Benseler *et al.* 2006). Angiography typically reveals vascular stenosis, beading, tortuosity, and/or arterial occlusion (Aviv *et al.* 2006, Aviv *et al.* 2007). Children typically present with stroke-like neurological deficits such as hemiparesis or hemisensory deficit, which may be preceded by periods of headache, cognitive dysfunction, seizures and behavioral abnormality (Benseler *et al.* 2006). It is a chronic, progressive inflammatory disease and hence the mainstay of treatment is immunosuppression with anti-platelet agents used for secondary stroke prophylaxis (Benseler *et al.* 2006, Cellucci and Benseler 2010, Sen *et al.* 2010).

1.4.2. *Genetic and Syndromic Arteriopathies*

This is a heterogeneous group of cerebral arteriopathies with a genetic basis. Arteriopathy may be non-progressive or progressive, moyamoya-like or non-moyamoya-like, clinically symptomatic or asymptomatic (Milewicz *et al.* 2010, Munot *et al.* 2011). The literature is often limited and based on case reports. Both occlusive and aneurysmal disease can occur within the same patient and family. An example of a single gene mutation which leads to both occlusive and arterial aneurysmal disease in multiple vascular beds is seen in ACTA2 vasculopathy (Guo *et al.* 2009, Milewicz *et al.* 2010). Despite the moyamoya-like appearances, the natural history of the vasculopathy is not yet well characterized (Munot *et al.* 2012). PHACE(S) (Posterior fossa malformations, Haemangiomas, Arterial anomalies, Cardiac defects and Coarctation of the Aorta, Eye abnormalities, and Sternal abnormalities or ventral developmental

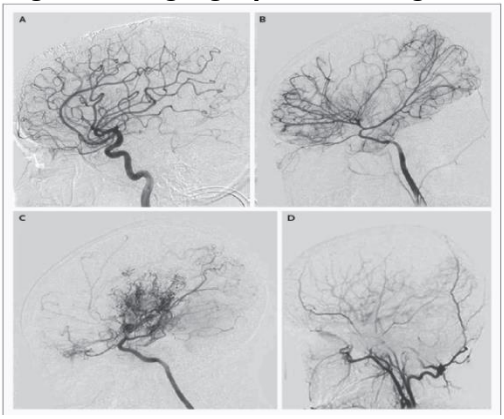
defects) is an example of a syndromic arteriopathy. The neurological features of PHACE(S) based on limited case reports can be divided into of two categories: i) congenital, structural anomalies which include malformations of the cerebrum, cerebellum and cerebral vasculature and ii) steno-occlusive arterial disease and a moyamoya-like vasculopathy. Cerebrovascular anomalies occurred more frequently on the side of the facial haemangioma, but not exclusively. Stroke may occur, although it is not commonly reported (Burrows *et al.* 1998, Drolet *et al.* 2006, Heyer *et al.* 2006, Heyer *et al.* 2008).

1.4.3.*Moyamoya*

1.4.3.1. Definitions

'Moyamoya' is the Japanese term for the angiographic 'puff of smoke' appearance (Suzuki and Takaku 1969) and is defined as a bilateral progressive intracranial arteriopathy involving the distal internal carotid (dICA), proximal middle cerebral (MCA) and proximal anterior cerebral (ACA) arteries of the circle of Willis (COW) (Amlie-Lefond *et al.* 2009). Unilateral cases are defined as probable moyamoya, 40% of which will progress to bilateral disease (Kelly *et al.* 2006). In a Japanese population, Suzuki defined 6 stages, from initial narrowing of the dICA to the 'puff of smoke' appearance of the lenticulostriate (LS) collaterals and finally to the disappearance of this network of collaterals (Figure 3) (Suzuki and Takaku 1969).

Figure 3 Angiographic Findings in Moyamoya



A. Normal

B. Narrowing of ICA

C. 'Puff of smoke' collaterals

D. Disappearance of 'puff of smoke' collaterals

Moyamoya disease (MMD) refers to patients, typically of Japanese or other Asian origin, who have no previously diagnosed condition, and are likely to have a family history and autosomally dominant or recessive genes as the aetiology of their cerebrovascular disease (Suzuki and Takaku 1969, Ikeda and Hosoda 1992, Hoffman 1997). Moyamoya syndrome (MMS) is the occurrence of moyamoya in association with an acquired or inherited disorder such as sickle cell disease, neurofibromatosis type-1 or Trisomy 21. MMS represents an heterogenous group of disorders. The prevalence and incidence of MMS is most likely related to the epidemiology and frequency of the underlying disease or risk factor and screening may be recommended in certain populations. (Rajakulasingam *et al.* 1979, Gadoth and Hirsch 1980, Pearson *et al.* 1985, Outwater *et al.* 1989, Rachmel *et al.* 1989, Drew *et al.* 1993, Ganesan and Kirkham 1997, Hoffman 1997, Matsuki *et al.* 1997, Lutterman *et al.* 1998, Rea *et al.* 2009, Duat-Rodriguez *et al.* 2014) MMS is proportionally more common in European and North American communities than MMD. Conversely in East Asian populations where this has been studied, the prevalence and incidence of MMS is up to ten times lower than that of MMD (Goto and Yonekawa 1992,

Yonekawa *et al.* 1997, Khan *et al.* 2003, Uchino *et al.* 2005, Hayashi *et al.* 2013)

1.4.3.2. Epidemiology and Genetics of Moyamoya

The relative frequency of MM varies between populations. Incidence of MM demonstrates geographic and ethnic variation and is estimated as 3/100 000 in the Japanese, and 0.086/100 000 in North America. In a Chinese study, more than 50% of the children with arteriopathy-associated AIS had Moyamoya disease (MMD) (Wang *et al.* 2009). Around 10% of Asian cases are familial (Kuriyama *et al.* 2008). Age at presentation is bi-modal, with peaks occurring at 5 and 40 years of age. However, the childhood age peak and male predominance are not replicated in comparative European or American populations (Kraemer *et al.* 2008).

Liu et al. (Liu *et al.* 2010, Liu *et al.* 2011) demonstrated a major founder susceptibility gene at p. R4810K on the ring finger protein 213 gene *(RNF 213).* However, this finding has not been replicated in the Caucasian Idiopathic moyamoya population and no major cofounder susceptibility genes have been identified. Candidate genes of interest include *MYRIP* which is highly expressed in the brain, located around the suggestive association region for cerebral infarction and has its C-terminal domain directly bound to actin which is encoded by *ACTA2* (Fukuda and Kuroda 2002, Liu *et al.* 2013). This could be responsible for the progression of vascular disease in Caucasian Idiopathic moyamoya.

Further novel genes of interest include the *BRCC3* and *MTCP1* genes. *BRCC3* encodes for a nuclear DNA repair complex and a cytoplasmic complex that might have a role in cardiomyocyte protection. Knockdown studies in Zebrafish suggest that *BRCC3* plays an important role in angiogenesis. The function of *MTCP1* is yet unknown (Herve *et al.* 2010, Miskinyte *et al.* 2011).

1.4.3.3. Natural History and Consequences of MMD

The natural history of moyamoya is not well understood. However, vascular progression often occurs in childhood even if asymptomatic (Suzuki and Takaku 1969, Imaizumi *et al.* 1998, Kuroda *et al.* 2000) (Moritake *et al.* 1986, Lin *et al.* 2011). Adult MMD is considered to be relatively more stable, although recent studies have challenged this notion. (Kuroda *et al.* 2007, Yang *et al.* 2014) Distal stenosis of the arteries of the COW results in reduction of cerebral perfusion pressure (CPP) and cerebral blood flow (CBF) distal to the stenosis. Collateral networks from the deep thalamoperforating and lenticulostriate perforating arteries – giving rise to the angiographic 'puff of smoke' appearance - form adjacent to the stenosed vessels; pial collateral arteries from the posterior circulation; and transdural collateral arteries from the external carotid arteries supply the compromised brain in an attempt to maintain adequate CPP. Reduction in CPP may ensue with or without a compensatory rise in systemic blood pressure. Subsequent failure of CPP may result in cortical and sub-cortical ischaemia, with the clinical consequence of covert or overt arterial ischaemic stroke (AIS).

Haemorrhagic syndromes dominate clinical presentation in Asian MMD in contrast to clinical presentation in non-Asian adults or children which is usually with ischaemic symptoms, including dysarthria, hemiparesis, choreiform movements, cognitive decline, headache and seizures (Hallemeier *et al.* 2006, Kraemer *et al.* 2008, Scott and Smith 2009). Precipitants include hyperventilation and dehydration.

1.4.3.4. Moyamoya and neurocognitive outcome

Cognitive decline over time is now well recognized in paediatric MMD (Ishii *et al.* 1984, Kurokawa *et al.* 1985, Imaizumi *et al.* 1998, Imaizumi *et al.* 1999, Lee *et al.* 2011). The majority of early studies examining neurocognitive outcome in moyamoya disease were conducted in Japanese populations. Factors consistent with a poor outcome include early age at onset, transient ischaemic attacks followed by stroke, diffuse arteriographic occlusion, and bilateral or left hemisphere lesions (Maki *et al.* 1976). In one of the first longitudinal studies of intelligence in MMD, Imaizumi et al demonstrated a drop in full scale intelligence quotient (FSIQ) in the first 5-9 years after symptom onset, followed by a plateauing of intellectual function (Imaizumi *et al.* 1998), replicating a previous observation (Kurokawa *et al.* 1985). A longitudinal study in a UK population of the effect of moyamoya on IQ over time was conducted by Hogan et al in children with and without sickle cell disease, mainly homozygous sickle cell anaemia (SCA) compared with controls matched for age, gender and ethnicity (Hogan *et al.* 2005). They demonstrated similar effects although follow-up did not go beyond 5 years for the majority of patients (Hogan *et al.* 2005). In addition, the majority of studies (including the above) have been

conducted in symptomatic children, i.e. those presenting with stroke or transient ischaemic attack (TIA). In Hogan's series, children with bilateral clinical strokes had lower IQs when compared to those with unilateral stroke (Hogan *et al.* 2005). A number of their children also had recurrent TIAs. A more recent study by Williams et al in patients with MM examined the relationship between MM, IQ and higher indices of cognitive outcome in patients with and without stroke and demonstrated that MM arteriopathy itself has a deleterious effect on cognitive outcome independent of stroke (Williams *et al.* 2012). However, the relative importance of MM angiopathy, the associated perfusion deficits and cerebral infarction on IQ and indices of higher cognitive function are not fully explored in these studies.

1.4.3.5. Moyamoya disease, baseline cerebral blood flow and cognition

Ishii et al.(Ishii *et al.* 1984) found that preoperative IQ was related to baseline regional cerebral blood flow (rCBF) measured using a ^{133}Xe inhalation method in children with MMD. There are few other data.

1.4.3.6. Conventional Imaging Techniques in moyamoya

Conventional anatomical imaging techniques focus on accurate neuroradiological identification of acute or chronic ischaemic parenchymal disease and the presence and extent of moyamoya arteriopathy. Brain CT and MRI are able to demonstrate areas of overt or covert parenchymal ischaemia recognised as areas of gliosis or encephalomalacia within vascular territories or

in the distal vascular bed supplied by penetrating arteries of the MCA and ACA and related to border-zone infarction. Altthough the geographic use of the term watershed relates to a ridge formed for example by a range of mountains, which sends water to different rivers on each side, the terms border-zone and watershed infarction, are often used interchangeably. They refer to the border between cerebral vascular territories where the tissue is furthest from arterial supply and thus most vulnerable to reductions in perfusion. However, the typical vascular territories shift in the face of the vascular occlusionand collateralisation seen in moyamoya (Lee *et al.* 2005) Acutely, brain CT and MRI detect cytotoxic oedema, or the effects thereof. CT, which is faster and usually obviates the need for sedation or general anaesthesia, requires ionising radiation with the associated small but significant risk of cancer and cataracts. Early (1 – 3 hours) non-contrast CT changes post stroke include loss of grey-white matter differentiation, hypoattenuation of deep nuclei and cortical hypodensity with associated parenchymal swelling and gyral effacement. These changes are evident in 60-70% of adults with stroke in the first 6 hours. (Lev *et al.* 1999) MRI has a higher sensitivity and specificity than CT in the early diagnosis of ischaemic stroke. The use of diffusion weighted imaging (DWI) and apparent diffusion co-efficient (ADC) allows for imaging detection of ischaemia within minutes of onset. (Srinivasan *et al.* 2006) Additional MRI sequences useful, but less sensitive, for the detection of ischaemia include T1-weighted, T2-weighted and FLAIR sequences.

The inferred mechanism of ischaemia by MRI or CT may be embolic and/or haemodynamic. Hence recognition of patterns of ischaemic stroke in

moyamoya may be informative of aetiology and direct management (Rafay *et al.* 2015).

Furthermore, inferences about cortical perfusion can be made from the linear hyperintensities in a sulcal pattern seen on FLAIR sequences and called the "ivy sign" (Chabbert *et al.* 1998, Maeda and Tsuchida 1999). Diffuse leptomeningeal enhancement was first reported in contrast-enhanced T1-weighted image in patients with moyamoya disease by Ohta et al (Ohta *et al.* 1995). Fronto-parietal predominance is reported. Studies have not found a significant correlation between the angiographic severity of moyamoya and the presence of the ivy sign. The presence of the ivy sign may be a useful indicator of moyamoya in the absence of moyamoya collaterals (Fujiwara *et al.* 2005). In addition, the presence of the ivy sign has been shown to be positively correlated with ischaemic symptoms and negatively correlated with CBF and CVR (Mori *et al.* 2009).

MRA tends to overestimate stenosis and underestimate collaterals (Yamada *et al.* 1992, Saeki *et al.* 2000). Plain CT, CT with contrast, T1 and T2-weighted MRI, MR angiography, CT angiography and conventional angiography provide indirect and direct information about the anatomical state of the vessels. Conventional angiography allows for a more dynamic and hence functional assessment by registration of the temporal arterial-venous cycle. However, interpretation of these findings with respect to the risk of stroke occurrence or recurrence is limited (Ganesan *et al.* 2006, Fullerton *et al.* 2007, Amlie-Lefond *et al.* 2009, Braun *et al.* 2009).

1.4.3.7. Treatment of moyamoya

Medical treatment strategies for the primary prevention of stroke are limited and include the use of Aspirin, Acetazolamide or anticoagulants such as Warfarin or Heparin (Monagle *et al.* 2008, Roach *et al.* 2008) and in children with sickle cell disease, blood transfusion (Dobson *et al.* 2002). Calcium antagonists may be used with caution to treat intractable headache (Hossain and Evers 1994). There are currently no known treatments to prevent the vascular progression, which occurs in the majority even if asymptomatic (Suzuki and Takaku 1969, Imaizumi *et al.* 1998, Kuroda *et al.* 2000).

Moyamoya disease spares the external carotid arteries (ECA) and its branches. The aim of surgical treatment is to improve CBF in the impaired region. Surgical methods aim to improve the cerebral circulation either by bypassing the area of occlusion (direct anastomosis) or encouraging the formation of collaterals (indirect anastomosis). Cerebral neovascularisation from the ECA is the basis for indirect revascularisation surgery (Kuroda and Houkin 2012). Indirect procedures are more commonly used in children and are typically a type of synangiosis procedure such as encephaloduroarteriosynangiosis (EDAS) or encephalomyosynangiosis (EMS). During these procedures, tissues containing ECA branch (dura mater, temporal muscle, galeal tissue, or superficial temporal artery) are put in direct contact with the ischaemic brain. Neovascularisation occurs with neoangiogenesis requiring a minimum period of 3 months. Direct procedures are more commonly used in adults and involve the anastomosis of a branch of the ECA, typically the superficial temporal artery (STA), and the MCA or ACA. Hence, improvement in CBF is immediate. There is however an increased risk of post-operative haemorrhagic infarction

presumed to be secondary to hyperperfusion. Combined (direct and indirect) revascularisation procedures may more effectively improve cerebral circulation than single direct or indirect procedures (Matsushima *et al.* 1992, Ikezaki *et al.* 1994).

Current indicators for surgery include acute arterial ischaemic stroke, transient ischaemic attack, cognitive decline, and evidence of vascular or parenchymal progression of disease (Kronenburg *et al.* 2014). Given that medical options are limited in number and efficacy, primary revascularization surgery is usually the intervention of choice. There appears to be a reduction in symptomatic progression from 66% in those receiving no treatment to 2.6% following surgery and there is a 96% probability of remaining stroke free 5 years following surgery (Choi *et al.* 1997, Fung *et al.* 2005). However, surgery is not without risk. In one study by Guzman et al of the effect of revascularization surgery on the clinical outcome of 450 patients with MMD they found a surgical morbidity rate of 3.5% and a mortality rate of 0.7% per treated hemisphere (Guzman *et al.* 2009). There are no published observational cohort studies comparing disease progression and outcome in patients with and without surgical revascularization. Demonstration of true benefit from revascularization surgery would require a randomized controlled trial with revascularization surgery as an intervention. Feasibility of this would be challenging.

1.4.3.8. Surgery for Primary Prevention of Stroke and Cognitive Decline in MMD

Pre and post-surgical SPECT studies in children with MMD demonstrated abnormalities in cerebral haemodynamics pre-operatively, even in the absence of infarction, with improvement post combination (direct and indirect) revascularisation surgery (Ohtaki *et al.* 1998). Improvements in CBF have also been documented in children with stroke (Matsushima *et al.* 1990, Bowen *et al.* 1998).

It has been proposed that the stage of cerebrovascular haemodynamic compromise at the time of operation may be an important determinant of surgical success. Patients with no clinical signs of infarction and a decreased regional cerebral blood flow (rCBF), increased regional oxygen extraction fraction (rOEF), and regional cerebral blood volume (rCBV) without any change in regional cerebral metabolic rate of oxygen consumption (rCMRO2) on PET scans may be good candidates for bypass surgery in MMD. Matsushima et al suggested that if surgery is performed in a brain with normal blood flow or following an extreme reduction in rCMRO2, i,e. too early or too late, collateral vessels may not form (Matsushima *et al.* 1990). A number of PET and SPECT studies have demonstrated improvement in regional CBF and CBV post revascularization surgery, mostly in the area surrounding the bypass (Ikezaki *et al.* 1994), coupled with improvements in clinical symptoms. In a recent large study, Kim et al found that baseline rCBV from perfusion MRI and rCBF using SPECT predicted surgical outcome in univariable analysis but they were not in the final multivariable model, although measurement of reserve using

acetazolamide (see 1.9.1, p.78) and SPECT remained an independent predictor (Tamaki and Yoji 2010).

Other studies have suggested that children may be more likely than adults to self-collateralize, as well as to develop neovascularisation from anastomosis, as cerebral circulation and oxygen metabolism tend to decline with age (Pantano *et al.* 1984, Kuwabara *et al.* 1990). A longitudinal study of children with asymptomatic and/or incidentally diagnosed moyamoya by Lin et al (Lin *et al.* 2011) demonstrated that radiographic progression occurs in the majority (SCD more than NF1) and heralds clinical progression. However, given that not all children with progressive moyamoya have acute clinically evident ischaemic events, tools to predict ischaemic risk and hence guide timing of surgery would be invaluable in this population.

1.5. Normal Cerebral Haemodynamics

1.5.1. Cerebral blood flow equation

Cerebral blood flow can be described as the relationship between cerebral perfusion pressure (CPP) and cerebrovascular resistance (CVRE):

$$CBF = CPP/CVRE$$

CPP is equal to mean arterial blood pressure (MABP) – intracranial pressure (ICP):

$$CPP=MABP-ICP$$

In the absence of pathologic cerebral conditions, ICP is negligible and CPP is roughly equivalent to MABP. CVRE is inversely proportional to blood viscosity

and proportional to the fourth power of the radius of the vessel (Poiseuille's Law). Poiseuille's Law can be applied to describe the relationship between factors which affect cerebral blood flow. It describes the laminar flow of an incompressible uniformly viscous fluid through a cylindrical tube with constant circular cross-section. Although this equation is not fully applicable to the cerebrovascular bed as it is not a rigid system, it's effective total length is not known, and blood is not a uniformly viscous (newtonian) fluid, it describes the relationship between CBF, blood pressure and resistance. It also serves to highlight how small changes in the radius of cerebral blood vessels can produce marked alterations of CVR, largely by variation in the degree of constriction of the cerebral resistance vessels.

1.5.2. Normal cerebral blood flow

The brain has high energy requirements with limited intracellular energy resources. Hence it is highly reliant on cerebral blood flow and perfusion to meet its metabolic requirements. Cerebral blood flow is regulated to match the regional metabolic demands of the brain. This essential nutrient and oxygen exchange occurs mainly in the capillary bed - at the level of the microcirculation. Penetrating arterioles branch perpendicularly from the pial arterioles and penetrate the parenchyma of the brain where they form an extensive network of capillaries within the brain parenchyma. Factors that effect this exchange and therefore microcirculatory CBF are the path length, red blood cell capillary transit time and neuronal activity (Zlokovic 2008).

Although there are several techniques for measuring CBF in animals and Man, there are problems with methodology (for review see Kirsch et al. 1985) and even if these can be solved, none is suitable for routine use in children because they are all complicated and time-consuming and require dedicated personnel. Most methods of measurement of CBF involve delivery of an amount of tracer substance to the brain and measurement of uptake or loss of tracer over a time period. There are serious doubts about the validity of techniques which rely upon the equilibration of a tracer between blood and brain, as the partition coefficient for damaged brain may well be different from that for normal brain and may change during the course of the illness. Several rely on the use of radioactive isotopes, and the techniques cannot therefore be repeated frequently because of the cumulative dosage and because the isotope must be cleared from the body before the next dose is given.

CBF in normal adults is approximately 50 ml $100g^{-1}$ min^{-1} whether measured by the Kety-Schmidt, intra-arterial, intravenous or inhalation ^{133}xenon techniques, or by near infrared spectroscopy. There are few data available in normal children (Kennedy and Sokoloff 1957, Settergren et al. 1980) because of the ethical difficulties so that the interpretation of abnormal results may be very difficult. Kennedy and Sokolof (1957) found CBF to be much higher in conscious normal children than in adults, in the order of 100 ml $100g^{-1}min^{-1}$, but as much of their data had to be discarded because the subjects were unco-operative; it is possiblethat their values were higher because of the childrens' anxiety. However, even under anaesthesia, values for CBF in children appear to be slightly higher than those for adults (Settergren et al, 1980) although there

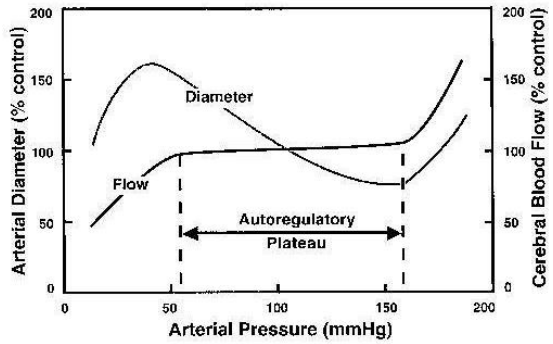
is considerable variation. Despite methodological concerns there is general agreement that in the healthy brain it appears that CBF is maximal, around 100ml/100g/min, in mid childhood, i.e. around 8 years of age, following which it declines, approaching adult values (50ml/100g/min) in the teenage years (Kennedy and Sokoloff 1957, Biagi *et al.* 2007). The Kety-Schmidt technique was modified for repeated use in intensive care and has been used to show low CBF in children who have recently had a traumatic brain injury (Sharples et al 1991). Cerebral metabolic rate and cerebral oxygen extraction are maximal shortly after injury in children (Sharples et al. 1995), which means that the brain is very vulnerable to ischaemic injury at this stage.

A number of intrinsic safeguarding mechanisms exist to ensure adequate cerebral blood supply. These include the vascular network which incorporates a high degree of redundancy (del Zoppo and Hallenbeck 2000, Blinder *et al.* 2010, Hirsch *et al.* 2012, Blinder *et al.* 2013) as well as cerebral autoregulation and functional hyperaemia.

1.5.2.1. *Cerebral autoregulation*

Cerebral autoregulation is one of the most important factors allowing CBF to remain relatively constant despite fluctuations in arterial blood pressure. This is thought to be mediated at the level of the smooth muscle arterioles in the vascular bed, which are exquisitely sensitive to changes in blood pressure and pCO_2 (Figure 4) (Ogawa *et al.* 1990).

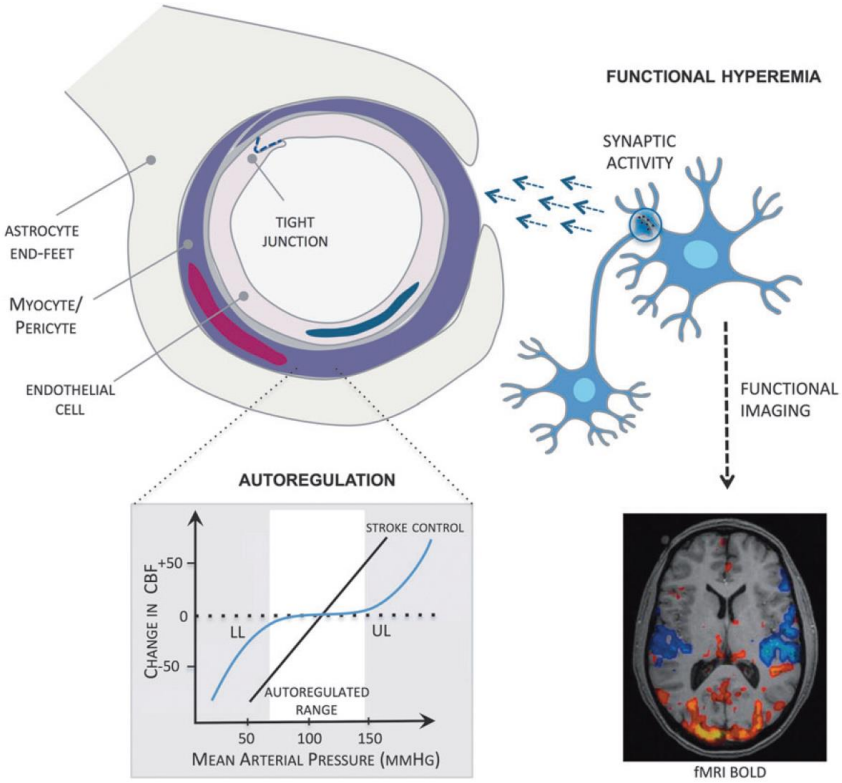
Figure 4 Normal Cerebral Blood Flow Autoregulation



1.5.2.2. *Functional hyperaemia or neurovascular coupling*

Functional hyperaemia or neurovascular coupling actively pairs cerebral blood flow to changes in neural activity. Synaptic activity stimulates the release of signalling molecules that initiate changes in vascular tone. Although neurovascular coupling remains incompletely understood it is known to be influenced by neurotransmitters, ion flux, endothelial cells, astrocytes and pericytes. (Hamel 2006, Lok *et al.* 2007, Jackman and Iadecola 2015) Several studies performed in steady-state animal preparations and in healthy human volunteers reported a linear relationship between neural activity and the BOLD signal (Logothetis and Wandell 2004). The haemodynamic response can vary across populations, cortical areas, and stimuli duration (Birn *et al.* 2001, D'Esposito *et al.* 2003). Hence functional hyperemia or neurovascular coupling also forms the basis of functional imaging (Figure 5).

Figure 5 Neurovascular Regulation in the Ischaemic Brain. From Jackman and Iadecola, Antiioxidants and Redox Signaling, Vol 22, Number 2, 2015



1.5.3. Cerebral Metabolism

Studies of healthy anaesthetized children suggest age-related increases in cerebral metabolic rate for oxygen (CMRO2) after infancy up to 14 years old (Biagi *et al.* 2007). Similar to CMRO2, cerebral metabolic rate for glucose (CMRglu) is lower in children at birth and increases during childhood but peaks by 3 years to 4 years, and remains high until 9 years of age. Thereafter CMRglu decreases and approaches adult rates (Chugani *et al.* 1987). Under normal conditions, CBF is tightly coupled to CMRO2, at global as well as regional levels. Central nervous system activation results in an increase in CBF more than CMRO2, resulting in a decrease in the cerebral oxygen extraction fraction (OEF) (Fox and Raichle 1986, Philip *et al.* 2009), a quantification of the

matching of CBF to cerebral metabolism and an indicator of brain tissue vulnerability to reduction in CPP.

1.6. Haemodynamics in Cerebral Arteriopathy

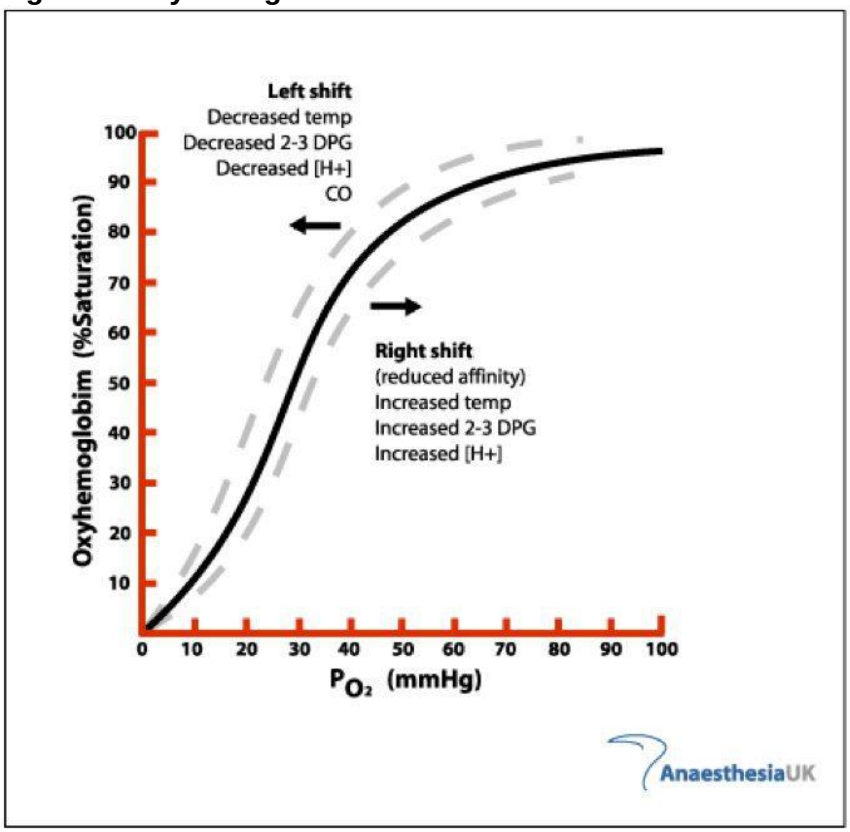
1.6.1. Effect of vascular disease on distal cerebral blood flow

Stenosis or occlusion of major cerebral arteries reduces the arterial pressure in the distal circulation which then becomes dependent on collateral vessels; if this is not sufficient to maintain cerebral perfusion pressure, the result is reflex vasodilatation (stage 1 haemodynamic compromise) (Derdeyn *et al.* 1999) If perfusion pressure falls further, there is an increase in the oxygen extracting capacity of the brain (the oxygen extraction fraction (OEF)) in order to maintain normal cerebral metabolic function (stage 2 haemodynamic compromise) (Gibbs *et al.* 1984) also described as 'misery perfusion' (Baron *et al.* 1981, Yamashita *et al.* 1996). Once OEF is maximal, further reductions in perfusion pressure lead to disruptions in tissue metabolism and ultimately to brain infarction.

The majority (~ 98%) of the oxygen in blood is reversibly bound to haemoglobin. This is also the case in the microcirculation. The oxyhaemaglobin curve describes the relationship between oxygen saturation and partial pressure of oxygen in the blood. It is a tool for understanding how blood carries and releases oxygen and is determined by the affinity of haemoglobin for oxygen. The 'driving force' for oxygen movement from blood to tissue is the partial pressure of oxygen of the tissue to which the haemoglobin is exposed. Hence when there is a drop in tissue partial oxygen pressure the affinity of Hb for

oxygen drops and the release of oxygen bound to Hb is triggered. Oxygen passes from blood to tissue cells by diffusion, and the amount of oxygen moving across the capillary wall is equal to the amount consumed by the tissue supplied by that capillary. The amount of oxygen consumed as a fraction of oxygen delivery defines the oxygen extraction ratio or fraction, which in turn is a quantification of CBF and oxygen exchange as invoked by CMRO2 (Duling et al 1970, Pittman et al 2005, Pittman 2013). Multiple factors can affect the affinity of Hb for oxygen, causing shifts of the oxyhaemaglobin curve to the left (increased oxygen affinity) or right (reduced oxygen affinity). Fig 6

Figure 6 Oxyhemoglobin Dissociation Curve



2-3 DPG – 2-3 diphosphoglycerate; CO - carbon monoxide

1.6.2. Effect of moyamoya on distal cerebral blood flow

In moyamoya, blood flow distal to the stenotic vessels is thought to be maintained by a drop in vascular resistance mediated by small artery and arteriolar vasodilation. Systemic hypertension is commonly seen in moyamoya and is probably compensatory in an attempt to maintain CPP and CBF. As the disease progresses, however, the ability of this autoregulatory system to preserve adequate perfusion is lost when compensatory arteriolar dilation reaches a maximum. Further increases in vascular resistance at the stenosis ultimately lead to tissue oligaemia and possible ischaemia (Ishii *et al.* 1984, Grant *et al.* 2005).

Reduced baseline rCBF distal to the stenosis in the arteries of the COW has been demonstrated in MMD. Early PET studies of cerebral circulation demonstrated disruption in cerebral haemodynamics even in the absence of infarction (Ohtaki *et al.* 1998). In pre-infarctive ischaemia, regional reduction in cortical CBF and vascular reserve correspond to areas of increased CBV and tissue OEF, compensating for low CPP and maintaining the rCMRO2, i.e. 'misery perfusion' (Ikezaki *et al.* 1994). Loss of vascular reserve and haemodynamic compromise, as seen on CBF-SPECT studies, is compatible with 'misery perfusion' as seen on PET (Baron *et al.* 1981). Such tissue with low or 'misery' perfusion can be considered as tissue at risk of silent or overt infarction, and most likely to benefit from early interventional strategies (Ohtaki *et al.* 1998).

There are many ways of defining penumbra (Symon *et al.* 2007). A pragmatic therapy directed definition is that of ischaemic tissue that is potentially

salvageable as distinguished from the ischaemic core that has already sustained irreversible injury (Fisher 1997). Modified criteria have been presented by (Donnan *et al.* 2007).

Figure 7 Criteria for the Ischaemic Penumbra

1	An area of hypoperfused, abnormal tissue with physiological and/or biochemical characteristics consistent with cellular dysfunction but not death.
2	The tissue is within the same ischaemic territory as the infarct core.
3	Demonstration that the tissue can survive or progress to pan-necrosis.
4	Salvage of this tissue is related to better clinical outcome.

There are multiple mechanisms of cell death that may occur in response to ischaemia: 1) necrosis 2) apoptosis and 3) autophagy.(Xu and Zhang 2011) Necrosis is the final endpoint of energy failure resulting in neuronal cell death secondary to ischaemia. Although traditionally thought to be a passive form of cell death, further research suggests the existence of a programmed necrosis, called 'necroptosis' by one group (Degterev *et al.* 2005) Delayed cell death caused by ischaemia is characterized by apoptosis – a process of cell suicide or programmed cell death, regulated by different cell signaling pathways Autophagy is a highly regulated cellular mechanism that leads to degradation of long-lived proteins and dysfunctional organelles. Whether it is protective or destructive appears to depend on the levels of hypoxia or ischemia. Whereby necrosis appears to dominate the ischaemic core, apoptosis and autophagy appear to dominate the penumbra. (Xu and Zhang 2011)

rCBF is typically reduced in children with MMD compared with controls in a regional (fronto-parietal) distribution, with concomitant increases in OEF and regional cerebral blood volume (rCBV) but maintenance of $rCMRO_2$ (Ikezaki *et*

al. 1994, Kuroda *et al.* 1995, Ishikawa *et al.* 1997). Increased rCBV represents both compensatory vasodilatation related to the reduced rCBF and the blood contained within the collateral circulation. In patients with more advanced disease, there is a progressive fall in rCBF and eventually a decrease in $rCMRO_2$.

Haemodynamic abnormalities improve but may still be outside the normal range after surgical revascularization (Nakagawara *et al.* 1997). Clinical improvement is not directly related to the improvement in haemodynamic studies. Similar changes do not appear to occur in the adult moyamoya population, although in this age group, there is a slight reduction in global rather than regional CBF and no significant increase in rCMRO2, OEF or rCBV (Kuwabara *et al.* 1990, Ishikawa *et al.* 1998).

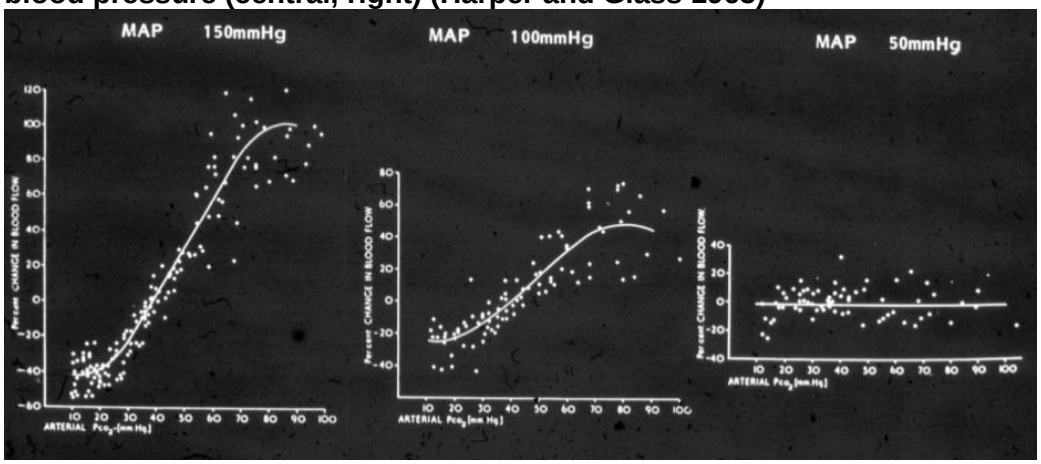
1.7. Cerebrovascular Reactivity

CVR is the measurement of vessel reactivity to changing partial pressure of carbon dioxide (pCO2) and is defined as the change in blood flow per unit change in pCO2. It is an important marker of brain vascular reserve and the ability to augment and maintain CBF under conditions of stress. CVR studies are the brain equivalent of a cardiac stress test. Impaired CVR is an indicator of tissue at risk of decline in function, typically but perhaps not always, related to ischaemia. There are relatively few studies in conditions affecting children (Davis *et al.* 1983, Clifton *et al.* 1988, Kuwabara *et al.* 1997, Prohovnik *et al.* 2009, Heyn *et al.* 2010).

As smooth-muscle tone in the arterioles is profoundly influenced by arterial carbon dioxide tension ($PaCO_2$), small vessel arterioles dilate in response to an

increase and constrict with a decrease in the $PaCO_2$, increasing or decreasing CBF in parallel (Figure 5 left) (Kety and Schmidt 1948). Under certain conditions such as hypotension (Harper and Glass 1965) (Fig 5 central, right), or after traumatic brain injury (TBI) (Philip *et al.* 2009), or in Sickle Cell Anaemia (SCA) (Prohovnik *et al.* 2009) arteriolar dilatation reaches a maximum and exhaustion of vasodilatory capacity may ensue resulting in flattening of the curve, with the risk of a decrease in CBF and CPP despite increasing $PaCO_2$, and consequent ischaemia (Philip *et al.* 2009, Prohovnik *et al.* 2009). Impaired CVR is associated with conditions of cognitive decline such as Alzheimer's disease (Marshall 2012). Patients with SCA have marked global cerebral hyperaemia secondary to anaemia, when compared to controls. Blood pressure is relatively low and autoregulation may be abnormal, although this is relatively difficult to test (Prengler *et al.* 2002). In addition, the response of the cerebral circulation to increasing carbon dioxide tension, which is normally positive, is often very abnormal in SCA, with negative reactivity (steal) reported in a quarter of patients in a recent study from Steven Pavlakis' group (Prohovnik *et al.* 2009). This abnormal response is related to the anaemia and the resultant adaptive vasodilatation. Untransfused children with SCA approach the upper limit of cerebral vasodilatation with chronically increased cerebral perfusion. Hence, despite increased globally increased CBF in the context of the anaemia, ischaemia may occur under any condition where there is an additional requirement for oxygen supply (e.g., increased metabolic demand due to fever or seizure, hypotension), putting the child at risk of brain damage, including progressive white matter injury and neurocognitive decline.

Figure 8 Percentage Change in cerebral blood flow with changing arterial carbon dioxide in dogs with normal (left) and progressively reducing blood pressure (central, right) (Harper and Glass 1965)



1.7.1. Measurement of Cerebrovascular Reactivity

These changes may be monitored using a number of techniques (Harper and Glass 1965, Kirkham *et al.* 1986).

Studies of cerebrovascular reactivity in childhood arteriopathy are limited. In a PET study of 20 patients with moyamoya (7 children), using ^{15}O H_2O as the tracer, Kuwabara et al found reduced cerebrovascular reactivity to changing PCO_2 compared with controls (Kuwabara *et al.* 1997). A recent study by Heyn et al of 9 adults and 2 children (aged 10 and 11 years respectively) demonstrated that cerebrovascular response to changing pCO_2, as measured using BOLD signal, correlated with the severity of moyamoya disease (Heyn *et al.* 2010). In addition Han et al demonstrated in children that CVR measures can provide information about cerebral haemodynamic compromise beyond ischaemic lesions, even into normal appearing brain parenchyma (Han *et al.* 2011).

1.8. Direct and Indirect Methods of Quantification of Cerebral Blood Flow

1.8.1. BOLD MRI

As it is non-invasive and does not require ionizing radiation, MRI is particularly well-suited for paediatric studies, which is especially advantageous for when repeated studies are required. Deoxyhaemoglobin (dHb) is paramagnetic and is hence a naturally occurring contrast agent for magnetic resonance imaging. Arterial blood comprises a relatively small fraction (~ 25%) of total CBV and contains negligible amounts of dHb. (Schmidt and Thews 1983)Therefore, MRI-relevant changes in total CBV are primarily due to passive inflation of venules caused by the elevation of venous blood pressure occurring when arteriolar resistance is lowered (Hoge *et al.* 1999, Mandeville *et al.* 1999) Deoxyhaemoglobin may be accentuated using gradient-echo techniques in high fields to image brain microvasculature. Oxyhaemoglobin (OxHb) has little effect on the signal (Ogawa *et al.* 1990, Buxton *et al.* 2004, He and Yablonskiy 2007).

The blood oxygenation level-dependent (BOLD) contrast follows blood oxygen changes induced under various circumstances, including alteration of inhaled gas mixtures that alter metabolic demand or blood flow, such as carbon dioxide (Graham *et al.* 1994). BOLD MR imaging is sensitive to the dHb concentration in blood (Ogawa *et al.* 1990) and it's signal is dependent on the concentration in the venous blood and the blood volume fraction of tissue. (Boxerman *et al.* 1995) An increase in dHb causes a reduction in MR signal due to magnetic susceptibility effects and increased T2* relaxation. In functional MR studies, increased CBF secondary to increased neuronal activation is associated with

decreased dHb and increased Hb concentrations. Under these conditions, oxygen delivery exceeds increased oxygen consumption by the tissue, resulting in increased BOLD signal intensity (Davis *et al.* 1998). A similar effect can be achieved in BOLD CVR experiments. Because increased PCO_2 causes vasodilation of cerebral blood vessels without increasing the metabolic rate of brain parenchyma, it can also be used to change the dHb concentrations within the cerebral vasculature. In healthy vessels, the MR signal intensity increases when pCO_2 increases due to dilution of dHb resulting from increased O2 delivery with constant tissue O2 utilization. In areas of negative reactivity, the MR signal decreases with increased PCO2. BOLD contrast can be used to provide in vivo real-time maps of blood oxygenation in the brain under normal physiological conditions and in relation to regional neural activity.

Using arterial spin labelling (ASL) as a reference standard for measuring CBF and comparing ASL CVR with BOLD CVR, BOLD MRI can accurately discriminate between normal and abnormal hemispheric CVR. Regions of paradoxical CVR on BOLD MRI have been shown to have a moderate positive predictive value (73%) for true paradoxical CVR. Importantly, complete lack of paradoxical CVR on BOLD MRI had a high negative predictive value (100%) (Hoge *et al.* 1999, Mandell *et al.* 2008).(Mandell *et al.* 2008).

Arterial partial pressure of carbon dioxide ($PaCO_2$) is directly proportional to end-tidal PCO_2 hence under normal physiological circumstances (Kirkham *et al.* 1986), control of end-tidal PCO_2 through manipulation of inhaled CO_2 (e.g. breath-holding, rebreathing circuit, ventilator adjustment) will result in proportional changes in CBF that can be indirectly imaged using BOLD MR sequences (Vesely *et al.* 2001). Quantitative MRI measures of CVR can

therefore provide ‘parametric maps’, which enables the interrogation of regional vascular dysfunction at the tissue level and reserve capacity of the cerebral vasculature through manipulation of alveolar PCO_2 (Graham *et al.* 1994, Detre and Wang 2002, Mandell *et al.* 2008, Kassner *et al.* 2010, Mandell *et al.* 2011, Donahue *et al.* 2013, Thomas *et al.* 2013).

1.8.2. CVR-ASL

In addition to semi-quantitative delineation of regional perfusion with gadolinium (Calamante *et al.* 2001, Kirkham *et al.* 2001), ASL can be used to assess CVR and CBF. ASL uses water protons in endogenous blood as a perfusion tracer. Labeled capillary intravascular spins do exchanges with brain tissue through the blood-brain barrier altering tissue magnetization making it possible to obtain images in which contrast is proportional to cerebral perfusion. Images are required distal to the labeling plane and perfusion maps obtained by subtracting labeled and control images. In contrast to BOLD MRI signal, ASL signal is independent of CBV, cerebral metabolic rate of oxygen consumption and PaO2 (Ogawa *et al.* 1993, Hoge *et al.* 1999, Thomas *et al.* 2001, Petersen *et al.* 2006).

Despite the advantages of ASL clinical application in the assessment of perfusion and CBF is problematic in steno-occlusive cerebrovascular disease. Limited signal-to-noise ratio and complex flow kinetics, requiring measurements of T1 values for blood and tissue, labeling efficiency and arterial transit time for absolute CBF quantification are barriers to the application of ASL for the assessment of CVR in moyamoya (Golay *et al.* 2007, Mandell *et al.* 2008).

T2* dynamic susceptibility contrast imaging (DSC) and T1 dynamic contrast enhancement (DCE) are MR perfusion techniques used in adults. However, concerns include the invasive nature of the contrast administration (high volume bolus in small venous channels), risk of anaphylaxis, nephrotoxicity, slow clearance and, in the case of repeated administrations, retention of gadolinium contrast in the brain (Levin, Kaufman et al. 1995, Kaewlai and Abujudeh 2012, Kanda, Ishii et al. 2014, Kanda, Oba et al. 2016).

Another MR based approach to measuring perfusion consists of a high-spatial-resolution 3D gradient echo MR sequence employing susceptibility weighted imaging (SWI). This technique has been employed to measure perfusion in healthy and asphyxiated newborns (Boudes, Gilbert et al. 2014) and recently, to measure penumbra in neonatal stroke (Watson, Dehaes et al. 2016). However, further exploration of this modality is required in the paediatric population.

1.8.3. Nuclear Medicine Techniques and CT

CT perfusion studies are widely used in adults, however they too require the use of contrast. In addition there is often a need for serial imaging in children with steno-occlusive arteriopathy, hence CT based techniques of measuring perfusion and cerebral blood flow are not usually employed in children.

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Techniques measuring CBF are based on original research by Kety and Schmidt, who developed a quantitative method to measure whole-brain CBF and the CMRO2 using nitrous oxide, in humans, based on the Fick principle. Fick's principle states that blood flow to an organ must equal the rate in which the organ metabolizes a blood constituent, divided by the constituent's vascular concentration — its arterial-venous difference. More simply put - that the amount of a substance taken up or eliminated by an organ is equal to the difference between the amount in the arterial blood and the amount in the venous blood supplying that organ, in the same time period.

The measurement of global cerebral blood flow with nitrous oxide required the following assumptions: (1) blood flow is in a steady state during the period of study and is not affected by the tracer. (2) the venous blood from the superior internal jugular vein is representative of the mixed cerebral venous blood with no contamination from extracerebral blood, (3) the period of measurement is long enough to allow equilibration of gas in the brain with the cerebral venous blood, (4) no significant arterial-venous shunts are present in the brain, and (5) the value of the partition coefficient of the inert gas is representative of the entire brain. The initial studies with nitrous oxide demonstrated that blood flow studies with this tracer did meet the above assumptions under most situations, the exception being in a region of disturbed physiology, where arteriovenous shunting may be significant and where the partition coefficient is likely to be altered.

Kety and Schmidt's (1948a,b) landmark papers established the foundational principle for most measurements of CBF ie. delivery of an amount of tracer

substance to the brain and measurement of uptake or loss of tracer over a time period, and provided the basis for the field of functional brain imaging.

Nuclear medicine techniques such as ^{133}Xenon clearance, positron emission tomography (PET), single photon emission computed tomography (SPECT), as well as stable Xenon-enhanced computed tomography (Xe/CT) have all been important in the early measurement and understanding of CVR. These techniques typically exploit the properties of the radioisotope. Although these techniques can provide accurate regional measures of baseline CBF and the CBF response to vasoactive stimuli, all require inhalation or injection (Ikezaki *et al.* 1994, Horowitz *et al.* 1995, Kuroda *et al.* 1995, McAuley *et al.* 2004) (Lee *et al.* 2009).

1.8.3.1. Single photon emission tomography (SPECT)

Repeated measures required for SPECT results in increased exposure to radiation and (again) increased errors due to head motion artifact and differences in washout of tracer. It too is often used in conjunction with a vasodilatory stimulus (Moretti *et al.* 1995).

1.8.3.2. Stable Xenon CT

1.8.3.3. ^{133} Xe/Stable Xenon CT

Xe/CT has been widely used in the study of moyamoya in conjunction with the acetazolamide challenge (see 1.9.1, p.78). ^{133}Xe has a low solubility in blood

and is rapidly cleared from the blood, thus allowing further studies to be performed within a short period of time (approximately 30 min). It does not require repeat blood sampling and allows for calculation of regional flow rates.

Disadvantages include a relatively long acquisition time therefore increasing the probability of motion artifacts and tolerability. Furthermore, due to regulatory issues, the Xe-CT CBF technique is not readily available at present in many countries, including the US (Horowitz *et al.* 1995, Nambu *et al.* 1995, Suzuki *et al.* 1996, McAuley *et al.* 2004). Further disadvantages include poor spatial and temporal resolution and a concomitant increase in CBF as a result of stable xenon inhalation.

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1.8.4. Transcranial Doppler (TCD)

Although the diameter of the vessel is typically not known, transcranial Doppler can measure CBF in conditions where diameter can be assumed to be constant (Kirkham *et al.* 1986) and quantification of CVR relies on measuring the change in mean blood flow velocity or pulsatility index within the major arteries of the

circle of Willis in response to a vasoactive stimulus such as carbon dioxide (Kirkham *et al.* 1986, Philip *et al.* 2009, Wu *et al.* 2011). However, TCD only provides an indirect measure of CVR in the large intracerebral vessels, one that is remote from the actual site of vascular dysfunction in the arteriolar bed. In childhood stroke, the large vessels are typically very abnormal in a variety of ways which are difficult to diagnose using TCD. As a result, TCD measures of CBF may include a large contribution from collateral sources, which can lead to false negative CVR measurements (Pindzola *et al.* 2001) and is unable to provide information about the extent of CVR impairment at the tissue level. The technique is very operator dependent and cannot delineate precise regions of compromised CVR.

1.9. Vasoactive Challenge Techniques for Mapping of Cerebrovascular Reactivity

The magnitude of the change in blood flow in response to vasoactive agents such as CO_2 or acetazolamide provides information concerning the intrinsic capacity of the cerebral vasculature to regulate blood flow. Hence, CVR response to a prescribed challenge can be documented CVR using a number of available techniques:

1.9.1. Acetazolamide challenge

This method requires intravenous injection of Acetazolamide, a selective carbonic anhydrase inhibitor. Acetazolamide reduces the conversion rate of

CO2 to bicarbonate resulting in increased CO2 in brain tissue, a change in extracellular pH and a rapid increase in CBF without a change in CMRO2 or arterial blood pressure (Vorstrup *et al.* 1984). However the need for injection, associated dizziness and TIA like symptoms and a poorly understood time to and mechanism for vasodilation remain as disadvantages to the use of this technique (Dahl *et al.* 1995, Saito *et al.* 2011).

1.9.2. Breath-holding

This is the simplest hypercapnic CVR technique. It does not require a source of CO_2 or special equipment. Underlying arteriopathy, in particular MMD, increases anaesthetic risk so one advantage is that the child does not require general anaesthetic (GA) as it is performed in the awake state. Breath-holding (to simulate apnoea) results in an increase in $PaCO_2$ and vasodilation (Thomason *et al.* 2005). Interspersing breath-holds with regular breathing for controlled periods of time and synchronization of these timelines with the BOLD signal generated on MRI allows for the generation of BOLD signal-CVR fit curves. However, as delivery of the vasoactive stimulus CO_2 is difficult to quantify and respiratory information is minimal, repeatability of such data is challenging.

With the breath-holding technique, patients breathe room air hence there is no control (between breath hold [BH] and normal respiration [NR]) for PaO_2 and any possible effects on CBF. The normal response to increase in inhaled CO_2 is increased tidal volume with a concomitant increase in $PaCO_2$. One study (Prisman *et al.* 2008) showed that the BOLD response to O_2 change is 0.006%/mmHg, with a sensitivity 50 times lower than that for CO_2

(0.3%/mmHg). Hence, the effect of increase in O_2 with the interspersed NRs is probably minimal. This minimal effect is likely to be because of the binding properties of haemoglobin and oxygen in the blood and the oxygen dissociation curve. Consequently apparently large increases of pulmonary O_2 are not mirrored in the blood or the cerebral circulation.

1.9.3. Fixed (5-7%) CO_2 inhalational techniques

Fixed CO_2 inhalational techniques with measurement of end-tidal CO_2 have increased the available respiratory data and improved the repeatability of studies. However, this technique is not always well tolerated in adults and, despite reductions in inhalational CO_2 time epochs, not well tolerated in children.

1.9.4. Hypercapnic CO_2 challenge under GA

Many children are too young or non-compliant for evaluation while awake and often require MRI examination under anesthesia. Hence, a modified version of the inhalation CO_2 technique has been simulated and successfully evaluated under these conditions whereby $EtCO_2$ levels checked at baseline are increased by reducing the ventilator rate during MRI acquisition, allowing for rise in CO2, followed by reinstitution of ventilatory settings so as to produce an end expiratory pCO_2 in the range of 35 – 40 mmHg. Effects of anaesthetic agents commonly used eg Propofol and inhalational anaesthetics are not fully understood.

1.9.5.Computer aided delivery of vasoactive substance

Quantitative techniques suitable for the awake state have been trialed with encouraging results in the adult cerebrovascular disease population (Mikulis *et al.* 2005, Heyn *et al.* 2010). One of the most promising of these is the RespirAct re-breathing system. This is a computer-controlled gas delivery system which allows the delivery of a quantifiable and repeatable vasoactive substance. Hence, targeted partial pressures of EtCO2 (e.g. hypercapnia) may be delivered to patients whilst simultaneously maintaining constant O2 levels, and hence eliminating changing pO2 as a confounder. The CVR maps generated are useful for inter-patient comparison.

1.10.Objectives

My primary objectives were to:

1) Validate the use of qualitative hypercapnic challenge BOLD MRI CVR (hBOLD CVR) using breath-hold and general anaesthetic methods as reliable and repeatable for use in the paediatric population

2) Assess the utility of hBOLD CVR as a tool for the identification of the risk of ischaemia in children with arteriopathy

A series of studies were hence conducted with the aim to answer specific questions to address the objectives of the research.

1.11. Research Questions

1.11.1. Study Questions and Study Chapters:

Chapter 2: Methodology

Chapter 3: Population Description and Clnical Presentation

Chapter 4: Reliability and Repeatability of hBOLD CVR Method of Analysis

<u>Study 1</u>

- Are direct and indirect quantitative measures of qualitative hBOLD CVR reliable and repeatable?

Chapter 5 Population hBOLD CVR Measures

- What range of quantitative hBOLD CVR measures are seen in a population of children with cerebrovascular disease?

Chapter 6: Comparison of hBOLD CVR with Conventional Angiography and Measures of Moyamoya Severity

<u>Study 2</u>

- Does CVR abnormality as demonstrated by quantitative hBOLD CVR measures correspond with the region of abnormality as demonstrated on conventional angiography
- Is hBOLD CVR abnormality associated with the severity of moyamoya as assessed by indices of disease severity?

Chapter 6: Pre and post-surgical hBOLD CVR

Study 3

- Do measures of hBOLD CVR change following revascularization surgery, and if they do are the changes concordant with change as demonstrated on conventional angiography and clinical change?

Chapter 7: hBOLD CVR and Neuropsychological Outcomes

Study 4

- Does hBOLD CVR predict neuropsychological outcome?

Chapter 8: hBOLD CVR and Cortical Thickness

Study 5

- Is abnormality in hBOLD CVR associated with abnormality in cortical thickness?

Chapter 9: hBOLD CVR and Chronic arterial stenosis

Study 6

- Does chronic non-progressive stenosis result in changes in hBOLD CVR beyond the stenosis?

1.12. Study Hypotheses:

1) Qualitative measures of hBOLD CVR studies are reproducible when repeated and reliable between observers.

2) Region of abnormality on hBOLD CVR corresponds with the region of abnormality as demonstrated on CA.

3) Measures of hBOLD CVR are proportional to severity of moyamoya as assessed by the modified Suzuki scale and grade of stenosis.

4) Changes in hBOLD CVR predict changes in clinical outcome.

5) Measures of hBOLD CVR are predictive of neurocognitive outcome in childhood MMD, specifically:

i) abnormal left hemisphere hBOLD CVR measures are associated with reduced verbal IQ

ii) abnormal right hemisphere hBOLD CVR measures are associated with reduced performance IQ

iii) abnormal hBOLD CVR measures are associated with reduction in processing speed

iv) abnormal hBOLD CVR measures are associated with abnormality in indices of executive function

6) Measures of hBOLD CVR are predictive of changes in cortical thickness

7) Chronic non-progressive steno-occlusive arteriopathy does not result in hBOLD CVR abnormality distal to the stenosis

2. Methodology

2.1. Introduction

In this chapter I describe the populations; study terms and definitions; and methodology common to all the studies.

2.2. Population

This was an unfunded study of a convenience sample of prospectively enrolled patients with clinically acquired hBOLD CVR studies. The data were analysed retrospectively. Hence, there was no sample size calculation.

hBOLD CVR became available at The Hospital for Sick Children, Toronto, Canada in 2000, after which the technique was employed in children with moyamoya referred to the Stroke Programme, as well is those returning for follow-up. hBOLD CVR studies were not conducted primarily when i) age of diagnosis and management decisions principally occurred before 1997 ii) revascularization surgery was not considered an option for management iii) consent was not obtained. In the early years of the study revascularization surgery was not considered an option for management for children with sickle cell disease. However, this changed in the latter years.

Children with moyamoya arteriopathy referred to the stroke clinic at The Hospital for Sick Children between August 2008 and December 2010 were prospectively enrolled in the Stroke Research Registry and had hBOLD CVR studies. Moyamoya patients had either unilateral or bilateral moyamoya arteriopathy. In parallel the Stroke Research Registry database was reviewed

to retrospectively identify patients with moyamoya and bilateral non-moyamoya arteriopathy who had hBOLD CVR studies. All symptomatic and asymptomatic children enrolled in Stroke Research Registry database and diagnosed with moyamoya arteriopathy were eligible for inclusion.

I decided to use children with a diagnosis of Transient Cerebral Arteriopathy as a model for unilateral non-moyamoya arteriopathy as it is the commonest unilateral intracranial arteriopathy of childhood. Hence, the brain MRI scans of all children on The Hospital for Sick Children Stroke Registry database with a previous diagnosis of Transient Cerebral Arteriopathy were reviewed. Children with a persistent moderate to severe stenosis (Grade 3 – 4) as defined below were approached for inclusion in the study. hBOLD CVR studies were conducted in those that consented. Clinical and radiographic information was obtained and reviewed in all. Medications at time of enrolment and hBOLD CVR study were noted.

Therefore, children were included according to 2 main arteriopathy groups i) and ii) and 1 sample of convenience iii):

i) Moyamoya arteriopathy – unilateral and bilateral

ii) Unilateral non-moyamoya arteriopathy

iii) Bilateral non-moyamoya arteriopathy

Specifically, studies included patients as follows:

1. Children with moyamoya and non-moyamoya arteriopathy who had hBOLD CVR studies. **(Study 1)**

2. Children with moyamoya arteriopathy who had hBOLD CVR studies and conventional angiograms. **(Study 2)**
3. Children with moyamoya arteriopathy who underwent revascularization surgery and had hBOLD CVR, MRI brain, MR angiography and/or conventional catheter angiogram (CA) studies prior to and following revascularisation surgery. **(Study 3)**
4. Children with moyamoya arteriopathy who had hBOLD CVR studies and neuropsychological assessment using standardized Wechsler scales of intelligence and Parent and Teacher BRIEF scores of executive function at at least one time point. **(Study 4)**
5. Children with moyamoya arteriopathy who had hBOLD CVR studies and no history of stroke at the time of the included hBOLD CVR study. **(Study 5)**
6. Children with unilateral persistent, non-progressive, moderate to severe, steno-occlusive arteriopathy and a history of unilateral stroke confined to the sub-cortical structures **(Study 6)**

2.3. Ethical Approval

Ethical permission was granted by the Institutional Review Board of the Hospital for Sick Children, Toronto, and consent was obtained from all families according to institutional guidelines.

2.4. Arteriopathy Diagnosis

2.4.1. Range of vasculopathies in childhood

2.4.1.1. Moyamoya Disease and Moyamoya Syndrome

Children were diagnosed with moyamoya arteriopathy if conventional angiography demonstrated stenosis or occlusion of the terminal ICA and/or the proximal MCA and/or MCA with the appearance of moyamoya/lenticulostriate collaterals. Children with unilateral moyamoya with collaterals (probable moyamoya) were included in this group (Fukui 1997).

Children without a previously diagnosed condition were diagnosed as having moyamoya disease (MMD) or idiopathic MM. Children with a previously diagnosed condition were diagnosed as having moyamoya syndrome (MMS).

Diseases associated with syndromic diagnosis included:

2.4.1.1.1. Neurofibromatosis Type 1

Children with Neurofibromatosis Type 1 (NF1) diagnosed according to accepted criteria (1988) and attending the Paediatric Neurofibromatosis Clinic at The Hospital for Sick Children, Toronto with MM were enrolled. Moyamoya diagnosis was made following targeted screening MRI and MRA imaging protocols based on the recommendations of the National Neurofibromatosis Foundation Optic Pathway Task Force and the American Academy of Pediatrics Committee on Genetics. Patients with a history of radiotherapy for optic pathway gliomas associated with NF1 were excluded from the group.

2.4.1.1.2. Sickle Cell Disease

Children with *HbSS* disease (SCD) with moyamoya and referred from The Hospital for Sick Children Haemoglobinopathy Clinic were included. *HbSS* diagnosis was confirmed by haemoglobin electrophoresis.

2.4.1.1.3. Trisomy 21 and other Chromosomal

Children referred to The Hospital for Sick Children Stroke Service with a diagnosis of Trisomy 21, Turner Syndrome, and other chromosomal abnormality MMS were included.

2.4.1.1.4. Radiation Vasculopathy

Children with a history of focussed cranial irradiation for treatment of a head and/or neck tumour were included.

A number of asymptomatic children were identified as a result of current vascular imaging surveillance protocols for children with NF-1 (Rea *et al.* 2009) and SCD and were included in the study.

2.4.1.2.Non-moyamoya Vasculopathy

2.4.1.2.1. Transient Cerebral Arteriopathy

Children diagnosed with Transient Cerebral Arteriopathy according to established criteria (Chabrier *et al.* 1998, Sebire 2006) were included as a non-moyamoya, non-progressive steno-occlusive arteriopathy control group. Specifically children with persistent moderate to severe stenosis on

conventional angiography of unilateral pMCA, pACA and/or dICA, plus infarcts limited to unilateral sub-cortical structures with spared cortex were enrolled.

2.4.1.2.2. Bilateral Non-moyamoya Arteriopathy

Children with bilateral non-moyamoya arteriopathies including PHACES syndrome (Posterior fossa malformations, Haemangiomas, Arterial anomalies, Cardiac defects and Coarctation of the Aorta, Eye abnormalities, and Sternal abnormalities or ventral developmental defects), bilateral Takayasu's arteritis and Sickle cell disease with bilateral non-moyamoya arteriopathy were enrolled in the study following referral to The Hospital for Sick Children Stroke Service, and included as a comparative sample of convenience.

2.4.2. Definitions of terms used are as follows:

i) Moyamoya arteriopathy (MM) – this was used to describe all patients with moyamoya arteriopathy

ii) Moyamoya disease (MMD) – this was used to describe patients with idiopathic moyamoya

iii) Moyamoya syndrome (MMS) – this was used to describe all patients with moyamoya in the context of another disease diagnosis eg Sickle Cell Disease (SCD-MM), Neurofibromatosis Type 1 (NF1-MM), a chromosomal disorder (Chromosomal-MM) or radiation induced moyamoya secondary to treatment for a head and/or neck tumour (Tumour-MM).

iv) Stroke (AIS) was defined as presence of a focal neurological deficit (which could be less than 24 hours) with radiological evidence of new focal area(s) of infarction on neuroimaging within a vascular territory

v) Transient ischaemic attack (TIA) was defined as presence of transient focal neurological deficits, lasting less than 24 hours, without radiological evidence of new focal area(s) of infarction on neuroimaging and that are not because of seizure or migraine.

vi) Recurrent ischaemic stroke was defined as a confirmed clinical and radiological ischaemic event that occurs following an initial stroke.

vii) Paediatric Stroke Outcome Measure (PSOM) - the neurological outcome was determined based on the data collected for the Canadian Paediatric Ischaemic Stroke Registry on the standardized Paediatric Stroke Outcome Measure examination, which has been validated for use in children (Kitchen *et al.* 2012). The PSOM represents a structured, classical, pediatric neurological examination containing 115 test items encompassing cognition, language, cranial nerve, motor, sensory, cerebellar, and gait functions. Test items are organized sequentially across development from early infancy to teenage years with a scoring option of “not age-appropriate” for each item. For infants 2 years, primitive reflexes and developmental examination items are included. On completion of the PSOM examination, the neurologist scores a Summary of Impressions containing 5 subscales: right sensorimotor, left sensorimotor (each with subcategories), language production, language comprehension, and cognitive/behavioral.

The PSOM has a five domain scoring system. Each domain can have a maximum of 2 points as follows:

0 = normal with no neurological deficits

1 = mild with no impact on function

2 = moderate with some functional limitation, and severe or profound with absence of function.

A summary of impressions is calculated out of 10 so that the PSOM total score is the sum of the 5 subscale scores. Ten is the worst possible score (maximum deficit) and 0 (no deficit) the best possible score.

2.4.3. Imaging techniques for diagnosis of vasculopathy

2.4.3.1. Brain MRI and 3D TOF MR Angiography

MRI was performed on a 1.5 or 3.0 Tesla scanner (Achieva, Philips, Best, Netherlands). For co-registration with the MRI CVR measures, 25 T1-weighted anatomical images were acquired using a three-dimensional spoiled gradient echo pulse sequence (slice thickness: 5 mm; no inter-slice gap). 25 BOLD MRI CVR images were acquired with a T2*-weighted single-shot gradient echo pulse sequence (field of view: 220 or 230 mm; matrix: 64x64; TR: 2000 ms; TE: 30 ms; flip angle: 85; slice thickness: 5.0 mm; no inter-slice gap; number of temporal frames = 180 or 240).

MRI brain scans conducted at the time closest to moyamoya diagnosis were reviewed by reviewers (GdV and ND) blinded to clinical information. Infarction was categorized as cortical ischaemic (implying a vaso-occlusive mechanism of

injury) or watershed (implying perfusion failure as the mechanism of injury). Watershed infarction was further divided into deep watershed when the ischaemic injury was predominantly within the white matter and cortical watershed when the ischaemic injury was predominantly cortical and at the border zones of the major cerebral arteries. The 'ivy sign' was noted as being present or absent on Axial T2 FLAIR sequences.

3D TOF MRA was conducted in all at the same time as the MRI. Staging and grading of stenosis on MRA was as described below.

2.4.3.2. Conventional catheter angiography

In all cases with MM diagnosis was made following 4 vessel conventional catheter angiography. Four vessel CAs were obtained under GA pre and (where applicable) post-operatively. In addition, those that had surgery had superselective angiography, where a smaller catheter is passed through a larger one into a branch artery supplying a small area of tissue. Lateral internal carotid artery, external carotid artery, superficial temporal artery, and moyamoya arteriopathy angiograms were analyzed in the late arterial phase to assess the relative contributions of the superficial temporal artery and moyamoya arteriopathy to overall revascularization as determined by the external carotid artery injection (King *et al.* 2010).

2.4.4. Staging of Moyamoya Arteriopathy

Moyamoya on CA and MRA was staged using a modified Suzuki (Suzuki and Takaku 1969, Tzika *et al.* 1997) staging scheme (Table 2) by ND and the study interventional radiologist (DA) blinded to the CVR data.

Table 2 Modified angiographic staging of moyamoya disease (Suzuki and Takaku 1969, Tzika *et al.* 1997)

Stage	Angiographic Findings
1	Narrowing of internal carotid artery bifurcation without Collaterals
2	Initiation of moyamoya collaterals
3	Intensification of moyamoya collaterals
4	Minimization of moyamoya collaterals
5	Reduction of moyamoya collaterals
6	Disappearance of moyamoya collaterals

2.4.5. Grading of Moyamoya Arteriopathy

Arterial stenosis on CA and where CA was not available, MRA, was scored according to the degree of vessel narrowing expressed as a percentage ie. 1 = 0-25%; 2 = 26-49%; 3=50-74%; 4=75-100%. If multiple vessels of the COW were involved, the worst vessel was scored and used for the purpose of analysis.

The moyamoya grading and staging system were used as imaging markers of the severity of moyamoya.

2.5. Revascularization Surgery

Clinical factors used to assess suitability for surgery included clinical presentation, MRI findings of ischaemic injury within a vascular territory, deep white matter or watershed gliosis, severity of vascular disease, progression of vascular disease and CVR. Children had a combination of direct and indirect procedures. Direct procedures included external carotid – internal carotid bypass (EC-IC). Indirect procedures included pial and inter-hemispheric synangiosis.

2.6. Cerebrovascular reactivity using a hypercapnic challenge

Three methods of increasing carbon dioxide tension were used, (a) breath-holding and (b) rebreathing in awake children and (c) altering carbon dioxide tension under general anaesthesia using ventilator changes.

2.6.1. Awake: Breath holding and Re-breathe mask

A block design was employed in the different methods described below (Fig 6):

2.6.1.1. Breath-holding (BH)

In a modification of the technique used in adults (Markus and Harrison 1992), children were instructed to hold their breath for as long as they could (usually between 15 and 30 seconds), then breathe for 30 seconds, hold their breath, then breathe for 60 seconds in repetitive cycles during MRI acquisition (Thomas *et al.* 2013).

2.6.1.2. Re-breathe mask (RM)

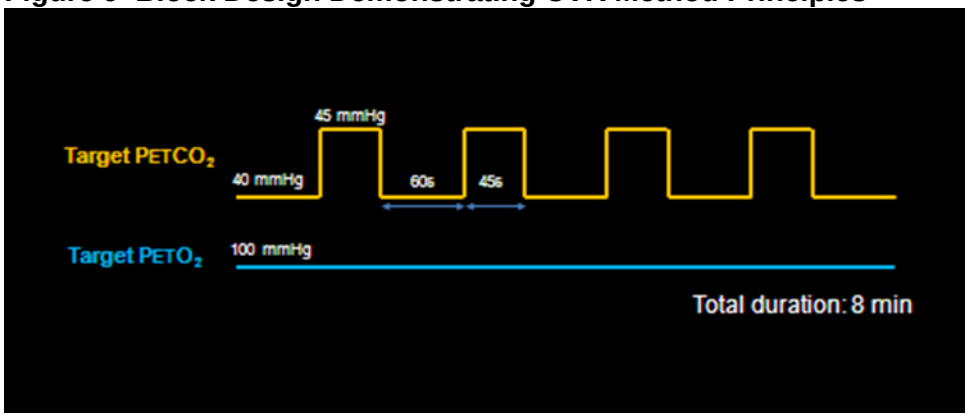
During BOLD-CVR imaging controlled amounts of CO2 mixed with medical air was delivered to subjects via an anaesthetic mask and rebreathing circuit. The circuit was connected to a computer-controlled gas sequencer (RespirAct; Thornhill Research Inc., Toronto, Canada) (Slessarev *et al.* 2007) that regulated both the quantity and composition of gas that was provided to the subject

(Prisman *et al.* 2008). The sequencer utilizes a prospective, feed-forward design that enables precise and independent targeting of partial pressures of end-tidal CO2 (PetCO2) and O2 (PetO2), irrespective of the subject's breathing patterns. Controlled changes to PetCO2 were achieved through a nine minute block sequence run in synchrony with the BOLD-CVR acquisition. The sequence alternated between 60 seconds of normocapnia (target PetCO2 = 40 mmHg) and 60 seconds of hypercapnia (target PetCO2 = 45 mmHg), while subject PetO2 levels were maintained at 100 mmHg. Partial pressures of CO2 and O2 exhaled by the subjects were monitored through sampling tubes in the rebreathing mask. The subsequent PetCO2 and PetO2 values, defined as the sampled pressures at the end of each expired breath, were plotted to generate end-tidal waveforms.

2.6.1.3.General Anaesthetic (GA)

Children were anaesthetized with propofol, intubated and paralyzed. The CO_2 level was increased by decreasing ventilation for 60-120 seconds two or three times during an MRI acquisition time of six to eight minutes. Between the episodes of CO_2 increase, the ventilation was reinstituted at the baseline level that had been found to produce an end expiratory pCO_2 in the range of 35 – 40 mmHg. The baseline epochs were 60-150 sec duration. There was electrocardiogram (ECG), blood pressure (BP), oxygen saturation ($SpO_{2)}$ and carbon dioxide (pCO_2) monitoring throughout the studies.

Figure 9 Block Design Demonstrating CVR Method Principles



The Respiract controls respiration so that end-tidal carbon dioxide ($ETCO_2$) steps between periods at 40 mmHg and at 45 mmHg. End-tidal oxygen (ETO_2) stays at 100 mmHg. Courtesy of Andrea Kassner

2.6.2. hBOLD CVR Mapping

Two different methods were applied depending on the hypercapnic challenge:

2.6.2.1. Breath-holding and general anaesthetic (BH and GA)

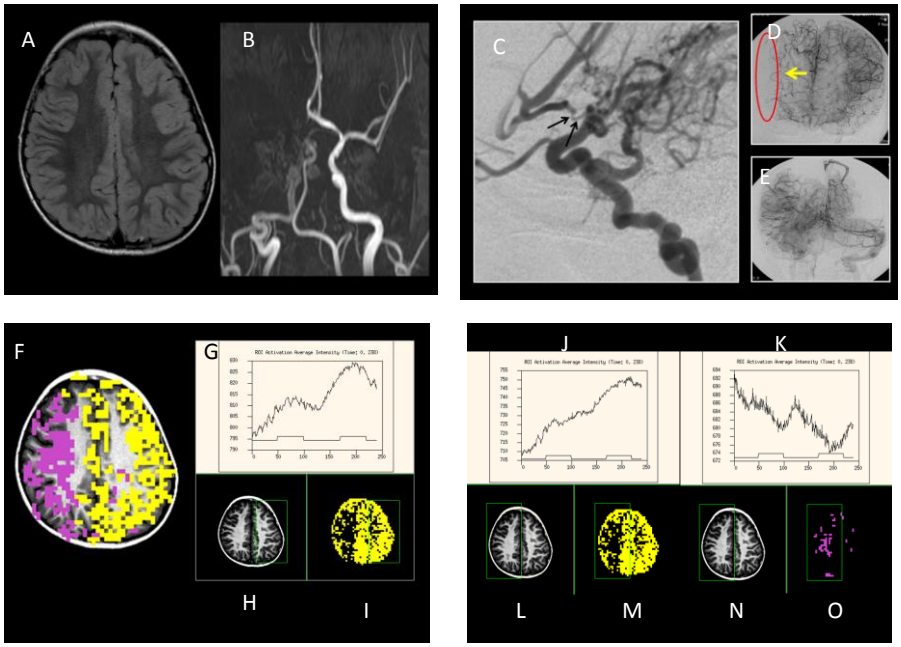
MRI data were analyzed using STIMULATE software. Motion correction, spatial and temporal filtering were performed automatically prior to analysis. Using correlation analysis, a co-efficient of correlation (r value) was calculated for each voxel between the time series data and the square wave reference form. The correlation (r) values above a given threshold were colour-coded. Positive correlation values above the threshold were colour-coded yellow, colourless indicated no correlation (i.e. no change in BOLD signal with changes in CO_2) and purple indicated negative correlation or paradoxical reactivity. A decrease in BOLD signal (purple) represented a redistribution of blood flow away from the corresponding vascular territory during the global vasodilatory stimulus of increased CO_2, thus indicating “vascular steal”. The colour coded BOLD signal

maps were then co-registered with the high resolution anatomical scans to produce the final CVR maps.

To determine the haemaodynamic response curve eight axial brain slices were chosen as regions of interest (ROI) and a corresponding time course of signal intensity plotted (Figure 10).

In neural activation functional MRI the timing of the neuronal activity is often assumed to the timing of the stimulus paradigm because the propagation of the neural signal (<100ms) is often much faster than the repetition time (TR) of the sequence. However, in breath-hold induced hypercapnia it would take 30 seconds for the $PaCO_2$ to change. Furthermore, there is a lag of the BOLD signal behind the change in $PaCO_2$, which is the time taken for blood to travel form the pulmonary vascular system to the heart and then to the brain vessels. Typically this time lag is around 15 seconds. (Yezhuvath *et al.* 2009, Blockley *et al.* 2011). To account for this for each individual child, preliminary analysis was performed by manually drawing a ROI in the posterior fossa (usually spared in MMD) and the BOLD time course within this region correlated with the CO_2 breath-hold/normal respiration epochs. The shift which produced the best BOLD fit curve was used as the comparative normal trace for each child. Not properly accounting for this time delay could result in false-positive or false-negative results leading to incorrect interpretation of CVR. We assumed that the arterial arrival time to the posterior fossa was similar to other brain regions, and any differences accounted for by the temporal resolution used in the study.

Figure 10 An Illustrative Case of hBOLD CVR CVR BOLD Signal Curves and Parametric Maps



A) Normal MRI of 5 year old Chinese girl with headache and paroxysmal episodes of limb paraesthesia. B) MRA showed signal drop-out in the right dICA, MCA and ACA. C) The conventional arteriogram showed right internal carotid artery stenosis with initial avascularity D) arrow and red circle then E) slow filling of the anterior circulation from the posterior circulation. The pre-surgery cerebrovascular reactivity is shown from F) to I). Hypercapnic BOLD CVR was colour coded so that yellow indicates positive (normal) response, and purple indicates abnormal (negative) 'steal'. G) is the hBOLD CVR signal and CO2 map for the left cerebral hemisphere (X axis – time and respiratory course, Y axis – BOLD signal). Inspection of the BOLD signal map I is suggestive of reduced positive pixels i.e. impaired positive CVR in the right hemisphere when compared to the left (unaffected) hemisphere. The linear BOLD MRI signal curves demonstrate a positive normal response for the left hemisphere. J is the linear BOLD MRI signal curve for the right (affected) hemisphere. It still demonstrates positive but attenuated reactivity. O and K Selection of the right hemisphere as the region of interest (ROI) demonstrated more negative pixels on the right than on the left, and an abnormal negative BOLD signal curve consistent with predominantly right sided reduction in positive reactivity and right sided steal.

2.6.2.2. Targeted end-tidal CO_2 (RM)

BOLD-CVR and end-tidal waveform data were transferred onto an independent workstation for further analysis using FSL v4.1.1 (FMRIB Software Library; FMRIB Analysis Group, Oxford, UK) (Smith *et al.* 2004). Images were first corrected for motion and then temporarily filtered to remove signal drift. To adjust for timing discrepancies between the end-tidal and BOLD-CVR data in each subject, the PetCO2 waveform was temporally shifted to maximize signal correlation with the whole-brain average of the BOLD-CVR data. Statistical parametric mapping (SPM) is a statistical tool that uses general linear modelling to analyze or characterize brain function. It allows the expression of a response variable such as the haemodynamic response in terms of a linear sum of explanatory variables or effects in a design matrix (Friston 1998). Using these principles a voxel-wise map of CVR values was computed by performing a least-squares fit of the BOLD-CVR data with the PetCO2 waveform on a voxel-by-voxel basis. The calculated correlation factors were further normalized by the time-averaged voxel signal intensities to express CVR as a percent signal change per mmHg of PetCO2. CVR maps were then co-registered to the high resolution anatomical images to allow for qualitative clinical assessment.

2.6.3. Qualitative and Quantitative Measures of hBOLD-CVR

Two quantitative methods, designed by ND and WL, were used to score the qualitative parametric maps (a) hemispheric pixel ratios (b) scoring by inspection. Infarcts were not excluded from BOLD analysis.

2.6.3.1.Hemispheric Pixels

The first axial slice of the hBOLD CVR parametric map above the ventricles was chosen for the purpose of scoring of the parametric maps containing cortical grey matter only (Thomas *et al.* 2014) However, the 5mm slice thickness of each slice means that it is possible for the parametric maps to have been subject to partial volume effects with each voxel containing several tissue types or substrate, such as CSF or white matter. Each axial slice was scored by the counting of hemispheric pixels at two thresholds. The first threshold was that at which a total of 200 (or as near to 200 as possible) pixels with a positive correlation to the time course were generated in the whole brain. The number of positive and negative pixels at this threshold were counted for each hemisphere and expressed as a ratio:

Number of positive pixels in hemisphere/200 positive pixels =

nP/200Ps and

Number of negative pixels in hemisphere/200 positive pixels =

nN/200P

The second threshold was that at which a total of 200 (or as near to 200 as possible) pixels with a negative correlation to the time course were generated. Not all cases had sufficient negative signal to produce 200 pixels even at the lowest correlation threshold. The number of negative pixels at this threshold were counted for each hemisphere and expressed as a ratio:

Number of negative pixels in hemisphere/200 negative pixels =

nN/200N

where n = number, P = positive pixels, N = negative pixels (Fig 11 and 12)

Figure 11 Example of Hemispheric Pixel Scoring (see text below for explanation)

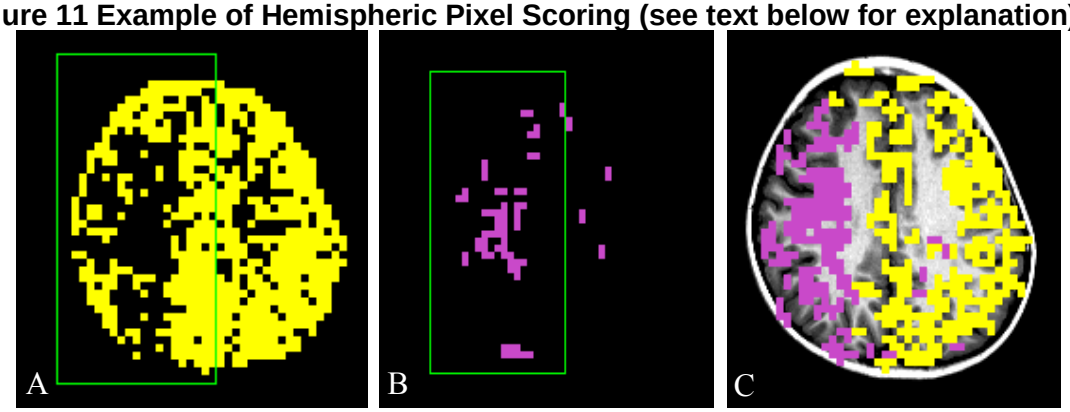
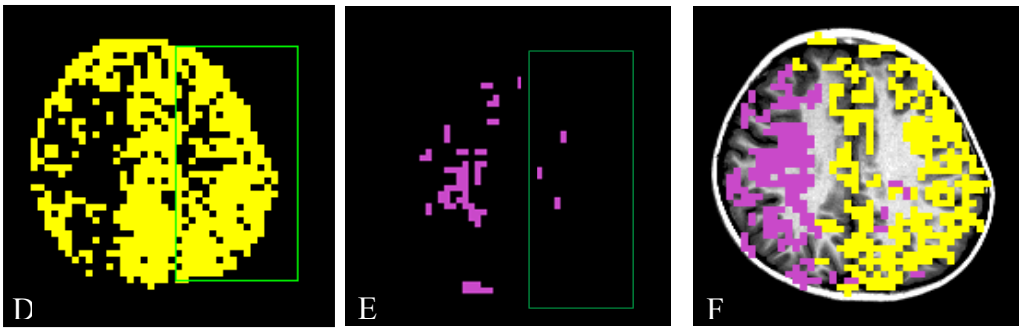


Figure 12 Example of Hemispheric Scoring by Pixels



The threshold was set so as to generate 200 positive pixels in the axial slice above the level of the ventricles:

A) Positive threshold positive pixels parametric map (affected side): the green box represents right hemisphere region of interest (side of moyamoya). Number of positive pixels in right hemisphere = 69.

Therefore nP/200Ps = 69/200 = .35

ie Right positive threshold positive pixel ratio = RPTPP ratio = .35

B) Positive threshold negative pixels parametric map: negative pixels counted at this threshold. The green box represents the same right hemisphere region of interest (side of moyamoya). Number of negative pixels in right hemisphere = 28.

Therefore nN/200Ps = 29/200 = .15

ie. Right positive threshold negative pixel ratio = RPTNP ratio = .15

C) Represents the composite parametric map.

D) Positive threshold positive pixels (unaffected side): Number of positive pixels in left hemisphere = 121.

Therefore nP/200Ps = 121/200 = .65

ie. Left sided positive threshold positive pixels = L PTPP ratio = .65

E) Positive threshold negative pixels (unaffected side): Number of negative pixels in left hemisphere = 3.

Therefore nN/200Ns = 3/200 = L PTNP ratio = .02

F) Represents the composite scoring map (same as C).

Therefore, in the presence of right sided tissue level CVR abnormality, the expected right sided PTPP hemispheric ratio would be less than 0.5, and the

further away from 0.5 toward 0 the greater the right sided CVR impairment. As right and left hemispheric ratio measures were reciprocal calculations (ratios added up to 1), the left sided PTPP hemispheric ratio would deviate from 0.5 toward 1.0. The reverse would apply for more left sided tissue level CVR abnormality i.e. right PTPP greater than 0.5 and left PTPP less than 0.5 (Table 3 and Fig11).

The presence of negative pixels (other than artefact) is indicative of negative reactivity and directly infers tissue level impairment of CVR. Therefore, the expected findings for negative pixels generated at thresholds targeting 200 negative pixels (NTNP) in a child with right sided tissue level CVR abnormality would be a higher NTNP ratio (greater than 0.5) on the right side, and lower on the left. The reverse would apply for more left sided tissue level CVR abnormality ie. right NTNP less than 0.5 and left NTNP greater than 0.5 (Fig 11 and Table 3).

Similar to NTNP, negative pixels generated at thresholds targeting 200 positive pixels ie. PTNP would be higher in the hemisphere with tissue level CVR impairment. However, the hemispheric ratios are not reciprocal measures and therefore would not necessarily add up to one (Fig 7, 8, 9 explanation of pixels)

Table 3 Examples of Hemispheric Pixel Ratios and Laterality of CVR Abnormality

	Laterality of CVR Abnormality			
	Normal	Right	Left	Bilateral
PTPP Right:Left	0.5:0.5	.25:.75	.25:.75	0.5:0.5
PTNP Right:Left	0:0	>0:0	0:>0	>0:>0
NTNP		.75:.25	.75:.25	.5:.5

Right:Left	0:0			

2.6.3.2. Scoring by Inspection

We devised the inspection scoring system in-house. Combined (positive and negative) BOLD parametric maps (A+B=C; D+E=F; C=F)) were generated and scored per hemisphere as follows:

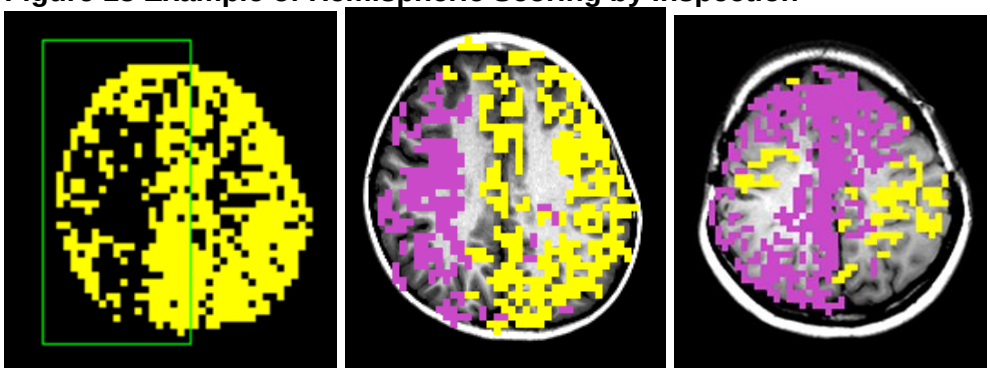
i) Normal CVR = 1

ii) Reduced positive reactivity +/- steal >2%<10% = 2

iii) Steal > 10% with or without reduced positive reactivity = 3

Therefore, the best possible total score would be 2 and the worst possible total score would be 6.

Figure 13 Example of Hemispheric Scoring by Inspection



A) B) C)

Therefore, in Fig 13 example A) the highlighted region of interest inspection score would be 2 (reduced positivity) for the right hemisphere and 1 for the left hemisphere (total score 3) B) the right hemispheric inspection score is 3 and left hemispheric inspection score 1 (total score 4) C) Bilateral hemispheric scores of 3 (total score 6)

2.6.3.2.1. Asymmetry index (AI)

Asymmetry indices (AI) were calculated for positive and negative pixels at positive and negative thresholds using the following formula:

Right hemisphere PTPP – Left hemisphere PTPP/Right hemisphere PTPP + Left hemisphere PTPP ie.

RPTPP – LPTPP/RPTPP+LPTPP

As RPTPP + LPTPP was preset at 200 (or as near to 200 as possible), then

RPTPP ratio – LPTPP ratio = PTPP AI

The same formula was applied for negative pixels at a positive and negative threshold ie. PTNP AI and NTNP AI.

The resulting scores range from -1 to 1 with values indicating the following:

Table 4 Interpretation of Asymmetry Index

	Positive Values (>0≤1) **Interpretation**	Negative Values (>-1<0) **Interpretation**

PTPP	Higher right hemispheric pixels **Better reactivity right hemisphere**	Higher left hemispheric pixels **Better reactivity left hemisphere**
PTNP	Higher right hemispheric pixels **Worse reactivity right hemisphere**	Higher left hemispheric pixels **Worse reactivity left hemisphere**
NTNP	Higher right hemispheric pixels **Worse reactivity right hemisphere**	Higher left hemispheric pixels **Worse reactivity left hemisphere**

2.6.3.2.2. Quantitative Measures of hBOLD CVR

The BOLD MRI signal time course was inspected and Δ BOLD MRI signal per ΔPCO2 and BOLD MRI signal baseline recorded for bilateral hemispheres (or 'whole brain') and right and left hemispheres.

Cerebrovascular reactivity was calculated (where possible) using the following formula:

$$\% \Delta \text{ BOLD MRI signal} / \Delta PCO2$$

2.6.4. Statistical Analysis

Statistical analysis was performed using SPSS version 20. Coefficient of variation was calculated as the ratio of the standard deviation to the mean, expressed as a percentage, to assess repeatability and kappa to assess reliability of method of analysis between observers. When data were normally

distributed T-test was used to compare 2 means and one-way ANOVA was used to compare means for >2 groups with post hoc analysis using Scheffe's test. Mann-Whitney or Kruskal-Wallis tests were used for comparison of non-parametric values. Pearson's correlation coefficient to assess association between parametric variables and Spearman's correlation coefficient for non-parametric variables. The Bonferroni correction was used to correct for multiple comparisons. Linear regression was used to explore associations with continuous variables. The value of $p<.05$ was used to indicate significance and $p<.1\geq.05$ to indicate a trend.

3. Population: Clinical and Radiological Characteristics

3.1. Abstract

3.1.1. Background

Moyamoya arteriopathy is an angiographic description of a steno-occlusive arteriopathy which typically involves the anterior circulation arteries of the circle of Willis. Characteristically the arteriopathy is bilateral and progressive, although unilateral (probable moyamoya) cases are included in the classification.

3.1.2. Objective

To describe and compare the clinical characteristics and radiological findings of children enrolled in the thesis studies with idiopathic moyamoya disease (MMD), moyamoya syndrome (MMS) and non-moyamoya arteriopathy who had hBOLD CVR studies.

3.1.3. Method

There were three study groups. Two main groups of children were enrolled from the stroke programme's database 1) children with moyamoya arteriopathy and representative of a progressive (often bilateral, ocassionally unilateral) arteriopathy and 2) children with Transient cerebral arteriopathy and representative of non-progressive unilateral arteriopathy. A third group of children with bilateral non-moyamoya arteriopathy were enrolled prospectively as sample of convenience for comparison with the other two groups. For all

patients, clinical and radiographic data were collected and hBOLD CVR studies were obtained.

3.1.4. Results

A total of 47 children (Group 1: 37 Bilateral or unilateral Moyamoya arteriopathy, Group 2: 6 Unilateral Non-moyamoya arteriopathy [Transient Cerebral Arteriopathy] and Group 3: 4 Bilateral Non-moyamoya arteriopathy [2 PHACE(S), 1 Takayasu arteritis, 1 Sickle Cell Disease]) were enrolled and had hBOLD CVR studies. The mean age of diagnosis of arteriopathy across all groups was 8.1 years (SD 4.2) (range 7 months - 18 years). Clinical and radiographic features differed across arteriopathy groups. Most presented with acute stroke, however among children with NF1-MM (Unilateral Moyamoya) most were asymptomatic at presentation and diagnosed on screening MRIs (almost 50%) or presented with headache alone. Infarction patterns differed, with deep watershed infarction being the typical pattern in the moyamoya group in contrast to thrombotic vaso-occlusive infarction pattern in the Non-moyamoya groups.

3.1.5. Conclusions

In a single center study I was able to differentiate two sub-groups of arteriopathy representative of the two main mechanisms of brain ischaemia in arterial ischaemic stroke. Specifically these were the moyamoya group as a prototype of progressive arteriopathy causing *chronic hypoperfusion mediated ischaemic injury* and Transient cerebral arteriopathy as a prototype of unilateral non-progressive arteriopathy causing *acute vaso-occlusive ischaemic injury*.

In this study clinical presentation with acute stroke (abrupt focal deficits) alone did not predict stroke aetiology or discriiminate between the moyamoya and non-moyamoya arteriopathy groups. A notable exception to this was the that children with NF1-MM had a more benign and indolent course. Radiographic presentation did differentiate across all three groups, with the imaging signature of deep watershed infarction seen within the moyamoya arteriopathy group. The latter is in keeping with hypoperfusion as the main mechanism of injury in moyamoya proposed in my thesis and supported by the published literature. Current medical treatments to prevent stroke (antithrombotic agents) are aimed at reducing thrombotic vaso-occlusive mechanisms and cannot inadequate adequately reduce the ongoing risk of ischaemic demise in this group. This treatment concern highlights the need to explore tools that can identify children at risk of ischaemia at the tissue level in the context of chronic hypoperfusion as seen in our population, the main motivation for my thesis studies.

3.2. Introduction

Arteriopathy is common in childhood stroke occurring in up to 80% of otherwise healthy children (Danchaivijitr, Cox et al. 2006, Amlie-Lefond, Bernard et al. 2009). It is a predictor of recurrence and poor short-term outcome (Ganesan, Prengler et al. 2003, Amlie-Lefond, Bernard et al. 2009, Goldenberg, Bernard et al. 2009). Arteriopathies can be logically divided into progressive or non-progressive types (Braun, Bulder et al. 2009) (Sebire, Fullerton et al. 2004). Diffuse cerebral vasculitis and moyamoya disease are examples of the progressive childhood arteriopathies (Fung, Thompson et al. 2005, Benseler,

Silverman et al. 2006), whereas TCA is an example of a non-progressive arteriopathy. Efforts continue to improve understanding of the aetiologic nature of the arteriopathies linking them with putative mechanisms including chromosomal and genetic abnormalities and inflammation (infectious and non-infectious (Munot, Crow et al. 2011, Mineyko and Kirton 2013, Wintermark, Hills et al. 2014). However therapeutic strategies are thus far inadequate at preventing the adverse outcomes associated with both progressive and non-progressive arteriopathy groups. The progressive arteriopathy group in particular, has an exceedingly high rate of recurrent stroke and poor neurological outcomes.(Amlie-Lefond, Bernard et al. 2009, Braun, Bulder et al. 2009).

Within the moyamoya arteriopathy group there appear to be subgroups with a different nature of and natural history of moyamoya disease (Fung, Thompson et al. 2005, Benseler, Silverman et al. 2006, Braun, Bulder et al. 2009). Children with progressive bilateral moyamoya with recurrent ischaemic events are more likely to be of South East Asian origin. Experience in our institution and published data suggests that children with Neurofibromatosis Type 1 moyamoya are more likely to have a unilateral moyamoya arteriopathy with a more indolent course and are frequently of Caucasian origin, and typically (Cairns and North 2008, Rea, Brandsema et al. 2009, Lin, Baird et al. 2011, Duat-Rodriguez, Carceller et al. 2014). Children with Sickle Cell Disease and moyamoya are more likely to be of African and/or Caribbean origin and they, similar to children with certain chromosomal syndromes such as Downs syndrome, tend to have a more typical bilateral (moyamoya disease-like) progressive arteriopathy with recurrent ischaemic events (Dobson, Holden et al.

2002, Jea, Smith et al. 2005, Pysden, Fallon et al. 2010, See, Ropper et al. 2015). Another sub-group are children with infantile moyamoya, who appear to have a very aggressive form of the disease, with fulminant recurrent large ischaemic strokes (Jackson, Lin et al. 2014, Al-Yassin, Saunders et al. 2015). More severe subgroups may warrant earlier surgical intervention. However, in determining the appropriateness and timing of surgical intervention multiple additional factors are relevant beyond clinical presentation, whether aggressive or benign. These include case-by-case consideration of increased peri-operative risk associated with disease severity, technical aspects of vascular surgery and anaesthetic risk (Soriano, Sethna et al. 1993, Imaizumi, Hayashi et al. 1998, Kim, Seol et al. 2004, Heyer, Dowling et al. 2008). Improved understanding of the natural history, clinical and radiological features of the moyamoya arteriopathies will provide further insight into the mechanisms of injury and allow for improved therapeutic strategies (Braun, Rafay et al. 2007, Mineyko and Kirton 2013, Rafay, Armstrong et al. 2015). Beyond these factors, there is a need for a valid and reliable method of measuring ischaemic brain risk in individual children with moyamoya to provide relevant and specific data to guide selection for revascularization surgery. The hBold-CVR method warrants consideration as such a method, as studied in this thesis.

In this chapter I describe the frequency, clinical and radiological characteristics of our study population which includes children with a progressive steno-occlusive arteriopathy: moyamoya arteriopathy (idiopathic and syndromic moyamoya) and non-progressive arteriopathy controls: non-moyamoya arteriopathy (TCA, PHACE(S), Takayasu arteritis and SCD). I do this to demonstrate the similarities and differences between these sub-groups

highlighting the complexity in managing children with arteriopathy for whom the most frequently asked question by parent and medical professional alike is, '"What is my/this child's risk of a stroke?". The assessment of CVR provides an opportunity to answer this question for these individual children.

3.3. Population Ascertainment

The study population is a combination of prospectively enrolled patients and a retrospective sample of patients with clinically acquired hBOLD CVR studies as outlined in Figure 10.

Between August 2008 and December 2010, 17 children with moyamoya arteriopathy (idiopathic and syndromic) referred to the stroke clinic at The Hospital for Sick Children had clinical hBOLD CVR studies. Consent was not obtained in four; hence 13 were consecutively enrolled in the Stroke Registry database.

Retrospective review of children on the Stroke Registry database prior to August 2008 (with children enrolled from 1992 – 2008) identified 73 children with a recorded diagnosis of moyamoya arteriopathy. Following further review of clinical and imaging data, the arteriopathy was confirmed to be moyamoya in 63 children. hBOLD CVR studies were not conducted primarily when i) age of diagnosis and management decisions principally occurred before 1997 ii) revascularization surgery was not considered an option for management iii) consent was not obtained. Consequently, of the children with moyamoya arteriopathy on the Stroke Registry database from 1992 to 2008, thirty-three (median age 6.5, range 1.17-17.5 years) did not have hBOLD CVR studies for

reasons listed above. hBOLD CVR studies were hence available in 30 children with moyamoya arteriopathy. Three of these studies were not analysable due to technical difficulties and consent was not obtained in three. Therefore, following retrospective review of the Stroke Registry database hBOLD CVR studies in 24 children with moyamoya arteriopathy were available for analysis.

Four children with Bilateral Non-moyamoya arteriopathy (Bilateral Non-MM) were retrospectively identified as having hBOLD CVR studies and included as a sample of convenience for comparison.

We also conducted prospective hBOLD CVR studies on six children with a diagnosis of Transient Cerebral Arteriopathy that were previously enrolled in the Stroke Registry database. To select these children for hBOLD CVR studies, we screened 45 children with TCA reviewing MRI brain, MR angiography and conventional angiograms to identify children with 1) confirmed TCA 2) moderate to severe (Grade 3 – 4) stenosis of the MCA +/- ACA and 3) infarction sparing the cortex (because hBOLD CVR is non-informative in zones of infarction). Eleven children were approached for inclusion and 6 consented to undergo hBOLD CVR studies. These 6 were included as non-progressive, Unilateral Non-moyamoya arteriopathy (TCA, Unilateral Non-MM) controls.

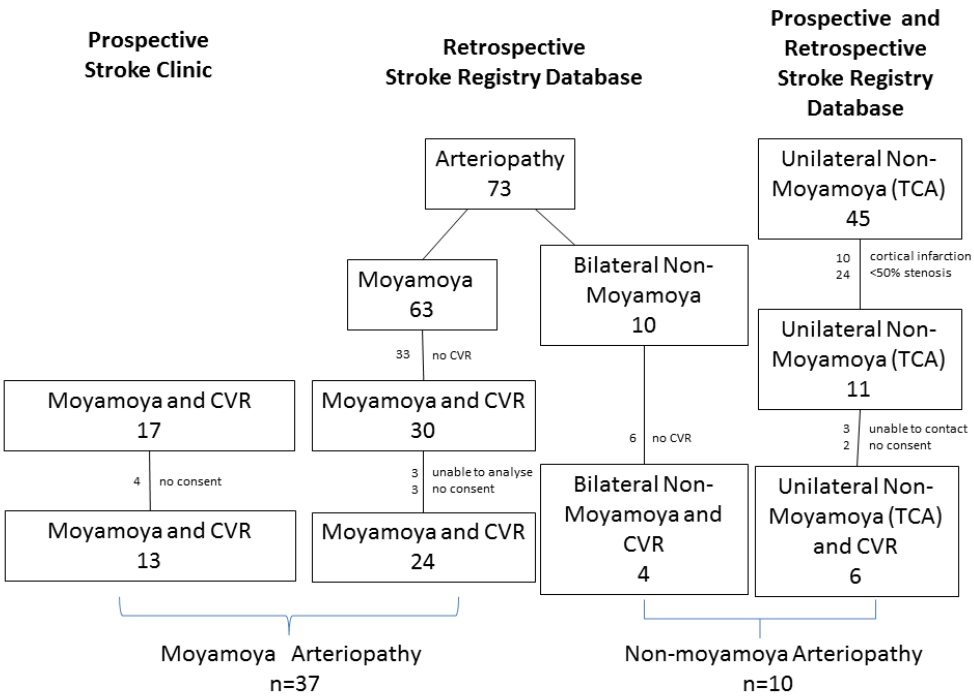
In summary 37 children with moyamoya arteriopathy (21 female, 16 male) had initial or follow-up hBOLD CVR studies (109 total studies, 54 under General Anaesthesia (GA); 49 Breath-hold (BH); 6 Re-breathe Mask (RM)) between November 2000 and December 2010 are reported and included in the study.

In addition, ten children were included as either Bilateral (4) or Unilateral (6) Non-MM controls. These 10 children (5 female, 5 male) had hBOLD CVR studies (3 under GA; 1 BH and 6 RM) which are also reported (Figure 11).

3.4. Statistical Analysis

One way ANOVA and the T-test were used to compare means when the data were distributed normally, and Kruskal-Wallis and Mann-Whitney U test used to compare medians when the data were not normally distributed. When some data, e.g. pixel counts, were distributed both normally and non-normally in different analyses, parametric and non-parametric statistics are reported. Categorical data were analysed using X^2 or, if there were <5 in a cell, with Fisher's exact test. A significance level of $p<.05$ was accepted.

Figure 14 Flow Diagram of Study Population



3.5. Results

3.5.1. Group Description

The total number of children with arteriopathy was 47 (25 male). Thirty seven had moyamoya arteriopathy (16 male, 21 females, 13 prospective and 24 retrospective). The proportional distribution of all arteriopathies (non-moyamoya and moyamoya) and moyamoya arteriopathies (idiopathic and syndromic) are presented in Figure 15 and Figure 16 respectively. The largest arteriopathy group was of those with idiopathic moyamoya (MMD) (n=13) followed by Neurofibromatosis type 1 (NF1-MM) (n=9) and sickle cell disease (SCD-MM) (n=5) moyamoya.

Figure 15 Pie Chart of Arteriopathies (non-moyamoya and moyamoya)

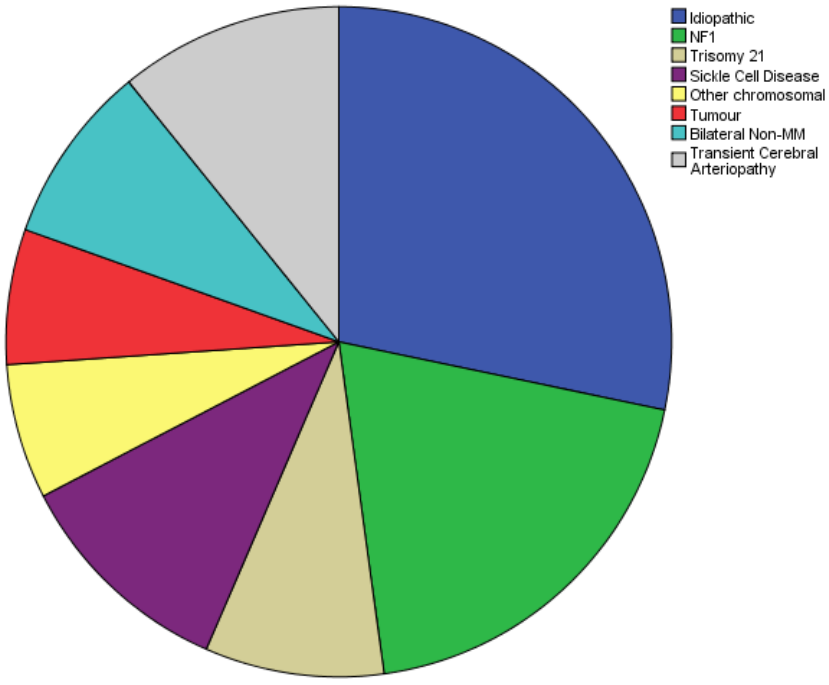
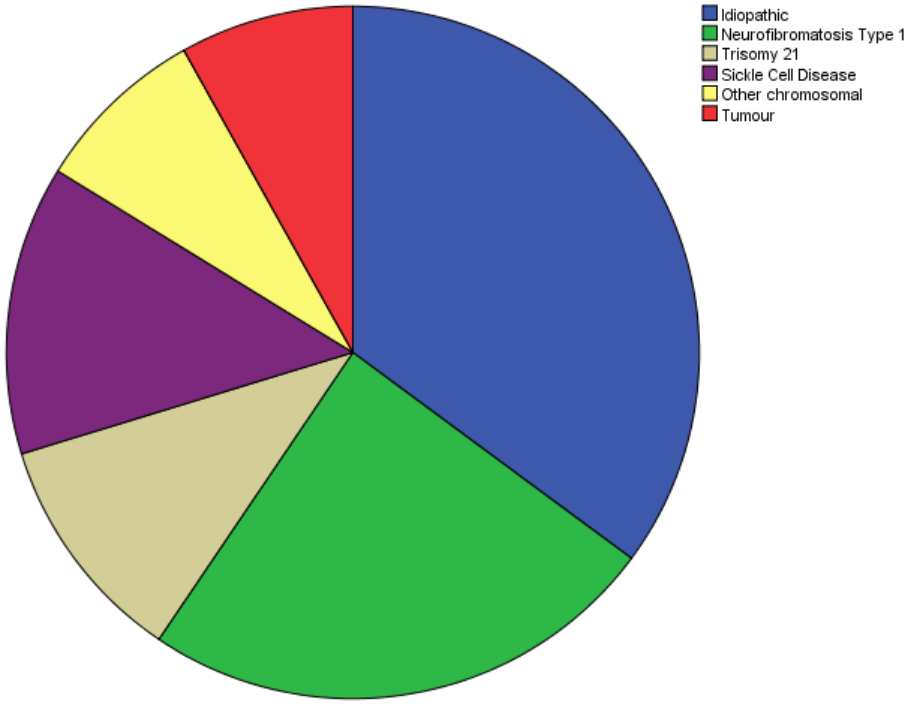


Figure 16 Pie chart of Idiopathic and Syndromic Moyamoyas



3.5.2. Clinical and Radiological Description of All Arteriopathy Groups

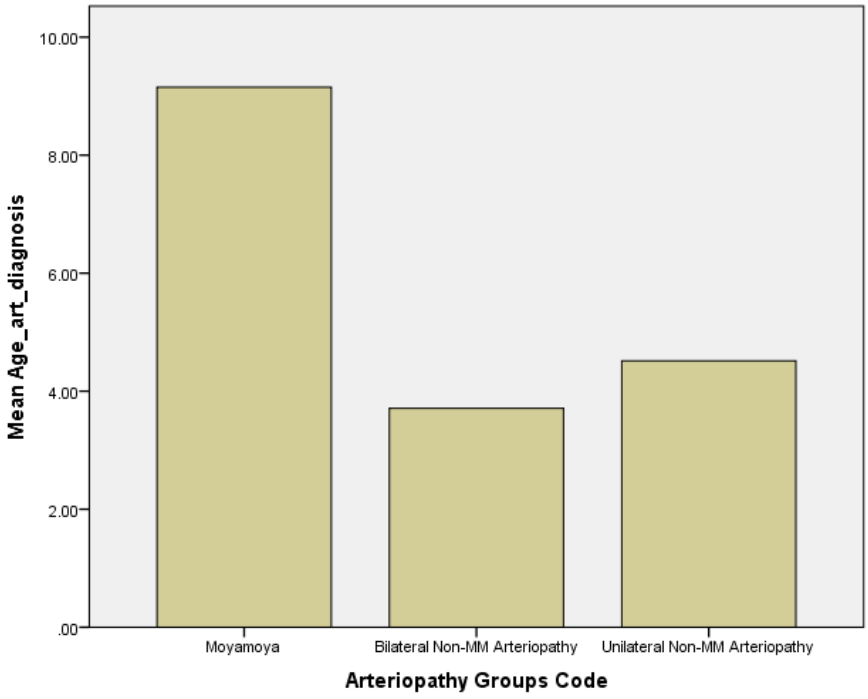
Table 4 describes the clinical features of the Moyamoya, Bilateral Non-MM and Unilateral Non-MM Arteriopathy Groups at time of arteriopathy diagnosis.

3.5.2.1. Age at Arteriopathy Diagnosis

The mean age at arteriopathy diagnosis (moyamoya and non-moyamoya) was 8.1 years (SD 4.2). The mean age of diagnoses was 8.6, 3.7 and 4.5 years for the Moyamoya, Bilateral Non-MM and Unilateral Non-MM Arteriopathy groups respectively. The Kruskal-Wallis test demonstrated a statistically significant difference in age of arteriopathy diagnosis between the Moyamoya, Bilateral Non-MM and Unilateral Non-MM arteriopathy groups $\chi^2(2) = 11.6$, $p = 0.003$. Post-hoc tests by Mann-Whitney U test (chosen because the group sizes were disparate) revealed that the mean age of diagnosis of arteriopathy was

statistically higher in the Moyamoya arteriopathy group compared to the Bilateral Non-MM arteriopathy *U*=22, p = 0.02 and Unilateral Non-MM arteriopathy groups *U*=39.5, p=.012. There was no statistically significant difference in age of arteriopathy diagnosis between the Bilateral and Unilateral Non-MM arteriopathy groups *U*=10.5, p=.75. (Table 4)

Figure 17 Mean Age of Diagnosis by Arteriopathy



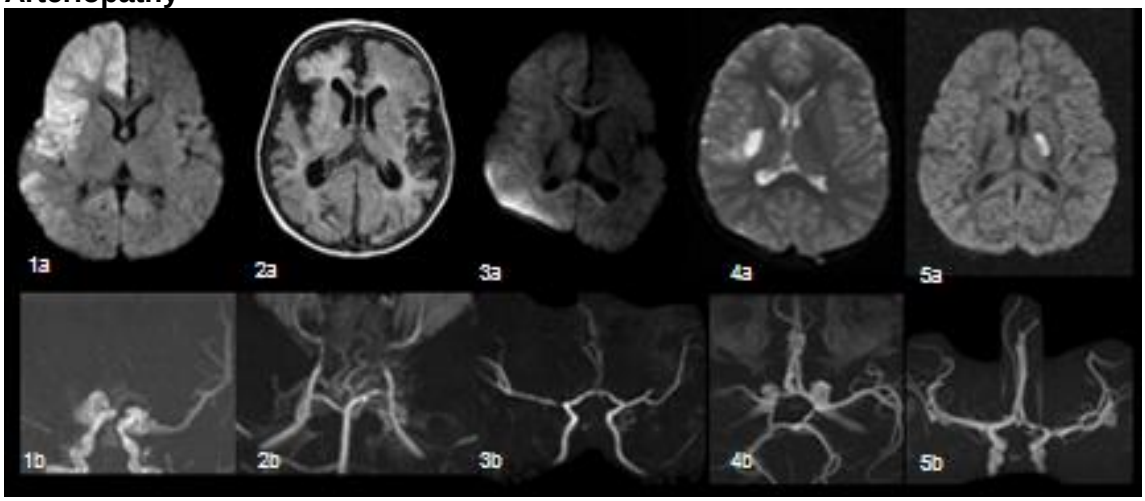
3.5.2.1.1. Infantile arteriopathy

Out of 47 children, five (1 Moyamoya disease, 1 SCD-MM, 1 Takayasu arteritis and 2 TCA) had a diagnosis under the age of two years. The youngest child aged two months, had a diagnosis of Takayasu arteritis (Bilateral Non-moyamoya). The earliest age of diagnosis in the moyamoya arteriopathy group was seven months.

Both of the very young children with MM arteriopathy presented with seizures. All the very young children with non-moyamoya arteriopathy presented with a

focal hemiparesis (Fig 18). All received anti-coagulation, one (Moyamoya disease) had revascularisation surgery, one (SCD moyamoya syndrome) a bone marrow transplant and one immunosuppressive therapy.

Figure 18 Parenchymal and Vascular Imaging of Children with Infantile Arteriopathy



Figures show Axial MRI DWI (1a, 3a, 4a and 5a) and FLAIR sequences (2a) in first row, and 3D TOF MRA in the second row (1b-5b). Figs 1and 2: Bilateral MM arteriopathy; Fig 3: Bilateral Non-MM arteriopathy (Takayasu arteritis); Figs 4 and 5: Unilateral Non-MM arteriopathy (Transient Cerebral Arteriopathy).

3.5.2.2. Clinical Presentation

Clinical records were reviewed and data collected on clinical status at time of diagnosis of arteriopathy.

3.5.2.2.1. Moyamoya arteriopathy

Twelve of the thirty-seven children (32%) presented with acute stroke (10 ischaemic, 2 haemorrhagic); 8/37 TIA; 4/37 seizures; 12/37 headache; and 2/37 other paroxysmal sensory symptoms at the time of moyamoya diagnosis. Six (16%) were asymptomatic at the time of diagnosis.

3.5.2.2.2. Bilateral Non-MM arteriopathy

Of the 4 children with Bilateral Non-MM arteriopathy, one child presented with a stroke and seizures, one with headache and two were asymptomatic at time of diagnosis of arteriopathy. Diagnosis in the asymptomatic children (one SCD, one PHACEs) was made following disease specific MRI brain screening protocols.

3.5.2.2.3. Unilateral Non-MM arteriopathy

All (6/6) of the children with Unilateral Non-MM arteriopathy presented with acute arterial ischaemic stroke. In addition, one had seizures and another headache at the time of presentation.

3.5.2.3. Arteriopathy Laterality

Arteriopathy was mostly left sided in the children with Unilateral Non-MM arteriopathy (4/6 left sided, 2/6 right sided); bilateral (23/37) in the children with Moyamoya arteriopathy and (by definition) bilateral in all (4/4) the Bilateral Non-MM arteriopathy group.

3.5.2.4. Radiographic Findings

3.5.2.4.1. Moyamoya arteriopathy group

Fourteen of the thirty-seven children had a pattern suggestive of watershed and cortical infarction, 12 watershed only, 3 cortical only and 7 not ischaemic (4 NF1 lesions, 3 normal). One child had hemiatrophy of the cerebellum. In total

twenty six of the 37 children (70%) had watershed infarcts (21 deep watershed, 4 deep and cortical watershed, 1 cortical watershed).

3.5.2.4.2. Bilateral Non-MM arteriopathy group

Two of the four children had a cortical vaso-occlusive pattern of infarcts (one PHACE(S) and one Takayasu's disease); one deep white matter watershed ischaemia (SCD) and one had no radiological evidence of ischaemia, but a dysplastic and hypoplastic cerebellum (PHACE(S)).

3.5.2.4.3. Unilateral Non-MM arteriopathy

Five of the six of the children had a pattern of middle cerebral artery perforator territory ischaemia, with sparing of the cortex. One child had a normal brain MRI scan.

3.5.2.5. Grade of Stenosis

Conventional angiograms (available in 34 with Moyamoya arteriopathy, all with Unilateral Non-MM and two with Bilateral Non-MM) one CT angiogram (Bilateral Non-MM) and four 3D TOF MR angiograms (3 with Moyamoya arteriopathy, 1 with Bilateral Non-MM) were reviewed and vessels graded from 0-4 according to the degree of stenosis by myself (ND) and the study radiologist (DA)(Ref Methods, Grading of Stenosis).

3.5.2.5.1. Moyamoya arteriopathy

Grade of stenosis was moderate to severe (Grade 3-4) in all 37.

3.5.2.5.2. Bilateral Non-MM arteriopathy

Two children (one PHACE(S), one Takayasu's disease) had Grade 4 stenosis on conventional angiography. One child with SCD had tortuosity of the vessels of the circle of Willis and a hypoplastic anterior cerebral artery on MR angiography. One child with PHACE(S) had bilateral agenesis of the internal carotid arteries on CT angiography. Arteriopathy was hence graded as 4.

3.5.2.5.3. Unilateral Non-MM arteriopathy

Three of the six children had severe stenosis (Grade 4), one moderate (Grade 3), one mild (Grade 1) and one no stenosis (Grade 1).

3.5.2.5.4. Medication Use

The majority of the children were on an anti-platelet agent: 38/47 Aspirin and 3/47 Clopidogrel. Other moyamoya related medications included Acetazolamide, Carbamazepine for seizures and Topiramate for seizures or headache.

Table 5 Population Demographics *Recoded as Idiopathic moyamoya ** Recoded as Other Chromosomal moyamoya
Two following radiation for rhabdomyosarcoma and a suprasellar germinoma. One hypothalamic hamartoma

Clinical Information	Moyamoya with CVR (37)	Bilateral non moyamoya arteriopathy (4)	Unilateral focal arteriopathy (6)	No consent (10)	Not found (3)	Not done (33)
Age diagnosis years (median, range)	8.61 (0.6-16.7)	3.7 (.17-8.25)	4.51 (0.54-9.7)	9.12 (0.9-17.0)	15 (14.1-16.2)	6.5 (1.17-17.5)
Age first CVR years (median, range)	10.76 (1.08-17.7)	6.0 (4.0-8.78)	15.1 (7.44-27.9)	11.0 (1.9-18.0)	16.0 (15.5-16.7)	-
Male	16 (43%)	0	5 (83%)	4 (40%)	0	10 (33%)
Co-morbidities						
-Trisomy 21	4				1	
-Neurofibromatosis I	9			2		1
-Hypomelanosis of Ito						1
-Turner's	1**					
-β-thalassaemia				1		
-Sickle cell disease	5	1		1	1	5
-Tumour	3#				1	1
-Schimke's						2
-Chromosomal	2**					1
-Biliary atresia						1
-Epilepsy	1*					
-Hyperprolactinaemia	1*					
-Factor IX deficiency	1*					
-Takayashu arteritis		1				

	Moyamoya with CVR (37)	Bilateral non moyamoya arteriopathy (4)	Unilateral focal arteriopathy (6)	No consent (10)	Not found (3)	Not done (33)
Treatment						
-PHACES		2				
-Familial	2*					
-Idiopathic	8*	0	6	6	0	21
Treatment						
Aspirin	28	4	6			
Clopidogrel	3					
Acetazolamide	2					
Carbamazepine	4	1				
Topiramate	1					
Other						
Blood Transfusion	5	1				
Penicillin V	5					
Enalapril	1					
DDAVP	1					
Thyroxine	1					
Vasotec	1					
Propranolol		1				
Radiotherapy	1					
Chemotherapy	1					
		1				
Immunosuppression		1				
Clobazam						

Table 6 Clinical and Radiographic Description of All Arteriopathy Groups

	Moyamoya arteriopathy n=37 (%)	Bilateral Non-MM arteriopathy n= 4 (%)	Unilateral Non-MM arteriopathy n=6 (%)
Clinical Presentation			
Yes (%)			
Stroke	12 (32)	*1 (25)	5 (83)
(Yes:No)			
TIA	8 (22)	0 (0)	1 (17)
Seizures	4 (11)	*1 (25)	1 (17)
Headache	12 (32)	1 (25)	0
Other	2 (5)	0	0
Asymptomatic	6 (16)	**2 (50)	0
Arteriopathy Laterality			
Bilateral	23 (62)	4 (0)	0
Right	6 (16)	0 (0)	2 (33)
Left	8 (22)	0 (0)	4 (66)
Radiographic Findings (Y:N)			
Not ischaemic	7	1	1
Cortical	3	2	5†
Watershed	12	1	0
(Deep white matter)	(12)		
(Cortical)	(1)		
Cortical ischaemic and Watershed	14	0	0
IVY	26:7 (79:21)	0	0
Vascular Findings			
Grade of Stenosis			
1	0	1	2
2	0	0	1
3	4	0	0
4	30	3	3
CVR Method	18 GA 17 BH 2RM	3 GA 1 BH	6 RM

* same patient
** SCD patient – MRI pick up because of raised TCD
** PHACES patient – MRI pick up on screening
† sub-cortical and/or deep grey matter
GA General anaesthetic BH Breath hold RM Re-breathing Mask

3.5.2.6.Ethnicity

Of the 47 children the majority were Caucasian (53.2%) followed by South East Asian (19.1%) (Table 7, Figure 18). Similar proportions remained in the moyamoya arteriopathy group (n=37) (Table 8, Figure 19). Children of South East Asian origin were of Japanese, Chinese and/or Korean parentage. Children of South Asian origin were of Indian sub-continent origin.

Table 7 Ethnicity Frequencies in All arteriopathy (n=47)

Ethnicity	Frequency	Percent
Caucasian	25	53.2
South East Asian	9	19.1
Black	6	12.8
South Asian	4	8.5
Middle Eastern	2	4.3
Caucasian/ South East Asian	1	2.1

Figure 19 Ethnicity Frequency Pie Chart in All Arteriopathy

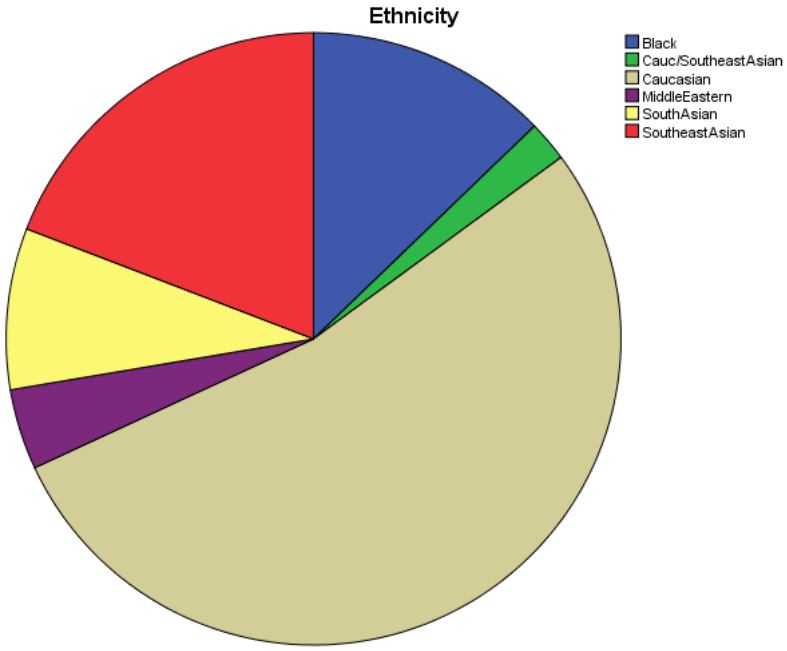


Table 8 Ethnicity Frequencies in Moyamoya Group (n=37)

Ethnicity	Frequency	Percent
Caucasian	17	45.9
South East Asian	8	21.6
Black	5	13.5
South Asian	4	10.8
Middle Eastern	2	5.4
Caucasian/ South East Asian	1	2.7

Figure 20 Ethnicity Frequency Pie Chart in Moyamoya Arteriopathy Group

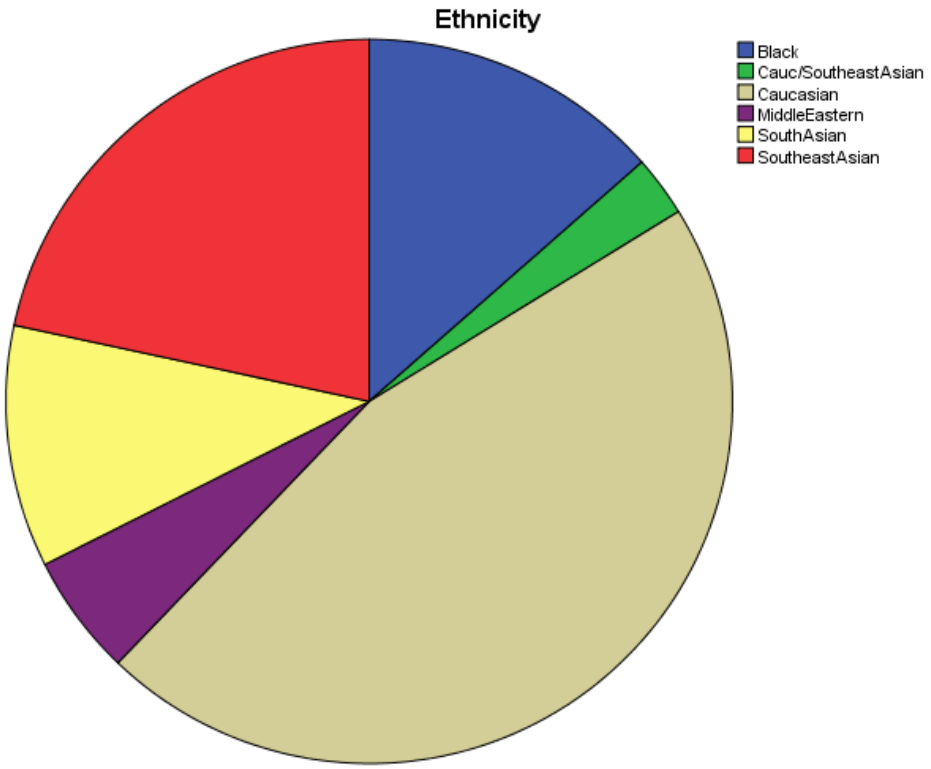
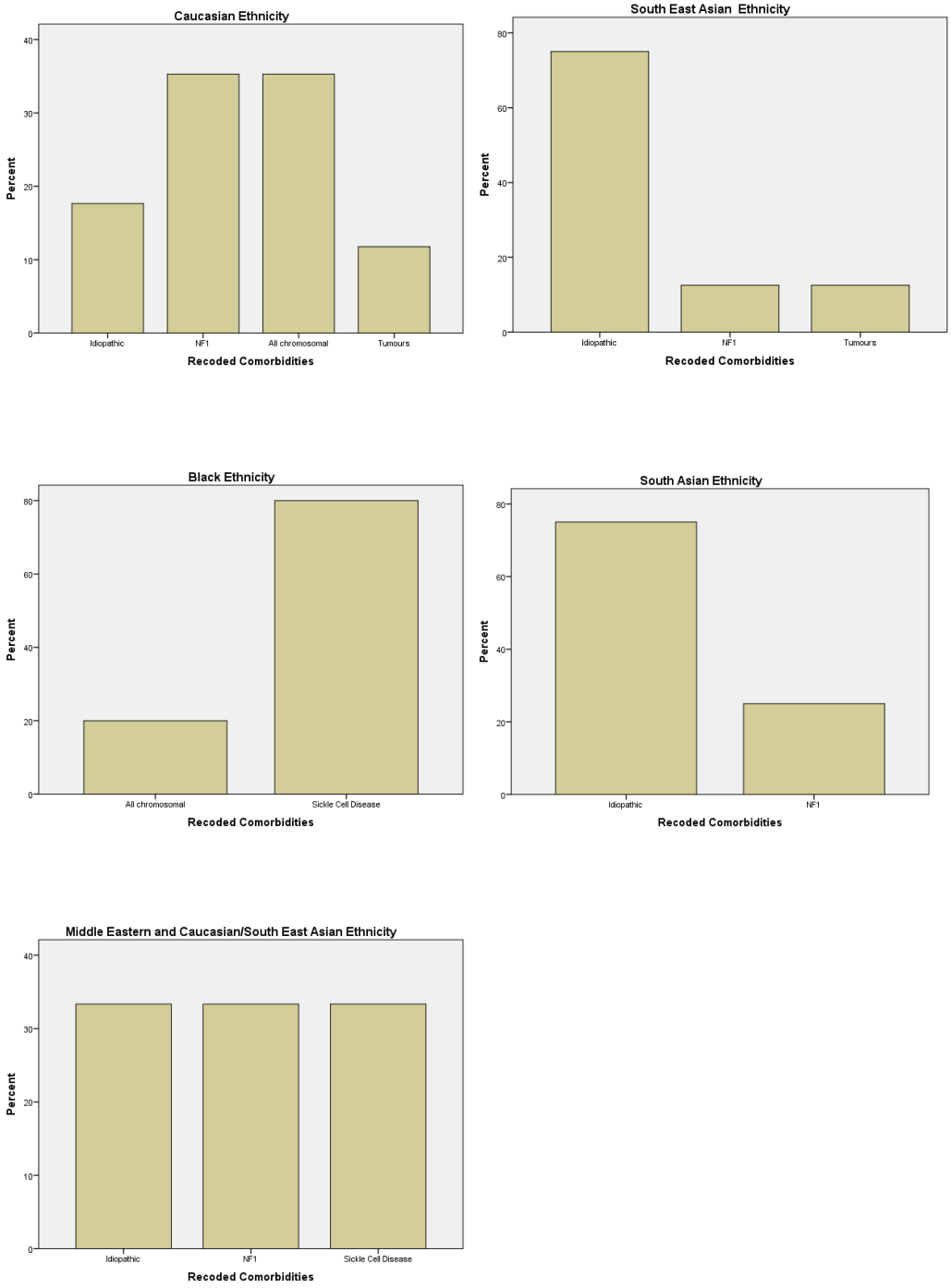


Table 9 Moyamoya Classification by Ethnicity

	Moyamoya Classification					
Ethnicity	Idiopathic N=13	Neurofibromatosis Type 1 N=9	Sickle Cell Disease N=5	All Chromosomal N=7	Tumours N=3	Total
Caucasian	3	6	-	6	2	17
South East Asian	6	1	-	-	1	8
Black	-	-	4	1	-	5
South Asian	3	1	-	-	-	4
Middle Eastern and Caucasian/South East Asian	1	1	1	-	-	3
Total	13	9	5	7	3	37

Figure 21 Moyamoya Classification by Ethnicity



3.5.3. Clinical and Radiological Description Moyamoya Arteriopathy Group

3.5.3.1. Moyamoya Classification

Moyamoya arteriopathy was classified as idiopathic (MMD) or syndromic (MMS). The syndromic groups were further divided by comorbidity. Initial syndromic categories were Neurofibromatosis Type 1 (NF1-MM), Sickle Cell Disease (SCD-MM), Trisomy 21 (Trisomy 21-MM), Other chromosomal (Other Chromosomal-MM) and Tumours (Tumour-MM). Thirteen children had MMD, two (out of two tested) within the same family had confirmed *RNF213* mutation analysis. Nine children had NF1-MM, five SCD-MM, four Trisomy 21-MM, three other chromosomal abnormalities and three Tumour related MMS (Tumour-MMS). Due to small numbers the Trisomy 21 and Other Chromosomal categories were collapsed into one All Chromosomal group (All Chromosomal-MM) (Table 9).

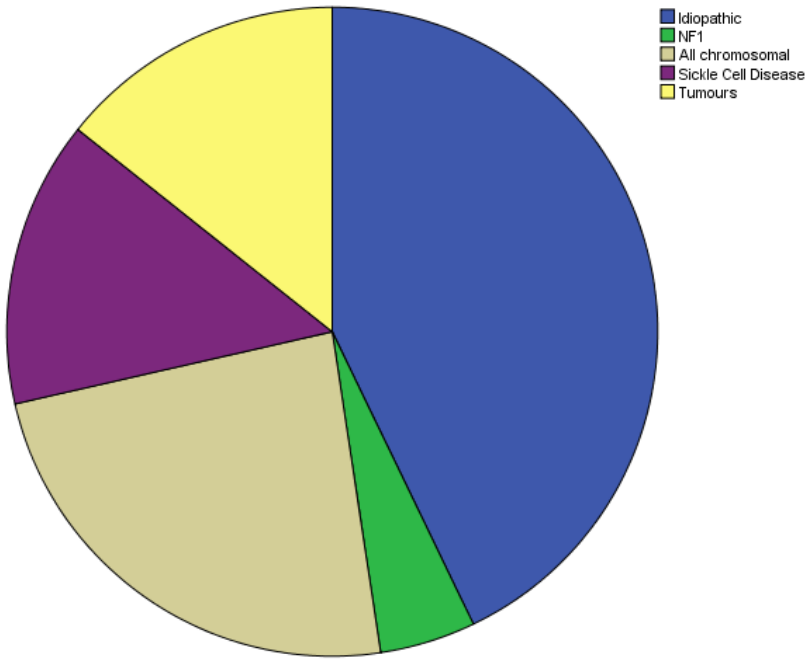
3.5.3.2. Age at Moyamoya (idiopathic and syndromic) Diagnosis

The mean age of MM diagnosis was 9.1 (SD 3.9) years. There was no statistically significant difference in age of MM diagnosis between the MM arteriopathy idiopathic and syndromic groups $\chi^2(4) = 4$, $p > 0.05$ (Table 9).

3.5.3.3. Clinical Presentation

Twenty-three of 37 (62%) children had an arterial ischaemic stroke at at least one time point (12 acute at time of diagnosis, 3 remote at time of diagnosis, 8 new [7/8 recurrent]; 7 male; 11 right, 7 left and 5 bilateral; mean age at stroke 8.3; SD 4.7; range 0.58 – 16.58) years. Ten of the 23 (43%) had Moyamoya Disease and the rest Moyamoya Syndrome (5/23 [22%] All Chromosomal; 3/23 [13%] Sickle Cell Disease; 3 [13%] Tumour; and 2 [9%] Neurofibromatosis Type 1 (Table 9 and Figure 21).

Figure 22 Frequency of Comorbidities in Stroke Group (n=23)



Clinical presentation was examined by ethnicity and moyamoya classification. Ethnicity groups were collapsed into Caucasian, Black and Asian (10) which

included South East Asian, South Asian, Middle Eastern and Caucasian/South East Asian.

Figure 23 Clinical Presentation within Moyamoya Groups by Ethnicity

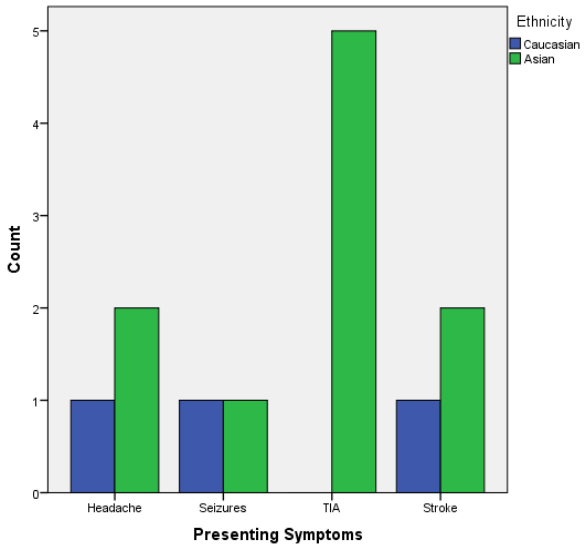


Table 10 Clinical and Radiological Characteristics of Moyamoya Arteriopathy Groups

	Moyamoya Arteriopathy n=37					
	Idiopathic n=13	NF-1 n=9	SCD n=5	Trisomy 21 n=4	Other Chromosomal n=3	Tumour n=3
Female: Male	7:6	3:6	3:2	4:0	2:1	2:1
Mean Age at MM Diagnosis	8.2	9.4	7.8	11.8	8.4	12.2
MM Laterality						
Bilateral	10	2	5	3	1	3
Right	2	2	0	1	1	-
Left	1	5	0	0	1	-
Clinical Presentation						
Stroke	3	1	3	4	1	0
Laterality						
Bilateral	1	0	1	0	0	0
Right	0	0	1	4	1	0
Left	2	1	1	0	0	0
TIA	5	1	0	0	0	2
Headache	3	3	1	0	1	1

Seizures Other Asymptomatic	2† 2 ^ 1	0 0 4	0 0 1	0 0 0	0 0 1	0 0 0
	Moyamoya Arteriopathy n=37					
	Idiopathic n=13	NF-1 n=9	SCD n=5	Trisomy 21 n=4	Other Chromosomal n=3	Tumour n=3
Course						
Surgery	11	7	0 *	3	2	2
Recurrent stroke	2	1 (new)	1	0	0	1
Recurrent TIA	3	0	0	0	0	0
PSOM	1.4	1.1	.75	2.8	3	2
Conventional Angiography						
Vessels						
Stage						
1	0	0	1	1	-	0
2	3	4	1	2	2	0
3	9	3	1	1	-	2
4	1	1	-	0	-	0
5	0	1	-	0	-	0
6	0	0	-	0	-	1
Grade						
3	1	1	0	2	-	-
4	12	8	3	2	2	3
MRI						
Not ischaemic	2	5	0	0	0	0
Watershed	3	2	3	1	1	2
Deep	(3)	(4)	(4)	(4)	(2)	(3)
Cortical	(3)	(0)	(2)	(0)	(0)	(0)
Cortical	1	0	1	0	1	0
ischaemic	6	2	1	3	1	1
Watershed and cortical	11	7	2	4	1	2
ischaemic	1	0	0	0	0	0
Ivy	(hemiatrophy					
Other	)					

* blood transfusion, 1 bone marrow transplantation, ^ 1 paroxysmal sensory symptoms, 1 remote AIS on investigation for hyperprolactinemia, † one with remote AIS NF-1 Neurofibromatosis type 1 SCD sickle cell disease

3.5.3.3.1. Idiopathic moyamoya

Ten (10/13; 77%) of the children were Asian and three (3/13; 23%) Caucasian.

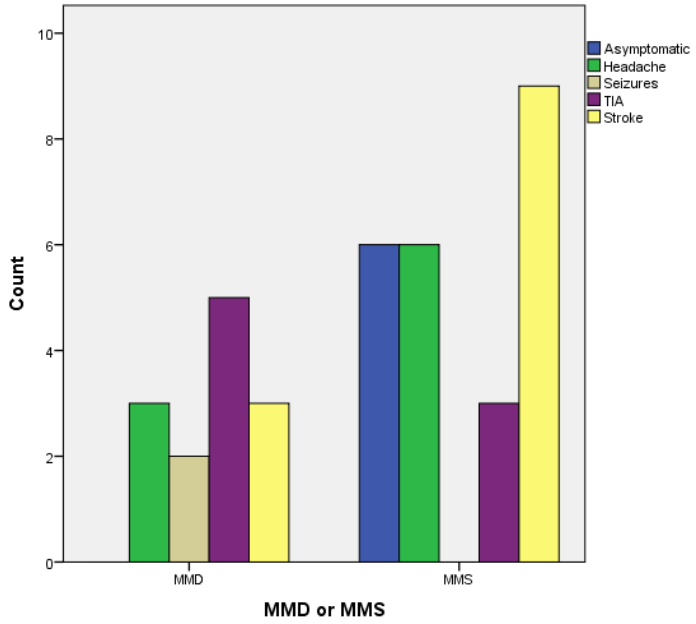
Of the Asian children 5/10 (50%); 2/10 (20%); 2/10 (20%) and 1/10 (10%) presented with TIA, stroke, headache and seizures respectively. Of the Caucasian children, 1/3 (33%) presented with stroke, headache and seizures. One child (Asian) presented with haemorrhagic stroke at fourteen years of age.

Three (3/13; 23%) presented with an acute arterial ischaemic stroke (one haemorrhagic). Two (2/13; 15%) had remote strokes diagnosed at the time of investigation of seizures (1) and hyperprolactinemia (1). Five (38%) children presented with TIAs (1 only; 1 with headache and seizures; 3 with headache). One (1/13; 8%) presented with headache only, one (1/13; 8%) with paroxysmal sensory symptoms only, and one (1/13; 8%) with seizures only (Fig 23).

3.5.3.3.2. Moyamoya syndromes

Nine (9/24; 38% [4/4 Trisomy 21-MM; 3/5 SCD-MM; 1/3 Other chromosomal; 1/9 NF1-MM]) presented with an acute arterial stroke; three TIA (3/24; 13% [1/9 NF1-MM; 2/3 Tumour-MM]); five with headache only (5/24; 21% [2/9 NF1-MM; 1/5 SCD-MM; 1/3 Other chromosomal; 1/3 Tumour-MM]); one (1/24; 4% [1/9 NF1-MM]) presented with headache and seizures and six (6/24; 25% [4/9 NF1-MM; 1/5 SCD-MM; 1/3 Other chromosomal-MM]) were asymptomatic at presentation (Fig 24).

Figure 24 Clinical Presentation by Moyamoya Classification



There was a difference in frequency of MMD and MMS between the stroke and no stroke group but this was not significant (p=0.166) (Table 11). Platelet numbers were significantly lower in the stroke than in the no stroke group (p=0.024) (Table 10). There was no significant difference in mean age at MM diagnosis, age at first hBOLD CVR study, age at revascularization surgery, haemoglobin or haematocrit between the stroke and no stroke groups in the children (Table 11).

Table 11 Population by Stroke Occurrence *significant at a level of p<0.05

	Stroke n=23	No Stroke n=14	t-test p value
MMD: MMS (%)	10:13	3:11	.166
Age at MM Diagnosis	9.03	9.3	>0.05
Age at First hBOLD CVR Study	11	10.2	>0.05
Age at Revascularization Surgery	9.6	9.7	>0.05
Haemoglobin	126	127	>0.05
Haematocrit	.374	.38	>0.05
Platelets	287	352	.024*

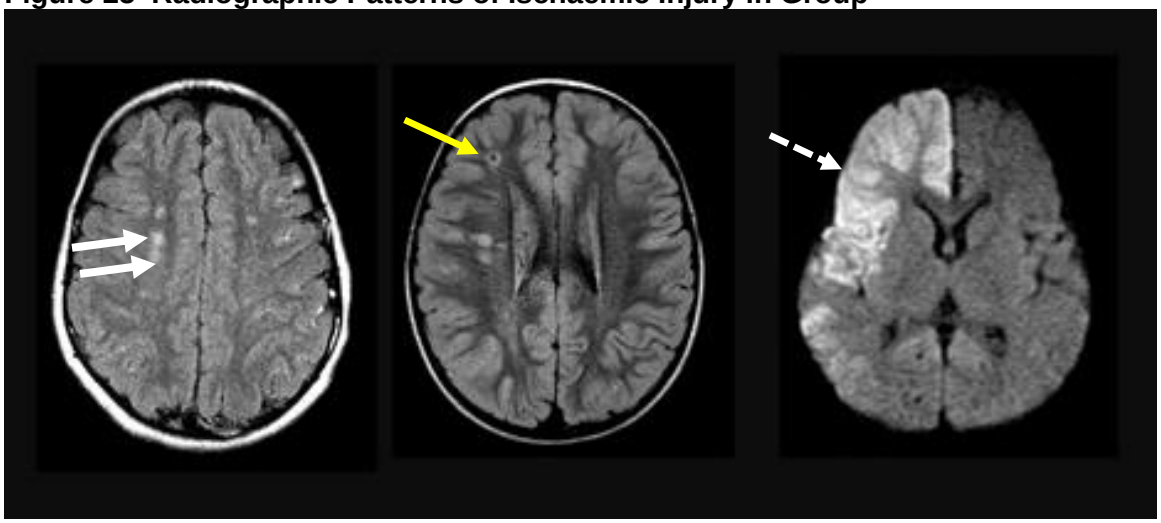
3.5.3.4. Laterality of Arteriopathy

Moyamoya laterality was bilateral in 10/13 of the idiopathic moyamoya group, 2/9 of the NF1-MM group, 5/5 of the SCD-MM group, 3/4 of the Trisomy 21-MM group, 1/3 of the Other chromosomal-MM group and 3/3 of the Tumour group. The NF1 and Other chromosomal-MM groups had a principally unilateral moyamoya which was left sided in 5/9 NF1-MM and 1/3 Other chromosomal-MM.

3.5.3.5. Radiographic Findings

Thirty-six children had an MRI brain scan and one CT followed by MRI at the time of clinical presentation and moyamoya diagnosis. MRI demonstrated no ischaemic changes in 8/37 (22%: 3/13 Idiopathic moyamoya and 5/9 NF1-MM). The pattern of ischaemic changes was a combination of watershed and cortical ischaemic in the majority (14/37, 38%: 6/13 Idiopathic-MM; 2/9 NF1-MM; 1/5 SCD-MM; 3 Trisomy 21-MM; 1 Other Chromosomal-MM; 1 Tumour-MM). The pattern of watershed infarction was mostly deep (20/37), and occurred in combination with cortical watershed ischaemia in 5/37 (Table 10 and Fig 24).

Figure 25 Radiographic Patterns of Ischaemic Injury in Group



A	B	C

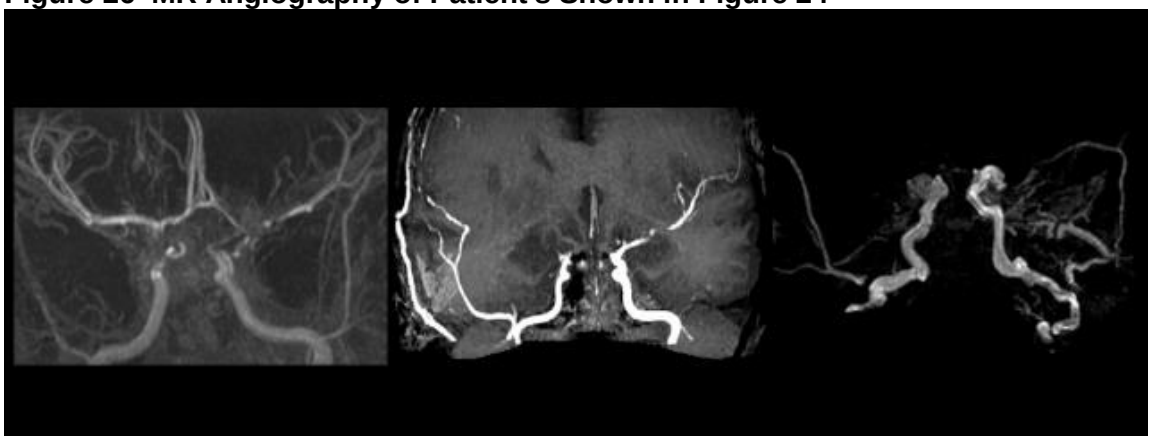
Axial FLAIR (A and B) and DWI (C) image showing deep watershed (white arrows), deep and cortical watershed (yellow arrow) and cortical (dashed white arrows) pattern of ischaemic infarction.

3.5.3.6. Moyamoya Stage and Grade of Stenosis

Conventional angiograms for staging of moyamoya disease were available in 34 children. Sixteen (16/34, 47%) had Modified Moyamoya Stage 3 disease, and 12 (12/34; 35%) Stage 2 disease. The rest were distributed between the remaining stages (Table 10).

Grade of stenosis was 4 (75-100% occluded) in 30/34 and 3 (50-74% occluded) in 4/34.

Figure 26 MR Angiography of Patient's Shown in Figure 24



A **B** **C**

Corresponding MR angiograms of MRIs shown in Figure 21 of three children with moyamoya.

3.5.3.7. Clinical Course and Outcome

Eight (8/37) children had new ischaemic (seven recurrent) events following diagnosis (5 stroke, 3 TIA). Five with Idiopathic moyamoya (three TIA, two stroke); one NF1-MM stroke (previously asymptomatic); one Tumour-MM stroke, and one SCD-MM stroke.

3.5.3.7.1. Pediatric Stroke Outcome Measure

Recurrence and outcome data was obtained from Pediatric Stroke Outcome Measure Clinical Outcome data routinely collected in the stroke clinic setting. Twenty-five children had Paediatric Stroke Outcome Measures data collected (mean time to PSOM from moyamoya diagnosis 1.96 years, SD 2.32, range 0-8.42 years) (Table 12). A score of 1 or above is given when there their function within a

specific domain is impaired to a level where there is impact on function, and is considered a poor outcome; higher scores indicate poorer outcome.

Children with Chromosomal-MM, followed by Tumour-MM had the highest mean and median scores (2.6:3.0; 2.0:2.0).

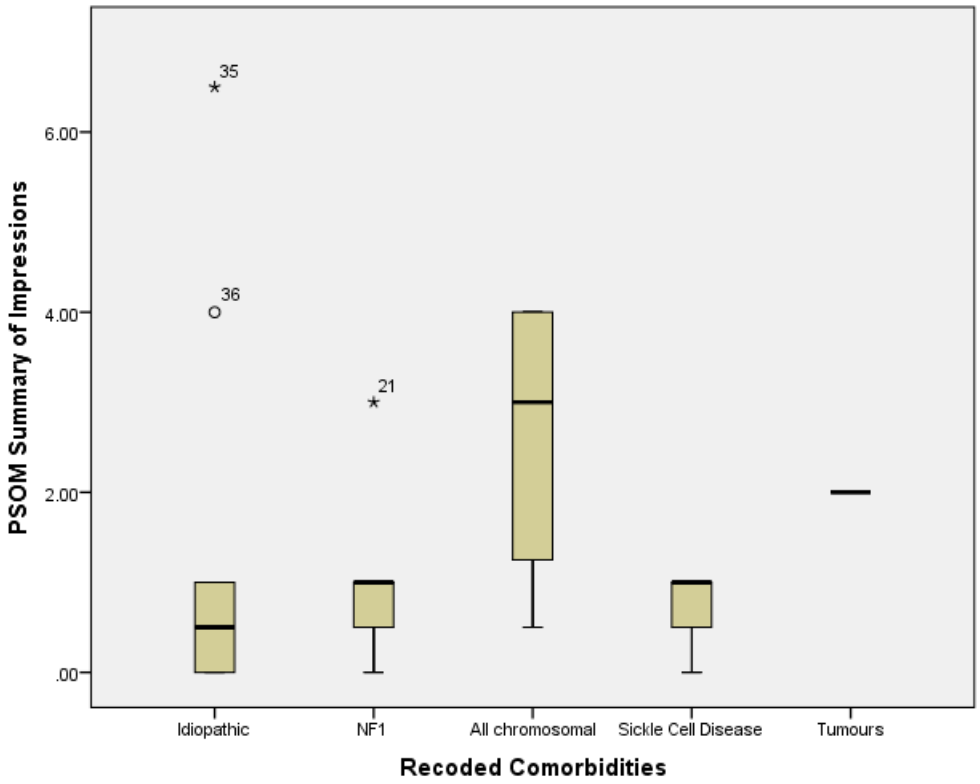
Table 12 Paediatric Stroke Outcome Measures by Moyamoya Classification

	Idiopathic	NF1	SCD	All Chr	Tumour
Mean	1.4	1.1	.75	2.6	2.0
Median	.5	1.0	1.0	3.0	2.0
Range	.0 - 6.5	.0 – 3.0	.00 – 1.0	.5 – 4.0	2.0

3.5.3.7.2. Revascularization Surgery

Twenty-five children had revascularization surgery (mean 9.63, SD 4 years; 6 bilateral, 10 right, 9 left procedures), ten had second surgeries (mean 8.9 years, SD 3.71 years). Pial synangiosis was the most commonly performed initial procedure (17/25). Other procedures included EC-IC bypass (3 only, 1 in combination with pial synangiosis); EDAS (225), EMS (2/25) and TMS (1/25). The mean time interval between surgeries was 0.56 years (6.7 months) with a maximum interval of 1.67 years. The youngest age at surgery was 2.17 years, oldest 16.42 years. Ten children (6 with stroke) had revascularization surgery prior to their first hBOLD CVR study. None of the children with SCD-MM had revascularization surgery. One had a bone marrow transplant for SCD.

Figure 27 Paediatric Stroke Outcome Measures by Moyamoya Classification



3.6. Discussion

3.6.1. Non-moyamoya Arteriopathy and Infantile Arteriopathy

Published reports on the natural history of arteriopathies (progressive and non-progressive) are variable and subject to many factors (Sebire *et al.* 2004, Braun *et al.* 2009, Beslow and Jordan 2010). The non-progressive arteriopathies are typically unilateral and the progressive arteriopathies bilateral, although they may start as unilateral. In my study the Non-moyamoya arteriopathy group represents the non-moyamoya, non-progressive (unilateral) and non-moyamoya, progressive (bilateral) arteriopathies.

The Unilateral Non-MM group all had a diagnosis of Transient Cerebral Arteriopathy (TCA). The majority were male and presented with an acute arterial stroke with a radiological pattern of injury suggestive of vaso-occlusion. Despite the similarity in degree of stenosis between the Unilateral Non-moyamoya/TCA and moyamoya arteriopathy groups the natural history of the groups differ in as much as TCA being a monophasic disease with an intial stuttering clinical course, which is typically not progressive with a low recurrence risk. Conversely the risk of stroke in moyamoya is lifelong, the disease itself progressive and the recurrence risk high. This group will be discussed in more detail in Chapter 9. All the children with Bilateral Non-MM were female. They are an aetiologically heterogenous group of children with bilateral arteriopathy, one of whom had a progressive vasculitis (Takayasu), all of whom had similar radiological appearances suggestive of vaso-occlusion. The stroke risk associated with vasculitis and SCD is high (Earley *et al.* 1998, Benseler *et al.* 2006, Switzer *et al.* 2006). However, published literature does not address the variable stroke risk associated with the range of vasculopathies seen in SCD which includes intra and extra-cranial tortuosity, aneurysms and dissection (Verlhac *et al.* 2014) (Kirkham and DeBaun 2004). Similarly PHACES syndrome is associated with a range of vasculopathies including moyamoya, and is therefore likely to be associated with a variable stroke risk. However the observed risk in Heyer's study was low (Heyer *et al.* 2008).

The infantile arteriopathy (moyamoya and non-moyamoya) represent a poorly characterized group of children who typically have a malignant course and poor outcomes (Jackson *et al.* 2014, Al-Yassin *et al.* 2015). The majority of our infantile

arteriopathy (diagnosed under two years of age) group did not have moyamoya arteriopathy and presented with an acute arterial ischaemic stroke.

Overall the non-moyamoya group appeared to share a similar clinical (focal motor stroke or TIA) and radiological (vaso-occlusive pattern) presentation, but have a variable natural history and stroke risk profile. This need to quantify stroke risk in a child with symptomatic or asymptomatic arteriopathy remains a challenge particularly in the asymptomatic patient and may be addressed by the assessment of CVR.

3.6.2. Moyamoya Arteriopathy

3.6.2.1. Moyamoya Disease

The childhood peak of presentation in moyamoya is five years of age, with a female preponderance (Wakai *et al.* 1997, Yonekawa *et al.* 1997, Chiu *et al.* 1998, Han *et al.* 2000, Baba *et al.* 2008). However, the epidemiological descriptions of the disease are mostly of Asian moyamoya disease or non-Asian (European or American) adult moyamoya disease (Hallemeier *et al.* 2006, Kraemer *et al.* 2008). The female preponderance is replicated in our population, however the later age of moyamoya diagnosis may reflect historical delays in diagnosis of the disease.

The majority of children with MMD were of South East Asian origin, whereas the majority of children with syndromic MM were Caucasian. The exception to this was the black children of African or Caribbean origin, who mostly had SCD-MM. None had MMD. The ethnic variability in our population is similar to that in published

literature which reports the highest prevalence of MMD in people of East Asian origin, with a relatively low prevalence in European and North American countries (Goto and Yonekawa 1992, Chiu *et al.* 1998, Hallemeier *et al.* 2006, Kraemer *et al.* 2008, Kuroda and Houkin 2008, Krischek *et al.* 2011, Liu *et al.* 2013). Children of African or Caribbean origin more commonly have SCD-MM. However, Urchino et al demonstrated that the prevalence of moyamoya in the Afro-American and Caucasian people was similar when patients with sickle cell disease were excluded (Uchino *et al.* 2005).

In adult literature important differences in clinical presentation are reported within the MMD group and ethnicity appears to be a major determinant of this. In the Kraemer et al study (Kraemer *et al.* 2008) of Caucasian adults with MMD, all patients presented with a cerebral ischaemic event, none presented with haemorrhagic stroke and none were asymptomatic. This is in contrast to the second peak of stroke in Asian MMD which typically presents with haemorrhage (Han *et al.* 1997, Han *et al.* 2000). The majority (77%) of our MMD population were Asian in ethnicity. The majority of the Asian MMD group in my study presented with focal motor deficits (TIA [50%] or stroke [20%]). The frequency of presentation with focal motor deficits was thus higher in the Asian MM group than the Caucasian MM group of whom 33% presented with focal motor deficits (stroke only). Seizures, headache and paroxysmal symptoms were present in the rest of the Caucasian MMD group. It is of interest that the only child to present with haemorrhagic stroke in our group was an Asian boy of fourteen years. The predominance of Asian children in our MMD group and the suggestion of a more

severe (as defined by TIA and stroke presentation) phenotype would be concordant with published literature (Kraemer *et al.* 2008, Scott and Smith 2009) (Guey *et al.* 2015). In a study of 21 Caucasian adults with moyamoya disease Kraemer et al demonstrated a mean age of onset of symptoms of 31 years. Only one presentation was at 4 years of age and four were 16 to 19 years. Therefore, differences by ethnicity in natural history and stroke risk seem to exist within the MMD group. However, publications on childhood Caucasian MMD are scarce and often embedded in adult reports (Graham and Matoba 1997, Yonekawa *et al.* 1997, Uchino *et al.* 2005, Kraemer *et al.* 2008, Kleinloog *et al.* 2012, Kossorotoff *et al.* 2012, Chen *et al.* 2014). Further exploration of the clinical features of MMD in in a large cohort of non-Asian children with MMD is thus warranted.

3.6.2.2. Moyamoya Syndrome

Presentation in the MMS group was variable and seemed dependent on syndromic association. The majority of children with SCD are diagnosed as a result of the newborn baby screening programme following which screening with TCD, MRI and MRA allows for early, pre-symptomatic detection of arteriopathy. However, despite the neonatal screening and post-natal TCD all the SCD-MM group in my study presented with AIS. Children with Trisomy 21-MM, Other chromosomal-MM and Tumour-MM mostly presented with ischaemic symptoms (stroke or TIA). In a report of the surgical experience in 32 children with Trisomy 21-MM a higher rate of pre-surgical stroke and higher age of diagnosis was noted (See *et al.* 2015). Age of diagnosis was higher than the average for our moyamoya population and all presented with ischaemic stroke. This is in contrast to the NF1-MM group in whom

almost half were asymptomatic at diagnosis, and only one progressed to having a stroke (prior to surgery). The cutaneous manifestations of NF1 coupled with recommendations for screening allows for pre-symptomatic detection of moyamoya arteriopathy and a true opportunity to prevent stroke and alter outcome (Rea *et al.* 2009, Lin *et al.* 2011).

3.6.3. Radiological and Vascular Findings

The predominant neuroimaging pattern of deep watershed infarction, suggestive of perfusion failure, in the moyamoya arteriopathy group is in striking contrast to the cortical ischaemia typically seen in vaso-occlusive arterial ischaemic stroke and exemplified by the non-progressive arteriopathies and PACNS. This has been noted in previous publications (Ringelstein *et al.* 1994, Rafay *et al.* 2015). Increased susceptibility to ischaemia as a result of perfusion failure and hypotension has been demonstrated in animal experimental models (Brierley and Excell 1966, Howard *et al.* 1987) Further studies, including those of autopsy series have demonstrated an important link between microemobli, hypoperfusion and infarction Hence, despite the common vascular pathology of steno-occlusion between the non-progressive arteriopathies, PACNS and moyamoya, the final implicated injurious mechanisms are of prinicipally of hypoperfusion causing ischaemia and deep border zone infarction or microembolism coupled with hypoperfusion or reduced washout in cortical border zone infarction (Romanul and Abramowicz 1964, Pollanen and Deck 1990, Masuda *et al.* 1994, Caplan and Hennerici 1998). Furthermore the presence of deep border zone infarction is

associated with a poor prognosis and clinical deterioration, in contrast to the finding of cortical border zone infarction which seemingly is associated with a more benign course. (Moriwaki *et al.* 1997, Krapf *et al.* 1998)

The timing of clinical presentation is in part thought to be related to the severity of vasculopathy and the occurrence of occlusion in the parent arteries (Kraemer *et al.* 2008, Sultan *et al.* 2015). However, despite the finding of moderate to severe stenosis in our group, there was no difference in vascular severity measures (Modified Moyamoya Stage or Grade of Stenosis) between the asymptomatic and symptomatic at time of moyamoya diagnosis. Hence, our arteriopathy severity measures alone could not be used as a reliable indicator of ischaemic risk and guide for timing of intervention.

Moyamoya laterality was predominantly bilateral or right sided. Unilateral, and in particular left sided moyamoya was a feature of the NF1-MM group. Duat-Rodriguez et al. (Duat-Rodriguez *et al.* 2014) reported a similar observation. Our findings are somewhat in contrast to the study by Koss et al, in which the reported NF1-MM is clinically and radiologically similar to that of idiopathic moyamoya (Koss *et al.* 2013). This was not our experience.

3.6.4. Treatment

The use of anticoagulants and anti-platelet agents in MM does not adequately address the primary pathological mechanism although anti-platelet treatment has been shown to be helpful (Scott *et al.* 2004, Roach *et al.* 2008, Research

Committee on the *et al.* 2012). The only significant difference between the stroke and no stroke groups was the platelet number which was unexpectedly higher in the non-stroke group. All children would have been on antiplatelet therapy. The platelet number itself was however normal for each group. Furthermore the use of tissue plasminogen activator in MM is contraindicated in acute stroke because of the risk of intracranial haemorrhage (Research Committee on the *et al.* 2012). Carbonic anhydrase inhibitors such as Acetazolamide and Topiramate are known for their vasodilatory effects and were used in addition to Aspirin in a few patients. In retrospective analysis medical treatments have been shown to not be as effective as surgical intervention.

Lin et al. (Lin *et al.* 2011) retrospectively reviewed the natural history of a cohort of 83 children who were asymptomatic at the time of diagnosis of moyamoya. All children had a syndromic diagnosis of either SCD-MM or NF1-MM. Radiographic progression occurred in 75% of the SCD-MM group and 59% of the NF1-MM group within a mean of 5.4 years of follow-up. Clinical progression was typically preceded by radiographic progression and occurred in 65% of the SCD moyamoya group and 44% of the NF1 moyamoya group (Lin *et al.* 2011). Consequently their suggestion was that the majority of children with moyamoya arteriopathy would benefit from revascularization surgery even if asymptomatic at presentation (Lin *et al.* 2011, Koss *et al.* 2013). However, revascularization surgery is not without risk which is highest in the first 30 days after surgery (approximately 4% per hemisphere) and decreases considerably after the initial period (Scott and Smith 2009). The same group reported a review of 477 hospitalizations for surgery and

demonstrated an increased risk of complication associated with a comorbid diagnosis of Down syndrome, Nf-1, SCD and pre-existing neurological deficits (Lin *et al.* 2011). The ultimate aim would be to prevent new overt or covert ischaemic demise within this population. Hence the opportunity to prevent occurrence of ischaemia in the early asymptomatic diagnosis of moyamoya or recurrence in the post ischaemic presentation of moyamoya is an important goal. A simple presumption of the earlier the better is complicated by the practical challenges of operating on small vascular calibre vessels, increased complication rates, lower cerebrovascular reactivity, difficulties with pain control made worse by age related limitations in communication, anaesthetic risk and comorbid associations (Soriano *et al.* 1993, Kim *et al.* 2004, Kim *et al.* 2005, Scott and Smith 2009, Jackson *et al.* 2014). Therefore there remains a need for a tool to help determine the time of surgery for the individual patient.

3.6.5. Course and Outcome

Stroke and TIA recurrence was highest in the MMD group. However, the MMS group was largely made up of children with NF1-MM. The other syndromic MM groups were relatively small and some heterogenous eg. the All-chromosomal group. The children in my study with Down syndrome had an aggressive course and poorer outcome when compared to the NF1-MM group. This more aggressive natural history is reported (Erguven *et al.* 2005, Jea *et al.* 2005, Kainth *et al.* 2013) ((See *et al.* 2015).

The PSOM summary of impression scores were poorest (high) in the All Chromosomal group. This group included children with Down syndrome, Turner syndrome and other chromosomal abnormalities all of which have a phenotype which includes learning difficulties. I did not conduct further analysis using the sub-categories due to numbers. However, the PSOM summary of impression scores may have been more reflective of the chromosomal comorbidity than poorer motor outcome. However, this remains to be explored.

In summary

In this descriptive study the non-moyamoya arteriopathy group presented with more recognizable features of stroke ie. focal motor weakness than did children with moyamoya arteriopathy regardless of age. The presentation with seizures in the under two moyamoya group may be secondary to the diffuse hypoperfusion related ischaemic process seen in moyamoya, rather than the characteristic thrombo-embolic vaso-occlusive process of the non-moyamoya arteriopathies. In addition neither the presentation (symptomatic or asymptomatic) of children with arteriopathy nor the laterality of arteriopathy were sensitive indicators of natural history or future stroke risk.

The observed imaging signature of deep watershed infarction seen within the moyamoya arteriopathy group is in keeping with the main proposed hypoperfusion mechanism of injury and published literature. Current treatments in stroke are aimed at thrombotic vaso-occlusive mechanisms and are hence inadequate to fully

address the ongoing risk of ischaemic demise in this group. This highlights the need to explore tools that can identify children at risk of ischaemia at the tissue level in the context of chronic hypoperfusion as seen in our population.

The children with TCA (unilateral non-progresive, non-moyamoya) all presented with an ishaemic event and none had a recurrence out of the expected 3 month period. Notably, and in contrast the majority of the mostly unilateral (left sided) NF1-MM group were asymptomatic or had headache at the time of diagnosis. A significant proportion of this group did not have an ischaemic event in the study period. Therefore the NF1-MM group had a mixed and in some more indolent clinical course.

3.6.6. Future Directions and Study Limitations

1. Population Ascertainment - Clinical and radiological comparisons between moyamoya and non-moyamoya arteriopathy groups are limited by the small numbers in the non-moyamoya arteriopathy categories as well as the heterogeneity of diagnosis in the Bilateral Non-MM group. The patient ascertainment method for the children with Unilateral Non-MM may have resulted in the selection of a more severe phenotype as they were selected for the presence of a moderate to severe stenosis at onset of the disease. A selection bias toward a more severe phenotype is possible but is less apparent in the Bilateral Non-MM group given the aetiologic and clinical heterogeneity. Therefore meaningful interpretation of these findings is limited.

2. Grading of Stenosis – the method used for classification of Grade of Stenosis may not have had sufficient discriminatory power at the moderate to severe end of the spectrum of vasculopathy. Sultan et al (Sultan *et al.* 2015) devised a three point measure of steno-occlusive severity in children with moyamoya arteriopathy which on preliminary analysis may be predictive of recurrence. Specifically our grading system did not discriminate between severe stenosis and occlusion. Hence I plan to revise and include the suggested criteria for the Grading of Stenosis in moyamoya arteriopathy and use it in future analysis.

3. Studies –

i) Understanding of the mechanisms that underpin differences within the MMD group and between the MMD and MMS groups would help inform the development of mechanism targeted therapies and guide management. Strides have been made within this field with recent discoveries of novel gene mutations (Milewicz *et al.* 2010, Miskinyte *et al.* 2011, Munot *et al.* 2011, Guey *et al.* 2015) (Liu *et al.* 2010, Liu *et al.* 2011)

Therefore exploration into the genetic mechanisms of disease may identify common or unique pathophysiological mechanisms and genetic biomarkers of disease which may lead to the development of novel targeted management approaches.

ii) Longitudinal large cohort studies of non-Asian MMD and single syndromic MMD would inform our practice, and ability to clinically differentiate between the aetiologically different progressive and non-progressive arteriopathies. This information will improve our ability to recognise the need for medical and surgical intervention and recommend it in a more timely manner.

3.6.7. Conclusion

I have hence demonstrated the similarities and differences between the various arteriopathy groups highlighting the challenge in managing children with arteriopathy for whom the better assessment of ischaemic risk would enable a more informed and individualised approach in the management of the child with arteriopathy. The measurement of CVR using hypercapnic challenge BOLD MRI provides such an opportunity.

4. Interobserver Reliability and Reproducibility of hBOLD CVR Measures

4.1. Abstract

4.1.2. Background

Qualitative hBOLD CVR with the use of carbon dioxide as a vasoactive stimulus (RespirAct) allows for the assessment of CVR in a non-invasive manner. However, application of this method is limited in the paediatric population, and particularly in the younger children. There are no published reports on the repeatability and reliability of qualitative hBOLD CVR techniques (breath-hold and general anaesthetic) in children.

4.1.3. Objective

To examine the feasibility of conducting qualitative hBOLD CVR studies in children. I examined whether qualitative measures of hBOLD CVR using endogenous CO2 in the awake (breath-hold) and sleep (general anaesthetic) state in children can be repeated reproducibly within a short time frame. I also sought to assess the reproducibility of scoring by inspection of hBOLD CVR parametric maps by different raters.

4.1.4. Method

Children with moyamoya were enrolled as described in Population Ascertainment Chapter 3. Children had qualitative hypercapnic challenge with endogenous delivery of carbon dioxide (breath-hold and general anaesthetic) and quantitative hypercapnic challenge with exogenous delivery of carbon dioxide (RespirAct, re-breathing mask) hBOLD CVR studies (see Chapter 2 for Methods).

Interobserver agreement for the categorical scoring (1=normal CVR, 2=reduced CVR or steal<10%, 3=steal>10%) was compared using Kappa scores. All repeat studies conducted on the same day in the same MRI session were compared with Kappa for repeat categorical scores by the same observer. Coefficient of variation, intra-class correlation and the Bland-Altman method were used to assess reproducibility of continuous hemispheric pixel measurements.

4.1.5. Results

In the 22 of 37 children in whom categorical scoring was undertaken by both observers, inter-rater reliability was excellent (kappa=1.0 for the right hemisphere and 0.8 for the left). Twenty nine of 37 children with moyamoya had repeat studies on the same day; 5 did not and 3 studies were excluded. Qualitative measures of hBOLD CVR demonstrated overall good reliability for scores by inspection, especially if studies deemed to be methodologically ‘poor’ were excluded. There was good to excellent intraclass correlation and no evidence of bias using the

Bland-Altman method for 2 of the 3 hemispheric pixel measures. Quantitative measures of hBOLD CVR were calculable for six children and ranged between 0.23 and 0.5 in the right hemisphere, and 0.23 and 0.8 in the left.

Conclusion

In this chapter I demonstrate that qualitative measures of hBOLD CVR under general anaesthetic and using the breath-hold technique can be scored with good interobserver reliability and interpretable for use in clinical practice. Quantitative measures appear reproducible. However, techniques to improve reliability and repeatability of patient breath-holding; standardization of the hBOLD CVR breath-hold and general-anaesthetic protocols to include improved acquisition of respiratory data need to be developed to allow more widespread reliable application of these tools for assessment of ischaemic risk in childhood cerebrovascular disease.

4.2. Introduction

Validation: interobserver reliability and reproducibility

To be used clinically to follow disease progression and predict outcome, a measure must have good interobserver reliability and reproducibility. Interobserver reliability and reproducibility of measures of CVR using a variety of techniques has been investigated in adult studies, but the evidence in the paediatric literature remains scarce. Adult CVR studies using quantitative MRI techniques such as rebreathing with the RespirAct device are done in the awake state (Heyn *et al.* 2010). In the paediatric population cerebrovascular disorders may be diagnosed in infancy when patient cooperation is not possible, necessitating an alternative means of assessing CVR reproducibly over time, such as either inspection and scoring or counting of the number of hemispheric pixels.

Interrater reliability of categorical variables, such as scoring by inspection of hBOLD CVR parametric maps, and reproducibility of repeat scores, may be assessed using Kappa's coefficient. Interpretation of score for Kappa are as follows (Landis and Koch 1977):

1. 0.01 indicates "poor" agreement
2. 0.01 to 0.20 indicate "slight" agreement
3. 0.21 to 0.40 indicate "fair" agreement
4. 0.41 to 0.60 indicate "moderate" agreement

5. 0.61 to 0.80 indicate "substantial" agreement
6. 0.81 to 1.00 indicate "almost perfect" agreement

Reproducibility of continuous variables, such as quantitative hypercapnic challenge with exogenous delivery of carbon dioxide (RespirAct, re-breathing mask) hBOLD CVR studies, or hemispheric pixel measurements on breath hold or during general anaesthetic, may be assessed using coefficient of variation, providing there are no negative values, or by intraclass correlation. Indices of reliability for ICC are as follows:

- < 0.7 poor reliability
- 0.7 adequate reliability
- 0.8 good reliability
- >0.9 excellent reliability

Reproducibility of rebreathing hBOLD CVR Measures in children

Computer controlled gas delivery using the rebreathing mask (RM) allows targeted manipulation of end tidal partial pressure of CO_2 consisting of a sequential gas delivery breathing circuit in combination with an automated gas blender (RespirAct TM, Thornhill Research Inc., Toronto, Canada) (Slessarev *et al.* 2007). The RespirAct device with a re-breathing mask has been validated in adults and children (Heyn *et al.* 2010, Han *et al.* 2011). A recent study in children by

Kassner's group demonstrated the reproducibility of quantitative CVR measures using the RespirAct device with a re-breathing mask in ten healthy older children (age 16.1 ± 1.6 years) (Leung *et al.* 2015). The coefficient of variation was 9.3+/5.9% for grey matter and 11.5+/-8.7% for white matter, while the within-day intraclass correlation was 0.857 (95% confidence intervals, CI 0.639, 0.943) and 0.895 (95%CI 0.734, 0.958) for grey and white matter respectively. However, as previously mentioned, this method has limited applicability in the paediatric population and there are no equivalent data in the younger paediatric population for the more appropriate CVR modalities such as hBOLD CVR under general anaesthetic (GA) and breath-holding (BH).

hBOLD CVR under general anaesthetic (GA)

hBOLD CVR studies conducted under GA is a feasible, yet previously unreported, method for the assessment of CVR in the very young, and/or those unable to tolerate alternative awake methods. It is relatively easy to measure carbon dioxide tension, either arterial or end-tidal, allowing quantitation of CVR, as well as inspection and scoring.

Breath holding

Breath-holding is a relatively well tolerated technique and has been used in adults as well as children. There is no need for mask ventilation, making the experience within the scanner less claustrophobic and obviating the need for an anaesthetist.

Longer breath-holds result in more robust and repeatable BOLD responses (Magon *et al.* 2009), and certain manoeuvres, such as paced breathing between challenges and breath-holds after expiration, may improve repeatability (Scouten and Constable 2008, Thomason and Glover 2008). In adult studies intraclass correlation for repeatability of CVR measurement for breath-holding ranges from not acceptable repeatability (ICC <0.4) to excellent repeatability (ICC=0.82) depending on the breath-hold challenge employed (Bright and Murphy 2013). Signal changes driven by BH range from 0.8% to 5.1% in a 1.5T MR system (Kastrup *et al.* 1998), with a similar or larger effect observed at 3T.

If carbon dioxide measurements are not feasible during breath hold, CVR cannot be quantitated and scoring systems to capture the relative hemispheric differences on the CVR parametric maps in a manner quantifiable as categorical variables and hence as indirect measures of hBOLD CVR are required (see Chapter 2 Methods Scoring). A scoring system was devised by the raters (Ref Methods Section). Scores for each hemisphere were between 1 and 3 according to the following schema:

1) Normal CVR = 1
2) Reduced positive reactivity +/- steal >2%<10% = 2
3) Steal > 10% with or without reduced positive reactivity = 3

4.3. Study Purpose and Hypothesis

In this section I sought to determine whether qualitative studies of hBOLD CVR in children using the devised scoring system is reliable between observers. I also hypothesized that indirect measures of hBOLD CVR conducted in children using breath hold or under general anaesthetic are reproducible.

4.4. Methods

Children with moyamoya and clinical hBOLD CVR studies were prospectively and retrospectively enrolled through the Children's Stroke Programme Clinic and using the Stroke Registry Database (Ref Chapter 3 Population). Children had qualitative (breath-hold and general anaesthetic) hBOLD CVR studies and a few had quantitative hBOLD CVR studies (Ref Chapter 2 Method Sections and Chapter 3 Population Attainment Section).

For interobserver reliability and reproducibility and assessment, hBOLD CVR studies of children with moyamoya arteriopathy were assessed for quality (good and poor). Patient and method/technical factors were recorded.

Interobserver reliability

If one patient had more than one successfully completed study then the first one was chosen for inclusion in analyses of interobserver reliability. Two raters (ND and WL)

blinded to clinical information independently scored each hemisphere of the hBOLD CVR parametric map in the same - first axial slice - above the ventricles.

Reproducibility

By using semi-quantitative scoring of the paremtric maps, we tested the reliability of method of analysis of hBOLD CVR. Blood flow measurements themselves, for example using ASL were not conducted. This could have allowed for testing of the reliability and reproducibility of the test itself although difficulties with the implementation of ASL in the pediatric population were not adequately resolved during the course of this study.

Repeat hBOLD CVR studies on the same day during the same MRI session were attempted in all children at all hBOLD CVR study time points. Successfully completed repeat studies on the same day during the same MRI session (1A and 1B) were included. Two methods of analysis of the scans were undertaken:

(i) Hemispheric inspection scores were agreed together by the two scorers (WL and ND) and were compared between studies 1A and 1B. Analysis was repeated for those with ‘good’ quality studies only (Thomas *et al.* 2013).

(ii) Positive and negative threshold hemispheric pixels of the hBOLD CVR parametric map in the first axial slice above the ventricles were counted as previously described (Method). Hemispheric pixels for studies 1A and 1B were compared so as to provide an index of reproducibility of measures – the intraclass correlation.

4.5. Statistics

The validation statistics used were:

1. Kappa's coefficient was used to assess:

(i) interobserver reliability of scoring by inspection of hBOLD CVR parametric maps and

(ii) reliability of repeat scores by one observer in studies 1A and 1B

2. Coefficient of variation for the hemispheric pixel measurements was calculated as standard deviation divided by mean.

3. Bland-Altman plots were used to assess the degree of agreement between hemispheric pixel measures (Bland and Altman 1986, Bartlett and Frost 2008).

4. Intraclass correlation was used to assess reproducibility of hemispheric pixel measurements. Absolute agreement analysis and the 2-way mixed effects model was chosen as the raters were fixed. Average measures intraclass correlation coefficient was reported (Shrout and Fleiss 1979). Mixed effect repeated measures ANOVA were implemented, with time (1A, 1B) as the within-subject variable, and method (BH, GA) as the between-subject variable.

Table 13 Overview of validation studies

	Interobserver for the same study	**Repeatibility on the same day**	
		Scores	**Pixels**
N included	22	28	29
Time points	1A or 1B	1A & 1B	1A and 1B
Statistics	Kappa	Kappa	Coefficient variation, Bland-Altman, Inter-class correlation

4.6. Results

4.6.2. Repeatability and Reliability of hBOLD CVR Measures

For the purpose of the interobserver reliability and reproducibility analyses the hBOLD CVR studies of children with moyamoya arteriopathy only (MMD or MMS) were considered (n=37). No repeat studies were done in five children, and a further three were excluded from repeated measure reproducibility as no scores were available for one or more of the hemisphere pixel analyses. Therefore 29/37 had a repeat study on the same day within the same session (1A, 1B; 29 repeat hemispheric pixel analysis, 28 repeat hemispheric inspection scores). Of the 29 (29/37) studies, one or both of 14/37 patient hBOLD CVR repeat (1A or 1B) studies had issues compromising quality (Table 14). Quality was significantly compromised in 10/14 of these studies and hence 10/29 studies were deemed poor quality and 19/29 good quality.

There was no statistically significant difference in age at diagnosis of MM, and age at the first hBOLD CVR study between the group who had repeat (1A, 1B) studies and those who did not. There was a statistically significant difference in age at diagnosis of MM and age at first hBOLD CVR study by General Anaesthetic and Breath-hold hBOLD hypercapnic challenge method (Table 14).

Figure 28 Flowchart for validation studies

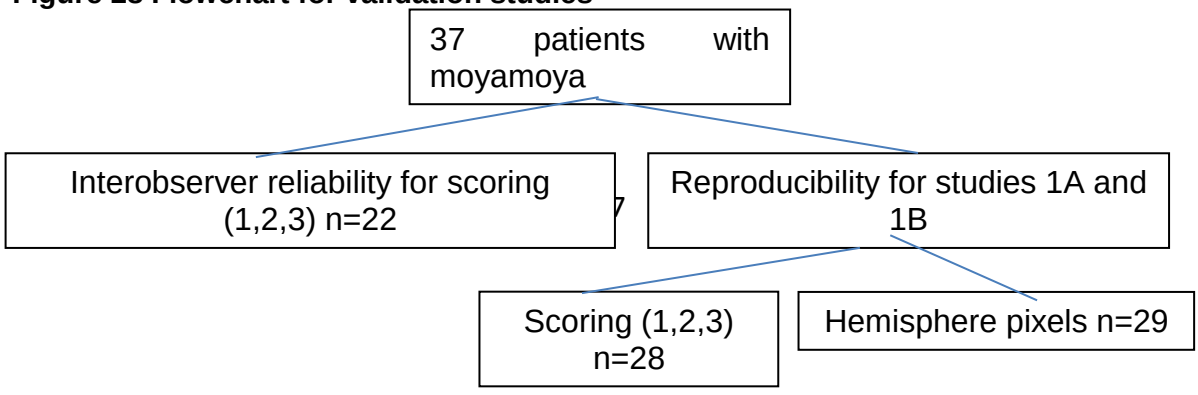


Table 14 Demographics of Patients by hBOLD CVR Method

	No Repeat Studies	Repeat Studies	t-test	Repeat Studies by Method			p value
				General Anaesthetic	Breath hold	Rebreath Mask	
N	8	29		14	15	2	
Age at Diagnosis Mean (SD)	7.27 (4.39)	9.65 (3.65)	.127†	7.74 (4.07)	11.42 (2.05)	12.13 (0.88)	0.004*
Gender M (%)	4 (50)	12 (41)		7 (50)	5 (33)	1(50)	0.371
Mean Age at hBOLD CVR (SD)	8.67 (3.66)	11.24 (4.34)	.136†	9.02 (4.84)	13.3 (2.55)	12.46 (.65)	0.006*
MM Stage							
1	0	2		1	1	0	
2	2	10		6	4	2	
3	5	11		5	6	0	
4	0	2		0	2	0	0.937
5	0	1		1	0	0	
6	0	1		0	1	0	
Degree of Stenosis 3/4 %	0/88	15/85		15/85	14/86		0.314
Stroke R/L/B	4/1/0	7/4/5		4/2/2	3/2/3	0/0/1	0.963

MM Stage: angiographic MM stage

* significant difference between age of diagnosis of MM and age at CVR between BH and GA groups †trend toward significant difference in age of CVR between BH and GA group

Table 15 Factors Affecting Quality in hBOLD MRI Studies

No of Patients	Comment (one or both studies)	Study Method (GA/BH)
4	Patient factors – movement artefact	1/3
1	Patient factors – prior medication*	1/0
9	hBOLD MRI method technical factors**	5/4

* patient on Diamox
** poor BOLD signal/Δ CO2 time course

Table 16 Anaesthetic agents used for Studies under General Anaesthetic

Number of patients	Propofol	Propofol and Sevoflurane	Propofol, Sevofluorane (+)	Thiopentone, Sevofluorane, Succinlycholine, Nitrous oxide, Lidocaine
17	5 (1/5 CG)	7	2 (C) 1 (A) 1 (FG)	1

P = Propofol; S = Sevoflurane; C = Carbamazepine; A = Acetazolamide; G = Glycopyrolate; L = Lidocaine; SC = Succinylcholine; N = Nitrous oxide; TH = Thiopentone

4.6.2.1. Inter-rater Reliability of Scores by Inspection

Twenty-two patients (48 hemispheres) were independently scored by WL and ND blinded to clinical information. Inter-rater hemispheric scoring of parametric maps was excellent; there was 100% agreement of right hemisphere scores (Kappa 1.00, p=0.0005) and excellent agreement (Kappa 0.862, p=0.0005) for the left hemisphere (Table 17).

Table 17 Right and Left Hemisphere Interater Crosstabulation and Kappa

	Right Hemisphere Rater 2 Score			Total		Left Hemisphere Score Rater 2			Total
Rater 1 Score	**1.00**	**2.00**	**3.00**		Rater 1 Score	**1.00**	**2.00**	**3.00**	
1.00	2	0	0	2	**1.00**	5	0	0	5
2.00	0	7	0	7	**2.00**	2	8	0	10
3.00	0	0	13	13	**3.00**	0	0	7	7
Total	2	7	13	22	Total	7	8	7	22

Kappa 1.00; p=0.0005 Kappa 0.862; p=0.005

4.6.3. Reproducibility of Repeat Measures by Hypercapnic Challenge Method

Of the 37 children with moyamoya and hBOLD CVR studies, 29 in total, 14 of 17 GA (82%; Propofol and Sevoflurane being the main anaesthetic agents used - Table 16) and 15 of 18 BH (83%) hBOLD CVR studies had repeat parametric maps scored by

(i) inspection by consensus of two observers

(ii) counting of hemispheric pixels.

4.6.4. Reproducibility of Repeat Hemispheric Scoring by Inspection of Parametric Maps

Assuming the study 1A score is the 'true' score, Cohen's Kappa analysis was used to assess the reliability of repeat hemispheric scoring by inspection (n=28; including both 'good' and 'poor' quality studies) (Thomas *et al.* 2013). Cohen's Kappa analysis was repeated for the 'good' (n=19) studies only.

4.6.4.1. Reliability of Hemispheric Repeat Scores by Study Quality

4.6.4.1.0. Reliability of 'All Good and Poor' Quality Hemispheric Repeat Scores (n=28)

Kappa was .557 and .523 for right and left hemispheric repeated measure scores respectively. In the right and left hemisphere, hBOLD CVR parametric maps were scored as normal at both time points (1A and 1B) for 6/8 (75%) and 7/8 (86%) of the normal (scored as 1 at study 1A) studies and 14/20 (70%) and 12/20 (60%) of the abnormal (scored as ≥2 at study 1A) studies respectively.

Out of 14 scores of 3, one child had a right hemispheric 1A score of 3, and 1B score of 1. The biggest difference in repeat measure scores was between scores 2 and 3 for both hemispheres (Table 18).

Table 18 Right and Left Hemispheric Scores Repeated Measures and Kappa (All repeat studies)

	Score Right 1A	Total		**Score Left 1A**	Total

Score Right 1B	1.00	2.00	3.00		Score Left 1B	1.00	2.00	3.00	
1.00	6	0	1	7	**1.00**	7	2	0	9
2.00	2	4	3	9	**2.00**	1	5	5	11
3.00	0	2	10	12	**3.00**	0	1	7	8
Total	8	6	14	28	Total	8	8	12	28

Kappa .557, p=0.000 Kappa .523, p=0.0005

4.6.4.1.1. Reproducibility of Good Quality Hemispheric Repeat Scores (n=19)

Kappa was 0.755 and 0.607 for right and left hemispheric repeated measure scores. In the right and left hemisphere hBOLD CVR parametric maps were scored as normal at both time points (1A and 1B) for 5/5 (100%) and 6/7 (86%) of the normal studies and 11/14 (79 %) and 8/12 (66%) of the abnormal (scored as ≥2) studies respectively. The biggest difference in repeat measure scores was between 2 and 3 for both hemispheres (Table 19).

Table 19 Right and Left Hemispheric Scores Repeated Measures and Kappa of Good Quality Studies

	Score Right 1A	Total		Score Left 1A	Total

Score Right 1B	**1.00**	**2.00**	**3.00**		**Score Left 1B**	**1.00**	**2.00**	**3.00**	
1.00	5	0	0	5	**1.00**	6	1	0	7
2.00	1	3	2	6	**2.00**	0	4	3	7
3.00	0	0	8	8	**3.00**	0	1	4	5
Total	6	3	10	19	Total	6	6	7	19

Kappa .755 p=.0005 Kappa .607 p=.0005

4.6.4.2. Reproducibility of Hemispheric agreement of GA and BH studies of Good Quality only:

Cohen's Kappa was 0.813 and 0.321 for right and left good quality repeat scores of hBOLD CVR done under GA (Table 20) and 0.6 and 0.844 for right and left repeat scores of hBOLD CVR done using the BH technique (Table 21).

Table 20 Right and Left Hemisphere Kappa for Good Quality Studies of CVR under General Anaesthetic

	Score Right 1A			Total		Score Left 1A			Total
Score Right 1B	**1.00**	**2.00**	**3.00**		**Score Left 1B**	**1.00**	**2.00**	**3.00**	
1.00	5	0	0	5	**1.00**	4	1	0	5
2.00	0	1	1	2	**2.00**	0	1	3	4
3.00	0	0	2	2	-	-	-	-	-
Total	5	1	3	9	Total	4	2	3	9

Kappa 0.813 p=.001 Kappa 0.321 p=.107

Table 21 Right and Left Hemispheric Kappa for Good Quality Studies of CVR by breath-holding

	Score Right 1A			Total		Score Left 1A			Total
Score Right 1B	**1.00**	**2.00**	**3.00**		**Score Left 1B**	**1.00**	**2.00**	**3.00**	
1.00	1	2	1	-	**1.00**	2	0	0	2
2.00	0	0	6	4	**2.00**	0	3	0	3
3.00	-	-	-	6	**3.00**	0	1	4	5
Total	1	2	7	10	Total	2	4	4	10

Kappa 0.6 p=.016 Kappa 0.844 p=0.0005

4.6.5. Reproducibility of Repeat Hemispheric Pixel Scoring

4.6.5.1. *Coefficient of variation for Reproducibility of Repeat Hemispheric Pixels*

The coefficient of variation was relatively high, especially for PTNP (Table 22)

Table 22 Coefficient of variation for hemispheric pixel measurements made for studies 1A and 1B on the same day

	n	Mean (%)	SD	Median (%)	Range
Right PTPP	29	18	17	13	0-83
Left PTPP	29	12	9	11	0-39
Right PTNP	26	87	48	88	0-141
Left PTNP	26	83	51	80	6-141
Right NTNP	29	13	13	11	0-57
Left NTNP	29	20	26	10	1-116

4.6.5.2. *Intraclass correlation coefficient for Reproducibility of Repeat Hemispheric Pixels*

Intraclass correlation coefficient analysis was used to analyse the reproducibility of repeat hBOLD-CVR hemispheric measures at one time point (1A and 1B) for each patient. Repeat hemispheric hBOLD MRI pixel measures were reliable for PTPP (good reliability; ICC .783 and .803 for right and left hemispheres respectively), left PTNP (good reliability .858) and NTNP (excellent reliability; ICC .910 and .889 for right and left hemispheres respectively). Repeat measures were less reliable for right PTNP (ICC .649) (Table 23).

Table 23 Intraclass Correlation of Repeat hBOLD MRI Measures of Hemispheric Pixels

CVR Pixels n=29	Timepoint		Intraclass	95% Confidence Interval	
	1A	1B	Correlation	Lower	Upper
	Mean (SD)	Mean (SD)			
PTPP					
R	.438 (.151)	.435 (.130)	.783	.534	.899
L	.563 (.146)	.563 (.130)	.803	.577	.908
PTNP					
R	.159 (.222)	.180 (.384)	.649	.245	.836
L	.162 (.277)	.14 (.294)	.858	.697	.933
NTNP					
R	.482 (.192)	.478 (.191)	.910	.807	.958
L	.457 (.189)	.462 (.18)	.889	.762	.948

4.6.5.3. Hemispheric Pixels Repeat Measures by hBOLD MRI Method

Six mixed effect repeated measures ANOVA were implemented, with time (1A, 1B) as the within-subject variable, and method (BH, GA) as the between-subject variable. Dependent variables for these analyses were right and left PTPP, right and left PTNP and right and left NTNP. There was no significant time*method interaction for any of the dependent variables.

The main effects of time and method were not significant for any of the dependent variables (all p values >.1). There were no significant differences in the hemispheric pixels either at different time points or by when using different hypercapnic challenge methods (Table 24).

Table 24 Repeat Measures of Hemispheric Pixels by hBOLD CVR Method

	General Anaesthetic (n=14)		Breath Hold (n=15)		t-test	
Method Timepoint	**1A**	**1B**	**1A**	**1B**	**1A**	**1B**
n=29	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	P	P
R PTPP	.455 (.163)	.446 (.162)	.423 (.143)	.424 (.096)	.581	.665
L PTPP	.550 (.155)	.554 (.162)	.575 (.141)	.571 (.10)	.652	.730
R PTNP	.151 (.235)	.070 (.101)	.167 (.218)	.284 (.511)	.854	.135†
L PTNP	.142 (.265)	.067 (.123)	.182 (.3)	.208 (.385)	.709	.202
R NTNP	.431 (.23)	.418 (.221)	.53 (.14)	.534 (.144)	.171†	.105†
L NTNP	.451 (.240)	.457 (.217)	.462 (.134)	.467 (.144)	.881	.893

4.6.5.4. Agreement between Right Repeat Hemispheric Pixels - Bland-Altman

As hemispheric pixel measures were reciprocal, Bland-Altman analysis was conducted for right hemispheric pixel measures only, by taking the difference in repeat measure hemispheric pixels (1A, 1B) and the average hemispheric pixel scores and plotting the values against each other. One sample t-test was conducted to compare mean repeat hemispheric pixel measures. Upper and lower confidence intervals were calculated as follows:

Upper interval: Mean difference + (standard deviation x 1.96)

Lower interval: Mean difference – (standard deviation x 1.96)

4.6.5.5. Right PTPP

There was no significant difference between 1A and 1B for RPTPP (p=.880). The mean difference was .0034 and standard deviation .120. Upper and lower confidence intervals were .239 and -.232 respectively. Linear regression analysis

demonstrated that there was no proportional bias in measures (t=1.006, p=.323) (Fig 29A).

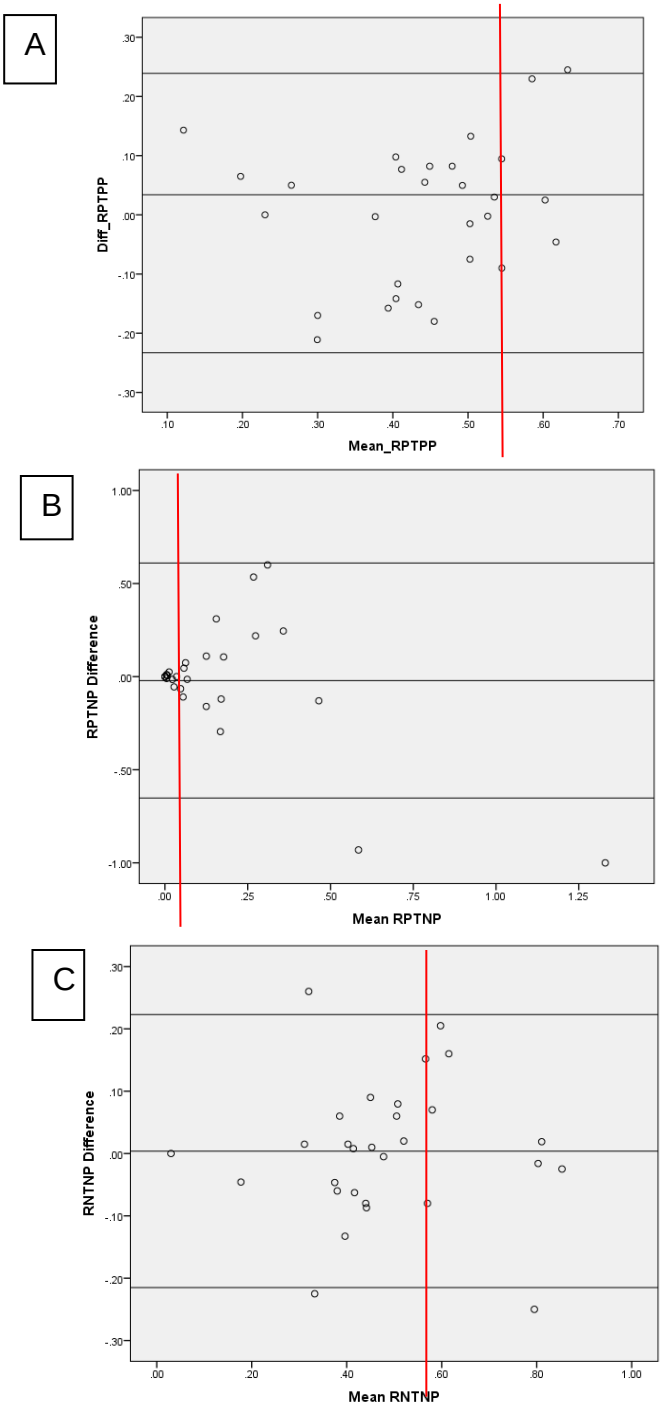
4.6.5.6. Right PTNP

Similarly, there was no significant difference between 1A and 1B for RPTNP (p=.727). The mean difference was -.0211 and standard deviation .322. Upper and lower confidence intervals were 0.60 and - 0.652 respectively. Linear regression analysis demonstrated proportional bias in measures (t -3.55, p=.001). Visual inspection suggests good agreement for repeat hemispheric measures when the mean PTNP lies between 0.00 and 0.125. The agreement above this level reduces as the mean difference in PTNP increases (Fig 29B).

4.6.5.7. Right NTNP

There was no significant difference between 1A and 1B for RNTNP (p=.859). The mean difference was .0037 and standard deviation .112. Upper and lower confidence intervals were 0.223 and - 0.215 respectively. Linear regression analysis demonstrated that there was no proportional bias in measures (t=.011, p=.991) (Fig 29C).

Figure 29 Bland-Altman plots for agreement between right hemispheric pixel measures. The central horizontal line represents a mean difference of zero, and the lines above and below are the 95% confidence intervals



4.6.5.8. hBOLD Cerebrovascular Reactivity

Twenty seven children had a spot CO2 with their first hBOLD CVR study and 14 children with a second hBOLD CVR study. All children who successfully underwent GA had a spot CO2. The mean spot CO2 for all the first available hBOLD CVR studies was 37.41, SD 5.116 mmHg. The mean spot CO2 for all the second available hBOLD CVR studies was 36.07, SD 3.931 mmHg. These values are not significantly different.

Six children (4 female, mean age first hBOLD CVR study 9.03, SD 4.657 years; mean age second hBOLD CVR study 9.06, SD 4.261 years) were identified with reliable serial CO2 measures, measurable CO2 change and BOLD MRI curves. Quantitative whole brain or hemispheric hBOLD CVR values were calculated for these using the following formula:

$$\% \Delta \text{ BOLD MRI signal} / \Delta PCO2$$

Right sided hBOLD CVR1 in those who had right sided moyamoya (five of six: four bilateral and one right sided) ranged from 0.23 - 0.50 %/mmHg, mean 0.408 %/mmHg. Left sided hBOLD CVR1 on the unaffected hemisphere (one right sided only) ranged from 0.39 - 0.80 %/mmHg, mean 0.6 %/mmHg.

Left sided hBOLD CVR1 in those who had left sided moyamoya (five of six, four bilateral and one left side only) ranged from 0.25 - 0.48 %/mmHg, mean 0.37 %/mmHg. hBOLD CVR 1 was not calculable pre-operatively on the unaffected hemisphere for the only patient with unilateral left sided moyamoya.

All second studies in this group were conducted following revascularization surgery so reproducibility data are not available. hBOLD CVR 2 was higher than CVR 1 in those that had a second study. Systematic evaluation of the effects of surgery on CVR are discussed in Chapter 7 (pre post). In the child with right sided MM hBOLD CVR was lower on the right than the left and improved following surgery. In the child with left sided moyamoya hBOLD CVR was lower on the left compared to the right.

Table 25 Calculable hBOLD Cerebrovascular Reactivity in 6 patients studied under GA with CO2 and Patient Demographics

Patient (M/F)	Idiopathic MM or Syndromic Diagnosis/ MM Laterality	Age at CVR 1	h BOLD Hemispheric Cerebrovascular Reactivity %/mmHg					Number of Surgeries (Surgery timing)	Stroke	Comments
		Age at CVR 2	W	R	L	R Ave	L Ave	a, b, n	a, b, n	
1 Female	Trisomy 21 Bilateral									
1A 1B		15.00	.26 .36	.31 .41	.23 .41	.39	.32	1(a)	b	1B better study
2A 2B		16.56	.72 .96	1.18 1.10	.57 .87	1.14	.72			
2 Female	Turner Syn Left									
2A 2B		6.61	.35 .44	.37 .50	.34 .42	.44	.38	1(b)	n	
3 Female	Idiopathic Bilateral									
1A 1B		7.50	.44 .45	.47 .50	.41 .43	.49	.42	2(b+a)	b	
2A 2B		8.13	.86 -	.89 -	.91 -	.89	.91			
4 Male	Idiopathic Bilateral									
1A 1B		7.17	.23 .39	.23 .23	.20 .33	.23	.27	1(a)	b	Poor signal change. Diamox. 1B better study

Patient (M/F)	Idiopathic MM or Syndromic Diagnosis/ MM Laterality	Age at CVR 1	h BOLD Hemispheric Cerebrovascular Reactivity %/mmHg					Number of Surgeries (Surgery timing)	Stroke	Comments
		Age at CVR 2	W	R	L	R Ave	L Ave	a, b, n	a, b, n	
5 Female	Idiopathic Right									
1A 1B		5.75	.42 .34	.46 .40	.39 .80	.43	.6	1(a)	n	
2A 2B		6.26	.59 .65	.50 .52	.59 .71	.51	.65			
6 Male	Chromosomal Bilateral									
1A 1B		14.67	.30 .44	.50 .50	.48 .48	.50	.48	N	b	

1A=first hBOLD CVR study of the first hBOLD CVR session; 1B=second hBOLD CVR study of the first hBOLD CVR session; 2A=first hBOLD CVR study of the second hBOLD CVR session; 2B=second hBOLD CVR study of the second hBOLD CVR session; W=whole brain, R=right hemisphere, L=left hemisphere; Ave=average; Timing of Revascularisation Surgery in Relation to hBOLD CVR study: a = after, b = before, n = no surgery; Timing of Stroke in Relation to hBOLD CVR study: a=after. b=before, n= no surgery.

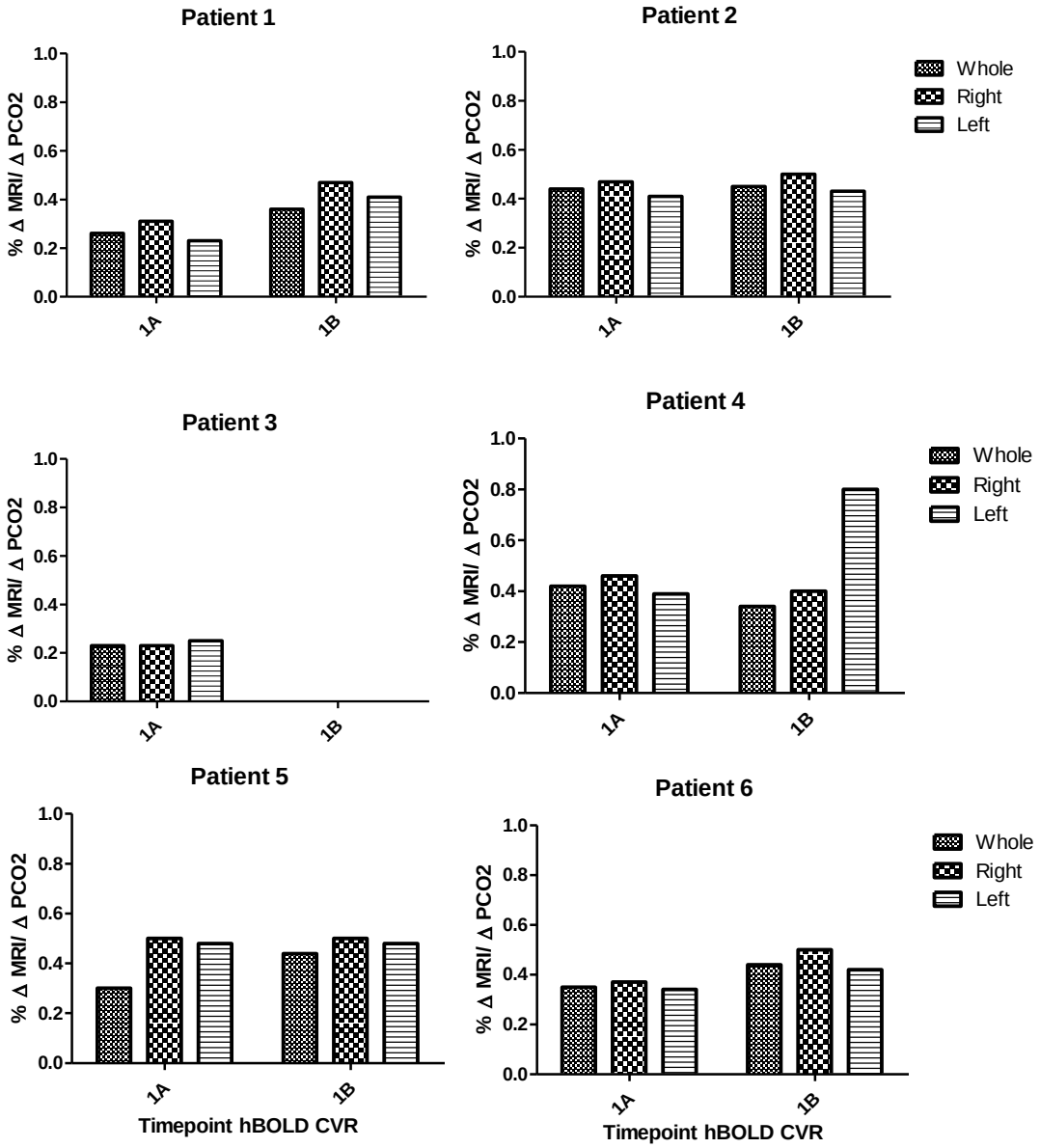
4.6.5.8.0. hBOLD CVR Repeat Measures

hBOLD CVR repeat measure means (1A and 1B, or when not available 2A and 2B) were correlated and compared using the paired t-test. There was a trend toward significant association between right repeat CVR measures. There was no statistically significant difference between the repeat measures. There was a trend toward significance in difference in whole brain and left hemisphere repeat CVR measures.

Table 26 hBOLD CVR Repeat Measure Comparison of Means

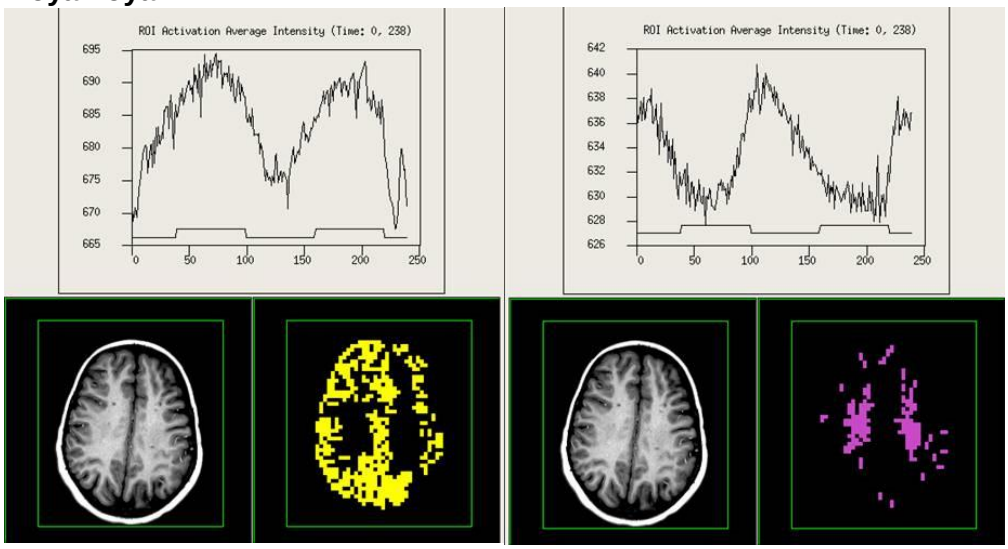
Hemisphere	h BOLD Cerebrovascular Reactivity %/mmHg					
	Correlation	p value	Mean Difference	SD	Confidence Intervals	paired t-test p value
Whole Brain	.172	.744	-.07	.09	-.164 - .024	.115†
Right	.778	.068†	-.033	.07	-.107 - .04	Ns
Left	.462	.357	-.137	.15	-.294 - .02	.076†

Figure 30 hBOLD CVR Values in Children with Moyamoya



1A=first hBOLD CVR study of first hBOLD CVR session; 1B=second hBOLD CVR study of first hBOLD CVR session

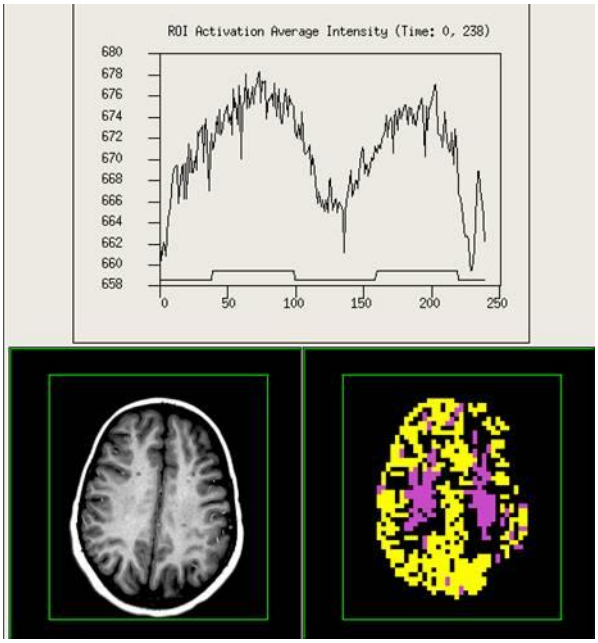
Figure 31 GA BOLD-CO2 fit curve and Parametric Maps in Child with Left Moyamoya



Whole brain T1 axial MRI at level above ventricle. ΔBOLD signal/Δ CO2 curves for positive pixels (yellow) and negative pixels (purple).

Left: BOLD-fit curve and positive pixel parametric map. Note reduced positive pixels (yellow) in the affected left hemisphere.

Right: BOLD-fit curve and negative pixel parametric map. Note presence of bilateral negative (purple) pixels.



Composite CVR parametric map showing areas of positive and negative reactivity. Note reduced positive reactivity in left hemisphere and bilateral steal.

4.7. Discussion

4.7.1. Patient Selection

The controlled delivery technique has utility in the older child, yet clinical applicability remains minimal due to set-up costs, time, requirement for ethical approval and - despite the older age group – tolerability. Furthermore the requirement for co-operation cannot be met by younger children, particularly those with challenging behaviors and developmental disorders.

In this study, almost an equal number of children had a study under general anaesthetic as had a breath-hold study. Only two children had studies using the rebreathing mask. These two children were adolescents and have been reported in the published literature (Heyn *et al.* 2010, Han *et al.* 2011). Hence this discussion will centre on the aforementioned general anaesthetic and breath-hold techniques.

Breath-holding and GA hBOLD CVR techniques are usable in the very young child to adulthood. Both methods use endogenous production of CO2 by breath-hold or ventilator simulation of apnea as the vasoactive stimulus. Use of these techniques allows for the assessment of CVR in age groups previously only amenable to assessment using techniques such as PET, SPECT and TCD. Children who had an hBOLD CVR study conducted under GA were significantly younger at diagnosis of MM and age of hBOLD CVR study and the youngest study was conducted at one year of age. This is in contrast to the youngest patient reported in current

literature aged 10 (Han *et al.* 2011) and 16 years (Mandell *et al.* 2008, Leung *et al.* 2015).

4.7.2. Reliability of Repeat Qualitative Measures of hBOLD CVR by Method

There are no published studies validating the use of ventilator induced hypercapnic challenge under GA in adults or children or validating the BH technique in children. There is one paediatric publication which discusses the feasibility and superior quality of GA studies in comparison to BH studies (Thomas *et al.* 2013). For a tool or technique to be valid it needs to measure what it sets out to measure. Construct validity is an overarching term used to assess the validity of a measurement tool or technique, for example in our case BH or GA hBOLD CVR, to measure a construct, for example tissue level microvascular health. It incorporates other forms of validity such as content validity, criterion validity, internal and external validity. To this end establishing construct validity is a process that requires testing of the other forms of validity in order for it to be demonstrated.

In our experience the use of these CVR methods has been informative and an important part of the decision making algorithm in this group of children.

Kappa scores demonstrating reliability of repeat scores by inspection for GA and BH good and poor quality studies were ‘fair’ to ‘almost perfect’ in the group with repeat studies on the same day. In studies conducted under GA, agreement between the right hemispheric repeat inspection scores was higher than for the left

hemispheric repeat inspection scores. The converse was true for the BH studies ie. Kappa scores were higher for the left hemispheric repeat measures than the right.

Using Kappa scores as the comparator, repeat measures for GA studies were not more reliable than BH repeat measures. However, as no one patient had two CVR studies using two different methods on the same day, and each hBOLD CVR by method group had different patients, direct comparison of reliability of method of analysis using test re-test analysis was not possible.

The BH study is clearly limited to patients who can understand and comply with instructions. As a more qualitative study, hBOLD CVR CVR studies are most useful for intra-subject analysis. The specifics of breath-hold execution have implications for the magnitude and reliability of the CVR response. A short breath-hold of 3 seconds is adequate to produce a BOLD response. However a longer breath-hold results in increased magnitude of effect because of the number of voxels exhibiting the response, and also more robust and repeatable measures of BOLD CVR. Good breath-hold practice guidelines for repeatable measures in healthy subjects include paced breathing between challenges and breath-holding after expiration (Bright and Murphy 2013).

Post processing of BH studies has to account for the hemodynamic response delay (HRD), which is incorporated into the analysis and based on assumptions of potential delay in CO2 increase and not on actual measurements of ETCO2. This is another potential source of error in the generation of quantitative and qualitative hBOLD CVR measures.

However, the applicability of these enhanced BH techniques in the younger child are limited. Hence our study reflects real world practice in this broad yet important age group. Of interest (Bright and Murphy 2013) set out to study the reproducibility of breath-hold CVR measures despite poor performance and demonstrated that with certain methods real world poor performance of BH can still result in reproducible results.

GA studies have the advantage of being usable in the very young and the uncooperative. GA studies were scored as being of better quality than BH as in a previous smaller series from the same centre (Thomas *et al.* 2013). The aim when changing the ventilator settings is to cause PETCO2 change of 7-10 torr. This was harder to achieve in our children on Diamox and Tegretol. This is presumably because of the independent effects of Diamox on PaC02 which are useful in the management of some children with moyamoya as the increase in PaCO2 results in a shift of the patient to the right on the autoregulatory curve. In some patients this may result in them reaching the peak of the vasodilatory capacity, resulting in an inability to further augment their CBF during hypercapnic stimulation.

4.7.3. Qualitative hBOLD CVR and Anaesthetic Agents

Cerebrovascular reactivity is affected by different anaesthetic agents. Propofol has cerebral vasoconstrictive properties and hence results in higher CVR in comparison to children anaesthetized using inhalational agents. The main anaesthetic agents used in our population were propofol and sevoflurane at

induction. Studies of the effects of various anaesthetics on middle cerebral artery blood flow and CVR have been conducted in healthy children using transcranial Doppler sonography (TCD). Although published literature on the effect of the combined agents on CVR is lacking and literature on their separate effects has to be interpreted with some caution because of the use of TCD to measure CVR, expected effects would be complementary and possibly allow for the truest measure of CVR in the individual. Propofol for neuroanaesthesia is well tolerated and CVR preserved in children as it is in adults. Its pharmacokinetic properties are desirable as they allow for rapid metabolic clearance and hence fast predictable recovery post anaesthetic which is essential in this group of patients. (Vandesteene *et al.* 1988, Rowney *et al.* 2002). This is contrast to the potentially undesirable cerebral vasodilatory effects of the volatile anaesthetic agents such as Sevoflurane (Matta *et al.* 1999, Fairgrieve *et al.* 2003, Karsli *et al.* 2003). The vasodilatory effect of volatile anaesthetics may result in the maximal cerebral vasodilatory response to increments in PET CO2 being achieved at an earlier stage resulting in a plateau effect at PETCO2 above 45mmHg. This in turn may result in less reliable assessment CVR, particularly at higher levels of PET CO2 and in comparison to propofol (Rowney *et al.* 2002). Furthermore there is a difference in magnitude of effect of specific volatile agents on CBF and hence CVR, with sevoflurane having the most potent effect when compared to isoflurane and halothane (Leon and Bissonnette 1991, Nishiyama *et al.* 1999). However, propofol has been shown to decrease cerebral metabolism and cerebral blood flow. This may have had an effect on absolute measures of CVR in our population.

Nitrous oxide in combination with propofol and sevoflurane was only used in one child. Nitrous oxide in anaesthetic and subanaesthetic doses (Dashdoj et al 2013) is known to increase CBF and CMRO2. When added to propofol or sevoflurane it does not affect CVR as measured by TCD in the normocapnic to hypercapnic range (Karsli *et al.* 2003, Wilson-Smith *et al.* 2003). It however does have an effect on CVR in the hypocapnic range when added to sevoflurane despite mean arterial pressures and heart rate remaining stable (Fairgrieve *et al.* 2003, Karsli *et al.* 2003).

Gender differences in CVR are described in adults; limited early studies in children have not demonstrated that for the hypercapnic challenge (Kassner *et al.* 2010, Siriussawakul *et al.* 2011).

4.7.4. Interobserver agreement of Scoring by 2 raters

Assessments of the quality of hBOLD CVR studies (Thomas *et al.* 2013) were made post-processing - at the time of scoring. Patient and hBOLD method factors were noted such as (when available) ΔCO2, hBOLD signal/ΔCO2 curve, motion artefact and other specific patient factors such as medication taken at time of hBOLD CVR study. Cohen's Kappa is an inter-rater agreement analysis that controls for the agreement expected by chance alone.

The inter-rater reliability of the expert raters measured by Kappa was excellent, a pre-requisite for using the technique clinically.

4.7.5. Repeat Measure Hemispheric Inspection Scores

Kappa calculated for all available repeat measure scores were generally fair to moderate. However, when calculated for studies assessed to be of good or reliable quality, Kappa scores were good to excellent. However, the repeat scores were least reliably discriminatory between inspection score 2 and 3. These scores were not sensitive enough to reliably discriminate between the abnormal studies where 2 represents reduced positive reactivity and 3 significant steal.

Despite the limitations of the GA and BH technique, the good interobserver reliability and reproducibility of the studies scored simply as 1, 2 and 3 is reassuring and warrants further study into improving the use of these techniques. This has important implications for the translation and incorporation of the results of hBOLD CVR studies into clinical practice.

4.7.6. Repeat Measure Hemispheric Pixels

Intraclass correlation is usually used to look for differences in measures between two different techniques. Like coefficient of variation, it can also be used to assess agreement between measures. Intraclass correlation was good to excellent for repeat hemispheric pixel measures, although the correlation was less for PTNP and the coefficient of variation was unacceptably high for this measure for both left and right hemispheres. Since high correlation may be explained by similar

variance, it does not necessarily imply a high level of agreement. Hence Bland-Altman analysis was used to investigate agreement between studies, any systematic difference between measurements and identify possible outliers. There was good agreement between the repeat hemispheric pixel measures except for PTNP. There was good to excellent level of reliability and agreement despite the inclusion of poor quality studies. The methodological difference in calculation of PTNP are likely to be the explanation for the wide confidence intervals and systematic bias seen for this measure, which is in line with the unacceptable coefficient of variability and worse intraclass correlation and means that PTNP is not likely to be useful clinically.

4.7.7. Quantitative Measures of hBOLD CVR

Quantitative expression of CVR requires knowledge of the change in $P_{ET}CO2$ which is not routinely measured in breath-hold studies, hence the limitation in applicability. We were able to calculate CVR measures in a small group of children who had GA studies. Direct CVR measures were not calculable for studies done by BH.

There is limited published data on CVR values in children. In a study comparing BOLD CVR to ASL CVR (Mandell *et al.* 2008) reported CVR measures in patients with arterial steno-occlusive disease. hBOLD CVR values in the younger (< 30 years) patients ranged from -.02 to .23 in the affected hemisphere of those with moyamoya, .37 in the unaffected hemisphere and .51 to 0.52 in a child with

subclavian steal syndrome. In our group, hBOLD CVR measures in the affected hemisphere ranged from .23 to .50 and .37 to .80 in the unaffected hemisphere.

Currently there are no qualitative CVR control data for healthy children, nor children with syndromic diagnosis commonly seen in moyamoya such as Sickle Cell Disease and NF1. However our data are promising in as much as much as support the use of hypercapnic challenge methods that are more tolerable and hence available to the child or infant affected by moyamoya.

4.8. Study Limitations and Future Directions

Future directions could include:

i) **Test-retest method agreement analysis** – this is standard practice in the comparative validation of different tools used to measure the same variable. BH and RM hBOLD CVR studies could be done in the same healthy patients with a brief 2-4 minute interval (Winter *et al.* 2010).

ii) **Anaesthetic agents for GA studies** – guidelines to improve uniformity in anaesthetic use would enable more reliable interpretation of CVR and BOLD data

iii) **Breath-hold studies** – use of forced expiration at the end of a breath-hold would enable quantitative assessment of CVR in more children In addition use of techniques known to improve reliability and repeatability such as paced breathing and end expiratory breath-holding,

measurement of end tidal CO2 and use of respiratory bellows or respiratory belt could help improve reliability of time course and quantitative CVR assessment, as the expired CO2 could be measured and used to quantify CVR, using the formula Δ CO2/Δ BOLD MRI..

iv) **Real-time analysis** – will reduce the time to reporting of results allowing for verification of data quality whilst the patient is in the scanner.

v) **Scoring by inspection** – this technique could be further explored, perhaps with a more discriminatory scoring system. However, the simple scoring system has good interobserver reliability, perhaps because of the few options for the raters to choose from, and currently has the greatest potential for clinical use as it appears to be reliably scored and reproducible but is not very labour intensive.

vi) **Longitudinal qualitative** and quantitative use of vailidated hBOLD CVR techniques in moyamoya arteriopathy and syndromic moyamoya arteriopathy studies.

4.9. Conclusion

Qualitiative CVR using breath-hold and general anaesthetic as the hypercapnic stimulus is feasible in paediatric practice, reliable and repeatable. Raters can also be trained to score CVR inspection maps reliably. At present, counting of hemispheric pixels does not appear to be worth the additional time as the reproducibility is not as good. However, quantitation of the change in CO2 as well as the change in hBOLD during GA studies might allow reliable and reproducible

measurement of CVR for comparison with studies using rebreathing techniques in older children and adults and for use in longitudinal studies of children with cerebrovascular disease.

5. Population hBOLD CVR Measures

5.1. Abstract

5.1.1. Background

Cerebrovascular reactivity (CVR) is an indicator of cerebrovascular reserve and a biomarker of cerebrovascular health and ischaemic risk. Adult studies of CVR using BOLD CVR are conducted in the awake state and require co-operation. However, diagnosis in moyamoya is often made in infancy and confers a lifelong risk of cerebral ischaemia, neurocognitive decline and stroke. hBOLD CVR using breath-hold or general anaesthetic as the hypercapnic challenge have the potential for use as a biomarker of ischaemic risk in this group. Yet reports of the use of these modalities remain scarce in the paediatric literature.

5.1.2. Objective

To describe the qualitative hypercapnic challenge BOLD (hBOLD CVR) measures of all the children enrolled in the study.

5.1.3. Method

Children with cerebral arteriopathy (moyamoya and non-moyamoya) were enrolled and had hBOLD CVR studies in addition to MRI/MRA brain and conventional angiography (See Population Ascertainment Chapter 3). Children had qualitative hypercapnic challenge with endogenous delivery of carbon dioxide (breath-hold and general anaesthetic) and quantitative hypercapnic challenge with exogenous

delivery of carbon dioxide (RespirAct, re-breathing mask) hBOLD CVR studies (See Method Chapter 2).

Measures of hBOLD CVR are presented in the moyamoya and non-moyamoya groups.

5.1.4. Results

Forty-seven children with arteriopathy had at least one hBOLD CVR study during the course of the study. Thirty seven had moyamoya arteriopathy, ten non-moyamoya arteriopathy (four Bilateral Non-moyamoya (Non-MM) arteriopathy and six Unilateral Non-moyamoya (Non-MM) arteriopathy. The mean age of first hBOLD CVR study was 10.4 years. The youngest child to have an hBOLD CVR study was one year of age. This study was conducted under general anaesthetic.

Mean hBOLD CVR inspection scores were higher in the moyamoya arteriopathy group compared to the non-moyamoya arteriopathy groups, suggesting more impaired hBOLD CVR and tissue level compromise. Within the moyamoya arteriopathy group, qualitative measures of hBOLD CVR were suggestive of greatest impairment and tissue level compromise in the Tumour-MM and Chromosomal group.

5.1.5. Conclusion

To my knowledge this is the largest series to describe qualitative hBOLD CVR findings in a population of children with moyamoya and non-moyamoya arteriopathy. I demonstrate that qualitative hBOLD CVR studies can be successfully conducted in children as young as one year and that those with more

severe cerebrovascular disease, i.e. moyamoya, have more compromise of the CVR. Furthermore there appear to be disease specific differences in generation of BOLD MRI signal and hence CVR. This requires further exploration in future studies.

5.2. Introduction

Quantitative BOLD MRI may be used to assess changes in blood flow by harnessing the paramagnetic properties of deoxygenated haemoglobin. Cerebrovascular reactivity (CVR) is the measurement of vessel reactivity to vasoactive stimuli such as exogenous drugs (eg acetazolamide) or changing arterial CO2 tension, and is defined as the change in blood flow per unit change in PCO2. It is an important marker for brain vascular reserve and the ability to augment and maintain CBF under conditions of stress. Cerebrovascular reactivity studies are the brain equivalent of a cardiac stress test. CVR in adults has been shown to predict ischaemic risk and impaired CVR is associated with conditions of cognitive decline such as Alzheimer's disease (Silvestrini *et al.* 2000, Markus and Cullinane 2001, Gupta *et al.* 2012, Marshall *et al.* 2012, Reinhard *et al.* 2014). Hence impaired CVR is an indicator of tissue at risk of ischaemia.

Breath-holding is a relatively well tolerated technique. There is no need for mask ventilation, making the experience within the scanner less claustrophobic and obviating the need for an anaesthetist. It however, requires co-operation from the child as it is conducted in the awake state.

In our group the youngest age of diagnosis of moyamoya was 6 months. hBOLD CVR studies conducted under GA is a previously unreported yet feasible method for the assessment of CVR in the very young, and/or those unable to tolerate alternative awake methods.

A barrier to the widespread use of BH qualitative hBOLD CVR in adult clinical practice and research has been the lack of end tidal CO2 measures which are required for quantitation of CVR. This was the case in my population. Inferences and qualitative reports could be made from the inspection of individual hBOLD CVR parametric maps based on colour-coded direction and relative magnitude of flow. Therefore, on a case by case basis, CVR was reported as demonstrating: normal (positive) reactivity (score 1), less than normal (positive) reactivity without steal or with steal up to 10% (score 2) and abnormal reactivity (negative with steal>10%) (score 3). The scoring system was designed to capture this in a quantifiable manner and hence serve as indirect measures of hBOLD CVR in this study (Chapter 2 Methods Scoring).

5.3. Study Purpose and Hypothesis

In this chapter I describe qualitative and quantitative hBOLD CVR measures in a population of children with moyamoya and non-moyamoya arteriopathy, and explore potential reasons for observed differences.

5.4. Methods

Children with arteriopathy and clinical hBOLD CVR studies were prospectively and retrospectively enrolled through the Children's Stroke Programme Clinic and using the Stroke Registry Database (Chapter 3 Population). Children had qualitative (breath-hold and general anaesthetic) hBOLD CVR studies and a few had quantitative hBOLD CVR studies (Chapter 2 Methods, Chapter 3 Population Attainment, Chapter 4 Validation).

Positive and negative threshold hemispheric pixels of the hBOLD CVR parametric map in the first axial slice above the ventricles were counted as previously described (Chapter 2 Methods).

hBOLD CVR hemispheric parametric maps in the same - first axial slice - above the ventricles were scored. As the pixel number was set to 100 in the slice above the ventricle, and the scoring was of raw (unsmoothed) data, it was possible to count individual pixels. The scoring system was devised by two raters (See Chapter 2 Methods). Scores for each hemisphere were between 1 and 3 according to the following schema:

- Normal CVR = 1
- Reduced positive reactivity +/- steal >2%<10% = 2
- Steal > 10% with or without reduced positive reactivity = 3

5.5. Statistics

One way ANOVA and the T-test were used to compare means where the data were normally distributed, and Kruskal-Wallis and Mann-Whitney U test used to compare medians where the data were not normally distributed. Categorical data were analysed using Fisher's exact test. A significance level of $p<.05$ was accepted.

5.6. Results

One hundred and nine hBOLD CVR studies of 218 hemispheres were conducted in total. Forty-seven children with moyamoya (37) and non-moyamoya (10) arteriopathy had at least one hBOLD CVR study.

5.6.1. Population

The first GA or BH hBOLD CVR study of each patient was included for analysis. For 2 patients this resulted in inclusion of the second and fourth hBOLD CVR studies respectively, as earlier studies used rebreathing with quantitative CVR measurement and hence had not been analysed using pixel analysis.

The mean age at time of first hBOLD CVR study was 10.4 (SD 4.5; range 1-17.7) years in the whole arteriopathy (n=47) group. There was a statistically significant difference in age of first CVR between arteriopathy groups (Kruskal-Wallis; $\chi^2(2) = 6.5$, $p = 0.04$). On post-hoc testing, there was a statistically significant difference in

age at first CVR between the Bilateral Non-MM arteriopathy group, Moyamoya (Mann-Witney *U* = 22, p=.02) and Unilateral Non-MM (*U*=1, p=.03) groups respectively. There was no statistically significant difference age at first CVR between the Moyamoya and Unilateral-Non-MM arteriopathy groups (*U*=68, p>.05).

5.6.2. Qualitative hBOLD CVR Measures by Arteriopathy Classification

5.6.2.1. Hemispheric pixels

Hemispheric pixels were available for analysis in 37/37 of the Moyamoya, 3/4 of the Bilateral Non-MM arteriopathy and 0/6 of the Unilateral Non-MM groups as these studies were conducted using the re-breathing mask (RespirAct). There was no significant difference between the mean PTPP hemispheric pixels by arteriopathy. Negative pixels (by positive and negative thresholds) were higher in the Moyamoya arteriopathy group. Mann-Whitney U Test demonstrated a trend toward significant difference in right and left PTNP, and right NTNP respectively (*U*=22.5, p=.093; *U*=21.00, p=.082; *U*=28.00, p=.172) (Table 26).

5.6.2.2. Asymmetry Indices

Asymmetry indices (AI) were calculated for the arteriopathy groups and plotted (Chapter 2 Methods Asymmetry Index). On inspection of the AI hemispheric plots, both Moyamoya and Bilateral Non-MM groups were predominantly right sided.

There was no statistically significant difference in mean AI (difference in hemispheric pixels/total pixels Ref Methods) for PTPP, PTNP or NTNP pixels between all three groups *U*=52, *U*=48, *U*=43 respectively, p>0.05 (Chapter 3 Clinical and Radiographic Description of Arteriopathy Groups, Table 26 and Figure 28).

5.6.2.3. Hemispheric Scores by Inspection

Mean hemispheric scores were higher in the Moyamoya arteriopathy group than the Bilateral Non-MM and Unilateral Non-MM groups. There was a trend toward significant difference in mean scores between the arteriopathy groups (Kruskal-Wallis; $\chi^2(2) = 4.34$, p=.10 and $\chi2(2) = 3.91$, p=.16 for right and left hemispheric scores respectively). On inspection the biggest difference in mean scores was between the Moyamoya and Bilateral Non-MM group scores (Table 27 and Figure 32).

Figure 32 Asymmetry Indices by Arteriopathy

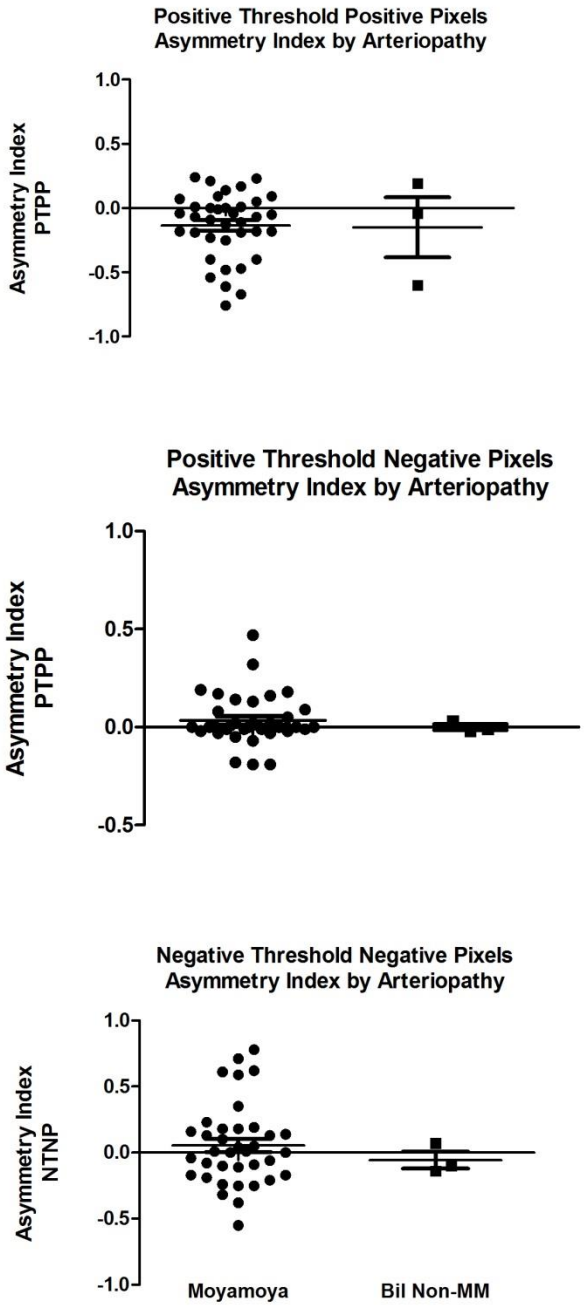


Figure 33 Mean Hemispheric Scores by Arteriopathy

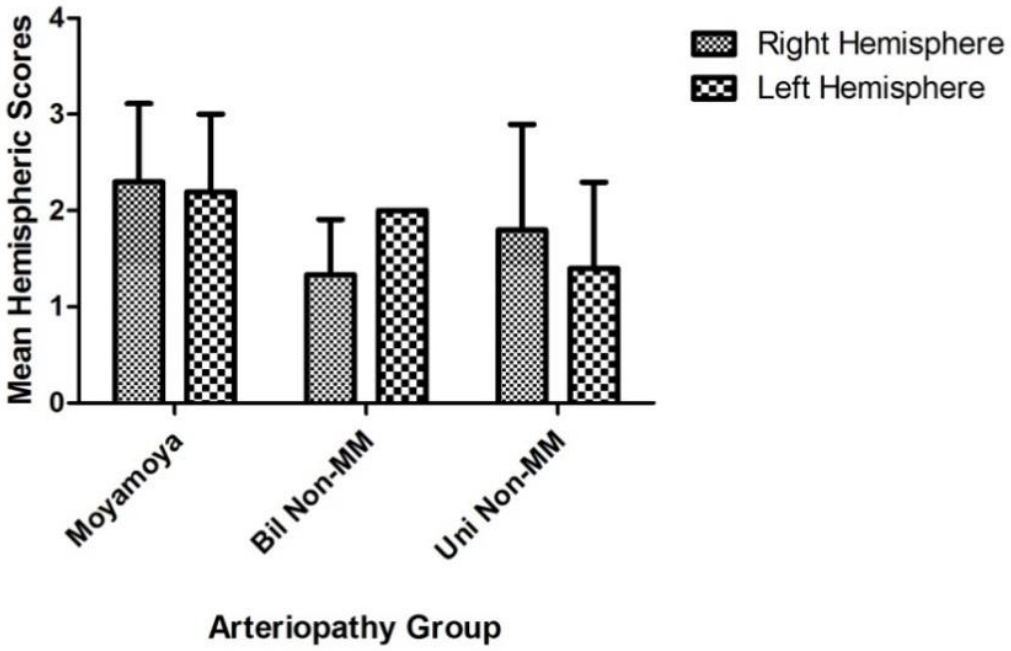


Table 27 Qualitative hBOLD CVR Measures by Arteriopathy

	Moyamoya arteriopathy n=37 (%)	Bilateral non moyamoya arteriopathy n= 4 (%)	Unilateral non-moyamoya arteriopathy n=6 (%)
Arteriopathy Laterality Bilateral Right Left	 23 (62) 6 (16) 8 (22)	 4 (0) 0 (0) 0 (0)	 0 2 (33) 4 (66)
CVR Method	18 GA 19 BH	3 GA 1 BH	6 RM
CVR Parametric Maps Steal (Yes/No) % PTPP (mean, range) Right Left PTNP Right Left NTNP Right Left	mean (range) .434 (.12 - .63) .571 (.38 - .90) .193 (.00 - 1.33) .159 (.00 – 1.32) .504 (.03 - .89) .450 (.03 - .73)	mean (range) .43 (.2-.6) .58 (.4-.8) .013 (.01-.03) .012 (.00-.03) .38 (.29-.43) .44 (.37-.58)	 N/A
Asymmetry Index PTPP PTNP NTNP	mean (range) -.134 (-.76 - .24) .033 (-.19 - .47) .054 (-.55 - .78)	mean (range) -.15(-.6 - .19) .002(-.02- 03) -.06(-.14-.07)	 N/A
CVR Hemispheric Inspection Scores (Total number of hemispheres = 92) 1 2 3	 Total (R:L) 17 (8:9) 22 (10:12) 35 (19:16)	 2 (1:1) 4 (2:2) 1 failed BH	 9 (4:5) 0 3 (2:1)
Mean scores (SD)	2.3 (.81)	1.33 (.58)	1.8 (1.1)

5.6.3. Qualitative hBOLD CVR Measures by Moyamoya Classification

Moyamoya arteriopathy was classified as idiopathic (MMD) or syndromic (MMS). Syndromic categories were Neurofibromatosis Type 1 (NF1-MM), Sickle Cell

Disease (SCD-MM), Trisomy 21 (Trisomy 21-MM), All chromosomal (All Chromosomal-MM) and Tumours (Tumour-MM) (Table 28).

5.6.3.1. Hemispheric Pixels and Aetiology

Mean right and left hemispheric PTPP were lowest in the Tumour-MM and NF1-MM groups respectively. Mean right and left hemispheric PTNP were highest in the Tumour-MM and SCD-MM groups respectively. Mean right hemisphere NTNP was highest in the Tumour-MM and mean left hemisphere NTNP was highest in the SCD-MM and NF1-MM groups respectively (Table 28 and Figure 34).

Kruskal-Wallis analysis demonstrated that right and left PTPP were significantly affected by comorbidity ($\chi2(4) = 11.69$, $p = .020$; $\chi2(4) = 13.35$, $p = .01$). Left NTNP had a trend toward being significantly affected by comorbidity $\chi2(4) = 8.0$, $p = .092$. Right and left PTNP and right NTNP were not significantly affected by comorbidity $\chi2(4) = 1.39$, $p = .846$; $\chi2(4) = 4.29$, $p = .368$; $\chi2(4) = 4.73$, $p = .316$ (Table 28 and Figure 34).

Table 28 Hemispheric Pixels by MM Arteriopathy Group

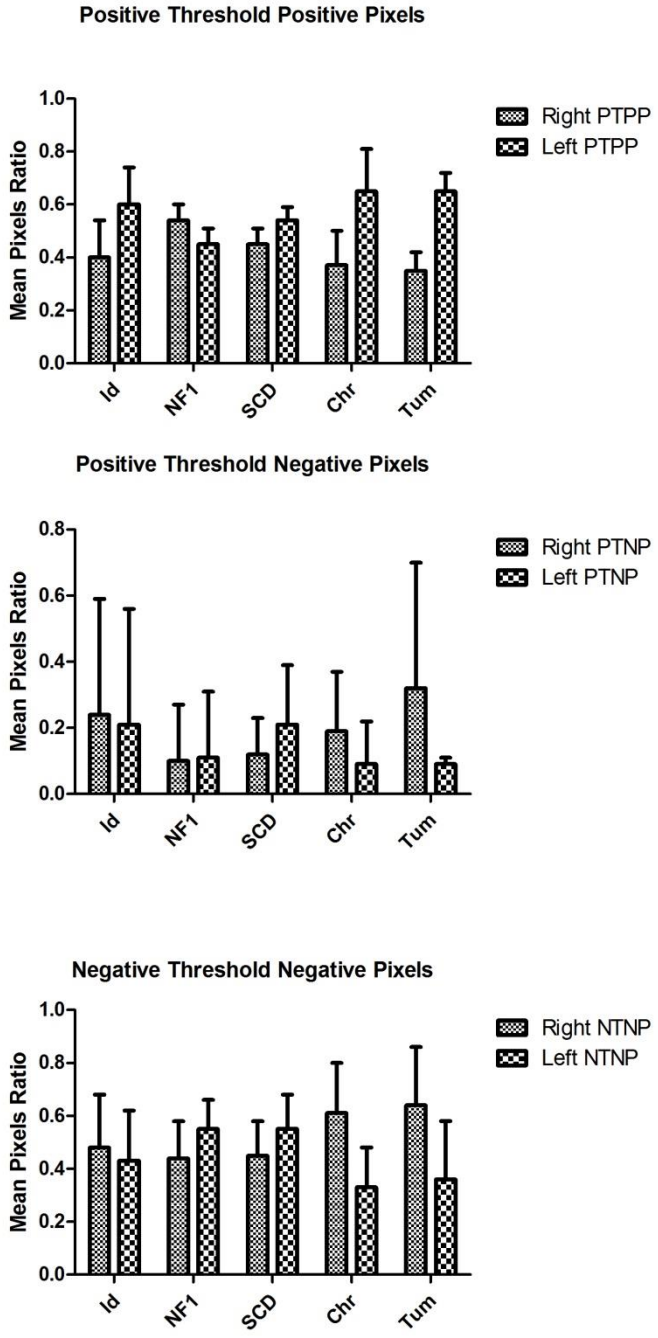
hBOLD CVR Pixels	Idiopathic n=13	Neurofibromatosis Type 1 n=9	Sickle Cell Disease n=5	All Chromosomal n=7	Tumour n=2	
Age at CVR	10.4	10.3	9.7	11.5	14.1	
Method						
BH	7	5	3	1	2	
GA	6	3	2	2	0	
RM	1	1	0	0	0	
Pixels	mean (SD)	mean (SD)	mean (SD)	mean (SD)	mean (SD)	
PTPP R	.40 (.14)	.54 (.06)	.45 (.06)	.37 (.13)	.35 (.07)	.02
L	.60 (.14)	.45 (.06)	.54 (.05)	.65 (.16)	.65 (.07)	.01
PTNP R	.24 (.35)	.1 (.17)	.12 (.11)	.19 (.18)	.32 (.38)	.846
L	.21 (.35)	.11 (.2)	.21 (.18)	.09 (.13)	.09 (.02)	.368
NTNP R	.48 (.20)	.44 (.14)	.45 (.13)	.61 (.19)	.64 (.22)	.316
L	.43 (.19)	.55 (.11)	.55 (.13)	.33 (.15)	.36 (.22)	.092

hBOLD CVR Pixels	Idiopathic n=13	Neurofibromatosis Type 1 n=9	Sickle Cell Disease n=5	All Chromosomal n=7	Tumour n=2	
Asymmetry Index	mean (SD)	mean (SD)	mean (SD)	mean (SD)	mean (SD)	x^2 (4)
PTPP	-.209 (.28)	.084 (.11)	-.091 (.09)	-.284 (.28)	-.3 (.15)	12.36*
PTNP	.02 (.09)	.018 (.07)	.10 (.12)	-.077 (.10)	.22 (.36)	7.37**
NTNP	.045 (.3)	-.046 (.24)	.284 (.31)	-.152 (.24)	.27 (.45)	7.55**
MM Laterality						
Bilateral	11	2	5	4	2	
Right	2	2	0	2	-	
Left	1	5	0	1	-	
Mean Hemispheric Pixel Scores	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
R	2.36 (.84)	1.89 (.93)	2.8 (.45)	2.43 (.79)	2.0 (.0)	
L	2.14 (.86)	2.44 (.73)	2.6 (.55)	1.86 (.9)	1.5 (.71)	

* significant at p<0.05

** trend toward significant at p</= .2

Figure 34 Mean Hemispheric Pixels by Moyamoya Arteriopathy Classification



Error bars represent standard deviation

5.6.3.2. Hemispheric Pixels and Asymmetry Indices

Analysis was repeated using asymmetry index. Kruskal-Wallis analysis demonstrated a statistically significant difference in PTPP and trend toward significance for PTNP and NTNP asymmetry index between the different comorbidities ($\chi^2(4) = 10.5$, $p = 0.021$; $\chi^2(4) = 8$, $p = 0.09$; $\chi^2(4) = 7.75$, $p = 0.1$) (Figure 35).

Following visual inspection of the graphs independent group comparisons of CVR asymmetry index was conducted using the Mann-Whitney test.

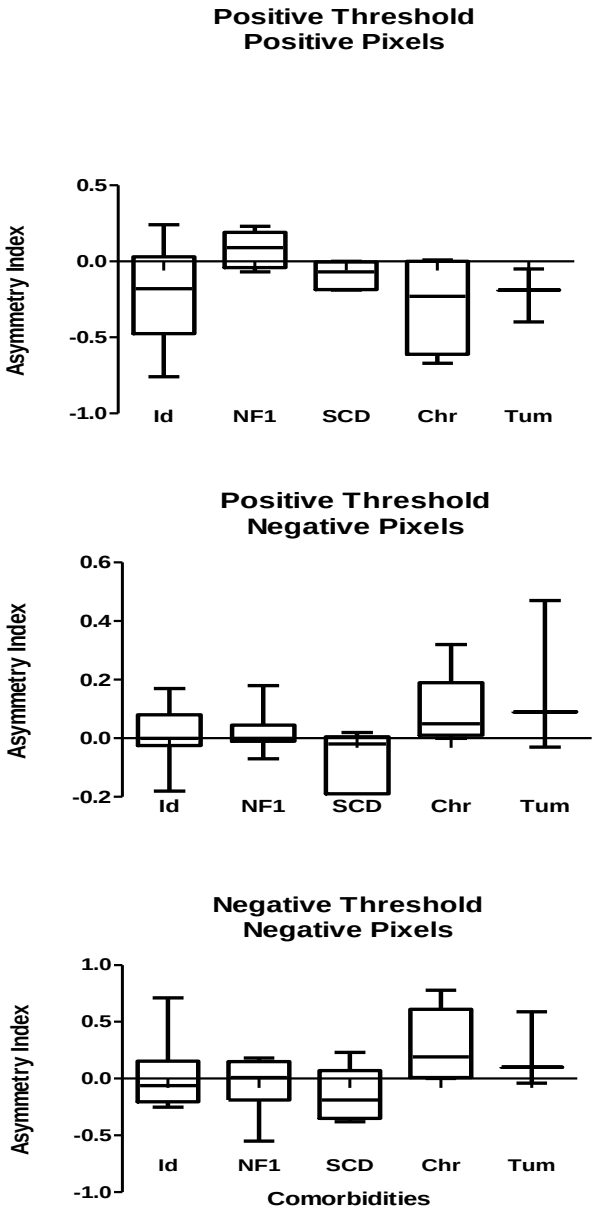
There was a statistically significant difference in PTPP AI between the NF1-MM and Idiopathic groups (U=21, p=0.007); PTPP and PTNP AI between the NF1-MM and chromosomal-MM groups (U=6, p=.007, U=11.5, p=.034); and a trend toward significant difference between the NF1-MM and chromosomal group-MM for NTNP (U=14, p=.064).

There was a statistically significant difference in PTPP AI between the SCD-MM and NF1-MM group (U=7, p=.04), Tumour-MM and NF1-MM group (U=0, p=.036); a statistically significant difference in PTNP and NTNP AI between the SCD-MM and chromosomal-MM group (U=3, p=.02, U=4, p=.03) respectively; and a trend toward significant difference in PTNP AI between the SCD and NF1-MM group, PTNP and NTNP AI between the SCD-MM and Idiopathic group (U=19, p=.16, U=20, p=.19).

NF1-MM AI demonstrated left hemispheric tissue level functional impairment (positive PTPP AI and negative NTNP AI). hBOLD CVR functional impairment was

right sided in the MMD, SCD-MM and Tumour-MM groups. Laterality of functional impairment was mixed (right on assessment of PTPP AI and left of NTNP AI) in the All Chromosomal group.

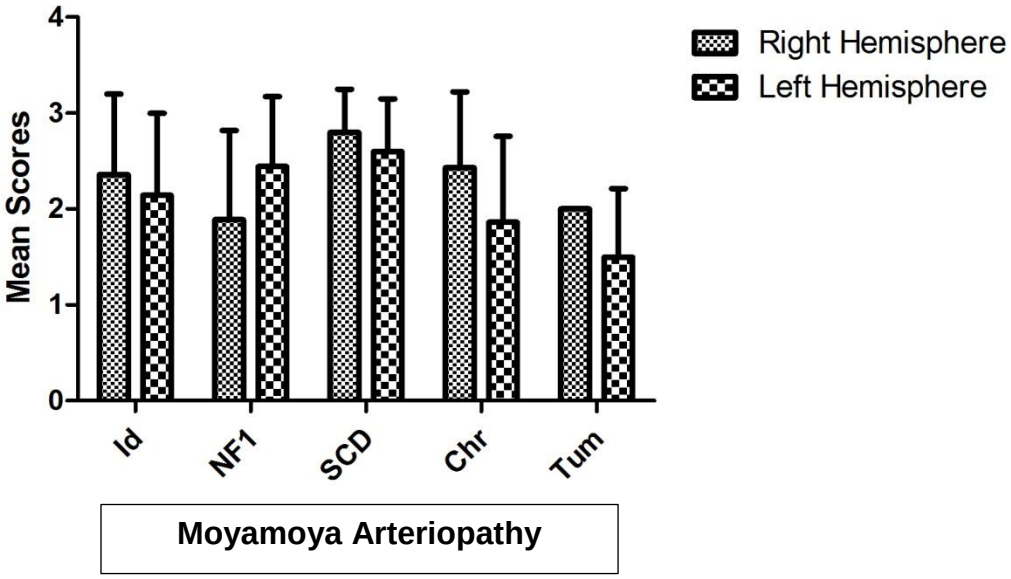
Figure 35 Comorbidity Pixel Asymmetry Index Id Idiopathic NF-1 Neurofibromatosis SCD Sickle cell disease Chr Chromosomal Tum Tumour



5.6.3.3. Hemispheric Pixels and Inspection Scores

Using the Kruskal-Wallis test there was no significant difference in mean inspection scores by comorbidiity ($\chi 2$ (4) = 4.79, p=.32; $\chi 2$ (4) = 4.66, p=.337 for right and left hemispheres respectively; Figure 36).

Figure 36 Mean Hemispheric Scores by Moyamoya Arteriopathy Classification



5.6.4. Population by hBOLD CVR Method

For this analysis the first hBOLD CVR (including RM where applicable) was included. Of the 37 children, the first available hBOLD CVR studies were under GA in 18, using BH in 17 and RM in two. Age at MM diagnosis was significantly lower in children who had a GA study when compared to children who had a BH or RM study (Kruskal-Wallis; χ^2 (2) = 11.61, p = .003; Figure 36; Table 29). Similarly there was a trend toward a lower age at first hBOLD CVR in children with GA studies when compared to children with BH studies (Figure 36; Table 29). The

two children who had RM studies were older at diagnosis and at first hBOLD CVR study. Mean right NTNP and right hemispheric inspection measures of hBOLD CVR had a trend toward being significantly higher in the BH group (Table 29 and Figure 39).

Figure 37 Age at Moyamoya Diagnosis by hBOLD CVR Method

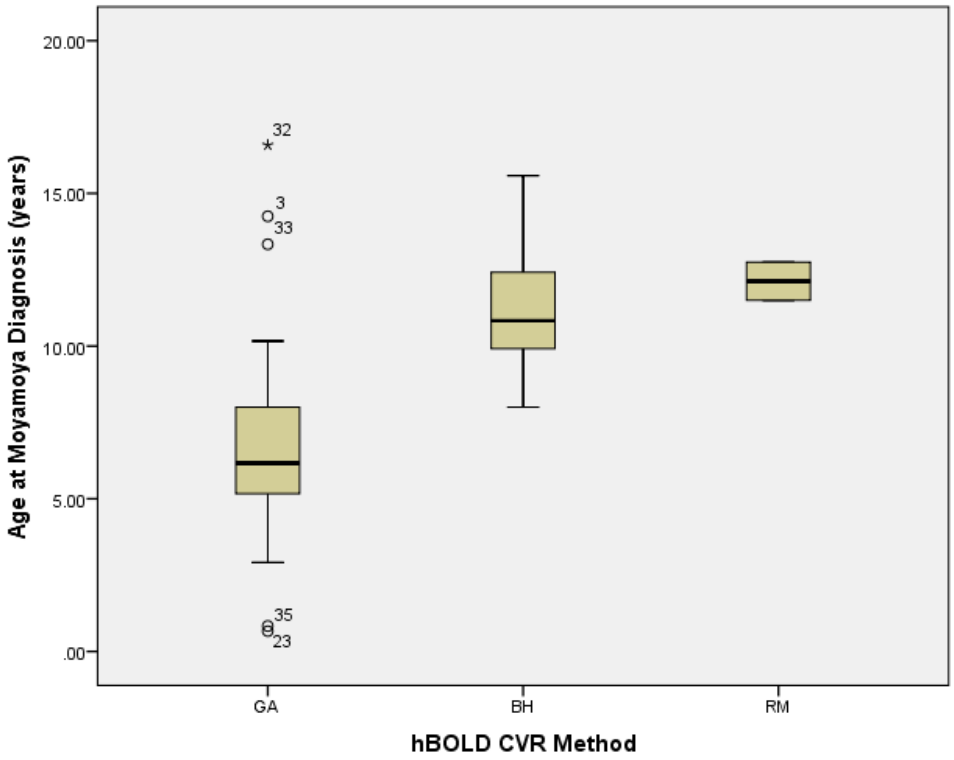


Table 29 Population by hBOLD CVR Method

	General Anaesthetic	Breath hold	Rebreath Mask	t-test
N	18	17	2	
Age at Diagnosis Mean (SD;range)	7.0 (4.3; .67-16.58)	11.03 (2.07; 8-15.58)	12.13 (.88; 11.5-12.75)	p=.003*
Gender M (%)	8 (44)	7 (41)	1(50)	p=.96
Age at hBOLD CVR Mean (range)	8.5 (1-17.67)	12.8 (8.08-17.08)	12.46 (12-12.2)	p=0.008*
MM Stage	n	N	n	
1	1	1	-	
2	7	3	2	
3	8	8	-	
4	0	2	-	
5	1	0	-	
6	0	1	-	
Stroke (%)	12 (67)	8 (47)	1(50)	
R/L/B (%)	36/18/12	27/11/11	0/0/100	
Mean Hemispheric Pixels	mean (SD)	mean (SD)	mean (SD)	
PTPP R	.43 (.151)	.44 (.09)	N/A	p>.05
L	.58 (.161)	.56 (.09)		p>.05
PTNP R	.15 (.167)	.18 (.17)		p>.05
L	.13 (.165)	.13 (.13)		p>.05
NTNP R	.46 (.224)	.56 (.121)		p=.123*
L	.45 (.214)	.45 (.117)		p>.05
Asymmetry Index	mean (SD)	mean (SD)	mean (SD)	
PTPP	-.15 (.31)	-.12 (.176)	N/A	p>.05
PTNP	.016 (.132)	.05 (.129)		p>.05
NTNP	.012 (.368)	.11 (.237		p>.05
Mean Hemispheric Scores	mean (SD) 2.06 (.938) 2.06 (.873)	mean (SD) 2.59 (.618) 2.24 (.752)	mean (SD) 2 3	p=.057† p>.05

Figure 38 Age at First hBOLD CVR by hBOLD CVR Method

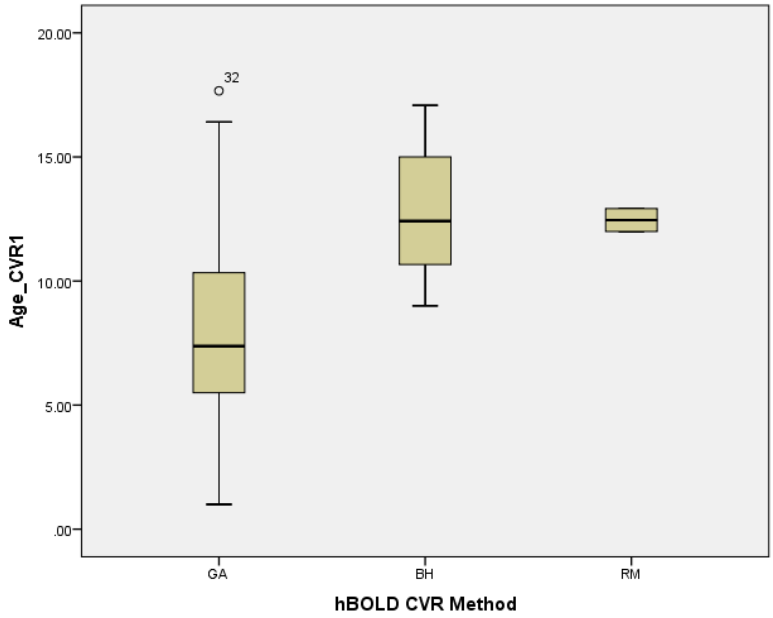
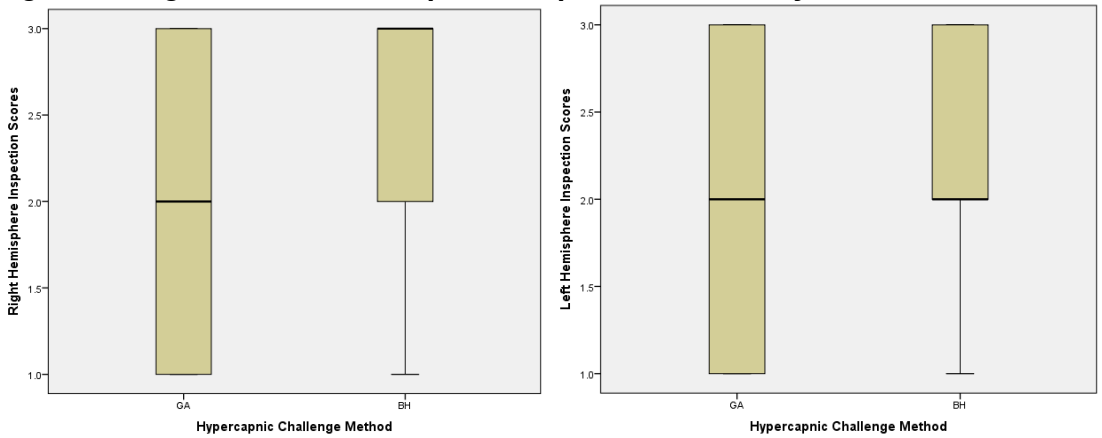


Figure 39 Right and Left Hemisphere Inspection Scores by hBOLD CVR Method



5.7. Discussion

5.7.1. Group Description

There are a number of published studies validating quantitative MRI CVR assessments of cerebral blood flow using hypercapnia as a vasoactive stimulus in adults (Ito *et al.* 2008, Bright and Murphy 2013, Tancredi and Hoge 2013) (Mikulis *et al.* 2005). However reports in children are limited and mostly refer to the use of controlled delivery of CO2 (Kirton *et al.* 2008, Han *et al.* 2011) (Thomas *et al.* 2013, Leung *et al.* 2015).

5.7.2. hBOLD CVR Measures

i) Moyamoya, Bilateral Non-MM and Unilateral Non-MM arteriopathy

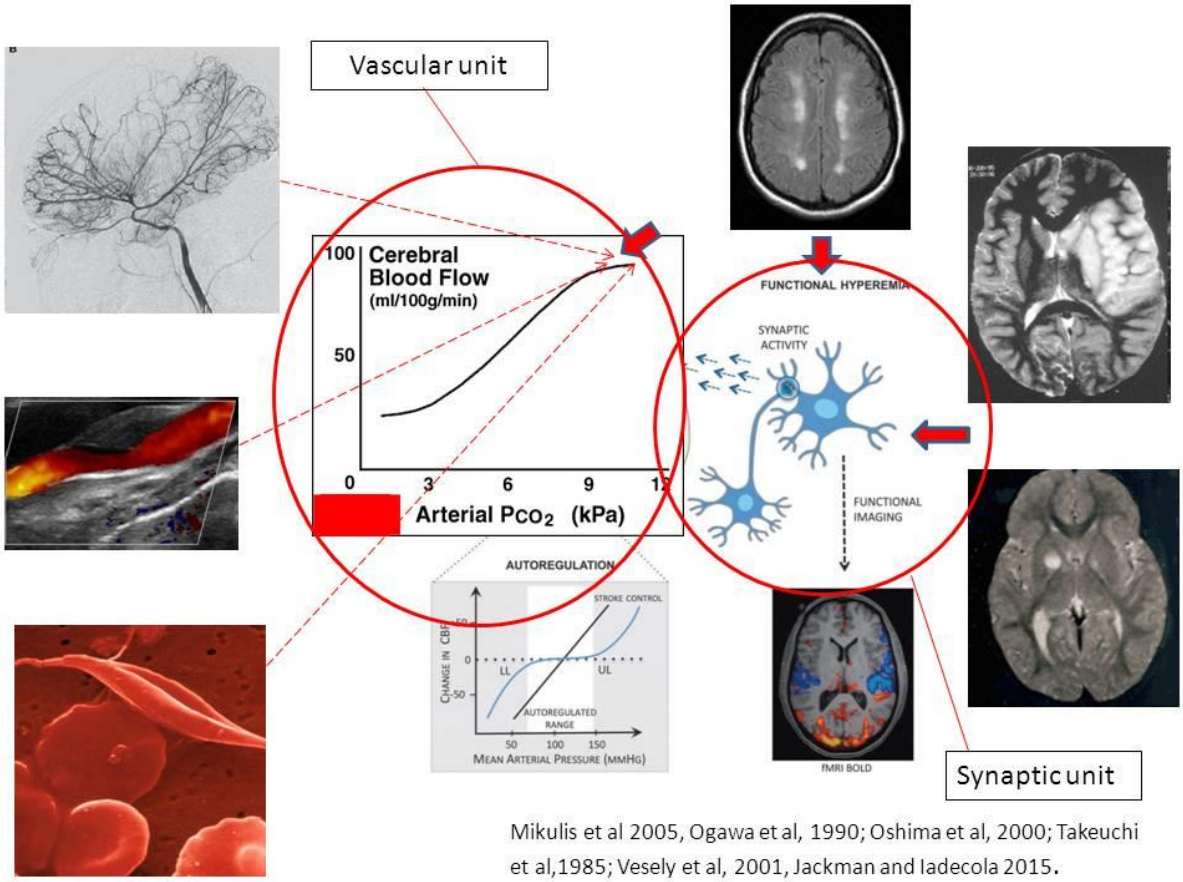
Qualitative hBOLD CVR studies were successfully conducted in 47 children. To my knowledge, this is the largest reported study of qualitative hBOLD CVR studies in children. The non-moyamoya arteriopathy groups were small; hence comparison of findings is limited.

However, negative pixels at the positive threshold were higher in those with moyamoya arteriopathy compared to those with Bilateral Non-MM. Negative pixels are suggestive of steal and ischaemic risk. This suggests more significantly impaired CVR and tissue level perfusion in the moyamoya arteriopathy group when compared to the Bilateral Non-MM group.

ii) Moyamoya Arteriopathy and Moyamoya Syndromic Arteriopathy

Thirty-seven of the 47 children with arteriopathy who successfully had qualitative hBOLD CVR studies had moyamoya arteriopathy (14 Moyamoya Syndrome, 9 NF1-MM, 5 SCD-MM, 7-All Chromosomal-MM and 2 Tumour-MM). hBOLD CVR measures were most severe, i.e. low PTPP, high PTNP and NTNP, in the Tumour-MM and SCD-MM groups. NF1 hBOLD CVR measures were more severe, i.e. low PTPP, high PTNP and NTNP, on the left compared with the right side.

Figure 40 Neurovascular Unit and Cerebral Autoregulation showing the interaction between the blood cells, the endothelium and the blood vessels (left) and the neurone, synapse and brain cells (right) involved in regulating the cerebral blood flow response to changing carbon dioxide and blood pressure



Mikulis et al 2005, Ogawa et al, 1990; Oshima et al, 2000; Takeuchi et al,1985; Vesely et al, 2001, Jackman and Iadecola 2015.

Neurovascular coupling and cerebral autoregulation are two vasoregulative mechanisms thought to be located at the level of the smooth muscle arteriole which functionally interact with each other (Figure 36). Neurovascular coupling describes the relationship between neuronal firing and haemodynamic changes in the vasculature adjacent to the activation areas. Synaptic activity stimulates the release of signaling molecules that initiate changes in vascular tone. Hence, neurovascular coupling – also known as functional hyperemia - forms the basis of BOLD functional imaging. It is a complex and incompletely understood process that involves multiple signaling pathways and chemical mediators (Koehler *et al.* 2009, Attwell *et al.* 2010, Jackman and Iadecola 2015). Cerebral autoregulation maintains constant cerebral blood flow despite perfusion pressure changes in the cerebral circulation (Azevedo *et al.* 2007) (Jackman and Iadecola 2015). Hence disruptions in this relationship may occur for any reason that affects the synaptic or vascular unit. Abnormalities in CVR caused by abnormal cerebrovascular autoregulation may occur due to chronic vasodilation in conditions such as chronic anaemia (SCD), or distal to a chronically fixed stenosis, as in moyamoya. In either situation there is a right sided shift on the cerebral autoregulatory curve with consequent attenuation of the vasodilatory response. The neuronal or synaptic contribution to functional hyperemia and the BOLD response is less explored and understood.

An example of how a specific disease may contribute to abnormality in functional hyperaemia is found with NF1. Neurofibromin is the product of the NF1 oncogene

and is a Ras signalling pathway inhibitory protein, dysregulation of which leads to excessive SMC proliferation and vascular occlusion (Hamilton and Friedman 2000, Dasgupta and Gutmann 2003). In addition, neurofibromin has been shown to modulate excitatory synaptic function (Costa and Silva 2002). The NF1 mouse models suggest increased perfusion on SPECT and increased density of microglial cells accompanied by enlarged capillaries in juvenile NF1 mice, specifically in the amygdala. Microglia survey the local microenvironment, and aid with the remodeling of neuronal circuits by making contact with synaptic spines (Tremblay and Majewska 2011, Siskova and Tremblay 2013, Welberg 2014, Apostolova *et al.* 2015). Activated microglia have higher energy demands, resulting in an upregulation of local cerebral blood flow. Neurofibromin has also been associated with brain tumorigenesis (Daginakatte *et al.* 2008). In the juvenile NF1 mouse model, the observation of regional hyperperfusion seemed age dependent and the increased microglial density suggestive of disease-specific pathology. Conversely regional cerebral hypoperfusion using Arterial Spin-labeled CBF was reported in children with NF1 by Yeom et al (2013), with similar findings in adult and childhood studies using FDG-PET (Balestri *et al.* 1994, Kaplan *et al.* 1997, Buchert *et al.* 2008, Yeom *et al.* 2013). These observations may explain difference between NF1-MM and non-NF1-MM, as well as differences within the group of children with NF1-MM. These observations require replication and exploration in future studies (Apostolova *et al.* 2015).

In my study, the lowest positive pixel and highest negative pixels measures – indicative of poor tissue level perfusion - were present in the Tumour-MM and

Chromosomal-MM group. The Tumour-MM group was very small (n=2) therefore the ability to draw conclusions from findings is limited. The tumour was resected in one. Both patients had radiotherapy induced moyamoya vasculopathy. Age at moyamoya diagnosis was the oldest in this group (14.1 years), and was four and ten years after tumour diagnosis and radiation. Hypercapnic challenge BOLD CVR is increasingly used in the preoperative mapping of brain tumours prior to resection; the main use in this regard is for the assessment of neurovascular uncoupling potential in the region of the brain tumour or vascular malformation (Pillai and Mikulis 2015). Neurovascular uncoupling can occur in high and low grade tumours. In high-grade glioma, tumour angiogenesis is associated with abnormal vasoactivity and permeability of the neovasculature. However the mechanism in low-grade tumours is not as well understood and may be associated with astrocytic dysfunction secondary to infiltration (Hou *et al.* 2006, Pillai and Zaca 2011, Zaca *et al.* 2014). The relative risk of stroke in childhood cancer survivors is 10 fold higher than sibling controls, and is highest in adult survivors of blood-borne cancers and brain tumours (Bowers *et al.* 2005, Bowers *et al.* 2006, Oeffinger *et al.* 2006). Radiotherapy for brain tumours confers additional risk. It is thought to result in a weakening of the blood-brain barrier in the microvasculature and the initiation of an inflammatory cascade and prothrombotic factors. There is a change in vascular integrity and function over time, leading to endothelial cell detachment and microthrombus formation on the subendothelium in the smallest vessels (Yuan *et al.* 2006, Wilson *et al.* 2009, Stewart *et al.* 2010). The compromised vascular wall is prone to steno-occlusive or dilatory vasculopathy (Fajardo 2005). In

addition secondary effects, such as the development of growth hormone deficiency at a young age (as in both of our children), is an independent additional stroke risk factor (de Haas *et al.* 2010, Green *et al.* 2012).

There are no published studies of CVR in association with chromosomal disorders. Children with Down syndrome have a predisposition to vascular disease in multiple vascular beds (Kontras and Bodenbender 1966, Chi 1975, Fleisher *et al.* 1978). The literature on moyamoya in Trisomy 21 is mostly limited to case reports, with minimal discussion regarding the underlying pathophysiological mechanisms. Impaired vascular endothelial function is reported, which could have an impact on cerebrovascular compliance and reactivity (Cappelli-Bigazzi *et al.* 2004). However, this is only speculative. Therefore the reason that tissue level CVR measures in the chromosomal disorders group should be amongst the worst in the moyamoya arteriopathy group is unexplained. Children with Down syndrome and moyamoya often present in infancy run a clinically severe course in terms of ischaemic events, and have a higher surgical complication rate (Dai *et al.* 2000, Jackson *et al.* 2014, See *et al.* 2015). Therefore clinical presentation and current understanding of the pathophysiological basis of tissue and vascular injury in Tumour-MM, and to a lesser extent Chromosomal-MM, is in keeping with the hBOLD CVR findings in my study, which are suggestive of higher ischaemic risk at the time of CVR than the other moyamoya arteriopathy groups. However, these findings need to be further explored in a large longitudinal cohort.

The SCD-MM group had high inspection scores, consistent with worse steal, when compared to the other moyamoya arteriopathy groups. They did not have

correspondingly low positive pixels and high negative pixel ratios, perhaps because of an insensitive inspection scoring system, or an oversensitive hemispheric pixel counting system. Alternatively, there may be inherent limitations in using hBOLD CVR in children with SCD, who may exhaust their cerebrovascular reserve due to the chronic hyperdynamic state caused by the anaemia, and in some, chronically elevated pCO2 and low pO2 often seen in this population, in addition to the arteriopathy (Prengler *et al.* 2002, Prohovnik *et al.* 2009). For children with SCD-MM, the ability to generate BOLD MRI signal on any given stimulus may be reduced by a combination of this chronic hyperdynamic state and the increased pulmonary vascular resistance (Prengler *et al.* 2002, Prohovnik *et al.* 2009).

The Idiopathic moyamoya group CVR measures were indicative of more right sided tissue level dysfunction. However their CVR measures were not the most severe in the moyamoya arteriopathy group. This is interesting and suggestive of a different ischaemic risk profile for children with moyamoya, which may be more dependent on their comorbidity than the primary arteriopathy.

Thus CVR of pre-ischaemic, normally perfused brains of children with syndromic arteriopathies may provide novel insights into the syndrome specific neuronal and vascular components of functional hyperaemia, and hence the pathological bases of difference in ischaemic risk between the moyamoya arteriopathy groups.

iii) hBOLD CVR Measures by Method

The youngest age at CVR was 1 year. This was done under GA which provides an approach to the assessment of tissue level ischaemic risk in the very young or uncooperative with moyamoya. Moyamoya severity by modified moyamoya stage was comparable between the GA and BH group. The GA group had a higher proportion of children who had a stroke. The observation that the right hemisphere scores were worse in the BH group compared to the GA group may be suggestive of poorer right hemisphere tissue level perfusion, and this may be related to the later age of diagnosis and hBOLD CVR in the BH group.

5.7.3. Study Limitations and Future Directions

5.7.4. Conclusion

Increased understanding of qualitative hBOLD CVR measures in Idiopathic MM and the MM syndromes may help identify patients at increased risk of ischaemia prior to clinical presentation. Inclusion of hBOLD CVR in longitudinal studies of all the moyamoya arteriopathy groups would provide relative, qualitative assessments of hBOLD CVR which could be adequate enough to guide the choice and timing of interventions such as revascularization surgery.

6. Comparison with Conventional Angiography and MM severity

6.1. Abstract

6.1.1. Background

Quantitative measures of hBOLD CVR in adults are shown to correlate with the presence of moyamoya and angiographic indices of moyamoya severity. There are no such studies for qualitative CVR using breath-holding or general anaesthetic in childhood. In addition there are a number of biological variables that are thought to influence BOLD MRI signal and hence may be important to take into account when interpreting hBOLD CVR measures.

6.1.2. Objective

To demonstrate whether hBOLD CVR measures using breath-hold and general anaesthetic are concordant with conventional angiography in children with moyamoya. In addition, to determine whether hBOLD CVR hemispheric pixel measures were affected by biological indices, such as haematocrit, which are important variables in cerebral blood flow and consequent BOLD MRI signal.

6.1.3. Method

Consecutive children diagnosed with moyamoya were enrolled. All conventional angiograms were staged for severity using modified Suzuki criteria and graded according to the degree of stenosis (Ref Methods). hBOLD CVR measures were compared to laterality and angiographic indicators of severity of moyamoya. hBOLD CVR measures were examined for correlation with biological variables.

6.1.4. Results

Thirty-seven children with moyamoya had qualitative hBOLD CVR assessments and 34 conventional angiography. Abnormality of qualitative hBOLD CVR measures was concordant with conventional angiographic abnormality. There was a statistically significant difference in hemispheric measures by grade of stenosis, and haematocrit and a trend toward statistically significant difference in hemispheric pixels by heart rate. There was no statistically significant association between hBOLD hemispheric measures and moyamoya severity using Modified Suzuki Staging.

6.1.5. Conclusion

Qualitative hBOLD CVR measures of impairment are concordant with abnormality as demonstrated on angiography and affected by the severity of disease as reflected by the severity of the stenosis. Biological indices such as haematocrit may have an effect on the BOLD signal and hence be an important variable to control for in the interpretation of hBOLD CVR results, in certain disease conditions commonly associated with moyamoya such as Sickle Cell Disease. Association of

qualitative CVR measures with other variables such as Suzuki Stage, heart rate and blood pressure requires further exploration.

6.2. Introduction

Conventional angiography is the standard tool for diagnosis of moyamoya and assessment of disease severity. There is an increasing trend to rely on MR angiography in the pre-surgical work-up of children with moyamoya and non-moyamoya arteriopathy. However MRA is unable to adequately assess arterial collaterals and may overcall main artery stenosis, especially if time-of flight is used (Saeki *et al.* 2000).

CVR is defined as change in BOLD MRI signal over change in the CO_2 tension. There are a number of factors which affect CVR. BOLD MRI signal is dependent on CBF and other variables such as cerebral blood volume (CBV), cerebral metabolic rate of oxygen consumption, arterial partial pressure of oxygen (PaO_2), and, to an unknown extent, hematocrit (Ogawa *et al.* 1993, Mandell *et al.* 2008). Another factor affecting CVR is blood pressure. Children and adolescents with untreated hypertension have diminished reactivity to hypercapnia, indicating abnormal vasodilatory reactivity (Settakis *et al.* 2003, Settakis *et al.* 2006, Wong *et al.* 2011). Furthermore, in studies using TCD to assess CVR, an inverse relationship between diastolic blood pressure indices and CVR has been demonstrated, suggesting that diastolic blood pressure may be a better predictor of cerebral end organ damage than systolic blood pressure (Settakis *et al.* 2003, Settakis *et al.* 2006, Wong *et al.* 2011). Therefore, I examined for the effect of these factors, namely hematocrit, diastolic and systolic blood pressure on hBOLD CVR measures.

Quantitative BOLD MRI CVR, using targeted control of end tidal CO_2 delivery in children and adults, has demonstrated concordance of angiographic abnormalities with CVR deficits, and demonstrated deficits beyond the primary area of angiographic abnormality and in regions of brain which appeared normal on MRI (Heyn *et al.* 2010, Han *et al.* 2011). Heyn also demonstrated a direct correlation between quantitative BOLD MRI CVR and measures of moyamoya severity, including the Suzuki score.

These studies–conducted mostly in adults, and using targeted controlled delivery of CO2–show that hBOLD CVR can provide additional functional information of the haemodynamic effect of steno-occlusive arteriopathy at the tissue level and complements both conventional angiography and more traditional structural and vascular MR imaging.

The studies described in this chapter were designed to establish the regional concordance of angiographic findings, to correlate indices of angiographic severity with qualitative hBOLD CVR, and to assess variables that may account for some difference in hBOLD signal in a group of children with moyamoya arteriopathy. Insights into any of these factors could improve our ability to assess ischaemic risk and direct management in this group of children.

6.3. Study Hypothesis

1) The region of abnormality on qualitative hBOLD CVR corresponds with the region of abnormality as demonstrated by conventional angiography.

2) Qualitative hBOLD CVR measures are associated with the severity of moyamoya as assessed by Modified Suzuki staging and grade of stenosis.

3) Qualitative hBOLD CVR measures are associated with biological indices such as haemoglobin, haematocrit, platelets, blood pressure and heart rate.

6.4. Methods

Children diagnosed with moyamoya arteriopathy (Idiopathic and Syndromic) were consecutively enrolled in the study and had a 6 vessel cerebral conventional angiogram, a brain MRI and a 3D TOF MRA. Conventional angiograms were staged using Modified Suzuki criteria and graded by the degree of stenosis by ND and DA (Chapter 2 Methods). In addition, 3D TOF MRA images were reviewed by ND and DA. Axial T2 FLAIR MRIs were also reviewed and the presence of Ivy noted. Qualitative hBOLD CVR studies were conducted in all (Chapter 2 Methods).

Haematologic and cardiovascular indices collected closest to the hBOLD CVR studies were recorded and used for the purpose of analysis. These included a complete blood count (haemaglobin, white cell count, platelets and haematocrit), blood pressure and heart rate.

6.5. Statistical Analysis

One way ANOVA and the independent t-test were used to compare group means of parametric data. Kruskal-Wallis and the Mann-Whitney U test were used to

compare non-parametric. Categorical data were analysed with Fisher's exact test. Statistical difference was deemed significant at a level of p<= 0.05.

Binomial regression analysis was used to assess the ability of hemispheric pixels to predict moyamoya laterality.

6.6. Results

6.6.1. Group Description

Moyamoya arteriopathy diagnosis and clinical characteristics are as described in Chapter 3. Thirty-seven children (mean age at diagnosis 9.3 +/- 3.88 years) had quantitative hemispheric hBOLD CVR studies and conventional angiography. The majority had bilateral moyamoya disease (Table 30).

Table 30 Arteriopathy Severity Measures and Biological Indices of Children with Moyamoya Arteriopathy

	Moyamoya Arteriopathy n=37
Mean Age at Diagnosis (SD)	9.13 (3.88)
Mean Age at CVR (SD)	10.68 (4.29)
Gender M(%)	16 (43)
Moyamoya Laterality (B/R/L)	23/6/8
Suzuki Scores (Frequency) 1 2 3 4 5 6	 2 12 16 2 1 1
Grade of Stenosis Frequency	Grade 3/4 4/30
Stroke Yes/No B/R/L	 21/16 5/11/5

Mean Biological Indices (SD)	
Hb	126.46 (18.89)
Hct	.377 (.054)
Platelets	311.73 (86.8)
Systolic BP	112.76 (16.96)
Diastolic BP	66.08 (12.54

M=male, B=bilateral, R=right, L=left, Hb=haemoglobin, Hct=haematocrit

6.6.2. Hemispheric Pixels and Moyamoya Laterality Whole Group (n=37)

6.6.2.1. Positive threshold positive pixels (PTPP)

There was a significant difference in right hemispheric PTPP between children with right moyamoya and left moyamoya, between children with right moyamoya and bilateral moyamoya, and between those with left moyamoya and bilateral moyamoya. Children with left moyamoya had higher right hemispheric PTPP than children with right or bilateral moyamoya (one way ANOVA; Table 31, Figure 41).

There was a significant difference in left hemispheric PTPP between children with left moyamoya and right moyamoya, between children with left moyamoya and bilateral moyamoya, and between those with left moyamoya and bilateral moyamoya. Apparently paradoxically, children with right moyamoya had higher numbers of left hemispheric PTPP than children with left or bilateral moyamoya (one way ANOVA; Table 31, Figure 41).

Children with right moyamoya had the lowest number of right hemispheric PTPP when compared to children with left and bilateral moyamoya. Children with left

moyamoya had the highest number of right hemispheric positive threshold positive pixels when compared to children with right and bilateral moyamoya.

6.6.2.2. Positive threshold negative pixels (PTNP)

There was no significant difference in PTNP in either hemisphere by moyamoya laterality (one way ANOVA; Table 31, Figure 41).

6.6.2.3. Negative threshold negative pixels (NTNP)

There was a significant difference in right hemispheric NTNP between children with right MM and left MM, and right MM and bilateral MM (one way ANOVA; Table 31, Figure 41). There was a significant difference in left hemispheric NTNP between children with right MM and left MM and children with right MM and bilateral MM (one way ANOVA; Table 31, Figure 41). Children with right MM had the highest number of NTNP in the right hemisphere. Similarly children with left MM had the highest number of NTNP in the left hemisphere.

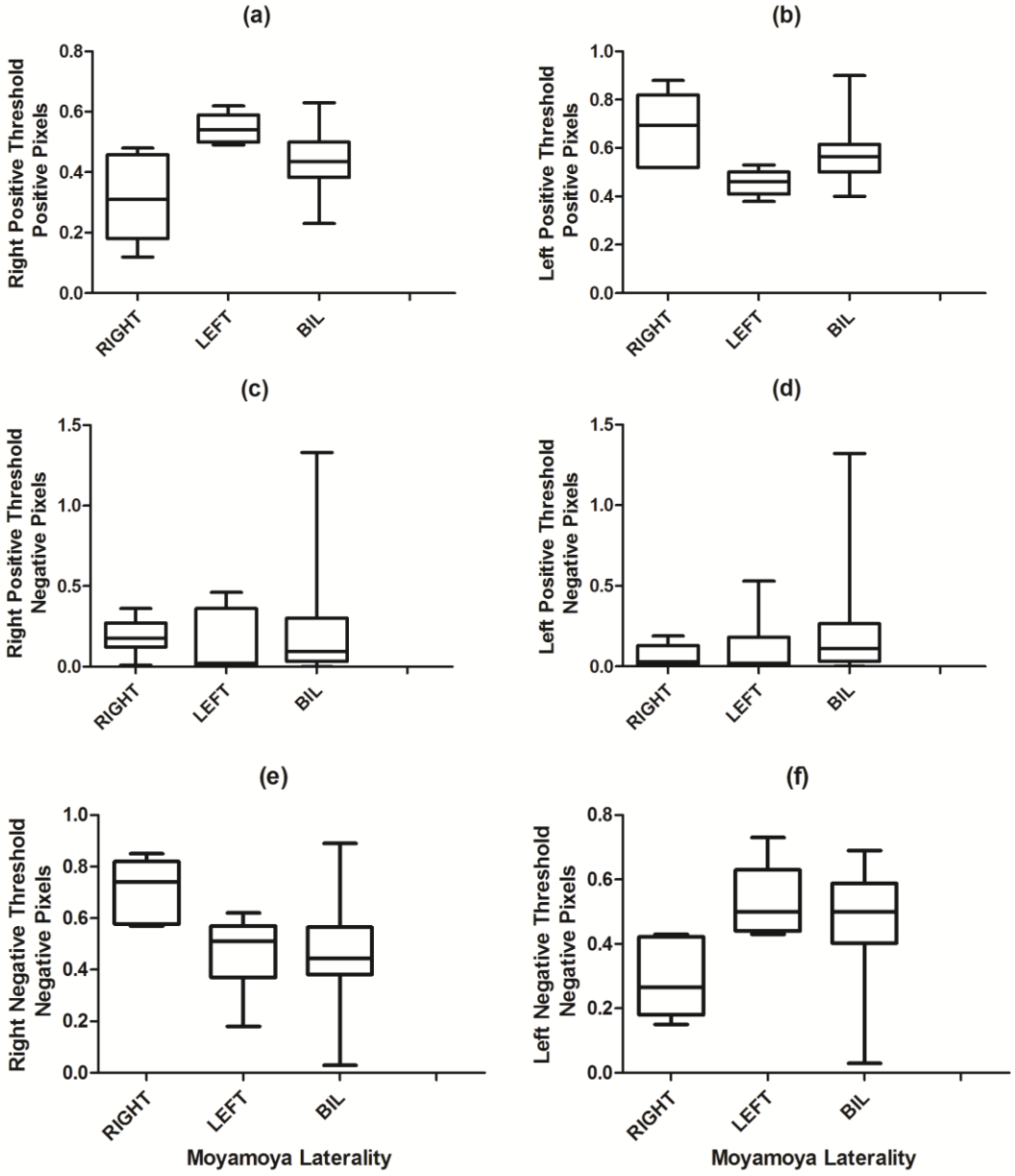
Table 31 Hemispheric Pixels and Moyamoya Laterality

hBOLD CVR Pixels	Moyamoya Laterality n=37						
	Right (n=6)		Left (n=7)		Bilateral (n=24)		
	mean, median	SD (range)	mean, median	SD (range)	mean, median	SD (range)	p value
PTPP R L	.31;.31*^ .69;.70**^^	.15 (.13-.48) .15 (.52-.88)	.55;.54*† .45;.46**††	.06(.49-.62) .05 (.38-.53)	.43;.44^† .58;.56^^ ††	.11 (.23-.63) .12 (.40-.90)	.001*;.05^;.04† .003**;.13^^;.06††
PTNP R L	.18;.17 .06;.03	.12 (.01-.36) .08 (.00-.19)	.14;.02 .12;.03	.19 (.00-.46) .19 (.01-.53)	.21;.1 .2;.11	.3 (.00-1.33) .28 (.00-1.32)	
NTNP R L	.72;.74^* .29;.26†**	.12 (.57-.85) .13 (.15-.43)	.45;.51^ .55;.5†	.15 (.18-.62) .11 (.43-.73)	.47;.45* .46;.5**	.17 (.03-.89) .17 (.03-.69)	.02^;.005* .01†;.05**

One way ANOVA

Significant difference in means at the p<0.01 level between * right and bilateral hemispheres,**right and left ^ p<=.05

Figure 41 Box-and-whisker plots showing hemispheric pixels and Moyamoya Laterality with medians, upper and lower quartiles (the edges of the boxes) and maximum and minimum excluding outliers (the ends of the whiskers)



Bivariate correlation was run for right MM and right hemispheric pixel measures, right MM and left hemispheric pixel measures. The same was repeated for left MM, and bilateral MM (Table 31). There was a significant negative correlation

between right PTPP and right MM, and significant positive correlation between right NTNP and right MM (p=.006 and p=.001 respectively). The direction of significant right MM correlation with left hemispheric measures was reversed. There was a significant negative correlation between left PTPP and left MM and a trend toward significant positive correlation between left NTNP and left MM (p=0.000 and p=.067 respectively). There was no statistically significant correlation between bilateral MM and hemispheric pixel measures. This indicates that hBOLD CVR, using hemispheric pixel measures, is concordant with moyamoya laterality.

Table 32 Moyamoya laterality correlation with Hemispheric Pixels

Moyamoya Laterality		Right Hemispheric Measures N=37			Left Hemispheric Measures N=37		
		PTPP	PTNP	NTNP	PTPP	PTNP	NTNP
Right	Pearson correlation	-.44	-.016	.518	.392	-.18	-.433
	P value	.006*	.925	.001*	.016*	.286	.007*
Left	Pearson correlation	.590	-.197	-.145	-.584	-.203	.304
	P value	.000*	.243	.393	.000*	.228	.067**
Bilateral	Pearson correlation	.028	-.091	.282	-.053	-.210	-.108
	P value	.870	.591	.091**	.757	.213	.525

* significant at p<0.05 level ** trend toward significant at p<0.1 level

Binomial regression analysis was used to assess the ability of hemispheric pixels to predict moyamoya laterality:

6.6.2.4. Right Moyamoya

Using binomial regression analysis, right PTPP and NTNP were significantly predictive of right moyamoya laterality (p=.002) and p=.053 respectively). The

model was able to predict MML 89.2% of the time compared to the null hypothesis prediction of 16.2%. Left NTNP was significantly predictive of right MM. Remaining left hemispheric pixel measures were not predictive of right MM laterality.

6.6.2.5. Left Moyamoya

Left PTPP and NTNP were significantly predictive of left MM (p=.017 and p=.031 respectively). There was a trend toward left PTNP being predictive of left MM (p=.157). Left hemispheric pixels were able to predict left MM 81.1 % of the time compared to the null hypothesis prediction of 21.6%. There was a trend toward right NTNP being negatively predictive of left MM (p=0.057). The remaining right hemispheric pixel measures were not predictive of left MM. Right hemispheric pixel measures were predictive of left MM 78.4% of the time compared to the null hypothesis prediction of 21.6%.

6.6.2.6. Bilateral Moyamoya

The right hemispheric measures were not predictive of bilateral MM. Right hemispheric pixel measures were predictive of bilateral MM 70.2% of the time compared to the null hypothesis prediction of 35.1%. Left hemispheric measures were not significantly predictive of bilateral MM laterality. Left hemispheric measures were predictive of bilateral MM 64.9% of the time compared to the null hypothesis prediction of 35.1%.

Table 33 B coefficient and Odds Ratios

Hemispheric Pixels	Right MM					Left MM				
	B	Sig	Exp(B)	Lower	Upper	B	Sig	Exp(B)	Lower	Upper
Right										
PTPP	-8.85	.002	.000	.000	.037	-11.08	.017	.000	.000	.134
PTNP	-2.6	.403	.075	.000	32.49	-5.07	.157	.006	.000	7.072
NTNP	4.2	.053	66.888	.949	4715.7	9.89	.031	19775.4	2.48	>>>*
Left										
PTPP	1.368	.295	3.928	.303	50.885	2.808	.205	16.57	.215	1274.5
PTNP	-2.074	.688	.126	.000	3085.45	-1.486	.541	.226	.002	26.53
NTNP	-5.725	.027	.003	.000	.521	-4.694	.057	.009	.000	1.16

6.6.3. Hemispheric Inspection Scores and Moyamoya Laterality

Thirty-seven children had at least one pair of hemispheric inspection scores. Frequency of distribution of scores by moyamoya laterality was explored by cross-tabulation (Tables 33-35). In the right hemisphere inspection, scores were abnormal for 20/24 (83%) of the bilateral moyamoya, 4/7 (43%) of the left-sided moyamoya, and 5/6 (83%) of right sided moyamoya groups. In the left hemisphere inspection, scores were abnormal in 19/24 (79%) of the bilateral moyamoya, in 7/7 (100%) of the left-sided moyamoya, and 2/6 (33%) of the right sided moyamoya groups. In children with unilateral moyamoya, none had a normal hemispheric score for the affected hemisphere. There was a significant difference in frequency of right and left hemispheric scores when right and left moyamoya groups were compared (Mann-Whitney U=7.5, p=.05 and U=5.5, p=0.02). There was a difference in right and left hemispheric scores when right and bilateral, and left and bilateral moyamoya groups were compared but this was not significant (Mann-Whitney U=46, p=.191, U=38.5, p=.082 and U=52, p=.139 and U=54, p=.167).

Right mean hemispheric scores by laterality were significantly different between children with right (mean 2.83+/-.408) and left (1.71+/-.951) moyamoya (p=.036). There was a difference in mean hemispheric scores between children with left (1.71+/-.951) and bilateral (2.33+/-.761) moyamoya but this was not significant (p=.199). There was no significant difference between mean scores for children with bilateral and right moyamoya.

Left mean hemispheric scores by laterality were significantly different between children with right (1.50+/-.837) and left (2.71+/-.488) moyamoya (p=.018). There

was a difference in mean hemispheric scores between children with right (1.50+/-.837) and bilateral (2.21+/-.779) moyamoya but this was not significant (p=.135). There was no significant difference between mean scores for children with bilateral and left moyamoya.

Table 34 Cross-tabulation of Right Hemispheric Scores and Moyamoya Laterality

	Moyamoya Laterality			
Right Hemispheric Scores	Bilateral	Left	Right	Total
1	4	4	0	8
2	8	1	1	10
3	12	2	5	19
Total	24	7	6	37

Table 35 Cross-tabulation of Left Hemispheric Scores and Moyamoya Laterality

	Moyamoya Laterality			
Left Hemispheric Scores	Bilateral	Left	Right	Total
1	5	0	4	9
2	9	2	1	12
3	10	5	1	16
Total	24	7	6	37

Table 36 Mean Hemispheric Scores by Moyamoya Laterality

Mean Hemispheric Scores	Moyamoya Laterality		
	Right	Left	Bilateral
Right (SD)	2.83* (.408)	1.71*† (.951)	2.33† (.761)
Left (SD)	1.50*† (.837)	2.71* (.488)	2.21† (.779)

One way ANOVA *significant difference in mean hemispheric scores between right and left moyamoya groups p=<0.5

† trend toward significant difference in hemispheric mean scores between left and bilateral, and right and bilateral moyamoya groups.

6.6.4. Hemispheric Pixels and Stroke

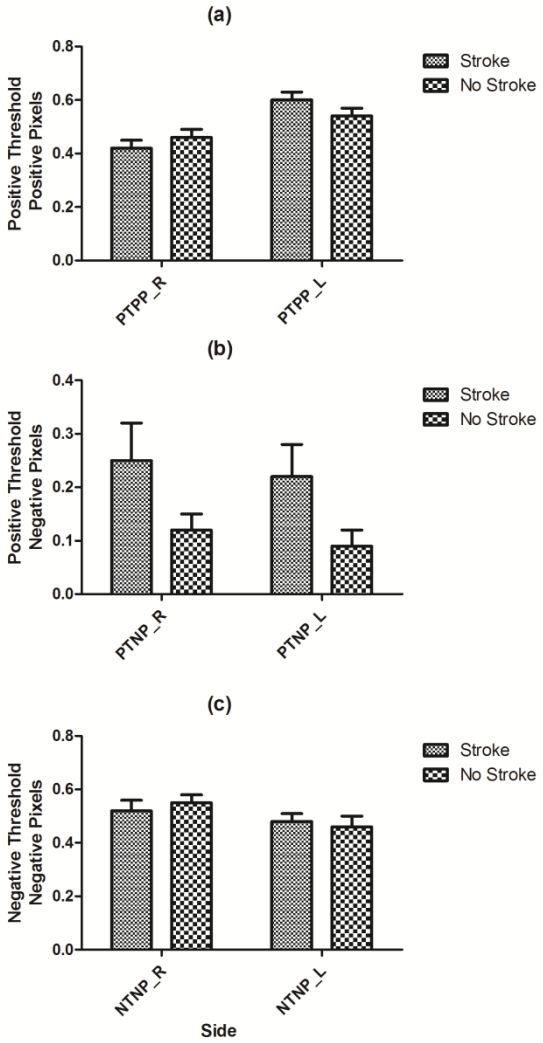
6.6.4.1. Hemispheric Pixels, Stroke and No Stroke

Of the thirty-seven children, 21 had had at least one stroke at the time of their hBOLD CVR study. There was a trend toward significance in difference between stroke and no stroke groups in PTNP with higher numbers in the stroke group (t-test; Table 37 middle row, Figure 42). There was no statistical difference in pixels between stroke and no stroke groups for positive and negative pixels generated at positive and negative thresholds respectively (t-test; Table 37, Figure 42).

Table 37 Comparison of Hemispheric Pixels between Stroke and No Stroke Groups

hBOLD CVR Pixels	Stroke (n=21)		No Stroke (n=16)		t-test
	Mean	SD	Mean	SD	p value
PTPP					
R	.42	.12	.46	.13	.378
L	.59	.13	.54	.13	.273
PTNP					
R	.25	.31	.12	.14	0.147†
L	.22	.29	.09	.14	0.107†
NTNP					
R	.50	.16	.51	.21	0.830
L	.47	.16	.43	.18	0.504

Figure 42 Comparison of Hemispheric Pixels for Stroke and No Stroke Groups. The error bars represent standard deviations



6.6.4.2. Hemispheric Pixels and Stroke Laterality

Of the children who had a stroke (21/37[56%]), 11 (52%) were right sided, 5 (24%) were left sided and 5 (24%) bilateral. On inspection in the children with unilateral moyamoya, PTPP were lower in the stroke hemisphere than the non-stroke hemisphere. Similarly NTNP were higher in the stroke hemisphere than the non-stroke hemisphere. In those with bilateral moyamoya, PTPP were lower in the right hemisphere, and NTNP higher in the right. Using the paired t-test, the within group difference between right PTPP in those with right stroke were significantly lower than left PTPP, and there was a trend toward right PTPP being significantly lower than left PTPP in those with bilateral stroke, and a trend toward right NTNP being significantly higher than left NTNP in those with right stroke. There was no statistically significant difference in hemispheric pixels between groups, by stroke laterality (one way ANOVA, p>0.5) (Table 38).

Table 38 Hemispheric Pixels and Stroke Laterality

hBOLD Pixels	Stroke Laterality					
	Right (n=11)		Left (n=5)		Bilateral (n=5)	
	mean, median	SD (range)	mean, median	SD (range)	mean, median	SD (range)
PTPP						
R	.40;.43	.13 (.2 -.63)	.45; .49	.13 (.26-.60)	.42; .41	.11 (.27-.55)
L	.62;.57	.15 (.4 -.90)	.55; .51	.13 (.40-.74)	.58; .59	.11 (.46-.74)
p value	.026*		.455		.180†	
PTNP						
R	.25; 27	.18 (.00-58)	.17; .05	.22 (.0-.51)	.32; .06	.57 (.03-1.33)
L	.17; 12	.15 (.00-45)	.18; .08	.21 (.0-.46)	.35; .11	.55 (.03-1.32)
p value	.194†		.808		.498	
NTNP						
R	.57; 54	.19 (.32-89)	.47; .47	.07 (.40-.59)	.45; .45	.10 (.31-.57)
L	.43; 46	.18 (.11-64)	.53; .53	.07 (.41-.6)	.56; .55	.10 (.44-.69)
p value	.256		.430		.315	

6.6.5. Hemispheric Pixels in Surgery and No Pre-surgery groups

Ten of the 37 (27%) children had undergone previous revascularization surgery at the time of their first hBOLD CVR. Further analysis was conducted on the children who had not had revascularization surgery prior to their first hBOLD CVR study (n=27). There was no statistically significant difference in hemispheric PTPP, NTNP and right PTNP between those with and without prior revascularization surgery. There was a trend toward significant difference in left hemispheric PTNP between groups. There was no significant difference in mean scores between groups (Table 39).

Table 39 Hemispheric Pixels in Surgical and Surgically Naïve Children

hBOLD CVR Pixels	Surgery (n=10)		No Surgery (n=27)		t-test
	Mean	SD	Mean	SD	p value
PTPP					
R	.44	.151	.43	.117	.884
L	.57	.147	.57	.127	.893
PPPTNP					
R	.28	.403	.17	.170	.295
L	.24	.407	.13	.143	.198†
NTNP					
R	.45	.221	.52	.165	.267
L	.45	.210	.45	.155	.977

Table 40 Mean Hemispheric Scores by Surgical and Surgically Naive Children

Mean Hemispheric Scores	Surgery	No Surgery	t-test
Right (SD)	2.3 (.823)	2.3 (.823)	.990
Left (SD)	2.4 (.843)	2.1 (.801)	.343

6.6.5.1. Hemispheric Pixels and Moyamoya Laterality (no pre-surgery, n=27)

There were similar significant differences in hBOLD CVR measures by moyamoya laterality in the no pre-surgery group.

Table 41 hBOLD CVR Pixels and Moyamoya Laterality in No Pre-surgery Group

hBOLD CVR Pixels	Moyamoya Laterality						
	Right (n=5)		Left (n=5)		Bilateral (n=17)		
	mean, median	SD (range)	mean, median	SD (range)	mean, median	SD (range)	p value
PTPP R L	.33;.39* .67;.62**	.16 (.12-.48) .16 (.52-.88)	.53;.53 .47;.47	.04 (.49-.59) .04 (.41-.53)	.43;.46 .57;.55	.09 (.23-.60) .11 (.40-.90)	.014* .043**
PTNP R L	.19;.17 .07;.04	.13 (.01-.36) .08 (.00-.19)	.17;.02 .15;.02	.22 (.00-.46) .23 (.01-.53)	.16;.07 .13;.11	.17 (.00-.58) .13 (.00-.46)	0.7 0.5
NTNP R L	.7;.68^ .31;.33†	.13 (.57-.85) .13 (.15-.43)	.48;.5 .53;.5	.1 (.37-.62) .08 (.44-.63)	.52;.47 ^^ .48;.53 ††	.15 (.31-.89) .15 (.11-.69)	.062;.059 .044†;.058

*Significant difference in right PTPP in right MM compared with left MM
** Significant difference in left PTPP in left MM compared with right MM
† Significant difference in left NTNP between right and left MM laterality

6.6.6. Hemispheric Pixels in Stroke and No Stroke Groups (without pre-surgery)

Fifteen of 27 children had had a stroke at the time of their first hBOLD CVR. There was no statistically significant difference in hemispheric pixels or scores between the stroke and no stroke groups. In the stroke group, right PTPP were significantly lower than left PTPP and there was a trend toward significantly higher PTNP and

NTNP in those with right stroke. Left NTNP were also significantly higher than right NTNP in the left stroke group.

Table 42 Hemispheric Pixels between Stroke and No Stroke Groups with No Pre-surgery

hBOLD CVR Pixels	Stroke (n=15)		No Stroke (n=12)		t-test
	Mean	SD	mean	SD	p value
PTPP					
R	.417	.114	.452	.121	.446
L	.594	.132	.546	.121	.344
PTNP					
R	.187	.187	.140	.150	.491
L	.144	.140	.107	.151	.511
NTNP					
R	.505	.189	.549	.134	.495
L	.444	.177	.456	.132	.845

Table 43 Mean Hemispheric Scores between Stroke and No Stroke Groups with No Pre-surgery

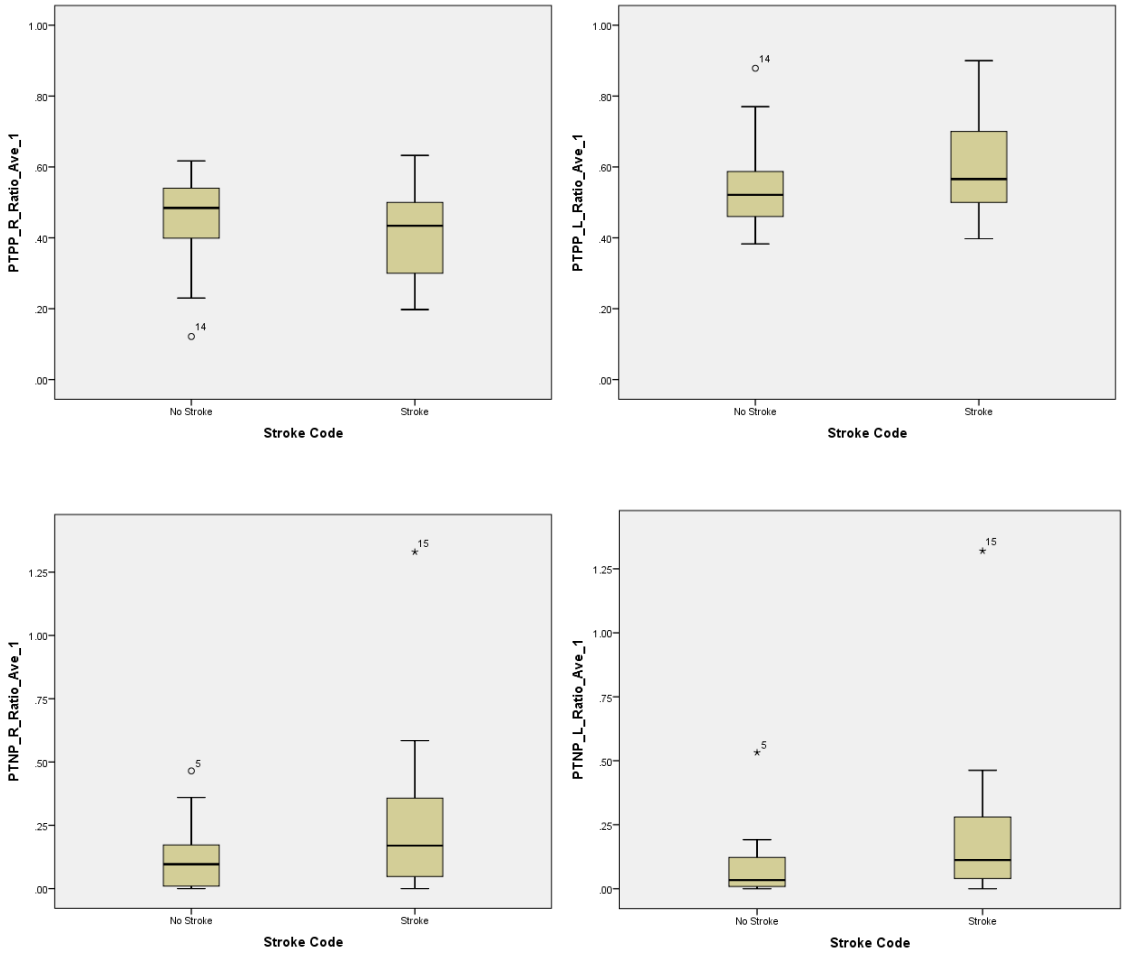
Mean Hemispheric Scores	Stroke	No Stroke	t-test
Right (SD)	2.33 (.724)	2.25 (.965)	.8
Left (SD)	2.00 (.845)	2.25 (.754)	.431

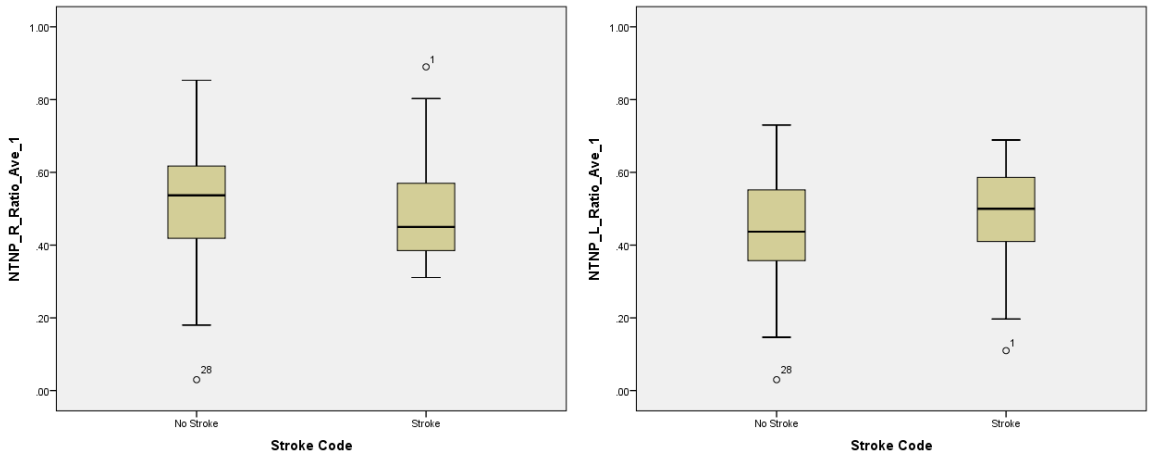
Table 44 Hemispheric Pixels by Stroke Laterality in No Pre-Surgery Group

hBOLD Pixels	Stroke Laterality					
	Right (n=9)		Left (n=4)		Bilateral (n=2)	
	Mean	SD	mean	SD	mean	SD
PTPP						
R	.37	.117	.51	.081	.44	.089
L	.64	.140	.5	.081	.56	.089
						.
p value	.013*		.99		.513	
PTNP						
R	.266	.192	.08	.13	.04	.019
L	.146	.125	.14	.216	.14	.108
p value	.071†		.253		.459	

NTNP R L	.577 .373	.213 .186	.434 .551	.033 .478	.32 .55	.013 .2
p value	.143†		.054*		.367	

Figure 43 Box-and-whisker plots showing Hemispheric Pixels between Stroke and No Stroke Groups with No Pre-surgery with medians, upper and lower quartiles (the edges of the boxes) and maximum and minimum excluding outliers (the ends of the whiskers)





6.6.7. Hemispheric Pixels and Radiographic Indices of Moyamoya Severity

6.6.7.1. Hemispheric Pixels and Scores by Modified Suzuki Stage

Conventional angiograms were available for review in 92% (34/37) children (Tables 44 and 45). Of these, most 55% (12/22) children with bilateral disease had Suzuki Stage 3 disease and 27% (6/22) had Suzuki Stage 2 disease. The rest (18%; 4/22) were spread between the remaining scores. Of the children with right sided moyamoya 2/22 had Suzuki Stage 2 disease and 1/22 Stage 1, 2 and 4 disease respectively. All children with left sided moyamoya had Suzuki Stage 1-3 disease. None had Stage 4, 5 or 6. There was no statistically significant difference in mean hemispheric pixels (Table 47) or scores (Table 48) by Modified Suzuki stage in the whole group (n=37), or in the No Pre-surgery Group (n=27) (Table 49). Linear regression analysis was run for Modified Suzuki Stage and hemispheric pixels. There was no statistically significant effect on hemispheric pixels by Modified Suzuki Stage (Table 50).

Table 45 Moyamoya Laterality by Modified Suzuki Stage in Whole Group (ConA)

Moyamoya Laterality	Modified Suzuki Stage (ConA)						Total
	1	2	3	4	5	6	
Bilateral	1	6	12	1	1	1	22
Left	0	5	2	0	0	0	7
Right	1	1	2	1	0	0	5
Total	2	12	16	2	1	1	34

Table 46 Moyamoya Laterality by Modified Suzuki Stage in No Presurgery Group

Moyamoya Laterality	Modified Suzuki Stage (ConA)						Total
	1	2	3	4	5		
Bilateral	1	5	7	0	1	1	15
Left	0	3	2	0	0	0	5
Right	1	1	1	1	0	0	4
Total	2	9	10	1	1	1	24

Table 47 Hemispheric Pixels by Modified Suzuki Stage in Whole Group

	Modified Suzuki Stage					
	1 n=2	2 n=12	3 n=16	4 n=2	5 n=1	6 n=1
PTPP Right Left	Mean (SD) .3 (.146) .7 (.146)	 .48 (.101) .52 (.103)	 .41 (.138) .60 (.147)	 .5 (.071) .49 (.045)	 .6 .4	 .3 .7
PTNP Right Left	 .24 (.164) .087 (.066)	 .2 (.38) .2 (.371)	 .18 (.165) .16 (.166)	 .06 (.088) .06 (.078)	 .01 .00	 .58. .11
NTNP Right Left	 .63 (.248) .37 (.248)	 .44 (.128) .51 (.131)	 .51 (.219) .42 (.197)	 .57 (.002) .44 (.004)	 .41 .59	 .80 .21

Table 48 Mean Hemispheric Scores by Suzuki Stage in Whole Group (n=37)

Mean Hemispheric Scores	Modified Suzuki Stage n=34					
	1 n=2	2 n=12	3 n=16	4 n=2	5 n=1	6 n=1
Right (SD)	3.00	2.00 (.853)	2.38 (.806)	2.5 (.707)	1	2
Left (SD)	1.50 (.707)	2.58 (.669)	2.00 (.816)	2.00 (1.41)	2	1

One way ANOVA p>0.5

Table 49 Mean Hemispheric Scores by Modified Suzuki Stage in No Presurgery Group (n=27)

Mean Hemispheric Scores	Modified Suzuki Stage n=24					
	1 n=2	2 n=9	3 n=10	4 n=1	5 n=1	6 n=1
Right (SD)	3.00 (0.00)	2.11 (.928)	2.30 (.823)	2	1	2
Left (SD)	1.50 (.707)	2.44 (.726)	2.00 (.816)	1	2	1

One way ANOVA p>0.5

Figure 44 Scatterplot of Suzuki Stage and Hemispheric Pixels in No Presurgery Group

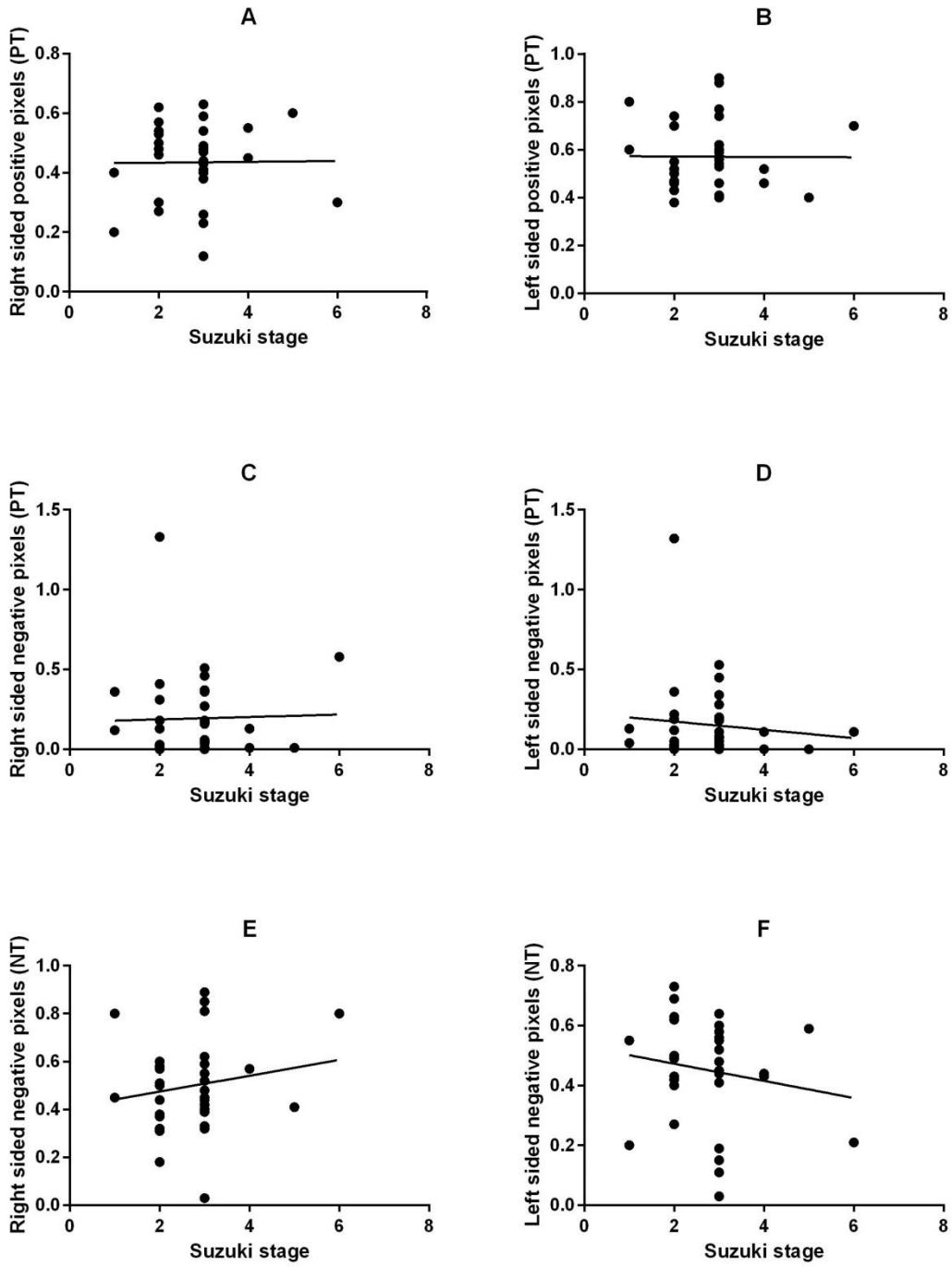


Table 50 Prediction of Hemispheric Pixels by Modified Suzuki Stage

	R	R^2	Adj R^2	F	B	SE for B	Beta	T	P
PTPP_R_ratio Modified moyamoya stage	0.01	0.0001	-0.031	0.003	0.001	0.023	0.010	0.056	0.956
PTPP_L_ratio Modified moyamoya stage	0.004	0.0001	-0.031	0.000	-0.001	0.024	-0.004	-0.024	0.983
PTNP_R_ratio Modified moyamoya stage	0.027	0.001	-0.030	0.002	0.007	0.047	0.027	0.156	0.877
PTNP_L_ratio Modified moyamoya stage	0.104	0.011	-0.020	0.352	-0.026	0.044	-0.104	-0.593	0.557
NTNP_R_ratio Modified moyamoya stage	0.173	0.030	0.000	0.992	0.032	0.033	0.173	0.996	0.327
NTNP_L_ratio Modified moyamoya stage	0.166	0.028	-0.003	0.905	-0.029	0.030	-0.166	-0.951	0.348

6.6.8. Hemispheric Pixels by Grade of Stenosis

In the thirty four children with quantitative hBOLD CVR measures and conventional cerebral angiograms, grading of stenosis was done on visual inspection of all conventional angiograms. Vessels were scored according to the degree of artery narrowing or occlusion in the affected hemisphere(s) and expressed as a percentage (Chapter 2 Methods for details):

Grade 1 = 0-25%; Grade 2 = 26-50%; Grade 3 = 51-75%; Grade 4 = 76-100% occlusion

All vessels of the COW were scored and the worst score used for the purpose of analysis. Four children had Grade 3 and 30 children had Grade 4 narrowing or

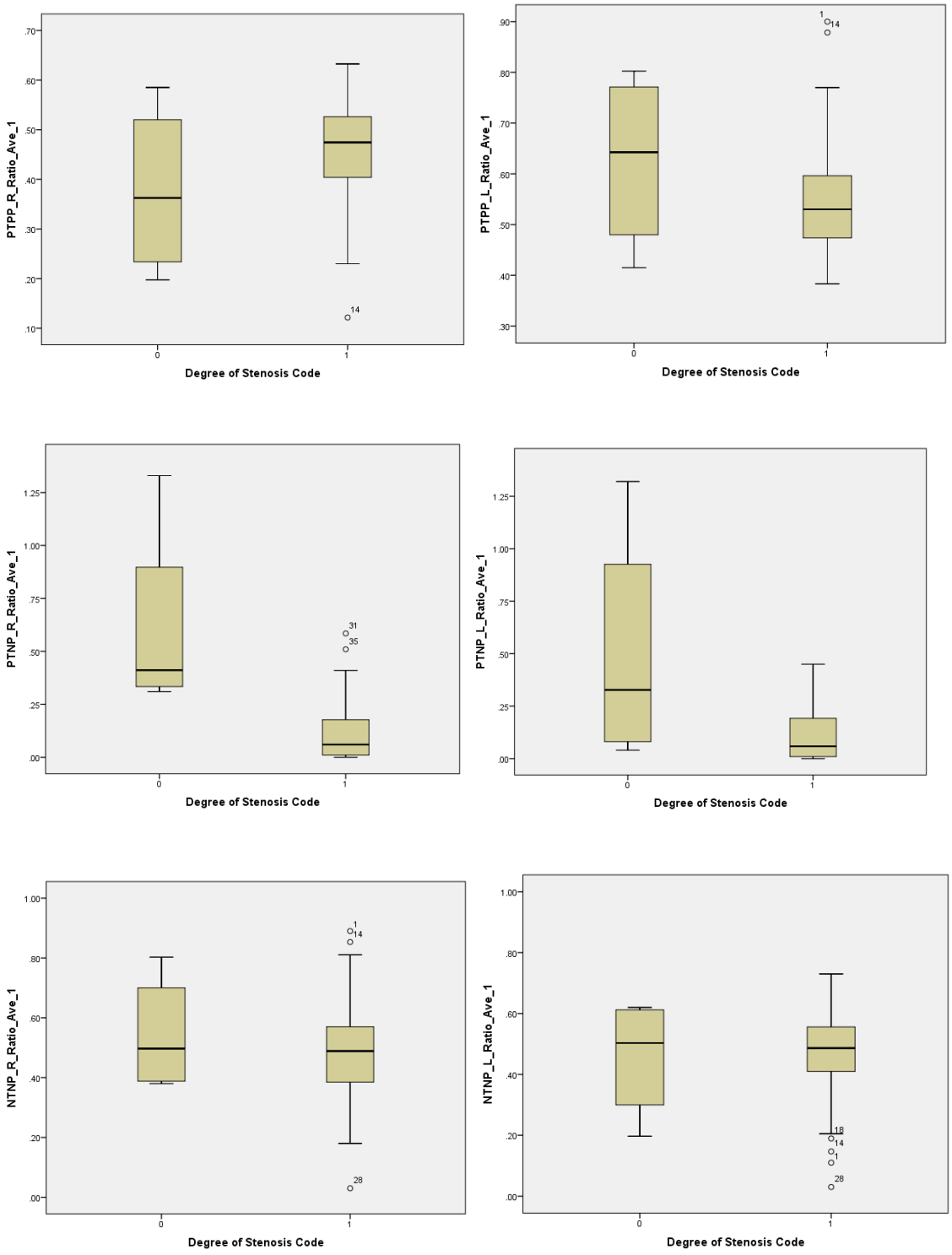
occlusion (n=34). Two of the four children with Grade 3 stenosis had Chromosomal (Trisomy 21)-MM.

Table 51 Mean Hemispheric Pixels by Grade of Stenosis

Pixels	Grade 3 n=4		Grade 4 n=30		p value
PTPP	**Mean**	**SD**	**Mean**	**SD**	
Right	.377	.176	..443	.124.	0.348
Left	.626	..178	.563	.131	0.398
PTNP					
Right	.616	.481	.136	.161	0.007*
Left	.504	.585	.109	.123	0.082
NTNP					
Right	.544	.199	.492	.187	0.608
Left	.456	.2	.451	.173	0.956

Mann-Whitney U * Significant at $p<0.05$ level;

Figure 45 Hemispheric Pixels by Grade of Stenosis (Code: 0=Grade 3; 1=Grade 4)



6.6.9. Hemispheric Pixels by IVY sign

Thirty six children had quantitative hBOLD CVR studies and MRIs reported for IVY. There was no statistically significant difference between the presence or absence of Ivy in hemispheric pixels (Table 52; Figure 46), nor the presence or absence of steal, as defined as reduction of pixels as an indicator of reduced CBF despite a vasodilatory stimulus, and total CVR score (Table 53; Figure 47).

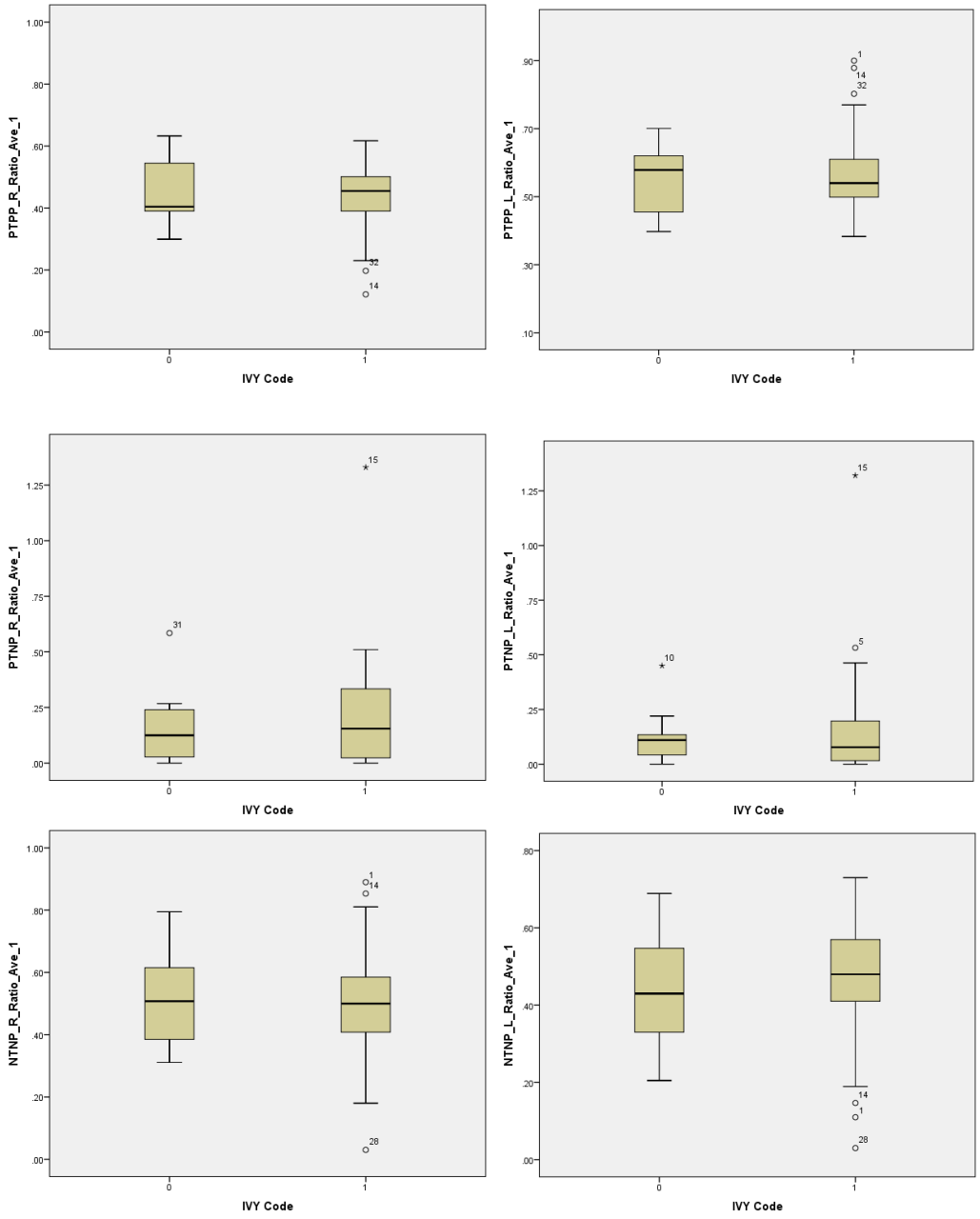
Table 52 Hemispheric Pixels by Presence of Ivy

Hemispheric Pixels	No Ivy n=9		Ivy n=27	
PTPP	**Mean**	**SD**	**Mean**	**SD**
Right	.449	.119	.426	.129
Left	.553	.113	.58	.138
PTNP				
Right	.161	.186	.21	.275
Left	.126	.14	.176	.273
NTNP				
Right	.515	.166	.505	.192
Left	.443	.163	.446	.173

Table 53 Measures of hBOLD CVR Abnormality by Presence of Ivy

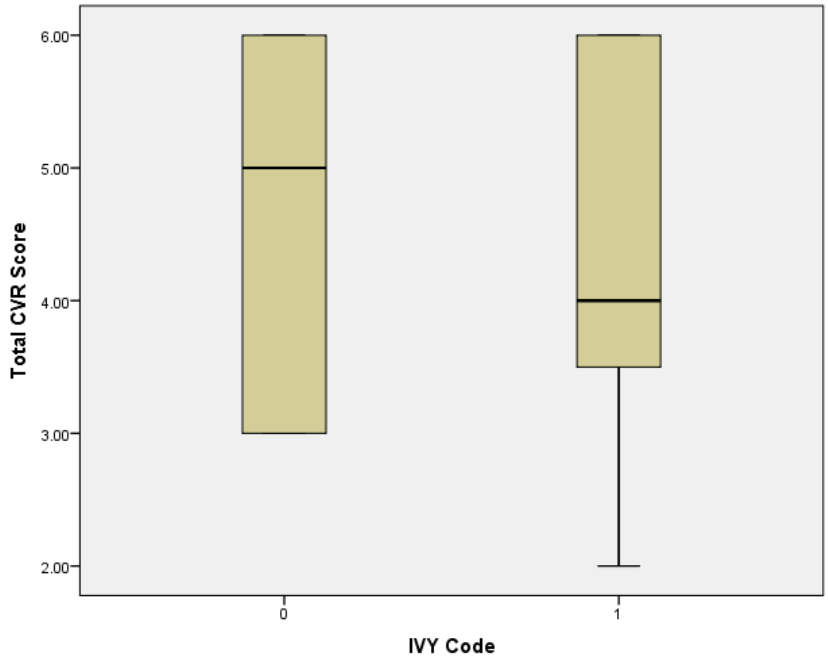
Measures of hBOLD Abnormality Using Inspection Scores	No Ivy n=9		Ivy n=27	
	Mean (Median)	**SD (IQR)**	**Mean**	**SD (IQR)**
Total CVR Score	4.67 (5.00)	1.323 (3.00)	4.44 (4.00)	1.31 (3.00)
	Yes (%)	**No (%)**	**Yes (%)**	**No (%)**
Steal	6 (67)	3 (33)	17 (63)	10 (37)

Figure 46 Hemispheric Pixels by Presence or Absence of the Ivy sign (prominent leptomeningeal collaterals result in vivid contrast enhancement)



Ivy Code: 0=No Ivy; 1=Ivy

Figure 47 Total hBOLD Score by Presence or Absence of Ivy (prominent leptomeningeal collaterals result in vivid contrast enhancement)



Ivy Code: 0=No Ivy; 1=Ivy

6.6.10. Hemispheric Pixels and Biological Indices

Spearman's non-parametric correlation was used to examine the association between potential biological indices of disease and hemispheric pixels in the no pre-surgery group (Table 54).

The mean interval between testing of haematologic indices, cardiovascular indices and hBOLD CVR was 1.2 (SD 5.16) and 1.7 (SD 5.88) months respectively. Group hematologic means were normal for age. Assuming the 50th centile for group height, the group mean systolic and diastolic BP was in the 50 – 90th centile using the National Heart, Blood and Lung Institute Tables and Guide (www.nhlbi.nih.gov). There was a significant inverse association between haematocrit and left hemispheric PTNP, and a trend toward a significant inverse

association between heart rate and PTNP. There was no statistically significant association between haemoglobin, platelets blood pressure and hemispheric pixels (Table 54).

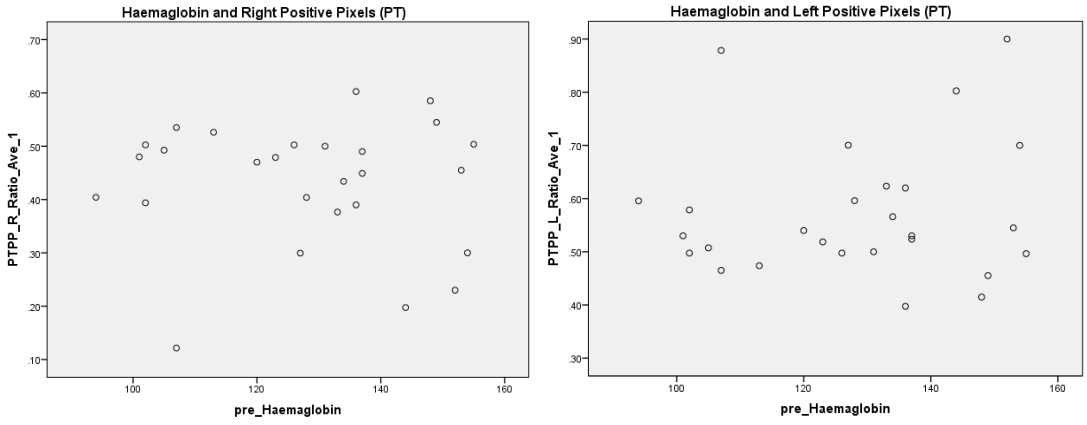
Table 54 Laboratory Data and Pixels In No Presurgery Group

hBOLD CVR Pixels n=27	Laboratory Data Correlation coefficient (p value)					
	Hb	Hct	Plts	BP Systolic	Diastolic	HR
PTPP R L	-.025 (.9) .039 (.85)	-.033 (.87) .03 (0.87)	.06 (.77) -.07 (.72)	-.23 (.25) .26 (.2) .	-.12 (.58) .17 (.42)	-.15 (.48) .14 (.49)
PTNP R L	-.22 (.26) -.37 (.06)	-.25 (.21) -.42 (.03)*	.08 (.69) .15 (.47)	.21(.28) .09 (.64)	.17 (.41) .1 (.63)	-.38 (.06)^ -.32 (.11)^
NTNP R L	-.21 (.30) -.23 (.26)	.23 (.26) -.23 (.25)	-.11 (.61) .12 (.55)	.11 (.59) -.07 (.72)	-.02 (.93) .06 (.77)	-.16 (.45) .15 (.47)

* Significant association between left sided negative pixels (PT) and haematocrit

^Trend toward significant association between right and left negative pixels (PT) and heart rate

Figure 48 Hemispheric Pixels and Haemoglobin



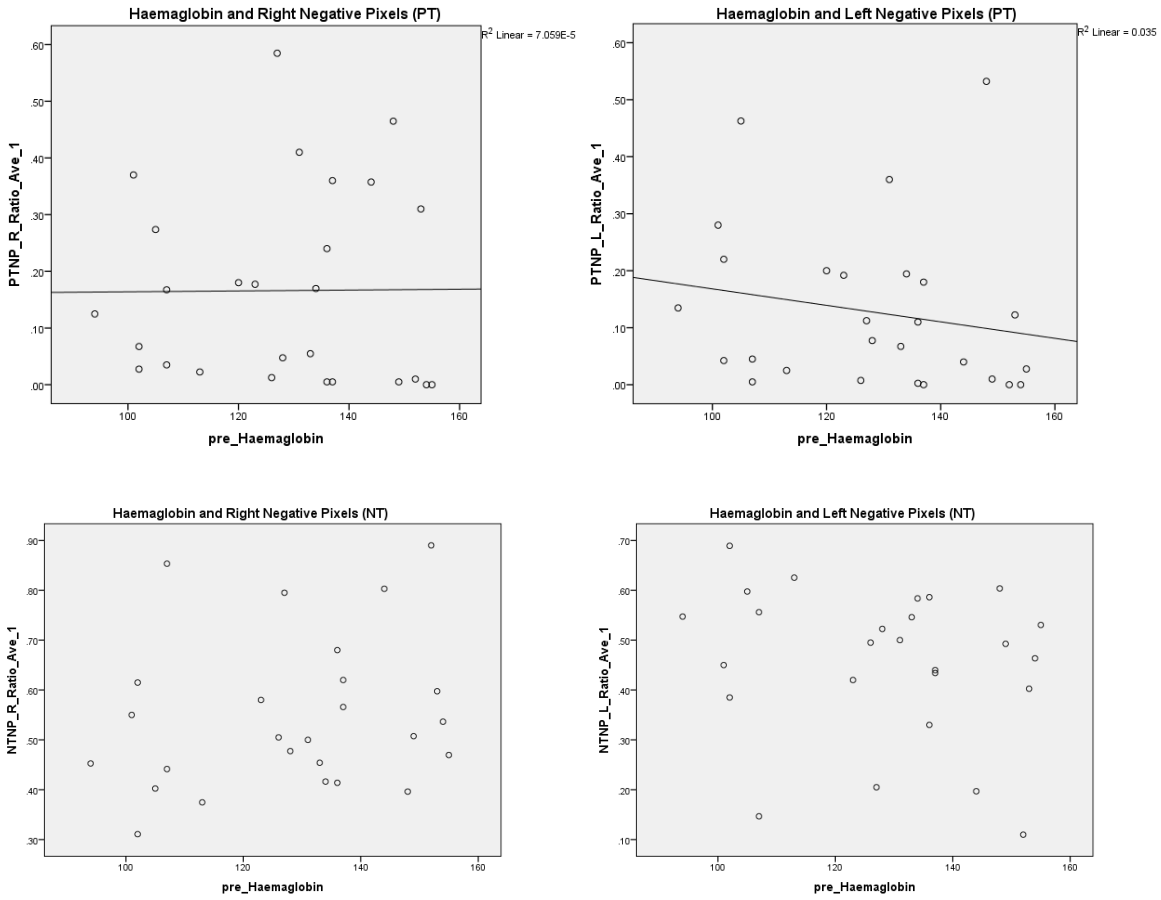
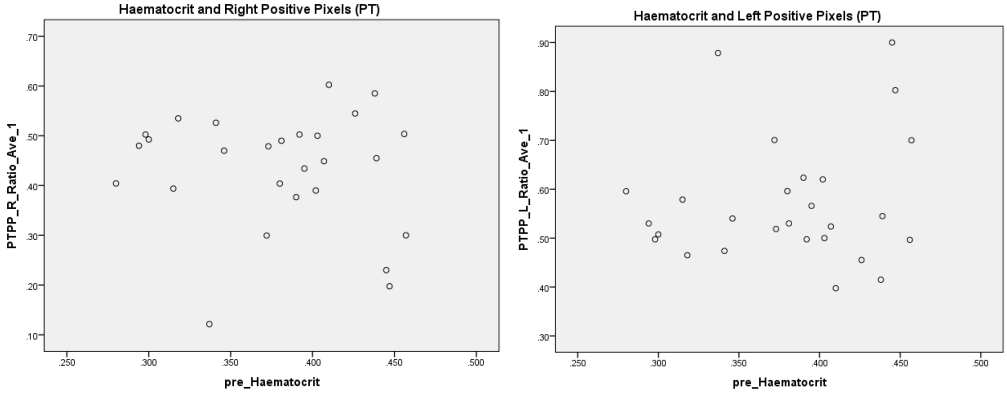


Figure 49 Hemispheric Pixels and Haematocrit



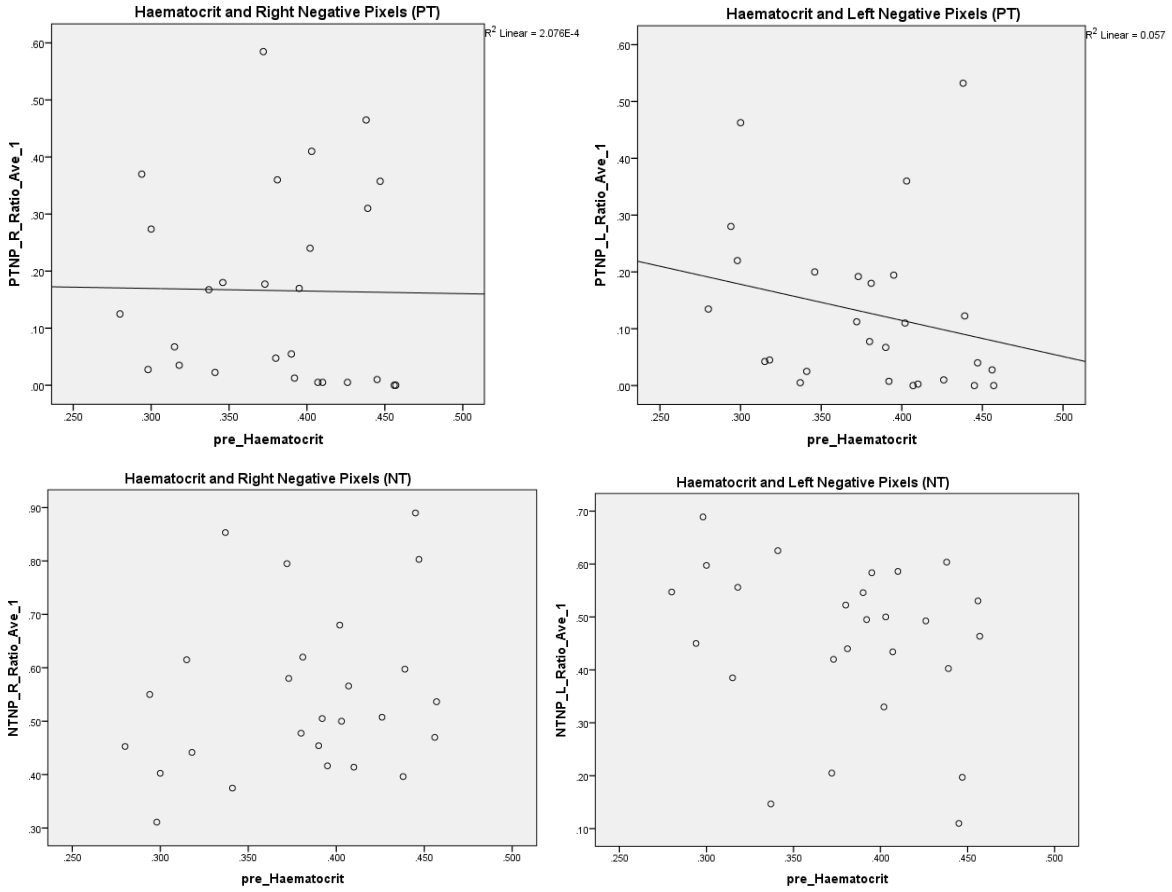
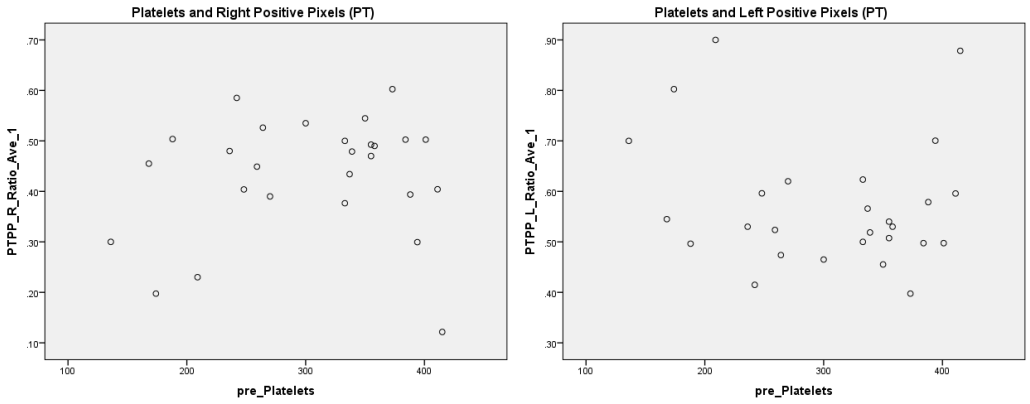


Figure 50 Hemispheric Pixels and Platelets



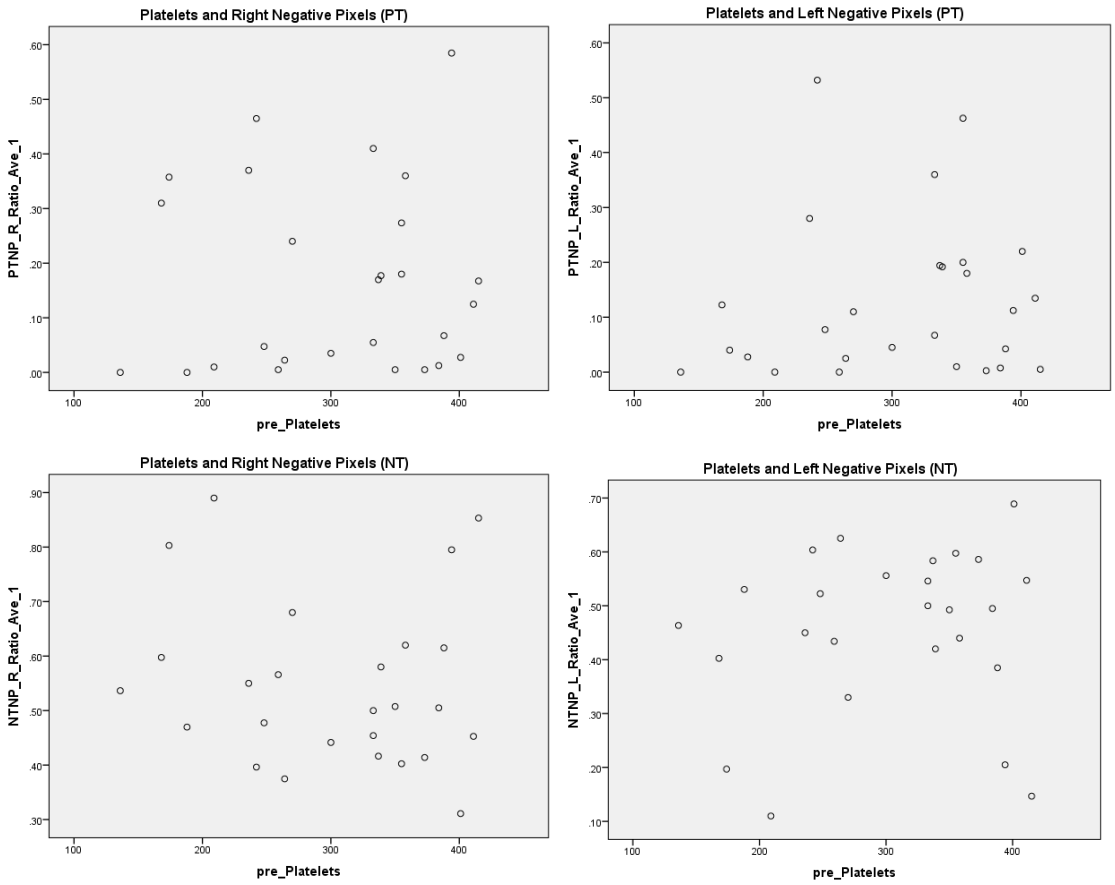
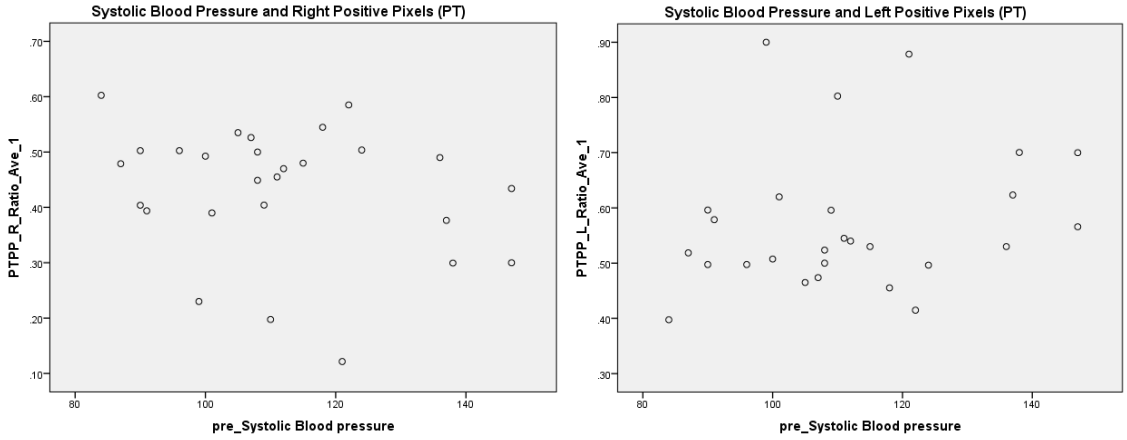


Figure 51 Hemispheric Pixels and Systolic Blood Pressure



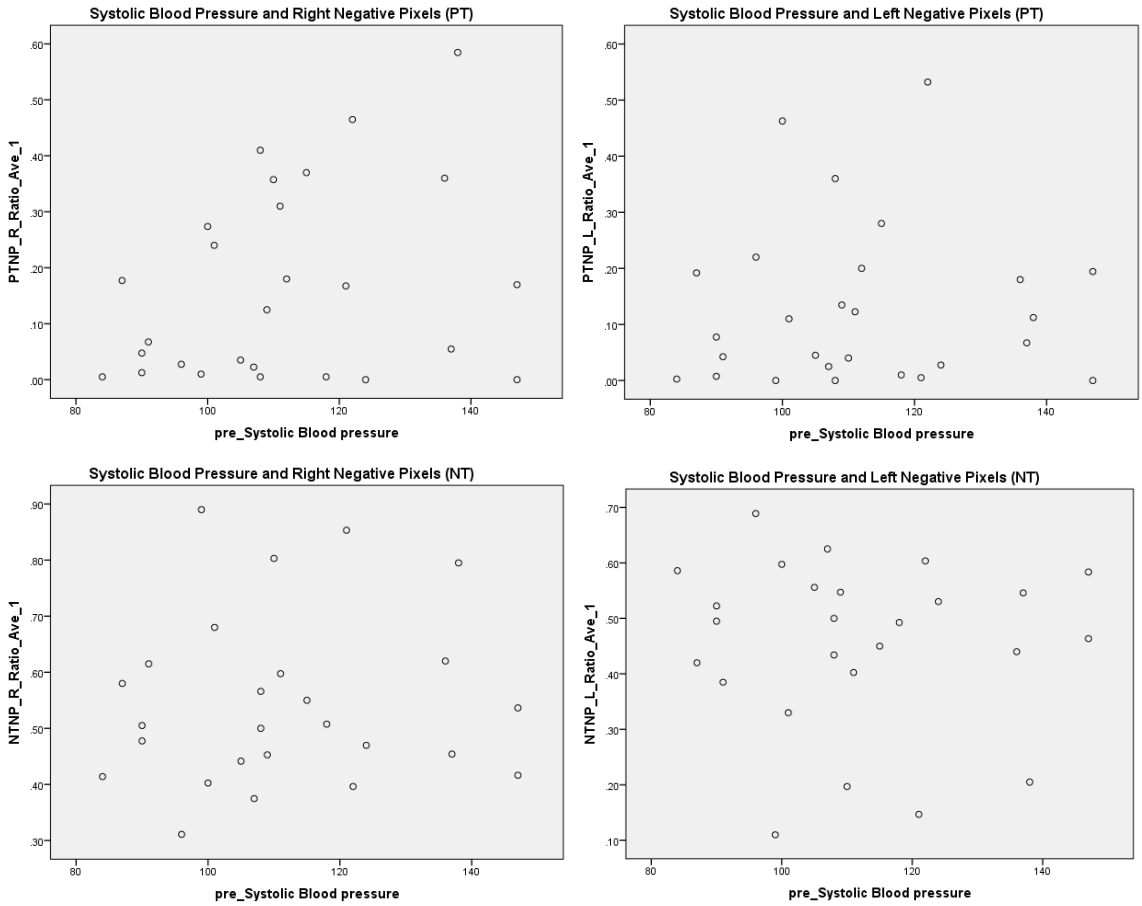
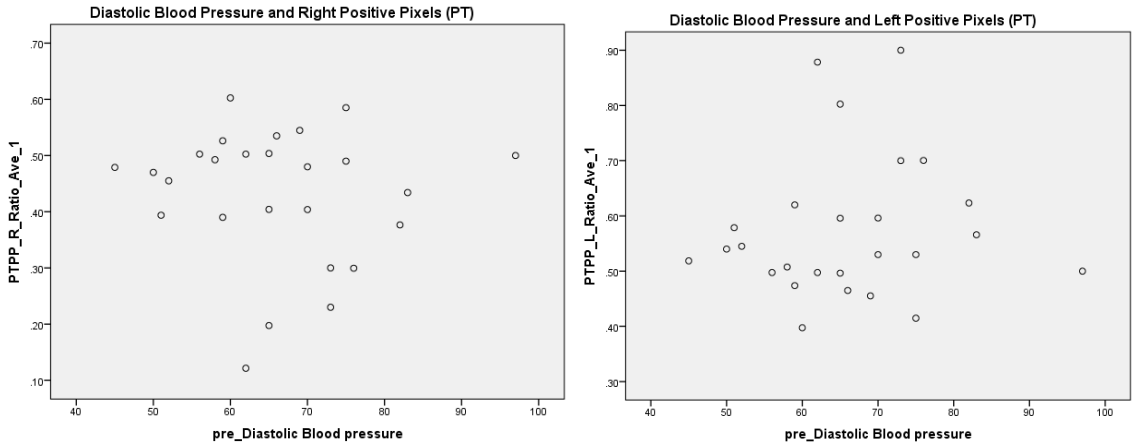
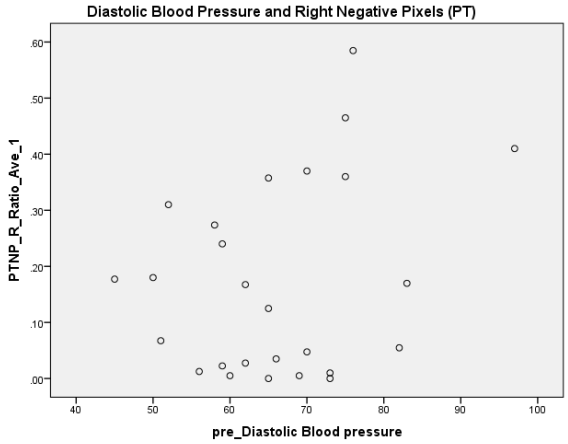
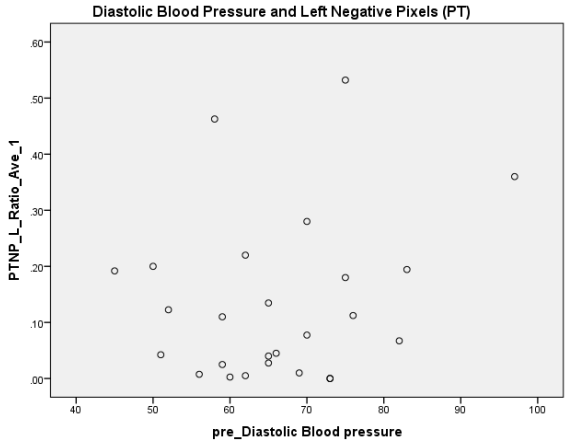
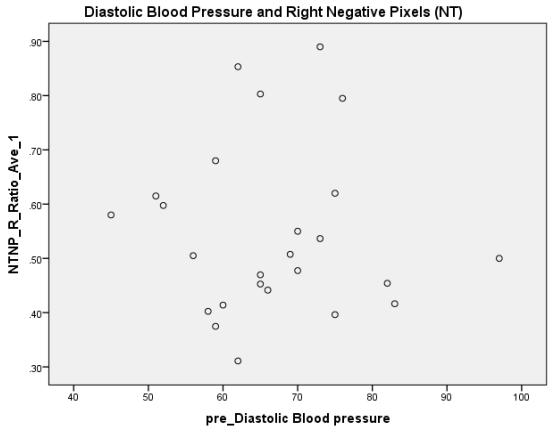


Figure 52 Hemispheric Pixels and Diastolic Blood Pressure




Diastolic Blood Pressure and Right Negative Pixels (PT)
PTNP_R_Ratio_Ave_1
.60
.50
.40
.30
.20
.10
.00
40
50
60
70
80
90
100
pre_Diastolic Blood pressure


Diastolic Blood Pressure and Left Negative Pixels (PT)
PTNP_L_Ratio_Ave_1
.60
.50
.40
.30
.20
.10
.00
40
50
60
70
80
90
100
pre_Diastolic Blood pressure


Diastolic Blood Pressure and Right Negative Pixels (NT)
NTNP_R_Ratio_Ave_1
.90
.80
.70
.60
.50
.40
.30
40
50
60
70
80
90
100
pre_Diastolic Blood pressure

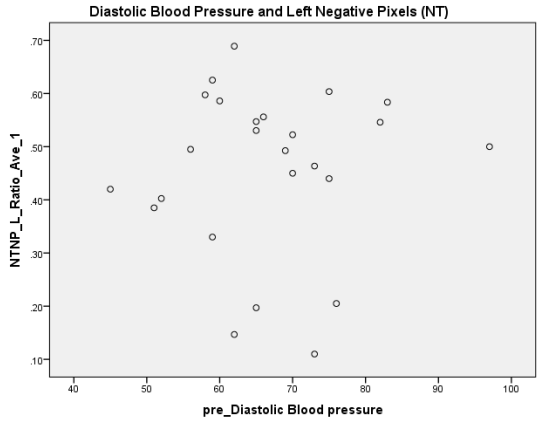

Diastolic Blood Pressure and Left Negative Pixels (NT)
NTNP_L_Ratio_Ave_1
.70
.60
.50
.40
.30
.20
.10
40
50
60
70
80
90
100
pre_Diastolic Blood pressure

Figure 53 Hemispheric Pixels and Heart Rate

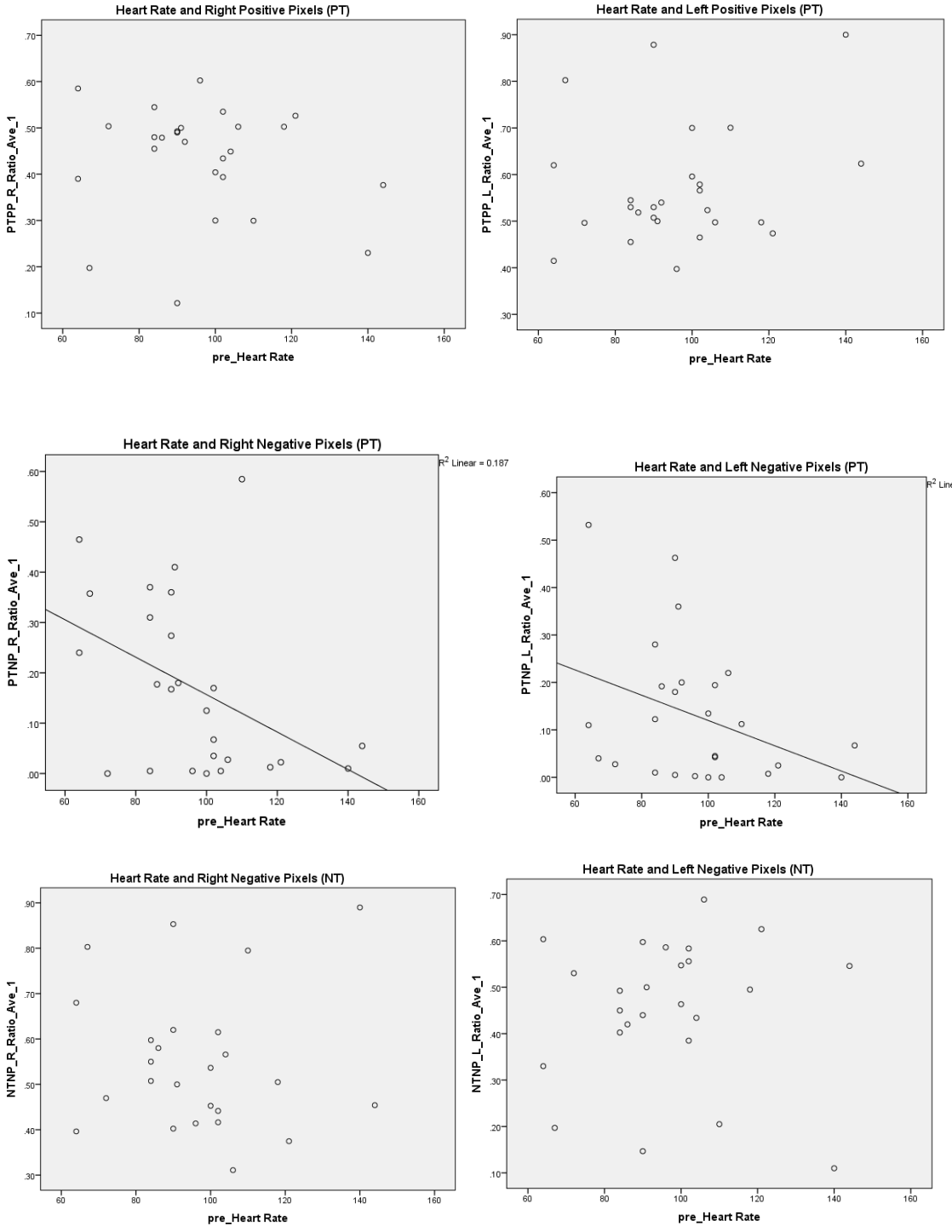
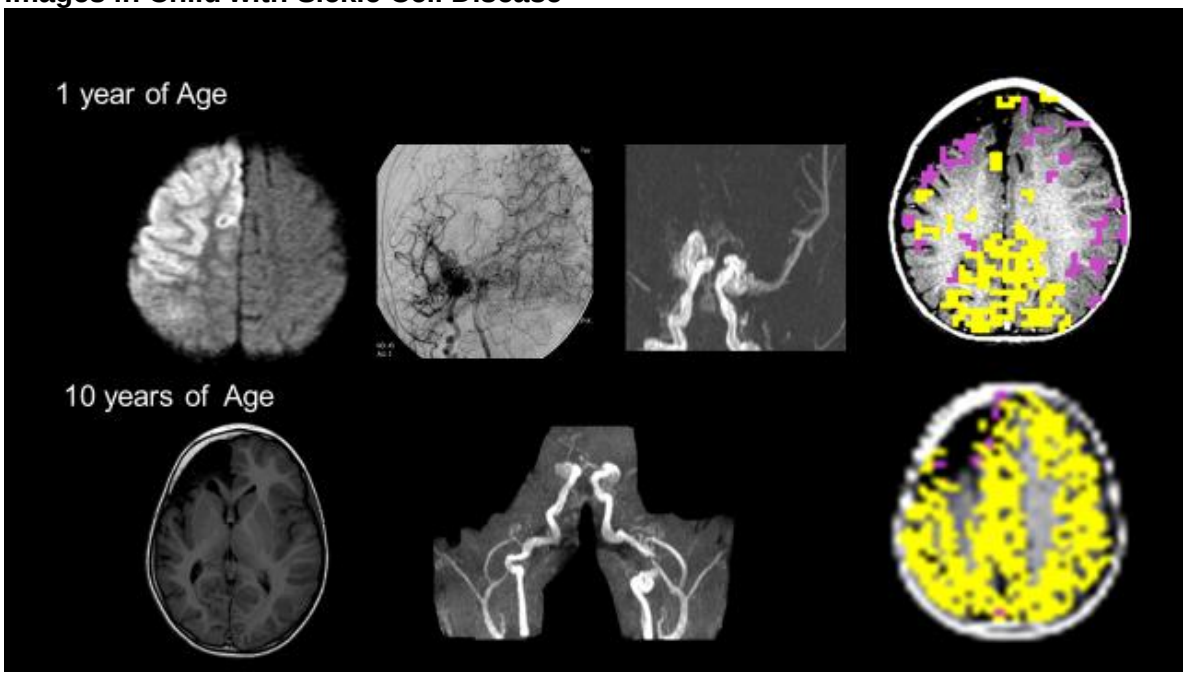


Figure 54 Pre and Post Bone Marrow Transplant Parenchymal, Vascular and CVR Images in Child with Sickle Cell Disease



Top Row: Pre-bone marrow transplant axial DWI image showing area of acute infarction. Conventional Angiogram and MR angiogram showing bilateral moyamoya arteriopathy. CVR parametric map demonstrating bilateral poor reactivity and steal.
Bottom Row: Post-bone marrow transplant axial FLAIR image showing area of encephalomalacia. MR angiogram showing bilateral moyamoya arteriopathy.
CVR parametric map demonstrating bilateral good reactivity and no significant steal.

6.7. Discussion

6.7.1. Concordance of Quantitative CVR measures and angiographic moyamoya laterality

Our study found concordance between regional abnormality of hBOLD CVR with the region of abnormality as depicted on conventional angiography.

In a normal brain hemispheric PTPP would be equally distributed between hemispheres. As all hemispheric pixels were calculated as a proportion of 200 total hemispheric pixels ie. right hemispheric pixels/200 and left hemispheric pixels/200, the right and left hemispheric pixel ratio should be 0.5:05 ie. one would expect 100 PTPP on the right and left hemisphere.

Examination of hBOLD CVR hemispheric pixels in the unilateral moyamoya group demonstrated a relationship between hBOLD CVR hemispheric pixel measures and moyamoya laterality, where positive pixels were lower in the diseased hemisphere and higher in the normal hemisphere, and negative pixels were higher in the diseased hemisphere and lower in the normal hemisphere. Mean PTPP were less than 0.5 in the affected hemisphere, and mean NTNP were greater than 0.5 in the affected hemisphere. This suggests that in unilateral moyamoya, hemispheric measures of hBOLD CVR may be used to predict moyamoya laterality and is indicative of tissue level microvascular dysfunction – the main aim of the tool. (Table 55).

Table 55 Example of Ratio of Hemispheric Pixels by Moyamoya Laterality

	Summary of Impressions Laterality of Arteriopathy by Conventional Angiography			
	No arteriopathy	Right	Left	Bilateral
PTPP Right:Left	0.5:0.5	.25:.75	.25:.75	.5:.5
PTNP Right:Left	0:0	>0:0	0:>0	>0:>0
NTNP Right:Left	0:0	.75:.25	.75:.25	.5:.5

Negative pixels are an indicator of abnormal paradoxical reactivity. In the normal brain, no negative pixels would be expected. The presence of negative pixels could therefore be used as a marker of disease severity. However, to account for circumstances where negative pixels may be apparent but not due to pathology, we set a threshold whereby negative pixels >2% of 200 positive pixels were considered abnormal (Chapter 2 methods).

We also found that the magnitude of difference between diseased and normal hemispheric pixel measures was higher in those with right sided moyamoya than left sided moyamoya.

Examination of hemispheric pixels by moyamoya laterality in the bilateral moyamoya group demonstrated a reduced proportion of right hemisphere positive pixels when compared to the left. However, hemispheric negative pixels measures were equally distributed between hemispheres.

In summary, since hemispheric positive pixels would be expected to be 0.5

respectively in a normal brain, deviation from this toward 0 would be indicative of disease in unilateral moyamoya, or the more diseased hemisphere in bilateral moyamoya; and deviation from 0.5 toward 1 would suggest a healthy or healthier hemisphere. Negative pixels could also be used an indicator of disease severity but may not be as sensitive an indicator of laterality.

6.7.2. Concordance of CVR measures and angiographic regional of abnormality

In the group of children with left sided MM on conventional angiography (n=7), three out of the seven had right and left hBOLD CVR abnormality. In those with right sided MM (n=6) two out of six had left and right hBOLD CVR abnormality. This is in keeping with study findings by Han et al (Han *et al.* 2011), and Heyn et al. (Heyn *et al.* 2010) where abnormality in quantitative measures of hBOLD CVR were demonstrated beyond the region of angiographic abnormality and in otherwise normal looking brain. This suggests that despite their more qualitative nature BH and GA studies of hBOLD CVR are useful for demonstrating existing or at risk regions of perfusion compromise beyond what can be seen on conventional angiography and MRI.

6.7.3. Laterality of Function

Our measures of CVR abnormality were consistently more abnormal on the right hemisphere than the left hemisphere. This was not simply explained by laterality

since in the bilateral moyamoya group, right hemisphere CVR measures were also worse than left CVR measures. These findings may relate to functional and anatomical asymmetries, which are described in humans and animals and seem to be advantageous for human and animal life (Duboc *et al.* 2015). Examples of this include cerebral lateralization of the autonomic nervous system and cardiovascular function (Hilz *et al.* 2001, Foster *et al.* 2008, Foster *et al.* 2013). BOLD fMRI studies have been used to investigate functional lateralization as a function of handedness and have shown that right-handed individuals have larger BOLD responses to hand movements in the dominant motor cortex compared with the non-dominant one (Bertolino *et al.* 2004, Haaland *et al.* 2004, Gut *et al.* 2007, Zeng *et al.* 2007). Whether this difference is due to neuronal responsiveness or vascular reactivity between the dominant and non-dominant hemispheres remains unclear. In a study of healthy right handed adult males, Driver et al recently demonstrated that there is hemispheric asymmetry in cerebrovascular reactivity, with CVR being higher and better on the left side. The higher CVR was associated with an increased density of large veins, something we were not able to control for in our study. Specifically the asymmetry in CVR was seen in the precentral gyrus but not evident when the whole hemisphere was compared. Whether this is as a result of lifelong use of the right hand, or that hemispheric dominance is innate and present from birth is not fully understood (Driver *et al.* 2015, Duboc *et al.* 2015).

Our study suggests preferential preservation of left hemispheric CVR. This may be an age related phenomenon as CVR asymmetries have not been identified in the literature based on the mostly adult studies (despite the functional and anatomical

differences noted in other eg. cognitive functions), or this finding may be due to the left hemisphere having more vascular reserve and resilience at the level of the capillary bed and neurovascular unit. In summary, whether there is preferential preservation of left hemispheric CVR requires further study and replication.

6.7.4. Hemispheric Pixels and Stroke

Although there was no statistically significant difference in quantitative CVR pixel measures between the stroke and no stroke group there was a significant difference between the stroke and non-stroke hemisphere within the stroke group. PTPP were lower in the stroke hemisphere in those with right and left stroke, and significantly so in those with right stroke. There was a trend for the right PTPP to be significantly lower on the right side in those with bilateral disease. Neurovascular activity as reflected by CVR is shown to be reduced to absent in the peri-infarct and infarcted regions. In contrast CVR appears to remain unaffected in healthy tissue both in patients with a history of stroke and healthy controls (Geranmayeh *et al.* 2015). Our method used to calculate the pixels i.e. adjusting the threshold to allow generation of 200 pixels in an axial slice, would negate the potential net effect of a large region of no reactivity on the hemispheric measures. Therefore, post processing lowering of the threshold to facilitate the generation of 200 pixels in some patients (eg large stroke) may have resulted in spurious or false positive findings and not be a reflection of active disease.

6.7.5. Hemispheric Pixels in Surgically Naïve Group

Group qualitative CVR measures were the same regardless of whether the patients had revascularization surgery or not. However, the difference in CVR measures in the stroke group, within the stroke and non-stroke hemisphere, was more pronounced in the surgically naïve group. Therefore, it is possible that there was a difference in CVR between the surgical groups which was not detected because of the difference in group size and the magnitude of CVR change.

The relationship between moyamoya laterality and hemispheric pixel measures was statistically significant in the children who had no history of prior revascularization surgery.

It is possible, however, that successful revascularization surgery does not always result in improved CVR. Direct or indirect bypass surgery improves the delivery of blood to the tissue. Improved perfusion distal to the stenosis should result in reduced vasodilation within the capillary bed, and therefore improved cerebrovascular responsiveness and reactivity. However, it is plausible that under certain circumstances, such chronic dilatation, hypertension, chronic anaemia and older age, this correction in cerebrovascular responsiveness may not occur. Therefore, perfusion studies alone may show improvement post revascularization surgery, but no improvement in CVR. In addition post-operative hyperperfusion, oedema and shifts in the post-operative watershed as demonstrated on SPECT has also been demonstrated in children with moyamoya, all of which could account for a lack of improvement in post-operative CVR. (Ogasawara *et al.* 2005, Ohue *et*

al. 2008, Fujimura *et al.* 2009, Hayashi *et al.* 2010) Longitudinal studies with larger cohorts are needed to further explore these theories.

6.7.6. Hemispheric Pixels and Angiographic Indices of Disease Severity

6.7.6.1. Modified Suzuki Stage

Donahue et al (Donahue *et al.* 2013) and Heyn et al. (Heyn *et al.* 2010) demonstrated an association between severity of moyamoya disease using Modified Suzuki staging, arterial circulation time (ACT) and hBOLD CVR. This was not replicated in the current study, and we did not quantitatively assess ACT. The majority of children in our study had Suzuki stage 2 or 3 disease. Modified Suzuki staging alone is likely not a good enough indicator of disease severity, or predictor of stroke risk. It is a description of the appearance of collaterals, ranging from no collaterals (Stage 1) to disappearance of collaterals (Stage 6). Stage 3 angiographic disease denotes the classic moyamoya or 'puff of smoke appearance' and is the most common stage reported in children presenting with moyamoya. It is difficult to differentiate between Stage 2 (initiation of collaterals), 4 and 5 (minimisation and reduction). Accurate staging requires serial conventional angiographic studies, which is a problem given the growing reluctance to perform conventional angiography in young children coupled with the limitations of MR angiography ie undercalling collaterals, and overcalling medium to large vessel stenois. In addition, current staging does not account for laterality of moyamoya;

therefore, using this method, unilateral Stage 6 moyamoya is deemed as severe as bilateral Stage 6 moyamoya.

Ischaemic injury in chronic hypoperfusion as seen in moyamoya occurs more gradually and over time. This is in contrast to the abrupt ischaemic injury caused in vaso-occlusive thromboembolic stroke. The rate of collateralization in response to parent artery stenosis is likely to be more important at a tissue level than the presence of collateralization. This is difficult to capture with the standard use of conventional angiography. Therefore the use of the traditional conventional angiography to infer disease severity and tissue level impairment is likely limited and the use of a non-invasive MR technique using conventional sequences could be of significant benefit in this population.

6.7.6.2. Grading of Stenosis

The majority (30/34 with conventional angiograms) of our children had Grade 4 (severe 75 – 100%) stenosis or occlusion. Four had Grade 3 (50 – 74% stenosis). Children with less severe stenosis had lower right PTPP and significantly higher right PTNP. Of the four children who had Grade 3 stenosis, two had Trisomy 21-MM and three had stroke (2 right sided, 1 bilateral). The children with chromosomal disorders, including Trisomy 21, had some of the lowest CVR measures (See Chapter 5). Hence the worse CVR measures of the less severe group are likely to be related to the underlying disease syndrome rather than a reflection of the Grade of stenosis itself.

The relationship between arteriopathy and tissue level impairment is unlikely to be linear. A study by Liu and Zhou in adults with ICA and MCA stenosis suggests that degree of stenosis may not be as good a predictor of stroke as CVR (Liu and Zhou 2014). The degree of stenosis is not necessarily correlated with the degree of regional blood flow, perfusion nor the risk of stroke. Adult studies of patients with carotid artery diseases report a relatively low risk of stroke in patients with severe stenosis (ie.90%) (Kasner *et al.* 2006) (Inzitari *et al.* 2000) The presence or absence of collaterals may influence this but not necessarily infer hemodynamic compromise at the tissue level. Collaterals may reflect either aggressive, unstable disease or alternatively protective compensatory mechanisms (Liebeskind *et al.* 2011). The integrity of the neurovascular unit may hence be maintained despite the presence of severe parent artery disease in the presence of adequate and timely collateralization. Detection of impending failure of this unit and stroke risk is possible with the use of qualitative hBOLD CVR. Deviation of hemispheric pixel CVR measures away from 0.5, the presence or increase of negative pixels, and relatively lower positive or higher negative CVR on a parametric map are all utilizable methods of qualitative CVR assessment in the childhood moyamoya population. Descriptions of longitudinal hBOLD CVR results which appear to predict ischaemic events are provided in the following chapter. However, this study was not powered to demonstrate whether abnormality in quantitative CVR was predictive of stroke.

6.7.6.3. Ivy Sign

In adults with moymoya disease, one study using single photon emission CT (SPECT) showed leptomeningeal high signal intensity (ivy sign) seen on fluid-attenuated inversion recovery images had a significant negative relationship with resting CBF and CVR, and a significantly positive relationship with ischaemic symptoms (Mori *et al.* 2009). In addition, the ivy sign has been shown to decrease post-operatively with corresponding increase in SPECT and MR perfusion changes. Ivy may be present in the absence of moyamoya vessels/collaterals and hence may be particularly useful sign when using conventional MRI imaging for the diagnosis and management of moyamoya disease (Fujiwara *et al.* 2005). However, there was no association between the presence of ivy and any hBOLD CVR measures of tissue level impairment.

6.7.7. hBOLD CVR Measures and Biological Indices of Disease

MRI BOLD signal is dependent on CBF and to unknown extent haematocrit (Ogawa *et al.* 1993, Hoge *et al.* 1999, Petersen *et al.* 2006), which is known to be correlated with CBF. Cerebral blood flow may also be related to haemoglobin, associated with oxygen content, and platelets, in relation to thrombosis. However, there are no published quantitative studies of hBOLD CVR examining the relationship between CVR and these biological measures. In addition systemic blood pressure is known to be an indicator of cerebrovascular disease and associated with compromise of CVR Ref (Settakis *et al.* 2003, Settakis *et al.* 2006, Wong *et al.* 2011). Hence I sought to examine the relationship between

haemoglobin, haematocrit, platelets and blood pressure with hBOLD CVR measures.

A statistically significant relationship between biological indices of disease and hBOLD CVR measures was only found for haematocrit and left PTNP, and a trend toward significance for heart rate and PTNP. The results are limited by the chosen methodology for measurement of pixels as a maximum number of 200 pixels is predetermined for each patient PTPP and NTNP are unlikely to be sensitive enough to demonstrate small to moderate effect of the biological parameters on hemispheric measures of qualitative CVR. As PTNP values were generated differently ie. number of negative pixels/200 positive pixels, this is likely to be more sensitive for across group analysis. Despite this the observed suggestion of association with heart rate is in keeping with heart rate being a marker of cardiovascular and cerebral autonomic function and therefore may be a real finding (Settakis *et al.* 2003, Wong *et al.* 2011, Mitra *et al.* 2014, Mitra *et al.* 2014).

6.8. Study Limitations and Future Directions

A number of the limitations of my study have already been mentioned. Further limitations and future directions are as follows:

1) Population size - The small numbers with Modified Suzuki Stage 1, 4, 5 and 6 meant that I was underpowered to make any conclusions about the association between Modified Suzuki Staging and hBOLD CVR as a measure of tissue level impairment. Similarly I did not have enough children with grade 1, 2 or 3 stenosis

to test whether grade of stenosis is associated with tissue level impairment as measured by hBOLD CVR. Our grading system did not distinguish between a 75% stenosis and a totally occluded vessel, which may have different upstream effects on CVR.

2) Longitudinal studies – analysis of hBOLD CVR studies in a longitudinal cohort would allow for the generation of quantitative CVR measure normal ranges, and quantification of ischaemic risk much like is done in stroke risk assessment in SCD.

3) Indicators of disease severity and biological indices - Modified Suzuki staging is limited as a sole marker of disease severity. Review of conventional angiograms to calculate ACT and modification of the scoring system to account for laterality of Suzuki stage; severity of parent artery stenosis; involvement of posterior circulation disease and regional distribution of ivy and non-moyamoya collaterals would be more representative of disease severity. This supplemented with the development of a more comprehensive scoring system which included clinical information would provide a better means for comparison with measures of CVR and improve our understanding of the ability of CVR to inform of ischaemic risk in this population.

Improved methodology in the collection of biological data, specifically BP would allow for a more robust assessment of association with CVR.

4) Laterality of function – resting state fMRI, CVR, EEG and PET studies conducted at the same time at different ages could help elucidate whether, and if so, to what extent there is lateralization of CVR in childhood and any impact on stroke risk in the developing brain.

5) It may be possible to investigate changes in cerebral blood flow with age in normal children in relation to cognition and behaviour

6.9. Conclusion

1) Abnormalities of qualitative measures of hBOLD CVR ie. hemispheric pixels and scores are concordant with laterality of angiographic abnormality.

2) Qualitative measures of hBOLD CVR demonstrate functional impairment of the cerebral vasculature beyond the region of angiographic abnormality.

3) Measures of hBOLD CVR are correlated with angiographic measures of moyamoya severity as determined by Grade of Stenosis.

7. Pre and post-surgical hypercapnic BOLD CVR

7.1. Abstract

7.1.1. Background

Moyamoya causes recurrent ischaemic events and progressive neurological decline. Revascularization surgery may prevent this decline. However indicators for selection of patients and optimal timing of surgery are not established. Abnormalities of hypercapnic challenge blood oxygen level dependent (hBOLD) CVR cerebrovascular reactivity (CVR) are predictive of brain-at-risk for ischaemia in steno-occlusive cerebral arteriopathy.

7.1.2. Objective

To determine whether qualitative hBOLD CVR identifies children at risk of cerebral ischaemic events and demonstrates changes associated with revascularization surgery in moyamoya.

7.1.3. Method

Children with moyamoya disease (MMD) or moyamoya syndrome (MMS) were assessed with MRI/MRA, conventional angiography and hBOLD CVR. Those who underwent revascularization surgery also had post-operative studies. Children with

serial hBOLD CVR studies but not recommended for surgery were included as a control group.

7.1.4. Results

Thirteen children (5 male, mean age diagnosis 9.1, SD 3.27 years, bilateral moyamoya in 6) underwent hBOLD CVR pre- and post-revascularization surgery (26 hemispheric studies). Of the surgical group five children had MMD and 8 MMS (5 with NF1). Seven children (2 male, mean age diagnosis 7.96, SD 4.02 years, bilateral moyamoya in 5) did not have intervening surgery and were a non-surgical control group with follow-up. Two of the non-surgical controls had MMD and 5 MMS (2 NF1, 2 SCD and 1 Trisomy 21).

Four children were asymptomatic at the time of diagnosis. Symptomatic children presented with headache (2), seizures (2), TIA's or stroke (3) and paroxysmal episodes (1). Pre-surgical hBOLD CVR was abnormal in hemispheres affected by moyamoya in all children. Three children developed de novo TIA/stroke prior to surgery. In all children follow-up CVR (1-6 months post-surgery) improved on the side of surgery following surgery. Four of seven children with worsened CVR measures (3 post-operatively, 1 serial measures) proceeded to become clinically symptomatic, and required further revascularization surgery in all 4.

7.1.5. Conclusions

The study results suggest that CVR has potential as a role for evaluating ischaemic risk in childhood moyamoya. It demonstrates clinically meaningful CVR

improvement post-revascularization surgery in the majority, and worsening appears to be predictive of clinical deterioration and ischaemic risk.

7.2. Introduction

Diagnosis in MM is typically made after symptomatic presentation. However, due to disease specific surveillance protocols (Adams *et al.* 1998), there is an increase in diagnoses in asymptomatic patients. The presence of moyamoya angiopathy is associated with an increased risk of ischaemic or haemorrhagic injury to the brain, however understanding of the natural history of MM is limited by small sample studies and a lack of systematic follow-up imaging (Ezura *et al.* 1995, Kuroda and Houkin 2008). The course of disease is variable yet predictors of progression or severity of disease lacking. Symptomatic progression may be rapid - leading to disability or death - or slow. In addition the steno-occlusive arteriopathy appears to progress in asymptomatic and unilateral cases (Ezura *et al.* 1995, Kuroda *et al.* 2005, Lin *et al.* 2011).

There is no cure for MM. Current standard treatment strategies do not halt the primary vascular progression. Hence treatments are aimed at improving cerebral blood flow distal to the stenosis. Medical options include the use of anti-platelet agents such as Aspirin, vascular vasodilators such as Acetazolamide and Carbamazepine and simple measures such as good hydration. However revascularization surgery is the definitive approach improving blood flow distal to the stenosis by direct or indirect revascularization procedures. Current standard indications for revascularization surgery include clinical presentation with ischaemic symptoms, and/or progression in moyamoya angiopathy and/or progression in ischaemic parenchymal lesions. Studies of perfusion and

cerebrovascular reserve or reactivity to date have been used to corroborate clinical and standard radiological findings. However the current literature is limited to describing it's use to guide surgical decision making in the absence of traditional indicators in childhood moyamoya rather than in examining any association with status at follow-up.

In this chapter I sought to explore how hBOLD CVR changes in response to revascularization surgery and over time, and by so doing explore the utility of qualitative hBOLD CVR in informing decisions regarding surgery in children with moyamoya.

7.3. Study Hypothesis

1) Abnormality in hBOLD CVR corresponds with the vascular territories supplied by the narrowed vessels as demonstrated on CA.

2) Regional abnormality in hBOLD CVR improves following revascularization surgery in the corresponding vascular territory.

3) Change in hBOLD CVR predicts change in clinical outcome.

7.4. Method

Indicators for revascularization surgery included the following parameters:

i) Severity of clinical presentation ie. presence and frequency of TIAs; history of acute AIS, frequent accompaniment of other clinical presentations such as headache and/or paroxysmal symptoms

ii) Severity of radiographic presentation ie. presence and extent of ischaemic injury, presence of IVY and severity of moyamoya according to the Modified Suzuki Stage.

iii) Severity of abnormality on hBOLD CVR

Inclusion criteria

i) Diagnosis of MMD/MMS

ii) Revascularization surgery or serial hBOLD CVR studies

iii) Pre and post-operative:

MRI/MRA

conventional angiography

hBOLD CVR

Exclusion criteria

i) Children < 28 days >18 years

7.5. Parenchymal and Vascular Imaging

Over the study period 1.5T or 3T MRI (Achieva, Philips, Best, Netherlands) brain, 3D TOF MR angiography and 6 vessel conventional angiography were done in study participants before and after revascularization surgery. In addition

superselective angiography was conducted in patients after revascularization surgery. MRI/MRA and conventional angiograms were read by study investigator (ND) and study radiologist (DA).

Grading of arterial stenosis by the study investigator (ND) and study neuroradiologist (DA) was by visual inspection of the conventional angiograms and MRAs closest to the time of the hBOLD CVR studies. ND and DA were blinded to clinical information. Stenosis was graded as a percentage of the normal arterial diameter such that:

1= <25%; 2 = 25-49%; 3= 50-74%; 4=75-100% occlusion

If more than one vessel was involved all were graded and the worst vessel chosen for the purpose of analysis. Staging of the conventional angiograms and MRAs was done using Modified Suzuki criteria and the presence or absence of the ivy sign noted pre and post operatively (Chapter 2 Methods).

7.6. Pre and post hBOLD CVR Analysis

hBOLD CVR studies conducted before and after revascularization surgery were processed and analysed by ND and WL. hBOLD CVR parametric maps were scored by inspection and hemispheric pixels measured by WL and ND blinded to clinical information. (Chapter 2 Methods)

7.7. Statistical Analysis

Independent samples t-test to compare means for parametric data and one sample paired t-test to compare means for parametric data from the same sample analysis were used. Wilcoxon's-matched pair test was used for non-parametric categorical paired analysis. The Mann-Whitney test was used for non-parametric assessment of association and Fisher's exact test for comparison of categorical data. A p value of <0.05 was deemed statistically significant.

7.8. Results

7.8.1. Group Description

Of the 37 children with moyamoya, 13 (5 male, mean age diagnosis 9.1, SD 3.27 years) had pre and post-operative hBOLD CVR studies as described above. Seven children (2 male, mean age diagnosis 7.96, SD 4.02 years) with a minimum of two hBOLD CVR studies at different time points and without intervening surgery were included as non-surgical controls with follow-up. There was no statistically significant difference in age of moyamoya diagnosis, moyamoya arteriopathy diagnosis and moyamoya laterality between the revascularization group (n=13), the no intervening surgery group (n=24), and the non-surgical controls with follow-up (n=7). (Ref Figs 52a and 52b, and Table 56).

Figure 52a Pie charts of Comorbidity Frequencies and Moyamoya Laterality in Intervening Surgery Group compared with No Intervening Surgery Group

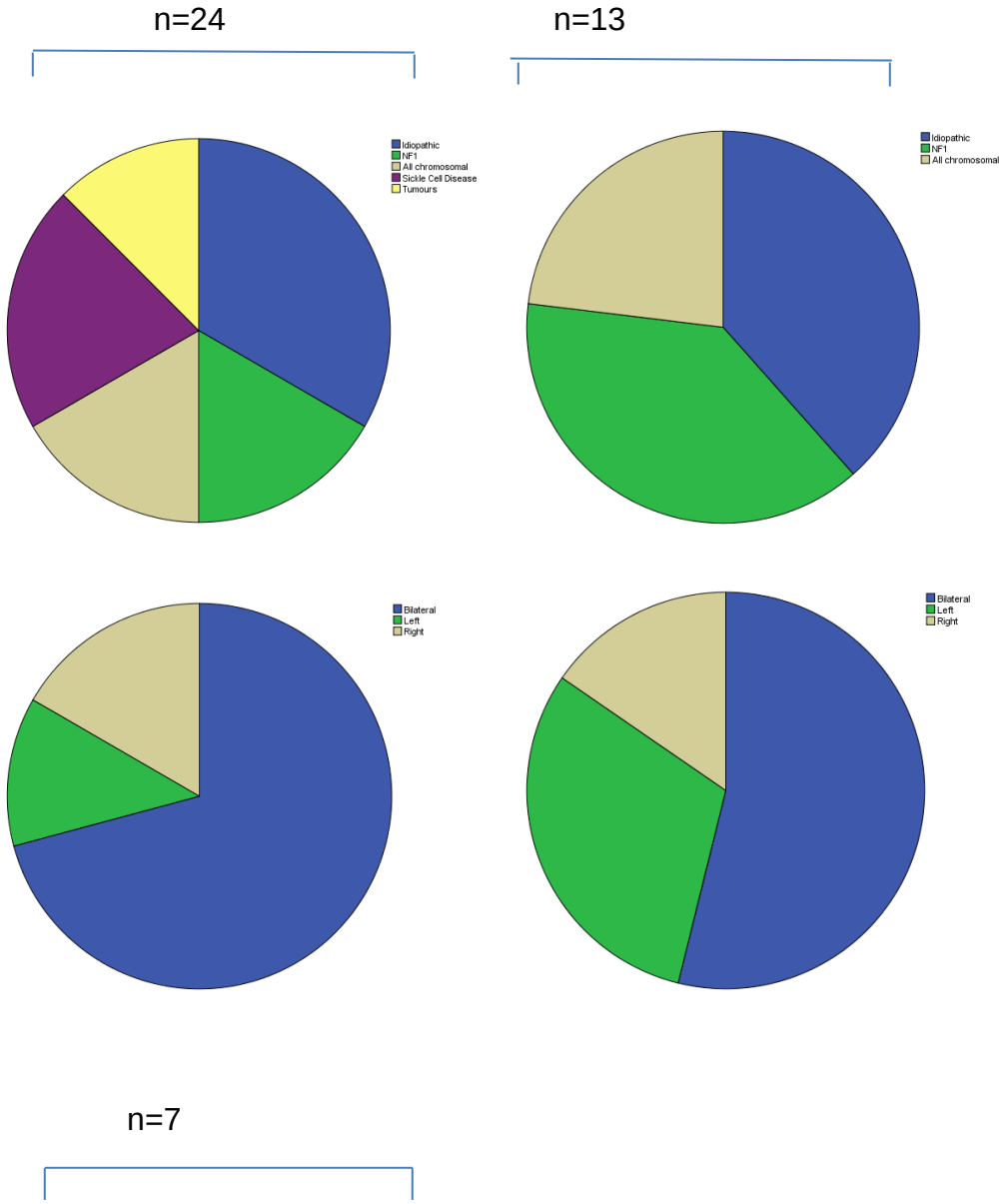


Figure 52b Pie Charts of No Intervening Surgery/Serial CVR Group

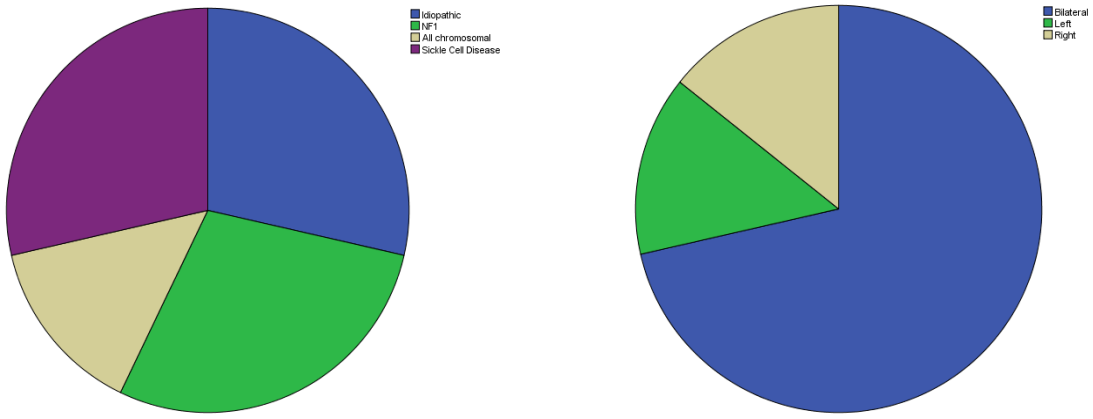


Table 56 Group Demographics of Non-surgical and Surgical Patients

	No Intervening Surgery	(a) t-test	Revascularization Surgery	(b) p value	No Surgery-Serial hBOLD CVR Controls
N	24		13		7
Mean Age at Diagnosis (SD)	9.1 (4.25)	.984	9.1 (3.27)	.496	7.96 (4.02)
Gender M (%)	11 (46)	-	5 (38)	-	2 (29)
Mean Age at hBOLD CVR 1 (SD)	11.1 (4.6)	.46	10 (3.73)	.961	10 (5.13)
Mean Age at hBOLD CVR 2 (SD)	12.5 (4.6)	.61	11.5 (3.87)	.504	12.5 (4.61)
Time between CVR1 and CVR2	-	-	1.47 (1.19)	.203	2.35 (1.72)

Moyamoya Laterality (B/R/L)	17/4/3	.499	6/2/5	.588	5/1/1
Comorbidity Code **Idiopathic** **NF1** **SCD** **Chromosomal** **Tumour**	 8 4 5 4 3	 .169	 5 5 0 3 0	 .351	 2 2 2 1 0
MM Stage **1** **2** **3** **4** **5** **6**	 2 8 10 0 0 1	 - 	 0 4 6 2 1 0	 - 	 1 3 3 0 0 0
Degree of Stenosis 1/2/3/4	0/0/1/20	-	0/0/3/10	-	0/0/0/7
Stroke (%) **B/R/L**	14 (58) 2/8/4	-	7 (54) 3/3/1	-	4 (57) 1/3/0

B=bilateral, R=right, L=left

7.8.2. Moyamoya Arteriopathy Diagnosis and Clinical Description in Surgical Group

Of the 13 children, five had Idiopathic-MM (two familial), five NF1-MM and three Chromosomal-MM (2 Trisomy 21 and one Turner's syndrome). Seven had bilateral, two right and four left sided moyamoya.

Four children were asymptomatic at the time of diagnosis and recommendation for surgery: 3 with NF1-MM and 1 Chromosomal-MM (Turner Syndrome). Diagnosis in these four was made following brain imaging indicated by their respective syndromic diagnosis. Of the remaining nine children, two presented with acute AIS; three with TIAs (two with headache and one with additional seizures); one with seizures and headache; and two with headache only. One child presented with paroxysmal sensory symptoms. Two of the four children who were asymptomatic at the time of diagnosis and recommendation for surgery had new overt ischaemic symptoms (1 AIS, 1 TIA) prior to surgery (Table 57).

Table 57 Moyamoya Classification and Clinical Description of Patients with Revascularization Surgery

Patient (M/F)	MM Disease or Syndromic Diagnosis	Moyamoya Laterality	Age at Moyamoya Diagnosis (years)	Moyamoya Presentation	hBOLD CVR 1 Age (years)	hBOLD CVR 1 Method	Age at Surgery (years)	hBOLD CVR 2 Age (years)	hBOLD CVR2 Method
1 (F)	Trisomy 21	Bilateral	10.17	Bil AIS	10.33	GA	10.58*	11.67	GA
2 (F)	Trisomy 21	Bilateral	14.25	R MCA AIS	15	GA	15.42	16.5	GA
3 (M)	NF1	Left	11.17	Asymptomatic	11.42	BH	11.83	12.33	BH
4 (M)	NF1	Left	11.50	Headache	12	RM	12	12.58	BH
5 (F)	NF1	Right	10.83	Headache	16.33	BH	16.42	16.92	BH
6 (F)	NF1	Left	5.17	Asymptomatic	5.58	GA	6.08	6.25	GA
7 (F)	Turners Syn	Left	3.83	Asymptomatic	4.08	GA	4.17	6.58	GA
8 (F)	Idiopathic	Bilateral	6.00	L TIAs	7.5	GA	7.5*	8.08	GA

Patient (M/F)	MM Disease or Syndromic Diagnosis	Moyamoya Laterality	Age at Moyamoya Diagnosis (years)	Moyamoya Presentation	hBOLD CVR 1 Age (years)	hBOLD CVR 1 Method	Age at Surgery (years)	hBOLD CVR 2 Age (years)	hBOLD CVR2 Method
9 (M)	Idiopathic	Bilateral	10.67	Headache Seizures Bil TIAs	10.75	BH	10.83	13.75	BH
10 (M)	Idiopathic	Bilateral	6.58	Seizures Headache	7.17	GA	7.25	7.67	GA
11 (F)	Idiopathic	Right	5.67	Paroxysmal sensory episodes	5.75	GA	5.75	7.17	GA
12 (F)	Idiopathic	Bilateral	12.75	R TIAs Headache	12.92	RM	13.08	14.17	RM
13 (M)	NF1	Left	9.92	Asymptomatic	10.67	BH	13.33	15.25	BH

* Prior right and left (respective) revascularization procedures

7.8.3. Moyamoya Arteriopathy Diagnosis and Clinical Description in No Intervening Surgery Group

Of 37 children, seven had moyamoya and repeat hBOLD CVR studies without intervening revascularization surgery or stroke. Two children had Idiopathic-MM; two NF1-MM; two SCD-MM and one Chromosomal-MM (Trisomy 21). Of these four presented with AIS; one headache and found to have a remote AIS on MRI; and two were asymptomatic at diagnosis. Diagnosis in the latter two was made following syndromic disease targeted screening protocols.

Two children had revascularization surgery prior to their serial studies and one after. One child had a bone marrow transplant between studies. The two children with SCD were also on a chronic blood transfusion programme (Table 58).

Table 58 Non-surgical 'Controls' with follow-up: Demographics of Patients with no Revascularization Surgery between hBOLD CVR Studies

Patient (M/F)	MM Disease/ Syndromic Diagnosis	Moyamoya Laterality	Age at Moyamoya Diagnosis (years)	Moyamoya Presentation	hBOLD CVR 1 Age (years)	hBOLD CVR Method	hBOLD CVR 2 Age (years)	hBOLD CVR Method	Intervention (Age,years)
1 (F)	Idiopathic	Bilateral	9.5	Bilateral AIS	15	BH	16.83	BH	R EDAS (10.92) L EDAS (11.00)
2 (M)	NF1	Left	6.33	L AIS (movement disorder)	7.25	GA	8.08	GA	L pial (6.42)
3 (M)	Sickle Cell Disease	Bilateral	.83	R AIS	1.00	BH	6.17	GA	BMT (4.6)
4 (F)	Idiopathic	Bilateral	12.67	HA Remote R AIS	16.08	BH	17.33	BH	
5 (F)	NF1	Right	8.58	Asymptomatic	9.00	BH	9.92	BH	
6 (F)	Sickle Cell Disease	Bilateral	11.83	Asymptomatic	12.5	BH	16.83	BH	
7 (F)	Trisomy 21	Bilateral	6.00	R MCA AIS	9.58	GA	12.08	GA	BEMS (13.25)

Table 59 Clinical and Radiological Details of Children with Revascularization Procedures

No	Age (yrs)	Sex	Op	Surgery	Co-morbidity/ Surgical Indication	Conventional angiography				Magnetic resonance			Cerebro-vascular Reactivity
						Vessels	Grade Stenosis	Suzuki Stage	Anatomic adequacy Collaterals	Moya moya	Parenchyma	IVY	
1		F	1	R EDAS	Trisomy 21 R TIAs	L dICA L M1/A1 R dICA R M1/A1	4	3	Adeq colls Slow perf RMCA	2	Bil R>L encephalomalacia ; laminar scarring	Y	Impaired R reactivity and steal
					L vascular progression	U	U	2	Adeq	6	Bil R>L encephalomalacia ; laminar scarring	N	Improved R steal L impaired reactivity and steal
2	15	F	1	Bilateral pial syn	Trisomy 21 High F VIII MTHFR het: R MCA AIS	R dICA L dICA L M1/A1	3	2	Bil ant circ deficits	2	Bilateral watershed	Y-bil	Bilateral impairment and steal
					Asymptomatic	+RM1 +RA1	4	4	Good (STA>MMA)	1	Bilateral watershed	Y-less	Some improvement ,but still bilateral impairment w steal
3	11	M	1	L pial syn	NF1 Occ headaches R TIA	L dICA/A1	3	3	Good ant. (R to L) L temporal lobe deficit	2/4	Normal	Y	Impaired L reactivity with some steal
					Improved headache No TIA	U	4	4	Good (STA, MMA)	2/4	Normal	Y	Improved

No	Age	Sex	Op	Surgery	Co-morbidity/ Surgical Indication	Conventional angiography				Magnetic resonance			Cerebro-vascular Reactivity
						Vessels	Grade Stenosis	Suzuki Stage	Anatomic adequacy Collaterals	Moya moya	Parenchyma	IVY	
4	11	M	1	L pial syn	NF1	L M1 occ	4+	2	Adeq	2	L watershed	N	L steal Impaired R reactivity
							4+	3	Better but slow (min from STA)	2	L watershed	N	Improved L steal. Some persistent R impairment
5	16	F	1	R pial syn	NF1 Headache	R dICA R M1/A1	4 4	4 4	Good	4	R watershed	Y	Bilateral steal WM
					Improved headache	U	4+	4	Good (STA, MMA)	4	R watershed	Y	Bilateral improved reactivity, less steal
6	6	F	1	L pial syn	NF1 Asymptomatic until 6 days b4 planned sx	R A1 L dICA occ	3 4+	5	Good patchy frontal deficit	6	Hamartomas	Y	Impaired L anterior reactivity
					Post-op TIAs resolved.	U	4 4+	5	Improved (MMA, STA non-contrib) Patchy frontal deficit	6	Hamartomas	Y	Improved overall +/- new steal L frontal
7	4	F	1	L pial syn	Turner's Asymptomatic	L dICA/M1	4	2	Adequate but slow	2	L watershed	Y	Bilateral impaired reactivity L>R c with steal

No	Age	Sex	Op	Surgery	Co-morbidity/ Clinical presentation/Sx Indication	Conventional angiography				Magnetic resonance			Cerebro-vascular Reactivity
						Vessels	Grade Stenosis	Suzuki Stage	Anatomic adequacy Collaterals	Moya-moya	Parenchyma	IVY	
						U	4+	2	Adequate but slow (STA/MMA)	2	L watershed	Y	Improved – no steal
8	6	F	1	R pial syn	Factor IX def L TIAs	R dICA RM1/A1 L dICA L M1/A1	4	3	Adeq (Post/STA/MMA)	3	R watershed	N	Marked bilateral steal – mostly anterior (L>R)
			2	L pial and inter-hemisph syn	R TIAs and AIS	U	4	3	Adeq	6	R MCA AIS Bilateral watershed	N	Improved Small areas of persistent bilateral steal
9	6	M	1	L ECIC	NF1 L focal seizures Migraine	R dICA R M1/A1 R opth L dICA L M1/A1 L opth	4+	3	Inadeq R vasc	6	Bil w'shed L>R Bil volume loss L>R Acute R MCA AIS	Y	R motor strip suggestive of steal. Failed BH
			2	R EDAS		+RP1 +LP1	4+	4	Improved but patchy frontal perfusion deficit	4	Bil w'shed L>R Bil volume loss L>R Old R MCA AIS	N	Bilateral impairment with steal No direct comparison

No	Age	Sex	Op	Surgery	Co-morbidity/ Clinical presentation /Sx Indication	Conventional angiography				Magnetic resonance			Cerebro-vascular Reactivity
						Vessels	Grade Stenosis	Suzuki stage	Anatomic Adequacy Collaterals	Moya moya	Parenchyma	IVY	
10	5	M	1	Bilateral pial syn	Familial; Seizures Headache TIA LMCA AIS	L dICA occ R dICA R M1/A1	4+	3	Good (R to L: L post to ant)	5	L MCA atrophy	Y	Bilateral impaired reactivity with steal - frontal sparing
					Asymptomatic	Not done				6	R MCA AIS	Y	Improved; decreased steal
11	5	F	1	R pial syn	B-Thalassaemia Trait: Headache Paroxysmal episodes	R dICA R M1/A1				1	Normal	Y	Impaired reactivity and R steal
			2	R occ pial syn	Reduced paroxysmal episodes Persistent HA	U				1	Normal	Y	Improved R ant steal Persistent R post steal
12	13	F	1	L pial syn	Familial: TIAs++ (R>L) Headache	R M1/A1 RP1 L M1/A1	3	2	Good L hem perf from RICA colls, and LVA colls to ant circ	2	L watershed	Y	Impaired L reactivity

No	Age	Sex	Op	Surgery	Co-morbidity/ Clinical presentation/ Sx Indication	Conventional angiography				Magnetic resonance			Cerebro-vascular Reactivity
						Vessels	Grade Stenosis	Suzuki Stage	Anatomic Adequacy Collaterals	Moya moya	Parenchyma	IVY	
			2	R pial syn	Initially improved TIAs. Subsequent increase L TIAs	+RdICA	4	3	Good (STA, MMA)	2	L watershed R MCA, ACA AIS		Regional improvement L reactivity with new regional impairment L ACA territory and R hemisphere with steal
13		M	1	L EDAS	NF1	L dICA L M1occ LA1	4+	3	Inadeq L watershed	2	Hamartomas	Y	Bilateral steal
						ND				2	Hamartomas		Improvement but persistent scattered steal

M=male; F=female; L=left; R=right; Bil=bilateral; syn=synangiosis, dICA=distal internal carotid artery; M1=proximal middle cerebral artery; A1=proximal anterior cerebral artery; occ=occlusion; STA=superficial temporal artery; MMA=middle meningeal artery; Y=yes; N=no; ant=anterior; PCA=posterior cerebral artery; ↓=decreased; Adeq=adequate; BH=breath hold; EDAS= Encephaloduroarteriosynangiosis;

7.8.4. Conventional Angiographic Findings

All (n=20) children had a conventional angiogram at at least one time point. Pre and post-operative four vessel angiography and post-operative (where applicable) superselective angiography were conducted to assess the extent of involvement of the vessels. Vessels were staged and graded as described in Methods (Chapter 2 Methods). In addition details of anterior circulation contribution to the watershed was noted. Arterial transit times were noted to be slow or normal as determined by an experienced interventional radiologist on the basis of time taken from arterial to capillary phase on conventional angiography.

Grossly, adequate anatomic self-collateralisation and perfusion albeit slow, from lenticulostriate, thalamostriate and/or pial collaterals was seen in all children. Cross-filling from the contralateral unaffected (in unilateral MM) or less affected (in bilateral MM) anterior circulation to the ipsilateral affected hemisphere, and cross-filling from the posterior to anterior circulation was seen with shifting of the watershed zones toward the ipsilateral (affected) or bilateral (more affected) hemisphere ie. $_{\text{unaffected}}$ ACA/MCA to $_{\text{affected}}$ ACA/MCA;$_{\text{unaffected}}$ PCA to $_{\text{affected}}$ MCA/PCA was seen in all. Regression of the cross-filling and shifted watershed was seen post-operatively in all with intervening surgery. Subsequent review of conventional angiography in 2 patients demonstrated patchy areas of avascularity despite good ipsilateral and contralateral self-collateralisation in other vascular regions (Table 59).

CVR abnormality was concordant with moyamoya laterality in all. Six children had unilateral moyamoya. Bilateral CVR abnormality was observed on the parametric maps in four of the six children (Fig 55 and 56).

Figure 55 hBOLD CVR Parametric Map (breath-hold) for Patient 13 with Unilateral Left Sided Moyamoya. Note the bilateral presence of steal (purple pixels).

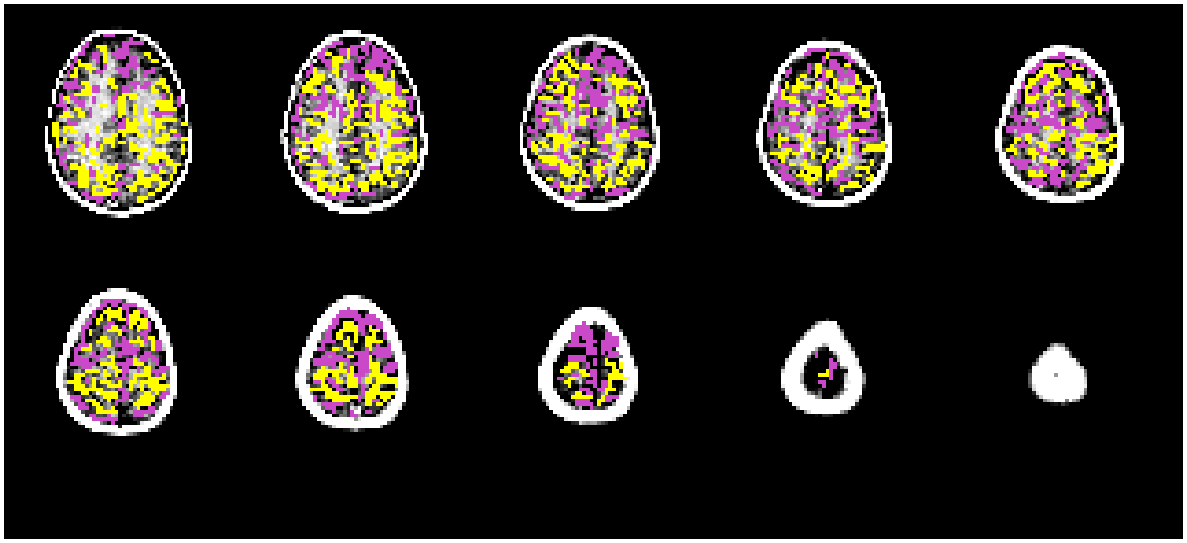
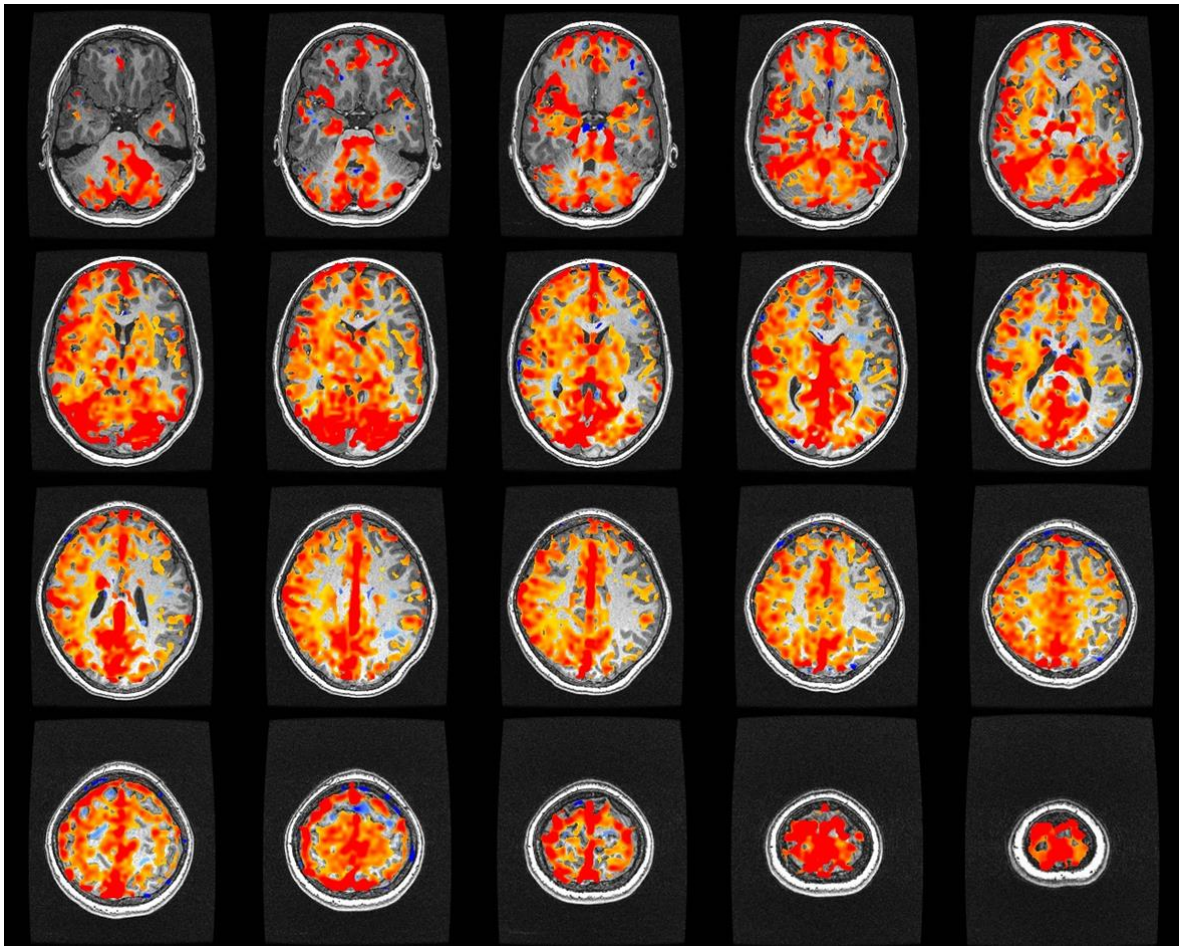


Figure 56 hBOLD Parametric Map (re-breathe mask) for Patient 4 with Unilateral Left Sided Moyamoya. Note the bilateral reduced reactivity (left more than right). Red denotes higher blood flow than yellow, blue or no colour



7.8.5. Revascularization Surgery and hBOLD CVR Studies

The mean age at revascularization surgery was 10.33 SD (3.87) years. Indications for revascularization surgery were as described above (Chapter 6 Methods)

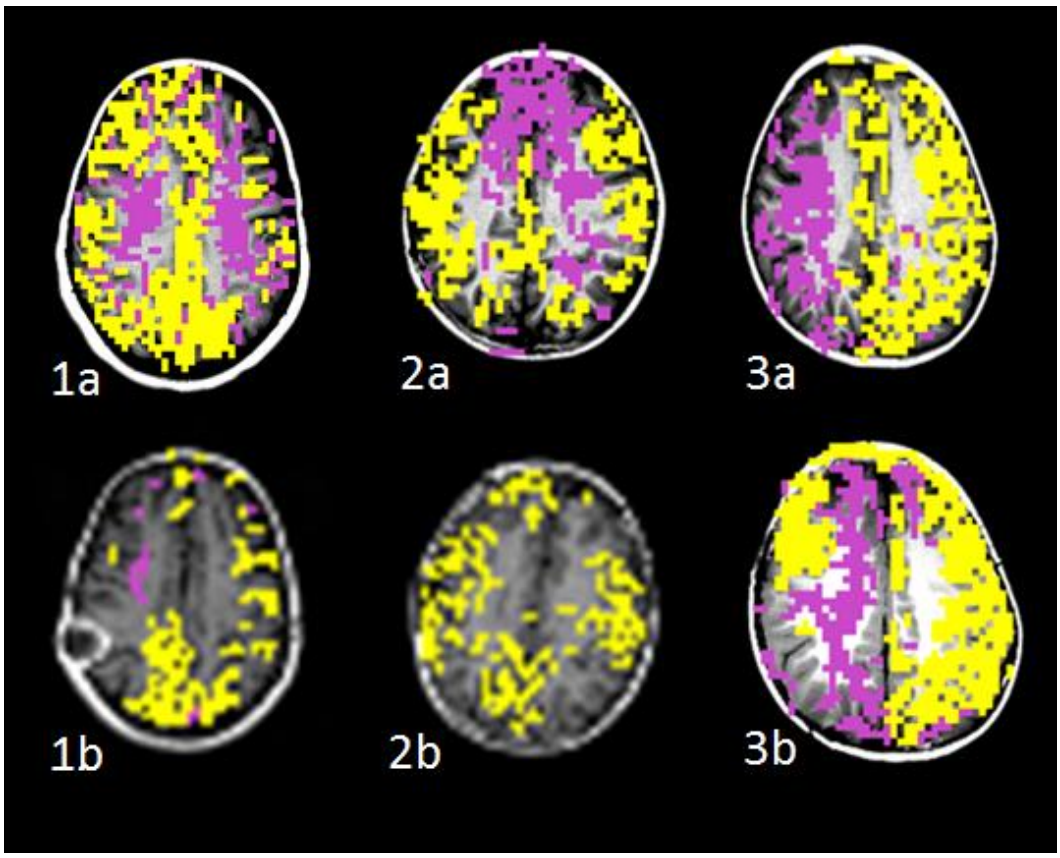
Thirteen children had, twenty-six hBOLD CVR studies (one pre-operative and one post-operative for each patient; 52 hemispheres): 7 GA-GA, 4 BH-BH, 1 RM-BH, 1 RM-RM.

In the revascularization surgery group the mean time between the first included hBOLD CVR study and revascularization surgery was 0.35 years (4.2 months), SD .689 years. The mean time between revascularization surgery and the second (follow-up) hBOLD CVR study was 1.08 years (13 months), SD 0.815 years.

In the non-surgical control with follow-up group the mean time between the first and second hBOLD CVR study was 2.35 years, 1.72 SD.

Six of 37 children had additional CO2 measurements, three of these (3/13) pre and post-operatively. Hence, quantitative hBOLD CVR was calculated pre and post-operatively for this sub-group.

Figure 57 Pre and Post-operative hBOLD Parametric Maps in 3 Children



1-3 a: Pre-operative CVR parametric maps demonstrating a) bilateral steal, left more than right, and reduced positive reactivity left more than right b) bilateral steal and c) right hemispheric steal

1-3 b: post-operative CVR parametric maps demonstrating reduced steal in all.

7.8.6. Serial hBOLD CVR Hemispheric Scores

Nineteen children had CVR inspection scores at two time-points (12/13 pre and post-operative; 7/7 no intervening surgery). Therefore 76 hemispheric scores were available for analysis.

7.8.6.1. Hemispheric Scores Operated, Operation Naïve and Control Hemispheres

7.8.6.1.1. Surgical Group

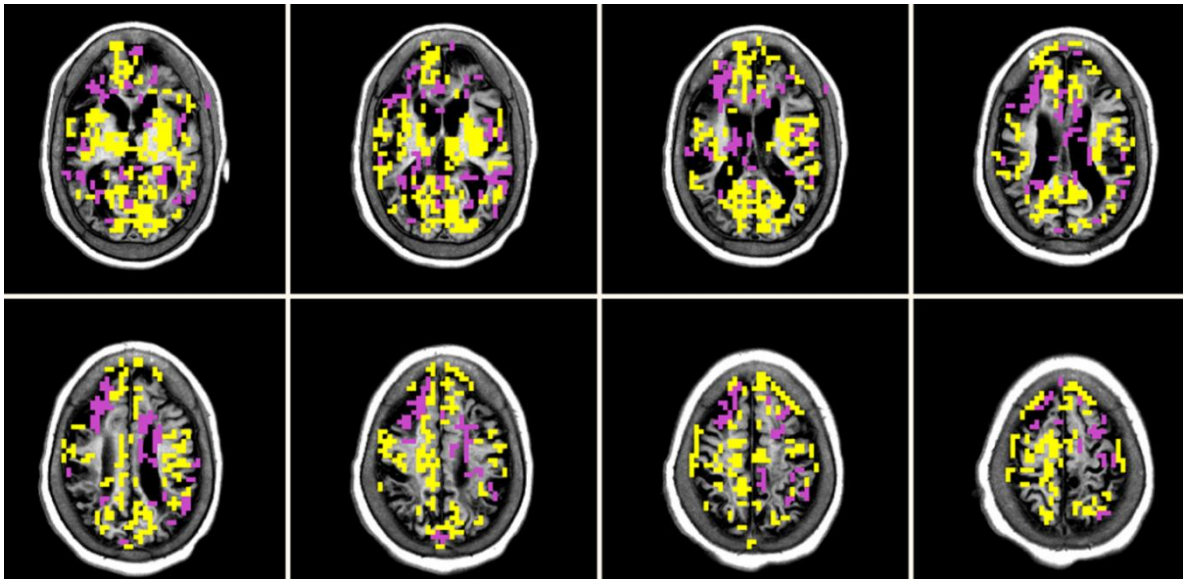
The mean (SD) of the pre- and post-operative scores of the operated hemisphere were 2.5 (0.555) and 2.4 (0.756). The mean (SD) pre- and post-operative score of the operation-naive hemisphere was 1.75 (0.858) and 1.9 (0.966) respectively. There was no statistically significant difference between pre and post-operative scores in the operated or operation naïve hemisphere. Pre-operative scores were higher in the operated hemisphere but this was not statistically significant. There was no statististically significant difference in scores by presence of unilateral or bilateral moyamoya (Table 60).

7.8.6.1.2. Control Group

The mean (SD) right hemispheric T1 and T2 scores were 2.6 (0.787) and 2.1 (0.9) respectively. The mean (SD) of the left hemispheric T1 and T2 scores were 2.6 (0.45) and 2.4 (0.748) respectively. There was a trend toward significant difference

in right hemispheric T1 and T2 scores (p=0.149). There was no significant difference between left hemispheric T1 and T2 scores, right and left T1 scores, or right and left T2 scores (Table 60 and Fig 60).

Figure 58 hBOLD CVR Parametric Map for Non-Surgical Control Patient 7. Patient became symptomatic and proceeded to have revascularization surgery.



Note bilateral reduced reactivity and scattered steal. Patient became symptomatic and proceeded to have revascularization surgery.

Figure 59 Pre and Post-operative Hemispheric Scores in Operated (n=7) and Operation- Naïve (n=6) Hemispheres

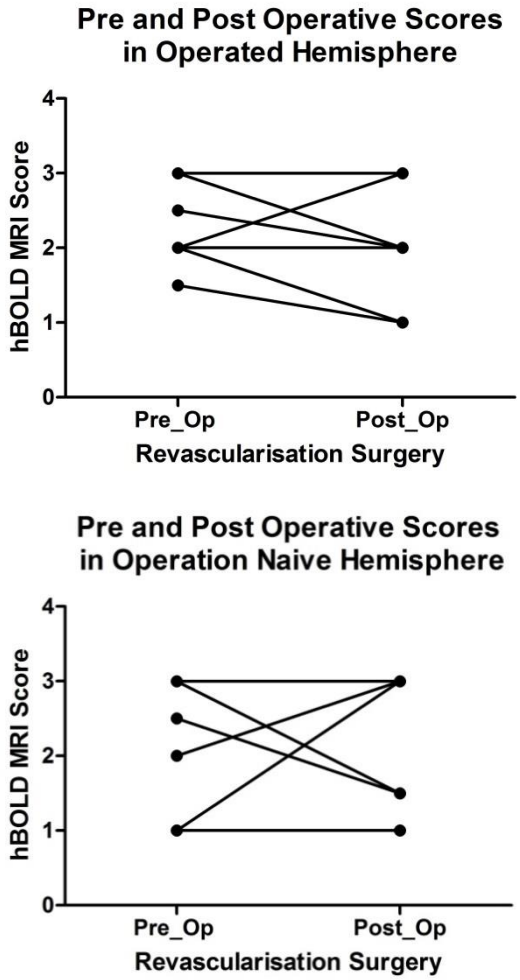
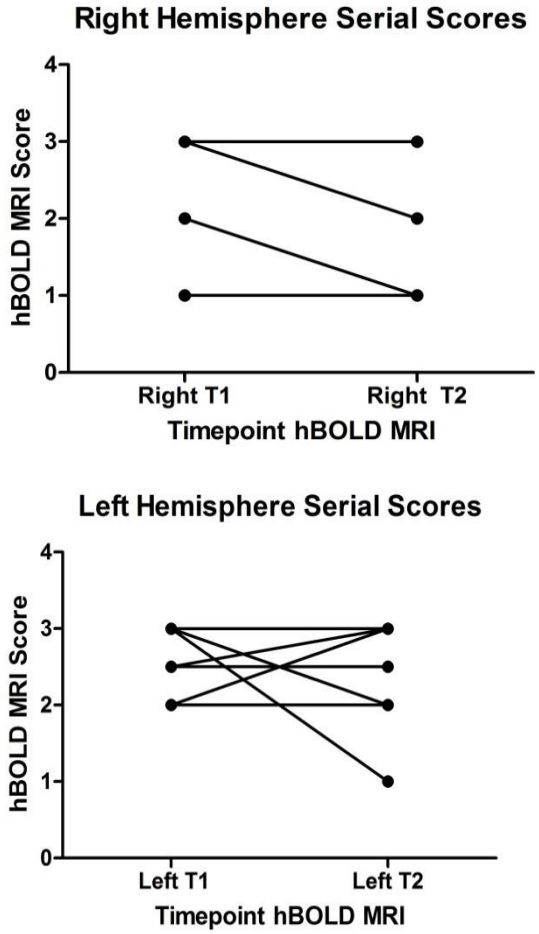


Figure 60 Timepoint 1 and 2 Hemispheric Scores (Right n=4; Left n=7) for Non-Surgical patients with Follow-up Control Group



7.8.6.2. Hemispheric Score Change and Clinical Description

Qualitative hBOLD CVR scores were divided into three categories better, same or worse depending on whether the scores respectively decreased, remained the same or increased respectively between the first and second study.

7.8.6.2.1. Operated Hemisphere Score Change and Clinical Description

In the operated hemisphere (n=14) scores remained the same pre and post-operatively for 8 hemispheres, were better (lower) in 4 hemispheres and worse (higher) in 2 hemispheres. The two children who had worse scores (score change 2 to 3) had NF1 (one right, one left moyamoya; Patients 5 and 6 respectively Table 56). Clinically Patient 5 improved following surgery, while Patient 6 had TIAs in the early post-operative period which resolved within 3 months, and persistent headache. (Table 60)

7.8.6.2.2. Operation Naive Hemisphere Score Change and Clinical Description

In the operation naïve hemisphere (n=10) scores remained the same pre and post-operatively in 5 hemispheres, were better (lower) in 2 and worse (higher) in 3. Of the children whose scores deteriorated one (Patient 12) had bilateral Idiopathic-MM, one (Patient 4) left NF1-MM and one (Patient 1) bilateral Chromosomal-MM (Trisomy 21). Score change was 1 to 3 in the first and 2 to 3 for the latter two

children. Patient 12 (Idiopathic, bilateral MM) had new onset left TIAs on the operation-naïve hemisphere. Patient 1 (Trisomy 21, bilateral MM) had de novo right TIAs and subsequently had left pial synangiosis. Patient 4 (NF1, unilateral MM) clinically improved following surgery. Pre and post-operative hypercapnic challenge method was different in this patient (RM first study, BH second study) (Table 60, 61 and 62; Fig 61).

7.8.6.2.3. Non-Surgical Control Group with Follow-up Hemisphere Score Change and Clinical Description

In the control (n=7) right hemisphere scores remained the same between T1 and T2 for 4 hemispheres, were better (lower) in 3 hemispheres and lower (worse) in 0 hemispheres. In the control (n=7) left hemisphere scores remained the same between T1 and T2 for 3 hemispheres, were better (lower) in 2 hemispheres and higher (worse) in 2 hemispheres.

The two patients who had worsening of scores had bilateral MM (one idiopathic, one SCD). Patient 1 had prior revascularization surgery with symptomatic improvement. Worsening in CVR scores was followed by clinical deterioration and increase in ischaemic symptoms. She subsequently had a further revascularization procedure. Patient 30 remained clinically stable on a regular transfusion programme. (Table 60, 62 and 63. Fig 61)

Table 60 Pre and Post Revascularization Hemispheric Scores and hBOLD MRI Timepoint 1 and Timepoint 2 Hemispheric Scores

CVR Scores	Surgery						No Surgery					
	Operated Hemispheres N=14		p	Operation Naïve N=10		p	Right hBOLD CVR N=7		p	Left hBOLD CVR N=7		p
	Pre	Post		Pre	Post		T1	T 2		T1	T2	
Mean	2.5	2.4	0.829	1.75	1.9	.784	2.6	2.1	0.149†	2.6	2.4	0.713
Score Change (%) B S W		4 (29) 8 (57) 2 (14)			2 (20) 5 (50) 3 (30)			3 (43) 4 (57) 0 (0)			2 (29) 3 (43) 2 (29)	

Wilcoxon matched-pair test p>0.5

Table 61 Deterioration in Score and Clinical Change in Operated Group

Patient	MM Diagnosis	MM Laterality	Op Hem	Score Change Op Hemisphere	Score Change Op Naïve Hemisphere	Hypercapnic Method Pre-post-op	Clinical Course Pre-post op	Progress
5	NF1	Right	Right	2 to 3	1 to 1	BH-BH	Improved HA	Stable
6	NF1	Left	Left	2 to 3	1 to 1	GA-GA	AIS pre-op TIA post-op	Persistent patchy frontal deficit on ConA and HA. LD
1	Trisomy 21	Bilateral	Right	2 to 1	1 to 3	GA-GA	R TIAs post-op	L pial
4	NF1	Left	Left	3 to 3	2 to 3	RM-BH	Improved HA	Stable
12	Idiopathic	Bilateral	Left	3 to 3	2 to 3	RM-RM	L TIAs post-op	R pial

Op Hem=operated hemisphere, Pre-post op=clinical course, HA=headache, LD=learning difficulties, L pial=left pial synangiosis, R pial=right pial synangiosis

Table 62 Deterioration in Score and Clinical Change in Non-Surgical Controls with Follow-up

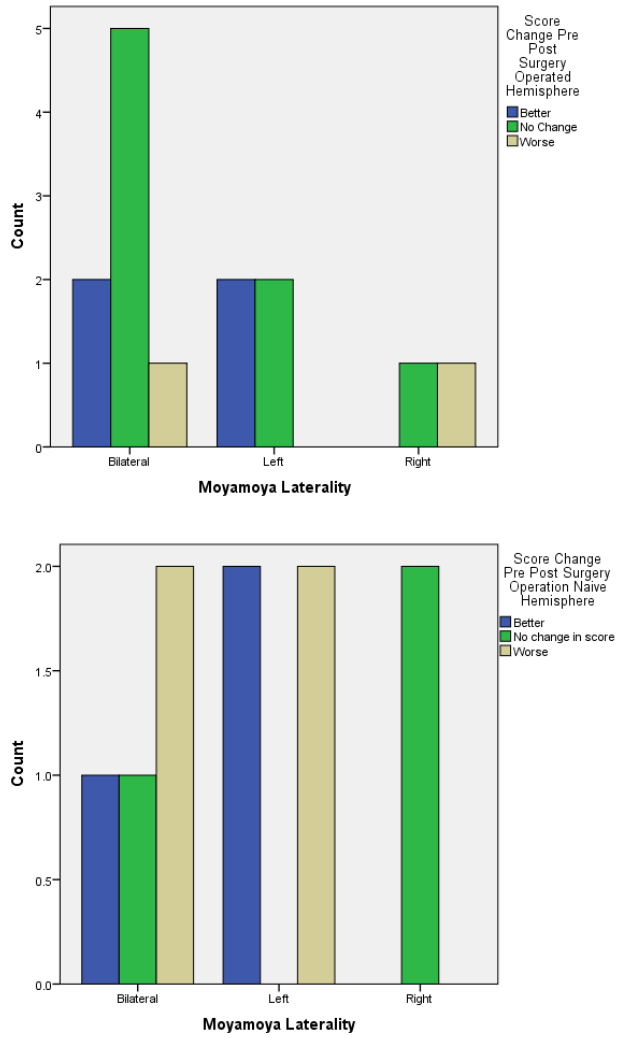
Patient	MM Diagnosis	MM Laterality	Score Change Right Hemisphere T1 T2	Score Change Left Hemisphere T1 T2	Hypercapnic Method T1 T2	Progress
1	Idiopathic	Bilateral	3 to 3	2 to 3	BH-BH	Clinical improvement following prior bilateral hemisphere revascularization surgery. Subsequent deterioration in clinical symptoms predated by deterioration in CVR. Omental free-flap procedure 2010
6	SCD	Bilateral	3 to 3	2.5 to 3	BH-BH	Stable

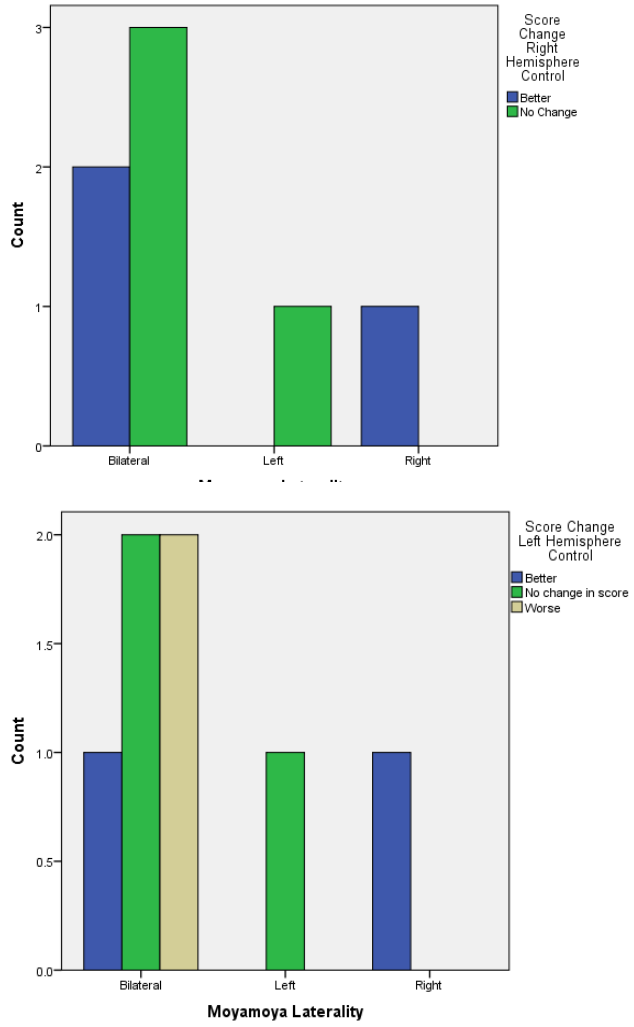
Table 63 Change in Scores in Revascularization Surgery Group and Non-Surgical Controls with Follow-up by Moyamoya Laterality

	hBOLD CVR Change											
Moyamoya Laterality	Revascularization Surgery						Controls					
	Operated N=14			Operation-Naïve N=10			T1 N=7			T2 N=7		
	Better	Same	Worse	Better	Same	Worse	Better	Same	Worse	Better	Same	Worse
Bilateral	2	5	1	1	1	2	2	3	0	1	2	2
Right	0	1	1	0	2	0	1	0	0	1	0	0
Left	2	2	0	2	0	2	0	1	0	0	1	0

Fisher's exact p= 0.556; 0.360; 1.0; 1.0

Figure 61 Hemispheric Score Change in Operative and Operation Naive Hemispheres in patients who were clinically better (blue bars), unchanged (green bars) or worse (light brown bars)





7.8.6.3. Pre and post-operative Hemispheric Score Change by Moyamoya Laterality

Cross-tabulation of change in hBOLD CVR scores by moyamoya laterality was performed. Using Fishers' exact test there was no statistically significant association between the hBOLD CVR change score and moyamoya laterality.

7.8.6.4. Moyamoya Laterality and Clinical Change in Surgical and Non Surgical Controls with Follow-up Group

To assess the association between clinical change and moyamoya laterality, clinical change between time of decision to proceed with surgery and post-surgical evaluation in the surgical group and clinical assessment closest to time point 1 and time point 2, clinical change was scored as follows and Fisher's exact test used for analysis:

1 = remained asymptomatic or improvement in symptoms

2 = no change/persistence of previous symptoms

3= worsening of previous or new/different symptoms

In the surgical group 3 children, all of whom had bilateral MM, had worsening of existent or new symptoms. The rest (n=9) of the children had improvement in symptoms or remained asymptomatic. Fisher's exact 3.08, p=.209 Spearman's correlation .548, p=.065 (Table 64 and 65).

Table 64 Clinical Change by Moyamoya Laterality in Surgical Group

	Clinical Change Score		
Moyamoya Laterality	Asymptomatic or Improved	New or Worse	Total
Right	2	0	2
Left	4	0	4
Bilateral	3	3	6
Total	9	3	12

All children in the no intervening surgery group had initial improvement in symptoms or remained asymptomatic. However, five (5/7) had a prior intervention (ie revacularization surgery) or non-surgical intervention (blood transfusion or bone marrow transplant) between hBOLD CVR studies. There was no clinically significant difference in clinical change score between the surgical and no intervening surgery group (Fisher's exact 1.53. p=1.00; Tables 64 and 65)

Table 65 Clinical Change by Moyamoya Laterality in Control Group

	Clinical Change Score		
Moyamoya Laterality	Asymptomatic or Improved	Same or Persistent	Total
Right	1	0	1
Left	1	0	1
Bilateral	4	1	5
Total	6	1	7

I sought to further explore whether comorbidity was associated with clinical change score. Using Fisher's exact test there was no significant association between comorbidity or moyamoya laterality and clinical change scores ($p > 0.5$ for all categories).

Table 66 Change in Scores in Revascularization Surgery Group and Non-Surgical Controls with Follow-up by Comorbidity

MM Disease or Syndromic Diagnosis	Revascularization Surgery						Controls					
	Clinical Symptoms						Clinical Symptoms					
	Operated			Operation-Naive			T1			T2		
	Better	Same	Worse	Better	Same	Worse	Better	Same	Worse	Better	Same	Worse
Idiopathic	1	4	0	1	1	1	0	2	0	0	1	1
NF1	1	2	2	2	2	1	1	1	0	1	1	0
SCD							1	1	0	0	1	1
Chromosomal	2	2	0	0	0	2	1	0	0	1	0	0

Fisher's exact test; p=.494; .771; .771; 1

7.8.7. Serial hBOLD CVR Hemispheric Pixels

Ten of thirty-seven children (twenty hemispheres) had pre and post-operative hemispheric pixels available or suitable for analysis. On inspection mean PTPP was lower in the 'to be' operated (ipsilateral) hemisphere (.418 +/- .13) than the 'not to be' operated (contralateral) hemisphere (.613 +/- .18), and the operated (ipsilateral) hemisphere (.463 +/- .16) than the operation-naïve (contralateral) hemisphere (.57 +/- .13). There was a correction toward a mean PTPP ratio of 0.5 in the operated and operation naïve hemispheres. Using the paired t-test there was a trend toward these differences being significant in all but the NTNP post operated and post operation-naive hemispheric pairings.

There was a trend toward significant difference in pre and post-operative NTNP pixel means in the operated hemisphere. There was no statistically significant difference in pre and post-operative hemispheric pixels within the surgical and non-surgical group for all other hemispheric pixel studies (all $p>0.05$). There was no statistically significant difference in mean pre and post-operative pixels in the surgical or no intervening surgery group by moyamoya laterality (Table 67 and Figs 62–65).

Table 67 Pre and Post-operative Mean Hemispheric Pixels and Non-Surgical Controls with Follow-up

CVR Pixels	Surgery						No Surgery					
	Operated N=12		t-test p value	Operation Naïve N=8		t-test p value	Right hBOLD CVR N=7		t-test p valuet	Left hBOLD CVR N=7		t-test p value
	Pre	Post		Pre	Post		1	2		1	2	
	mean (SD)	mean (SD)		mean (SD)	mean (SD)		mean (SD)	mean (SD)		mean (SD)	mean (SD)	
PTPP	.418 (.13)	.463 (.16)	.51	.613 (.18)	.57 (.13)	.64	.47 (.073)	.51 (.057)	.34	.53 (.073)	.48 (.056)	.32
PTNP	.16 (.18)	.24 (.37)	.37	.17 (.22)	.10 (.17)	.57	.16 (.128)	.1 (.095)	.4	.16 (.117)	.13 (.107)	.64
NTNP	.57 (.17)	.39 (.18)	.077†	.38 (.17)	.39 (.28)	.91	.424 (.136)	.373 (.139)	.21	.56 (.105)	.6 (.132)	.34

† trend toward significant difference

Figure 62 Pre and Post-operative Hemispheric Pixels in Operated (n=12) and Operation Naive (n=8) Hemispheres

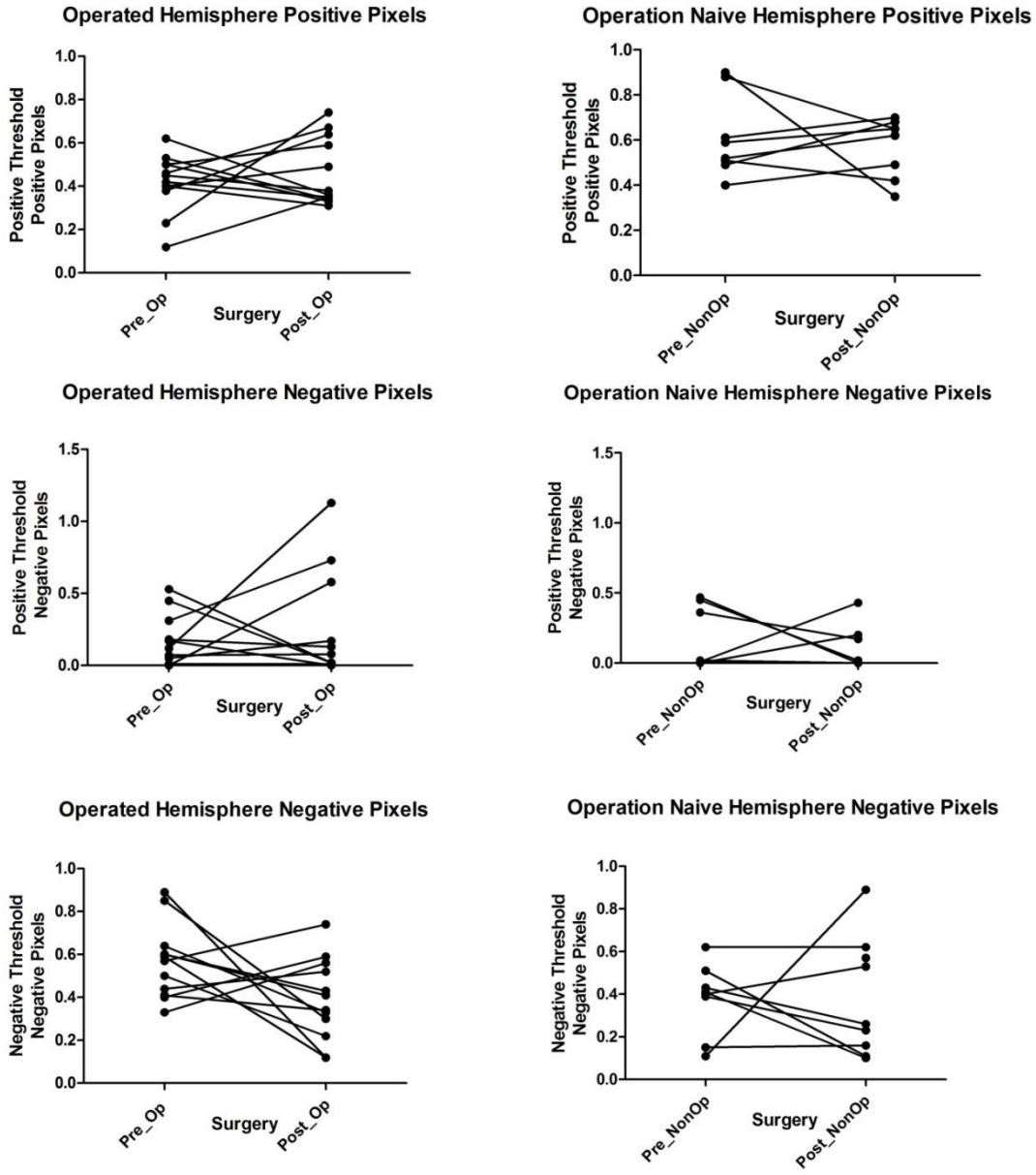


Figure 63 Pre and Post-operative Hemispheric Pixels in Bilateral MM Operated (n=6) and Operation Naive (n=2) Hemispheres

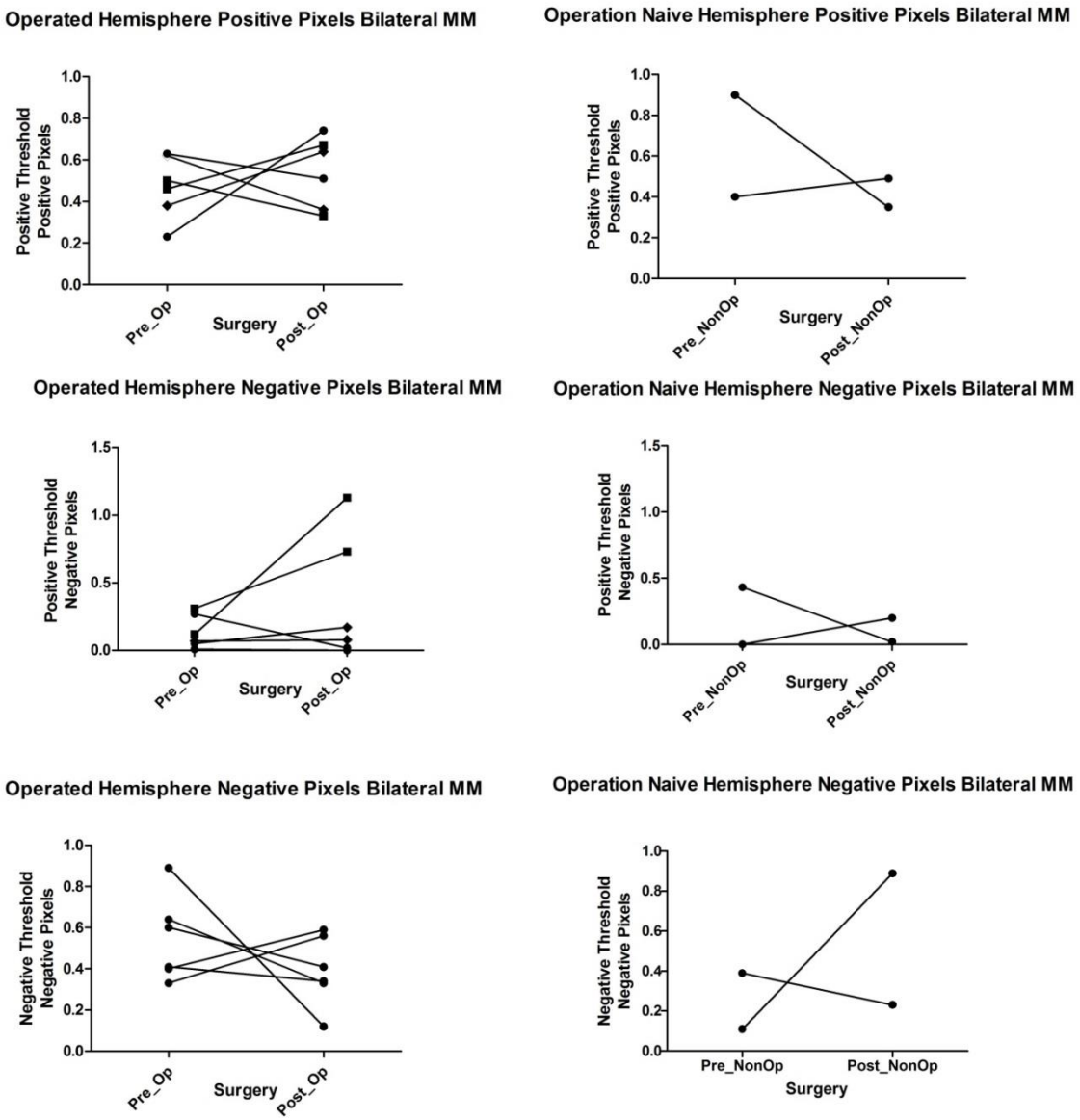
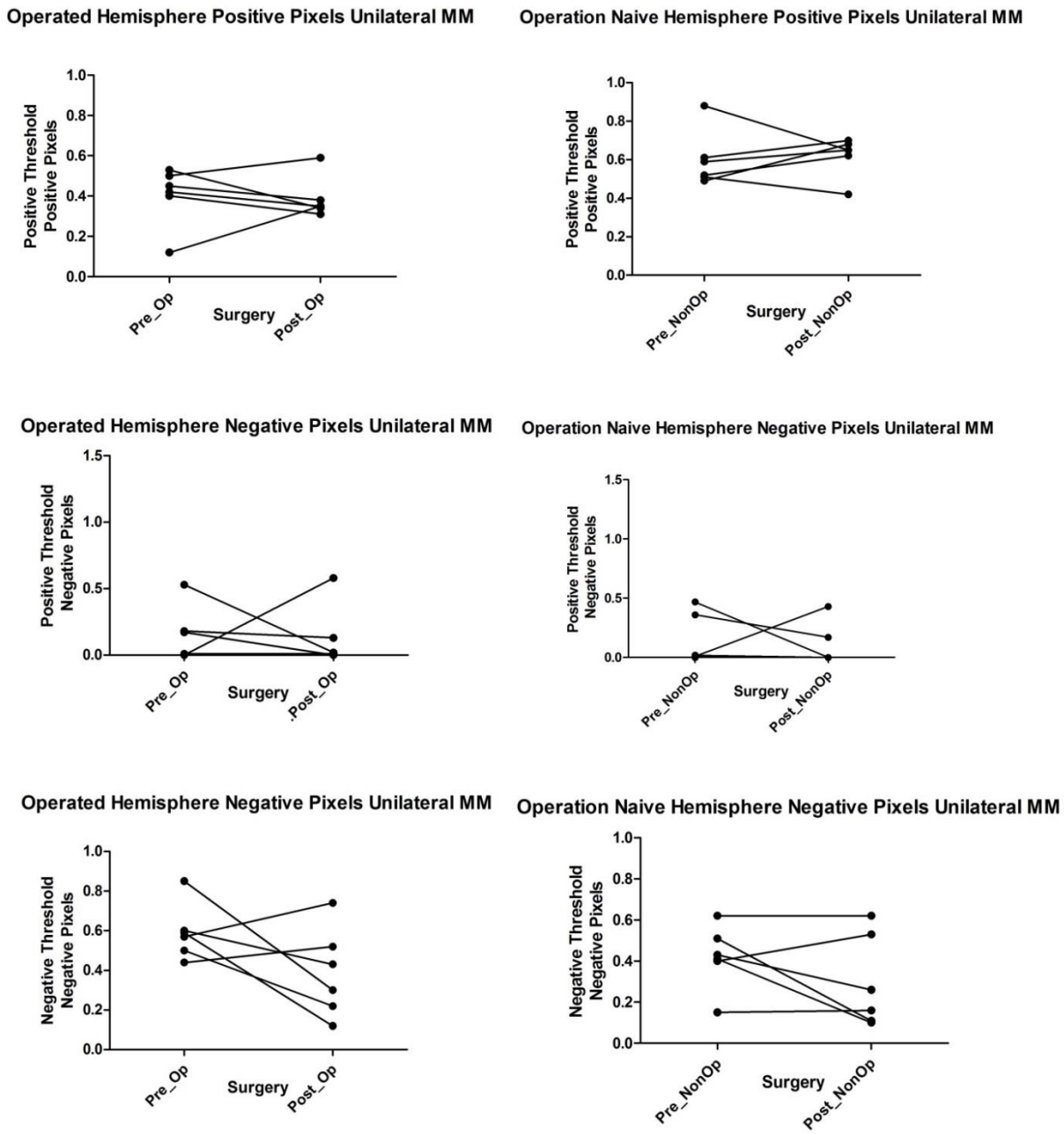
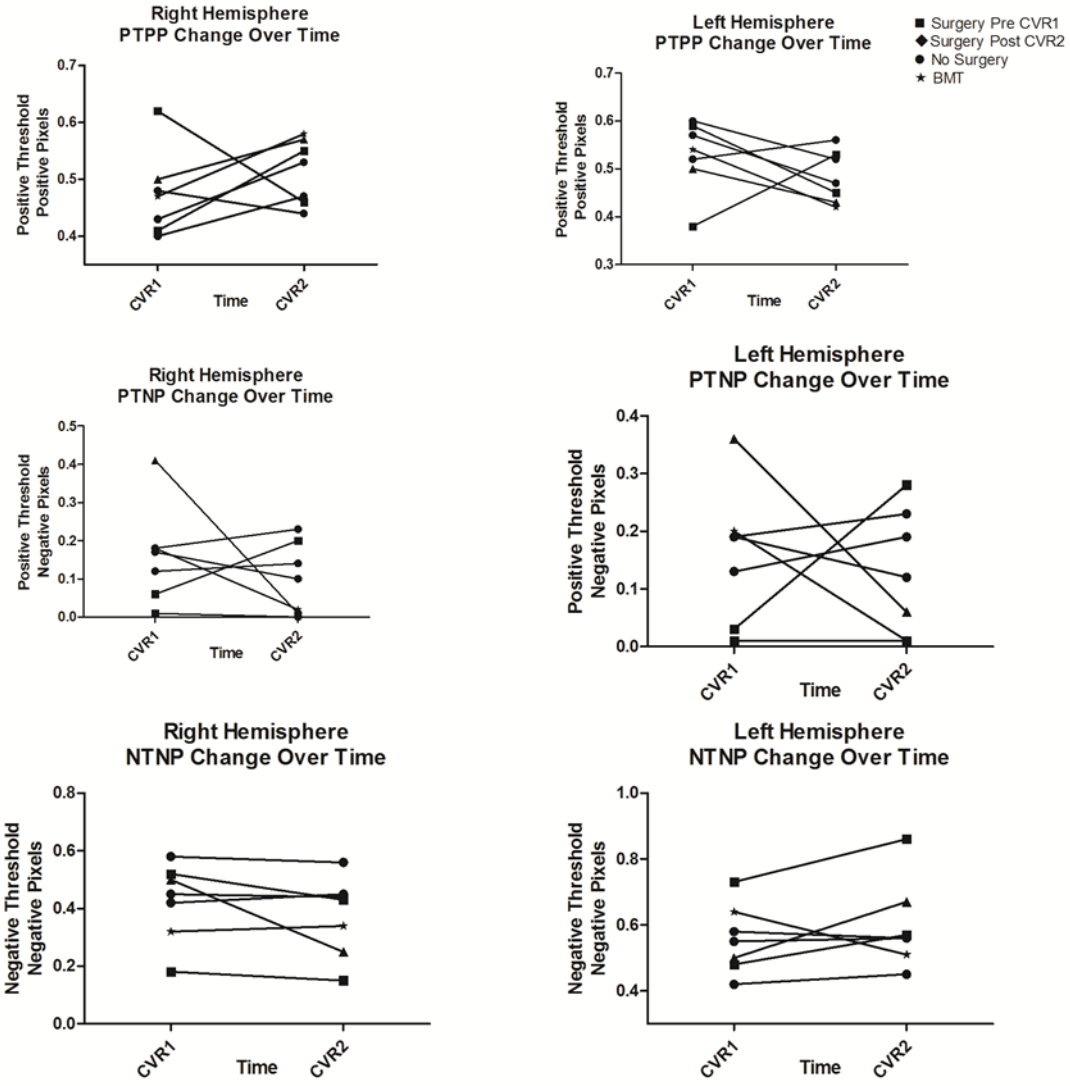


Figure 64 Pre and Post-operative Pixels in Unilateral MM Operated (n=6) and



Operation Naive (n=6) Hemispheres

Figure 65 Prepost Controls (n=7) - Change in Hemispheric Pixels Over Time Without Intervening Surgery



Clinical change scores were compared with hemispheric pixel change scores in the operated and operation naïve in the surgical group, and the right and left hemispheres in the no intervening surgery group. Using Fisher's exact test and Spearman's correlation there was a statistically significant association between operated hemisphere PTNP and clinical change score Fisher's exact 4.967, p=0.05. There was no other statistically significant association between the pre and post-operative CVR and clinical change scores (all p>0.5).

There was no significant difference in CVR score change or clinical score change in the operated and operation naïve hemispheres in the surgical group when divided by age of moyamoya diagnosis (less than compared to equal and more than 9 years; all p>0.05).

7.8.8. Quantitative hBOLD CVR Pre and Post Revascularization Surgery

Three of six children with calculable quantitative CVR had measures pre and post-surgery. Two (2/3) of the children had Idiopathic-MM (one bilateral, one unilateral), (1/3) Chromosomal-MM (Trisomy 21, unilateral). Pre-operative CVR in the 'to be' operated hemisphere ranged from .32 to .49. Patient 2 had bilateral hemispheric surgery at the same time point. Post-operative CVR in the operated hemisphere increased and ranged from .51 to 1.14. Notably pre-operative operation-naïve hemisphere CVR was .42 and .6 and post-operative CVR .65 to

.91. Hence there was post-operative improvement in CVR in the operation and operation-naive hemisphere. There was a trend toward the pre and post quantitative CVRs being significantly different. (Ref Tables 68- 70; Fig 66)

Table 68 Quantitative hBOLD CVR Pre and Post Revascularization Surgery

Patient	MM Class	MM Lat (B/R/L)	Surg Hem (B/R/L)	Operated Hemispheric hBOLD CVR n=4		p	Not Operated Hemispheric hBOLD CVR n=2	
				Pre	Post		Pre	Post
2	Tri 21	B	B	.39 .32	1.14 .72			
8	Id	B	R	.49	.89		.42	.91
11	Id	R	R	.43	.51		.6	.65
						.059		

Table 69 hBOLD CVR1 (baseline CVR) Values by Moyamoya Laterality

	hBOLD CVR1 %/mmHg mean		
CVR Brain Region	All	Moyamoya Laterality	
	n=3	Bilateral n=2	Right n=1
Whole	0.363	0.38	0.38
Right	0.401	0.44	0.43
Left	0.418	0.373	0.6

Table 70 hBOLD CVR2 (Follow-up CVR) Values by Moyamoya Laterality

	hBOLD CVR2 %/mmHg mean		
CVR Brain Region	Moyamoya Laterality		
	All n=3	Bilateral n=2	Right n=1
Whole	.735	.85	.62
Right	.762	1.015	.51
Left	.732	.815	.65

Figure 66 Pre and post-operative Hemispheric hBOLD CVR

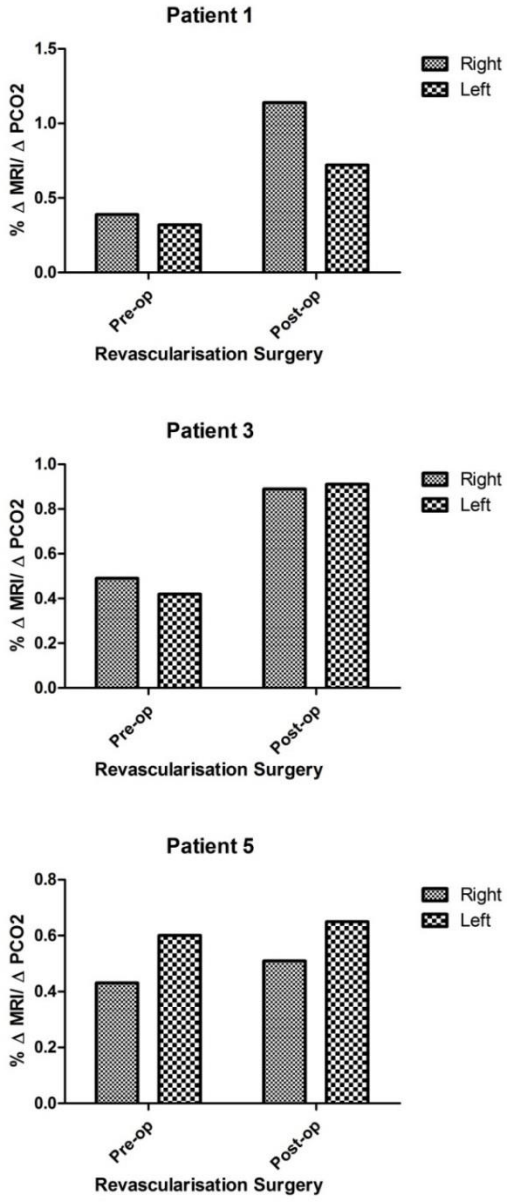
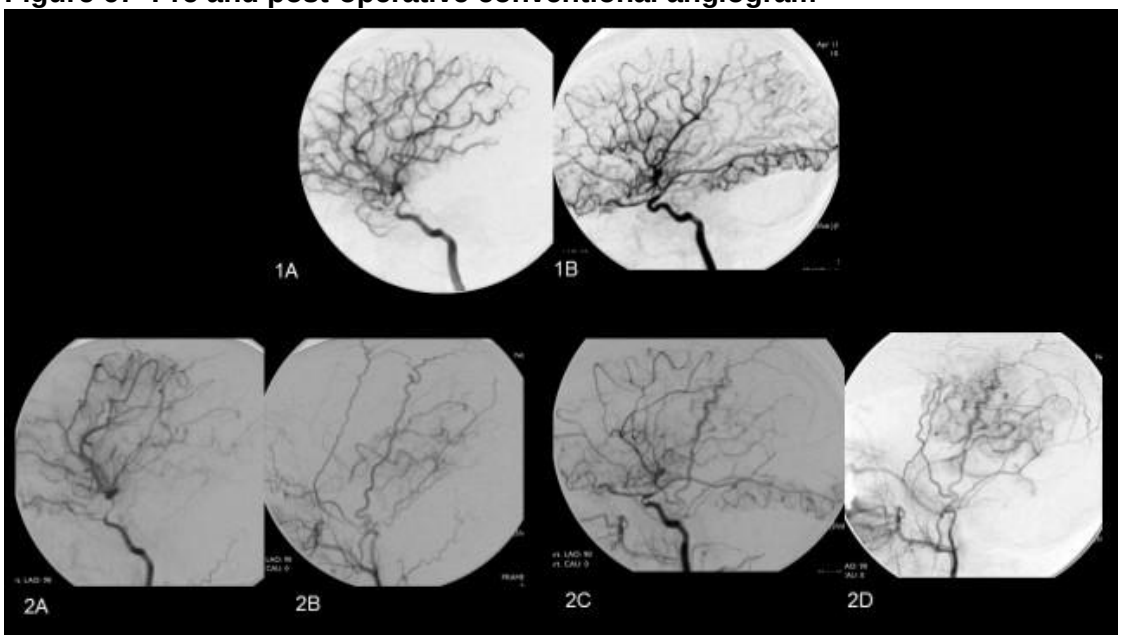
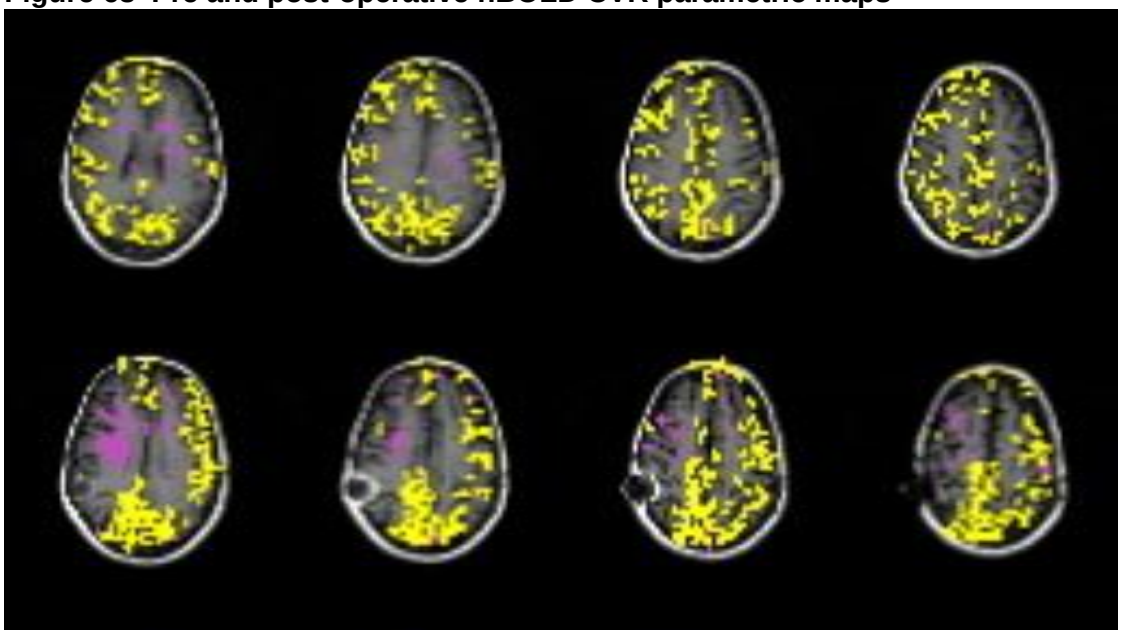


Figure 67 Pre and post-operative conventional angiogram



15 old girl with bilateral moyamoya. 1A: Right lateral ICA injection demonstrating reasonably good hemispheric perfusion prior to revascularization surgery
1B: Left lateral ICA injection demonstrating poor frontal and parieto-occipital perfusion prior to revascularization surgery
2A: Right lateral ICA injection demonstrating progressive occlusion and deterioration in right hemisphere perfusion
2B: Right lateral ECA injection demonstrating limited effect of synangiosis
2C: Left lateral ICA injection demonstrating progressive poor frontal and parieto-occipital perfusion
2D: Left lateral ECA injection demonstrating synangiosis within frontal and parietal regions

Figure 68 Pre and post-operative hBOLD CVR parametric maps



Top row: Pre-operative hBOLD CVR Parametric maps demonstrating bilateral reduced positive (yellow) reactivity (left more than right) and minimal bilateral steal.

Bottom row: Post-operative hBOLD CVR parametric maps demonstrating improved positive reactivity in left hemisphere and worsened positive reactivity (ie less yellow) and increased negative reactivity (purple) in right hemisphere in keeping with changes seen on conventional angiography and scoring.

7.9. Discussion

Standard indications for surgery include symptomatic presentation (stroke, TIA or seizures) progressive radiological findings ie vascular and or parenchymal. Ischaemic stroke – in particular bilateral – results in significant neurological injury, morbidity and mortality. In this study I sought to explore how hBOLD CVR changes in response to revascularisation surgery and over time.

7.9.1. Group Description and Clinical Presentation

The majority of children in this sub-population (n=20, surgical and control) had bilateral MM (6 Idiopathic-MM; 3 Trisomy 21; 2 SCD). The majority (5/7) of children with left MM had NF1-MM. Of the intervening surgery group, only two (Chromosomal-MM/ Trisomy 21) presented with AIS, three (Idiopathic-MM) TIAs. Almost half (6/13) had headache only or were asymptomatic at diagnosis (5 NF1-MM, 1 Turners Syndrome). One had seizures and headache, the other paroxysmal symptoms (both Idiopathic-MM). The majority in the control group had a history of ischaemic symptoms. Two out of the seven (1 with NF1-MM, 1 SCD-MM) were asymptomatic. All children in our group had adequate collateralisation on conventional angiography. Hence this is not a reliable sole discriminator of adequacy of tissue perfusion and indication for surgery.

Published Japanese and US indications for revascularization surgery include presentation with ischaemic symptoms; progressive vasculopathy; decreased

regional cerebral blood flow, vascular response and perfusion reserve, retrieved from cerebral circulation and metabolism studies (Roach *et al.* 2008, Research Committee on the *et al.* 2012, Smith and Scott 2012). These would be similar worldwide and applied according to regional resources. However, PET, SPECT and Tc-99m HMPO are all associated with a risk of radiation exposure, are technically challenging, have poor spatial resolution high capital costs (Lee *et al.* 2009);(Frush *et al.* 2003). Measures from conventional or digital subtraction angiography such as arterial circulation time (ACT) may be used to calculate haemodynamic status. However, there is a growing reluctance to perform ConA in children due to safety concerns compounded by an increase in early diagnosis of MM in asymptomatic children in whom the risk of an invasive angiographic procedure may be even less acceptable. Despite attempts to demonstrate parity in findings between these two angiographic modalities standard MRI and MRA ability to inform on haemodynamic status is confined to demonstration of parenchymal ischaemic injury, presence of the ivy sign, signal attenuation on TOF MRA and presence of collaterals (Saeki *et al.* 2000). MRA has been shown overcall degree of parent artery stenosis and under call collateralization again limiting it's utility in determining haemodynamic status. Based on their study of radiographic and clinical progression in children with asymptomatic MM and a demonstration of radiographic and clinical progression in their group comprised of children with SCD-MM and NF1-MM Lin et al recommended revascularization surgery in the majority even If asymptomatic (Lin *et al.* 2011). However, young

children (under 3 years) with a symptomatic presentation of moyamoya under have poor cognitive outcomes (Matsushima *et al.* 1990); and a more clinically fulminant course (Imaizumi *et al.* 1998, Kim *et al.* 2004) (Jackson *et al.* 2014). Furthermore specific indications to guide the timing and type (unilateral or bilateral) surgery in asymptomatic or incidentally diagnosed moyamoya are not well established. This is coupled with the absence of longitudinal outcome data in the young asymptomatic group. Therefore the potential risks of surgery in the young requires careful consideration (Soriano *et al.* 1993, Jackson *et al.* 2014).

7.9.2. Pre and post-operative concordance of CVR Abnormality and Angiography

As noted in the previous chapter (Chapter 5) hBOLD CVR abnormality was concordant with laterality of moyamoya pre-operatively and (from this study) post-operatively.

Pre-operative hBOLD CVR results are suggestive of disruption of CVR and consequent perfusion in the affected and un- or less severely affected hemisphere. The dynamic response and shifting of the un- or less affected watershed to support the abnormal or less affected hemisphere may itself be a stressor on the unaffected side. It is not known whether this shift in watershed to support the affected hemisphere may result in functional compromise on the unaffected side over time. The finding of abnormality of qualitative CVR, evident

beyond the ipsilateral diseased vascular territory into the non-infarcted, contralateral healthy appearing vascular territory and parenchymal tissue in unilateral moyamoya would suggest contralateral tissue level compromise. These regional abnormalities in hBOLD CVR were evident despite apparent adequate pre-operative self-collateralisation and are in keeping with the findings of Han *et al.* 2011.

Post-operatively there was a correction in region of hBOLD CVR abnormality toward the ipsilateral diseased hemisphere, such that the contralateral hemispheric abnormalities appeared to improve, again most clearly seen in those with unilateral disease, although numbers were small and the data must be interpreted with caution. This change is likely to be captured by measurement of angiographic arterial circulation time (ACT) not done in our group (Donahue *et al.* 2013).

7.9.3. Pre and post-operative hBOLD CVR scores and clinical change

7.9.3.1. Surgical Group

7.9.3.1.1. Operated hemisphere

Mean hemispheric scores in the pre-operative operated hemisphere were higher than in the operation naïve and healthy (in unilateral MM) or less diseased (in

bilateral MM) hemisphere. This would suggest concordance of tissue level microvascular dysfunction with clinical presentation.

The majority (8/14) of hemispheric scores on the surgical hemisphere remained the same post-operatively. Four of fourteen improved and two were worse. Of the two with worse post-operative scores one clinically improved and the other had progressive ischaemic symptoms which resolved in the early post-operative period. The latter child had persistent areas of avascularity on conventional angiography post-operatively, despite the improvement in some clinical symptomatology.

7.9.3.1.2. Operation naïve hemisphere

Five out of 10 hemispheric scores remained the same in the operation naïve hemisphere, two improved and three worsened. Two of the three children with worsened scores had bilateral MM and progressive ischaemic symptoms referable to the operation naïve hemisphere. Both children subsequently had revascularization surgery of the operation naïve hemisphere. One of the three children with worse scores had unilateral NF1-MM and clinically improved post-operatively. hBOLD CVR method was different between studies and score change was from 2-3.

Therefore post-operative worsening of scores occurred in five children (two in the operated hemisphere, three in the operation naïve hemisphere) and was predictive

of worsening ischaemic symptoms in 3/5 (60%). The two with worsening of scores but improvement in clinical symptoms probably reflect the previously noted limits of the scoring system to discriminate between degree of abnormality of hBOLD CVR ie between 2 and 3, and the additional difficulty in scoring different method serial hBOLD. Therefore in the majority the worsening of scores was indicative of tissue level functional cerebrovascular impairment despite operation in the operative hemisphere (unilateral MM) and contralateral operation naïve hemisphere (bilateral MM). This would support consideration of bilateral revascularization surgery in children with bilateral MM at the same surgery or closer time-points.

Just over half of the scores remained the same post-operatively in the operated and operation-naïve hemispheres despite the majority of children clinically improving post-operatively. This is somewhat unexpected for the operative hemisphere. This may be a limitation of the scoring system, method or real. If real this would suggest that the functional CVR at the tissue level did not significantly improve post-operatively. In all cases post-operative super-selective angiography demonstrated improved collateralization from the external circulation and hence improved perfusion. This should result in increased BOLD signal within the region of interest (the operative hemisphere) and reduced capillary bed vasodilatation given the improved distal perfusion, with consequent secondary improvement in cerebrovascular reactivity. Bowen et al (1998) demonstrated improved bloodflow on Xenon-CT CBF studies following revascularizaton surgery.

However, it is also possible that despite improvements in perfusion, vasodilatory capacity may not be regained in certain conditions where the post-stenotic dilatation has become more fixed, less compliant and and able to recover it's original diameter. This may occur when the fixed proximal steno-occlusive disease co-exists with for example, chronic anaemia or chronic hypertension. In addition recent studies of post-operative neurological decline have demonstrated that changes in cerebral haemodynamics are not always favourable. Post-operative cerebral hyperperfusion, oedema and shifting watershed have all been implicated – all of which would negatively affect CVR. (Hayashi *et al.* 2010) This requires further exploration in future studies.

7.9.3.2. Control Group

Most of the control group were pre-treated in as much as they had revascularization surgery (2/7), a BMT (1/7) and blood transfusion (2/7). Hence, the group was controlled for intervening surgery only.

Scores worsened in two (bilateral MM: one idiopathic, one SCD) of the 14 second time point control hBOLD CVRs. Of these two, one (idiopathic MM) had progressive worsening of CVR and ischaemic symptoms, and subsequently had further revascularization surgery. The other remained clinically stable on a regular blood transfusion programme. Blood transfusion occurred two days before the first hBOLD CVR study and one day before the second. Haematological

parameters on the respective transfusion days were Hb 94, HbS 26%, Hct .280, Platelets 411 and Hb 100, HbS 21.4%, Hct .308 and Platelets 409. Hence, worsening qualitative hBOLD CVR measures were indicative of clinical deterioration in children with bilateral disease one of whom was receiving ongoing treatment and the other had prior revascularization surgery and went on to have further revascularization surgery.

The mean interval between revascularization surgery and the second CVR study was 1.08 years (13 months), SD .815 years and 2.35 years SD 1.72 for the no-intervention group. This is a retrospective analysis and there was no protocol mandating a post-operative CVR at a specific timepoint. It is difficult to justify multiple CVR studies in children and there is no consensus on timing of post-operative investigations. SPECT and proton magnetic resonance spectroscopy studies have demonstrated that the efficacy of revascularization surgery as shown by it's effect on metabolism gradually appears within 3-6 months Uno et al. (Uno *et al.* 1996). Hence, in this study adequate time for the efficacy of revascularization surgery to be apparent elapsed by the second CVR. Arguably however, a more protracted interval may be confounded by the development of true new symptoms which is likely to have been the case in the two with worse CVRs who had concordant clinical deterioration.

7.9.4. Pre and post-operative hemispheric pixels and clinical change

Mean hemispheric PTPP were lower in the ‘to be operated’ hemisphere than the contralateral hemisphere suggesting impaired CVR on the ‘to be operated’ hemisphere. Observed correction toward mean hemispheric pixel ratio of 0.5:05 in the operation and operation-naïve hemispheres is in keeping with the biological expectation of improved perfusion and CVR on the operated hemisphere post revascularization surgery and the concordant hBOLD qualitative CVR improvement and was observed in the surgical group. There was a statistically significant association in clinical change scores for PTNP in the operated hemisphere of the surgical group. This association was not seen in the remaining hemispheric pixel change scores.

7.9.5. Pre and post-operative Quantitative hBOLD CVR change

Changes in quantitative CVR pre and post-operatively were quite striking. Pre-operative measures were lower pre-operatively and higher post-operatively. This is similar to the findings in the study by (Mikulis *et al.* 2005). This was However, in a small sub-group of children and needs to be replicated in other studies.

7.9.6. Clinical potential

This is the first reported study of children with moyamoya with consecutive qualitative CVR studies pre and post-operatively. This is also the first study to our knowledge which reports pre and post-operative quantitative measures of CVR. Han et al. (Han *et al.* 2011) reported on post-operative CVR change in three children using the targeted-controlled delivery of CO2 method. This study demonstrates that sequential and pre- and post- operative quantitative CVR studies are feasible in children and that region of abnormality as demonstrated on conventional angiography is concordant with region of abnormality on CVR. Furthermore in some cases the region of abnormality and suggestion of haemodynamic compromise at the tissue level extends beyond the region of abnormality as demonstrated on standard ConA, MRI or MRA. In Han et al's study (Han *et al.* 2011) two children had improvement in CVR and clinical symptoms post-operatively. One child with sequential studies had worsening of CVR and development of ischaemic symptoms over time. This is in keeping with the findings in our study.

This may reflect the limitation of the devised scoring systems as well as the need for caution when interpreting different modality qualitative CVRs.

Although there was a difference in qualitative and quantitative CVR measures between the 'to be operated' and operation naïve hemispheres, and pre and post-operatively, this was not statistically significant. This may be because of our sample size, the method devised to generate quantitative measures or the absence of a significant difference. This needs to be explored in future studies.

Revascularization surgery is not without risk of complication. Complications include stroke, transient neurological deterioration and death. In more recent literature transient neurological deterioration has been linked to post-operative focal hyperperfusion perhaps related to exhaustion of the cerebrovascular reserve, in part thought to be caused by a mismatch between OEF and CBV which both seem to be increased in pre and post-operative PET and SPECT studies. Postoperative cerebral hyperperfusion is defined as a major increase in ipsilateral cerebral blood flow (CBF) well above the metabolic demands of brain tissue (Sundt *et al.* 1981, Piepgras *et al.* 1988). It is well characterized in adult patients after carotid endarterectomy and more recently so in adult moyamoya (Furuya *et al.* 2004, Ogasawara *et al.* 2005, Kim *et al.* 2008, Kaku *et al.* 2012) Haemodynamically the risk appears to be represented by an increased OEF in the face of increased CBV which may be suggestive of maximized vasodilation reflecting exhausted autoregulatory reserve and hence higher risk of stroke. This is in comparison to the presence of increased OEF and normal CBV (Ogasawara *et al.* 2005) which may reflect preserved vasodilatory and hence autoregulatory capacity and hence a lower risk of stroke (Derdeyn *et al.* 2002). A combination of information derived from qualitative and quantitative CVR measures including absence of steal, low quantitative CVR measures and TIAs may be indicative of exhausted autoregulatory reserve and higher risk of stroke. However, to my knowledge, to date hBOLD CVR measures have not been used to assess operative risk and as hBOLD CVR does not provide a direct measure of CBV, and

quantitative ranges of CVR values are yet to be determined, this remains an area for further study and exploration.

7.10. Study Limitations and Future Directions

1) Development and validation of quantitative and qualitative CVR indicators for surgery.

2) Development of CVR measures of surgical risk to allow for improved selection for surgery and post-surgical risk management.

3) Longitudinal serial measurements of hBOLD CVR in a larger cohort of asymptomatic and symptomatic children with moyamoya.

7.11. Conclusions

1) In this relatively small sample, qualitative hBOLD CVR abnormality appears to be concordant with region of abnormality as demonstrated on conventional angiogram, and appears to extend beyond the primary region of arteriopathy into unaffected vascular beds in the contralateral hemisphere.

2) If confirmed in a larger sample of children, progression in hBOLD CVR abnormality with corresponding clinical deterioration may be able to be used to inform decision timing of surgery in symptomatic and asymptomatic moyamoya.

3) Regional abnormality in hBOLD CVR may improve following revascularization surgery in the corresponding vascular territory.

4) Lack of improvement of hBOLD CVR post revascularization surgery, despite clinical improvement, may reflect methodological limitations, exhausted autoregulatory capacity, or patchy tissue level functional compromise.

8. CVR and Neuropsychological Outcomes

8.1. Abstract

8.1.1. Background:

Moyamoya (MM) is a progressive cerebral vasculopathy associated with increased risk of arterial ischaemic stroke (AIS) and cognitive decline. Hypercapnic blood oxygen level dependent (hBOLD) CVR allows assessment of cerebrovascular reactivity (CVR) and reserve (CRes). There are few data on CVR and cognitive function.

8.1.2. Hypothesis:

We hypothesized that in childhood MM abnormalities of left, right and total CVR are associated with reduced verbal IQ (VIQ), performance IQ (PIQ) and processing speed (PS) respectively and that executive function would be abnormal in children with abnormal CVR.

8.1.3. Methods:

Children with MM, with or without AIS prospectively completed hBOLD CVR and Wechsler scales of intelligence. Parents and teachers completed the BRIEF. CVR studies were scored per hemisphere and grouped by presence of steal. Scores of intellectual and executive function were analysed for each group.

8.1.4. Results:

27 children with moyamoya completed an hBOLD CVR study and age-appropriate Wechsler Intelligence Scales (mean age 10.2 years, range 4.1-16.3 years; SD 3.9 years; 14 male, 13 female). In eleven children with no history of overt stroke of revascularization surgery abnormality of left CVR was associated with lower verbal IQ and bilateral steal was associated with lower intelligence scores in all domains apart from processing speed. Interestingly, indices of executive function were better (lower) in children with steal, although this result did not meet statistical significance.

8.1.5. Conclusion:

Abnormalities of CVR are associated with cognitive impairment in paediatric MM. Impairment of CVR may represent increased ischaemic risk as demonstrated by abnormality of CVR. However, the weighting of neurocognitive demise secondary to the chronic hypoperfusion of MM and neurocognitive demise secondary to disease specific factors in MMS and their relative manifestation in measures of CVR is yet unexplained. Further studies are required to explore the utility of hBOLD CVR as a biomarker for tissue level microvascular dysfunction In MMD and MMS so as to be able to determine risk of neurocognitive injury and ischaemia in children with MM.

8.2. Introduction

Untreated moyamoya disease and moyamoya syndrome may be associated with cognitive difficulties (Williams *et al.* 2012) and even decline (Imaizumi *et al.* 1999, Hogan *et al.* 2005) although this is not a universal finding (Lee *et al.* 2011). Intelligence quotient may be within the normal range in moyamoya disease in Asian children (Lee *et al.* 2011) but studies in North America have not found this (Williams *et al.* 2012). Traditional measures of cognitive ability such as IQ scores provide limited measures of cognitive function. A few studies have reported on more sensitive measures of brain injury and disease (Bowen *et al.* 1998, Williams *et al.* 2012).

The underlying disease in moyamoya syndrome may have an independent effect on cognitive outcome, e.g in Sickle Cell Disease or Neurofibromatosis Type I, even when children with severe learning difficulties, e.g with Down syndrome, are excluded. However, although one study found a difference in cognitive performance between children with Sickle Cell Disease and those with Idiopathic moyamoya (Hogan *et al.* 2005), another did not (Williams *et al.* 2012). The duration of symptoms probably plays a role, although this does not necessarily reflect the duration of vascular disease. Most studies have included children with stroke (Imaizumi *et al.* 1999, Hogan *et al.* 2005, Williams *et al.* 2012) and there are few data on the effect of isolated vasculopathy, e.g. bilateral vs unilateral, or Suzuki stage and these are typically limited to Japanese populations (Suzuki and Takaku 1969, Maki *et al.* 1976).

Bowen (Bowen *et al.* 1998) reported an improvement in overall cognitive ability following surgery, although this may not be seen in those with a 'poor intelligence quotient/severe learning disability' noted early on (Matsushima *et al.* 1997). In addition, Matsushima et al. (Matsushima *et al.* 1990) reported poorer cognitive outcomes in children presenting under the age of two years compared with those presenting after two years and treated before nine years of age.

Despite high individual variability, the left and right cerebral hemispheres display anatomical, molecular and functional left-right asymmetries that correlate with their functional specialization in particular cognitive processes. However, determination of the laterality of these cognitive functions in childhood is complex. Historical

descriptions of patients with lesioned hemispheres in the nineteenth century informed us of the neuroanatomical correlates of cognitive functions (Duboc *et al.* 2015). Language is probably the most studied of these functions. In 1967 Lenneberg suggested a critical period during which hemispheric dominance of language is established (2-12 year) (Lenneberg 1967). More recent neuroimaging studies would suggestive early bilateral representation of language with left sided dominance occurring between early childhood and adolescence with synaptic growth spurts and developmental peaks (Thatcher *et al.* 1997). In comparison studies about the lataralizaton of right hemispheric function in childhood are few and therefore based on adult literature and experience (Holland *et al.* 2001, Ressel *et al.* 2008, Everts *et al.* 2009, Everts *et al.* 2010). Cognitive functions such visuo-spatial perception, attention and performance IQ are more typically associated with the right hemisphere, whereas functions such as processing speed and executive function rely on recruitment of bilateral both cortical and sub-cortical networks.

In this study and based on the classic views on the lateralization of cognitive function, I sought to explore whether chronic hypoperfusion and abnormality in cerebrovascular reactivity, as seen in moyamoya, has ischaemic consequences measurable as neurocognitive outcome.

8.3. Study Hypothesis

I hypothesised that measures of hBOLD CVR can be used to predict neurocognitive outcome in childhood MM, specifically:

i) abnormal left hemispheric CVR is associated with reduced verbal IQ

ii) abnormal right hemispheric CVR is associated with reduced performance IQ

iii) abnormal CVR in either or both hemispheres is associated with reduction in processing speed

iv) abnormal CVR in either or both hemispheres is associated with abnormality in indices of executive function

8.4. Method

8.4.1. Inclusion criteria

Children with a diagnosis of MMD or MMS and a neuropsychological assessment as follows were included in the study:

Children aged between 6 and 16 years of age with cognitive assessments performed using the Wechsler Intelligence Scale for Children third edition (WISC-III) or the WISC fourth edition (WISC-IV). Children who were 16 years or older at

the time of assessment completed the Wechsler Adult Intelligence Scale, third edition (WAIS-III).

Participants who were 4 to 5 years old completed the Wechsler Preschool and Primary Scale of Intelligence, third edition (WPPSI-III), which provides index scores for overall intellectual ability (Full-scale IQ), verbal ability (Verbal IQ), and non-verbal ability (Performance IQ), with a mean score of 100 and an SD of 15. Average scores, as specified by test developers, range from 90 to 110, compared with a 'normal' test score range which reflects statistical consideration of one SD from the mean (e.g. 85–115). We did not include additional measures of memory, attention, or language, or formally test executive function because of time constraints in these patients attending for clinical purposes.

Executive function was assessed using the Behavior Rating Inventory of Executive Function, a standardized questionnaire completed by parents and teachers, which evaluates multiple aspects of executive function including emotional control, ability to transition between different activities, initiation, inhibitory control, working memory, planning, organization, and behavior self-monitoring (Gioia *et al.* 2000). The school-age version was used for participants aged 6 to 18 years, and the preschool version was used for participants aged 4 to 5 years. Both versions provide index scores for emotional–behavioral symptoms (Behavior Regulation ⁄ Inhibitory Self-Control Index [BRIS]) and cognitive symptoms (Metacognitive Index [MET]) of executive dysfunction, as well as an overall index of executive impairment (Global Executive Composite [GEC]). The normative sample has a

mean of 50 and an SD of 10. High scores are related to poorer functioning, with scores of 65 or greater considered significantly elevated and indicative of impairment (Williams *et al.* 2012).

8.4.2. Exclusion criteria

i) Children under the age of 4 years and over 18 years at the time of testing

ii) Children with a history of whole brain radiation therapy

iii) Children with a severe learning disability in the context of a genetic comorbidity such as Downs Syndrome were excluded from the analyses based on intelligence, as the underlying condition would have been a confounder and the focus was on the effect of moyamoya, but where possible parent and/or teacher reports of executive function were obtained.

8.5. Results

8.5.1. Group description

8.5.1.1. Intellectual Testing

27 children with moyamoya completed an hBOLD CVR study and age-appropriate Wechsler Intelligence Scales (mean age 10.2 years, range 4.1-16.3 years; SD 3.9 years; 14 male, 13 female). 26 had full scale intelligence quotient (FSIQ) and verbal IQ (VIQ), 27 performance IQ (PIQ), 21 Wechsler memory scales (WMS),

and 22 Processing speed (PS). Two children had a diagnosis of Trisomy 21 and significant learning difficulties hence their intellectual assessments were not usable. Reports of executive function were obtained from parents and teachers using the Behavior Rating Index of Executive Function (BRIEF).

The mean age of intellectual assessment of the whole group (n=27) was 10.44 years, range 4.08-16.33, SD 3.86 years.

The hBOLD CVR study closest to the time of intellectual assessment was chosen for analysis. The mean time between hBOLD CVR and IQ assessment was 8.7 months (range 15 days – 4 years; SD 12 months).

8.5.1.2. Clinical Description

The mean age at moyamoya diagnosis of the whole group with intellectual assessments (n=27) was 8.75 years, range 0.83-15.58 years, SD 3.57 years. Twelve children had a clinically overt stroke. The mean age of overt stroke was 7.6 years, range 0.75 – 14.2, SD 4.2years. Of the children with overt stroke two (overt strokes) occurred after the intellectual assessment so they were included in the no stroke group. Therefore 17 children with moyamoya and no antecedent history of overt stroke had an intellectual assessment.

The mean age of intellectual assessment in the group with overt stroke (n=10) was 11.1 years SD 3.69 years. The mean age of intellectual assessment in the group without an overt stroke (n=17) was 10.03 years SD 4 years. There was no

statistically significant difference in age of intellectual assessment between the overt stroke and no overt stroke groups independent of arteriopathy (disease or syndrome) diagnosis.

The mean interval between intellectual assessment and hBOLD CVR for the stroke and no stroke groups was 12.1 months, SD 11.8 months and 7.2 months, SD 12.1 months respectively (Table 71).

Table 71 Clinical Description of Patient's with Intellectual Assessments

	All patients n = 27	Moyamoya disease n = 11	Moyamoya syndrome n = 16
Arteriopathy Laterality Bilateral Right Left	 16 4 7	 8 2 1	 8 2 6
Mean age at diagnosis years (SD)	8.75 (3.57)	8.46 (3.18)	8.88 (3.78)^
Mean age at intellectual assessment years (SD)	10.44 (3.87)	10.13 (3.78)	10.65 (4.03)^
Stroke (%) Mean age at stroke years (SD)	10 (37) 7.15 (4.14)	7 (64) 7.55 (3.9)	5 (31) 7.58 (4.99)^
Radiographic Findings (Y:N) Not ischaemic Cortical Watershed (Deep white matter) (Cortical)	 7 1 8 (8) (1)	 2 0 3 (3) (0)	 5 1 5 (5) (1)

Cortical ischaemic and Watershed	10	5	5
Other	1	1*	0

^Mann-Whitney U, p>0.05 * left hemiatrophy

8.5.2. Steal, Intellectual and Executive Function in Whole Group

To test my hypothesis and examine for an association between CVR abnormality as demonstrated by the presence of steal, hypothesised to represent more severe haemodynamic compromise, and intellectual outcome BOLD CVR scores were recategorized into 2 groups: significant steal (CVR score 3 in either hemisphere) and no significant steal (CVR score of less than 3 in both hemispheres). Eighteen of twenty seven children had significant steal: 9 male, 9 bilateral, 4 right and 5 left sided steal. Of the 9 without steal 5 were male, 6 had bilateral, 1 right and 2 left sided moyamoya.

8.5.2.1. Effect of Steal on full scale, verbal and performance IQ, and on working memory index and processing speed index

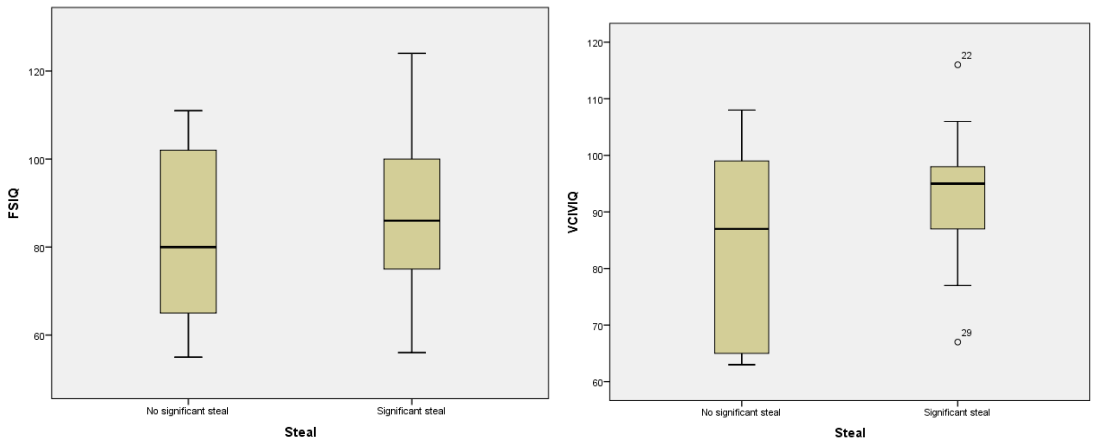
Although mean scores were mostly higher and within normal range in those with steal, two sided t-test revealed that the mean scores for FSIQ, VCI, PRI, WMS and PSI did not differ between children with steal and no significant steal (Table 72, Fig 69).

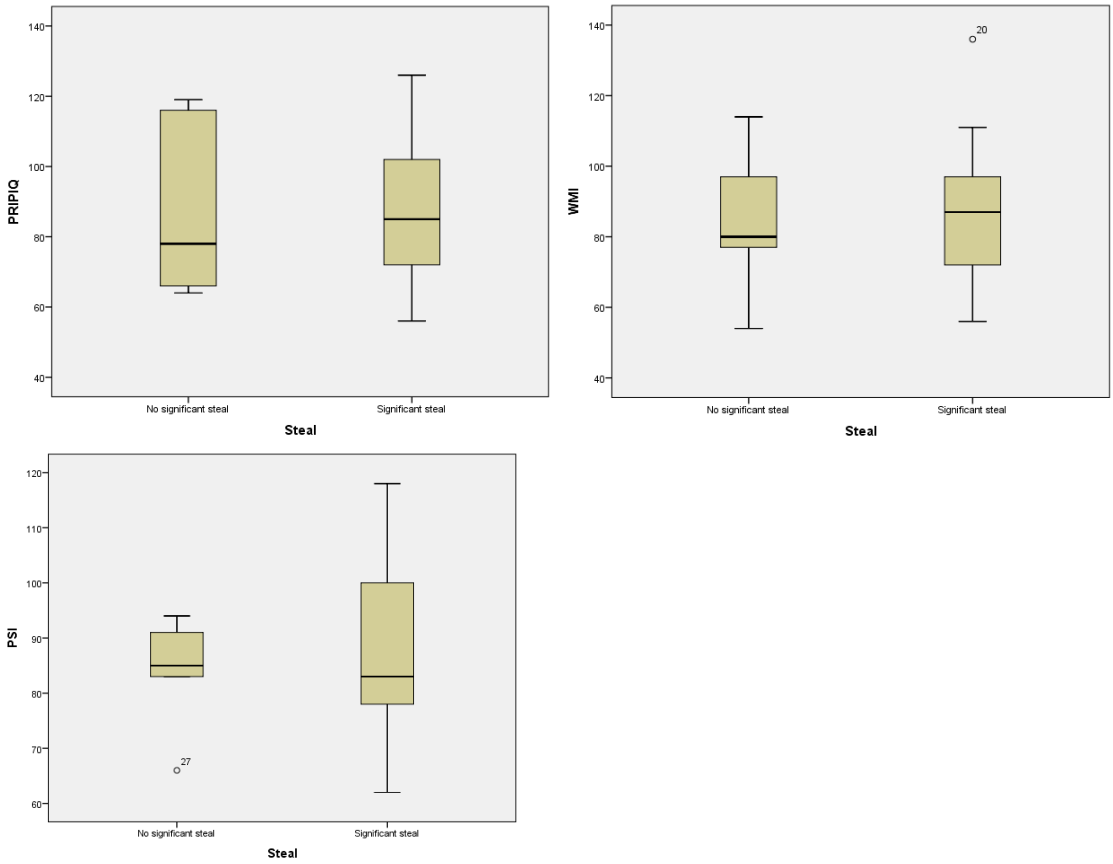
Table 72 Weschler Intelligence Scale Intelligence Scores (WISC) by Presence of Significant Steal

	Steal		Mean difference (CI)	p value
	Yes mean (SD) n=17	**No** mean (SD) n=8		
FSIQ	90.1 (20.09)	83.4 (19.72)	-6.63 (-25.47- 12.2)	ns
VCI	93.9 (13.4)	85.1 (16.6)	-8.8 (-22.37- 4.78)	ns
PRIQ	91.12 (23.6)	84.9 (21.9)	-6.24 (-26.72- 14.23)	ns
WMS	88.5 (21.32)	84.4 (22.5)	-4.14 (-28.27- 20)	ns
PS	91 (17.02)	83.8 (10.9)	-7.2 (-24.56- 10.16)	ns

t-test

Figure 69 Weschler Intelligence Scale Intelligence Scores (WISC) by Presence of Significant Steal





8.5.2.2. Effect of Steal on Parent and Teacher BRIEF scores of executive function

Available mean scores of executive function were lower for patients with steal than those without steal in both Parent and Teacher reports for two of the three composite scores (GEC and BRI). There was a trend toward significant difference in composite scores for MET with higher scores being in the no steal group.

Parent and Teacher mean scores for monitoring and Teacher mean scores for initiation were significantly lower in those with steal than those without steal.

There was a trend toward difference by steal for working memory and shift (Table 73 and Fig 70 and 71).

Table 73 Linear regression for effect of unilateral and bilateral steal vs no steal on scores of intelligence

	Parent				Teacher			
	Steal **Yes** mean (SD) n=16	**No** mean (SD) n=8	mean difference (CI)	p value	**Steal** **Yes** mean (SD) n=16	**No** mean (SD) n=7	mean difference (CI)	p value
Inh	54.8 (9.75)	64.6 (8.82)	9.8 (1.32-18.31)	.026*	53.4 (10.65)	62.6 (18.06)	9.2 (-3.24-21.66)	ns
Shift	55.8 (12.28)	68 (13.72)	12.25 (.79-23.71)	.057†	57.9 (12.25)	71.6 (18.81)	13.7 (.096-27.3)	.05*
EmC	56.3 (14.1)	61.8 (10.38)	5.5 (-6.2-17.2)	ns	54.4 (11.31)	70.4 (16.1)	16 (3.86-28.13)	.012*
Initi	57.6 (14)	66 (8)	8.36 (-3.7-20.41)	.16	60.2 (10.71)	72 (10.2)	11.79 (.527-23.05)	.035*
WMT	60.8 (12)	71.4 (13.14)	10.56 (-.54-21.66)	.06†	59.2 (12.46)	71.9 (17.91)	12.67 (-.742-26.08)	.063†
Plan	61.1 (11.71)	71.7 (9.41)	10.66 (.19-21.14)	.046*	59.7 (10.71)	65 (8.23)	5.31 (-4.17-14.79)	ns
Org	55.9 (9.54)	55.6 (5.68)	-.36 (-.86-7.89)	ns	55.3 (12.69)	75 (29.15)	19.71 (.473-39)	.045*
Mon	57.5 (9.5)	68.6 (10.11)	11.07 (1.68-20.47)	.023*	56.9 (11.65)	68.5 (11.1)	11.57 (-.20-23.35)	.054*
BRI	56.7 (11.67)	67.4 (9.75)	10.71 (.634-20.78)	.038*	55.7 (9.9)	70 (19)	14.27 (1.54-27)	.03*
MET	60.8 (12.42)	71.9 (10.12)	11.06 (-.19-22.3)	.054†	60.6 (10.94)	71.1 (13.51)	10.54 (-.7-21.78)	.064†
GEC	59.6 (11.4)	71 (7.81)	11.44 (1.55-21.33)	.026*	58.94 (11.15)	71.4 (16.23)	12.49 (.421-24.56)	.043*

Flex n=1	44	43			83	47		

t-test * significant at the p</=0.05 level †trend toward significance at the p<0.1 level

Figure 70 Mean Parent Scores of Scales of Executive Function by Steal

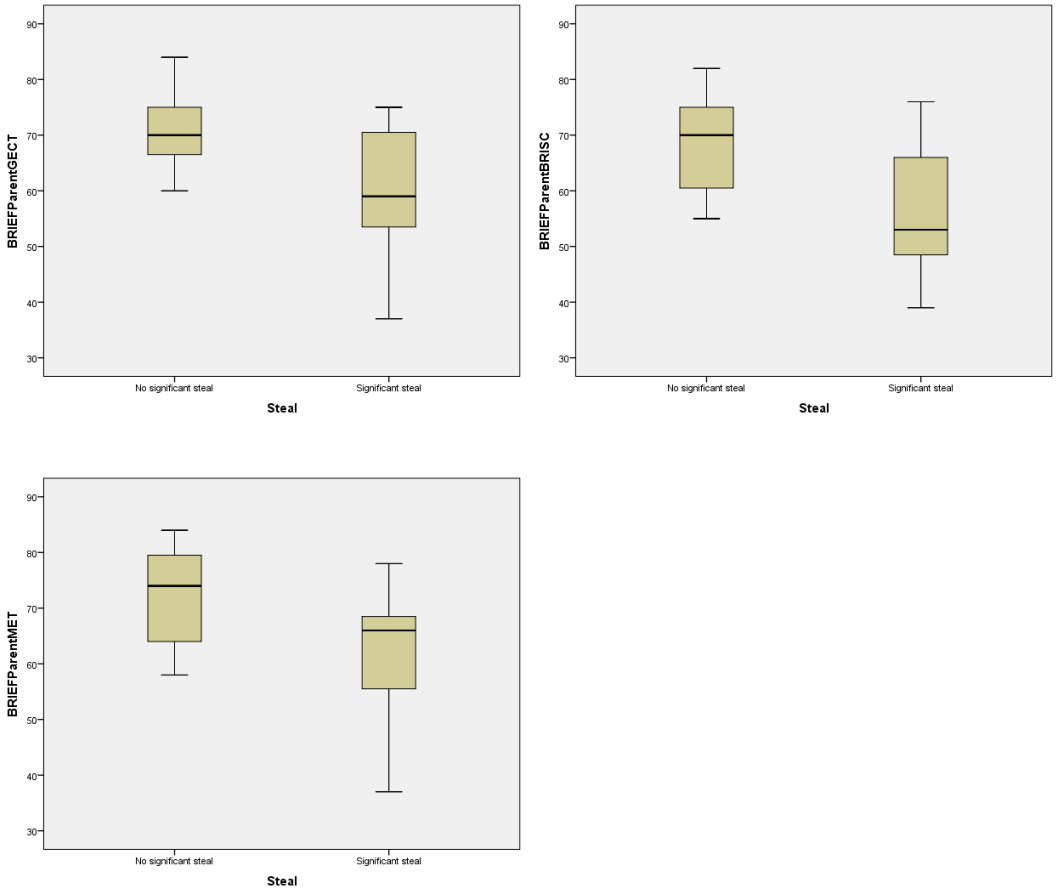
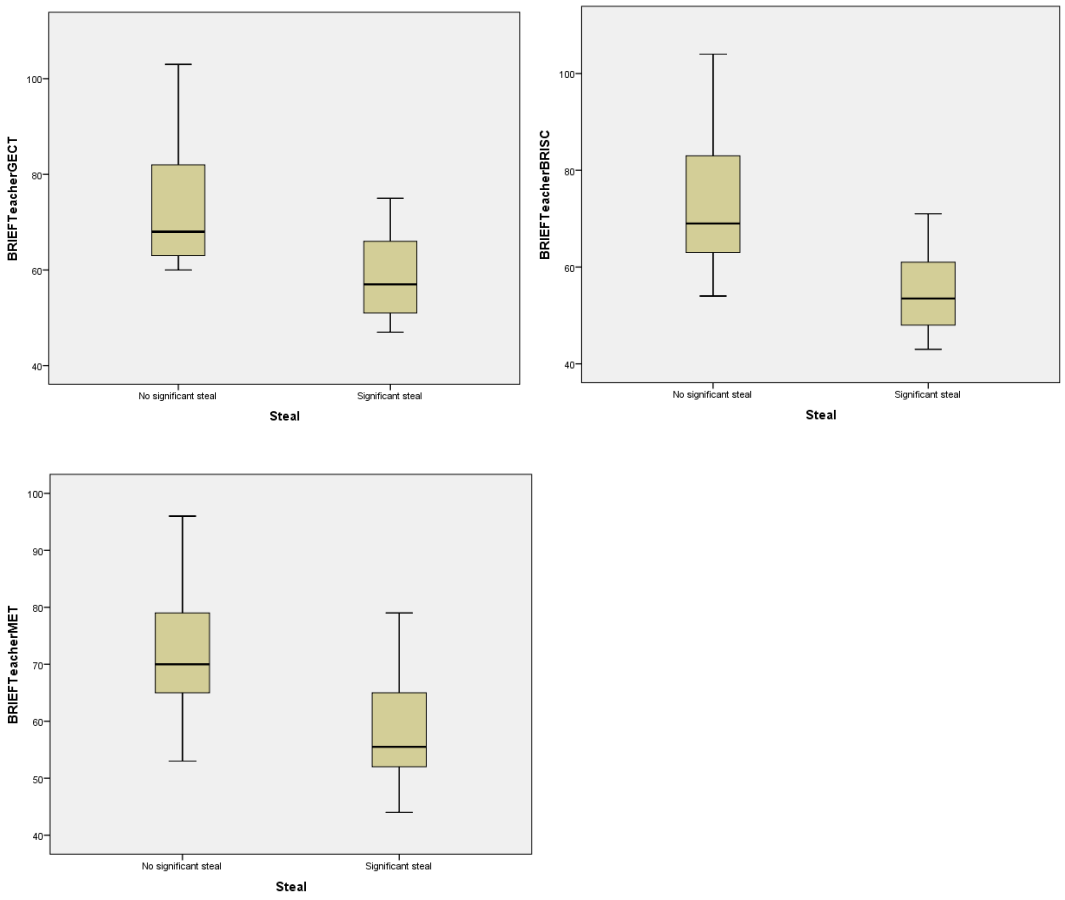


Figure 71 Mean Teacher Scores of Scales of Executive Function by Steal



8.5.2.3. Effect of Laterality of Steal on full scale, verbal and performance IQ, and on working memory index and processing speed index and on Parent and Teacher BRIEF scores of executive function

To examine whether hemispheric abnormality of CVR was associated with specific neurocognitive deficits, scores of intellectual and executive function I used linear regression analysis with laterality of steal as the CVR measure and predictor.

8.5.2.3.1. Right, Left and Bilateral Steal and Neurocognitive Function

Laterality of steal was not significantly predictive of Wechsler indices of intelligence scores. There was a trend toward right sided steal being inversely associated with VCI, i.e. the greater the steal, the lower the VCI. Similarly, WMI was lower when left sided steal was higher (Table 74 and Fig 72).

For indices of executive function, bilateral steal was significantly inversely associated with Parent and Teacher BRI, Parent MET and Parent GEC. There was a trend towards bilateral steal predicting Teacher MET and Teacher GEC. Right sided steal was significantly inversely associated with Teacher GEC and there was a trend toward a significant relationship with Teacher BRI (Tables 75 and 76; Fig 73).

Table 74 Linear regression for effect of right, left or bilateral steal vs no steal on IQ measures

	R	R^2	Adj R^2	F	B	SE for B	Beta	T	p
Full scale IQ									
Compared with no steal	0.343	0.117	-0.022	0.843					0.487
R Steal					11.321	12.529	0.222	0.904	0.378
L steal					13.971	11.705	0.298	1.194	0.247
Bilateral steal					-1.286	10.685	-0.031	-0.120	0.905
VCI Verbal IQ									
Compared with no steal	0.468	0.219	0.095	1.775					0.186
R Steal					17.107	8.744	0.452	1.956	0.065**
L steal					11.857	8.168	0.341	1.452	0.163
Bilateral steal					1.867	7.457	0.060	0.249	0.806
PRI Performance IQ	0.295	0.087	-0.044	0.666					0.582
Compared with no steal					9.375	14.251	0.154	0.657	0.518
R Steal					15.325	13.275	0.274	1.154	0.261
L steal					-1.000	11.644	-0.021	-0.086	0.932
Bilateral steal									
WMI									
Compared with no steal	0.538	0.289	0.137	1.898					0.176
R Steal					-8.400	16.379	-0.129	-0.513	0.616
L steal					23.100	13.133	0.469	1.759	0.100**
Bilateral steal					-3.114	11.463	-0.074	-0.272	0.790
PSI									
Compared with no steal	0.252	0.063	-0.124	0.339					0.798
R Steal					5.200	12.148	0.124	0.428	0.675
L steal					11.200	11.159	0.299	1.004	0.331
Bilateral steal					5.771	9.740	0.182	0.593	0.562

**Trend toward significance at level of p</=0.1

Table 75 Linear regression for effect of of right, left or bilateral steal vs no steal on Parent BRIEF measures of executive function

	R	R^2	Adj R^2	F	B	SE for B	Beta	T	P
Parent BRIS									
Compared with no steal	0.479	0.229	0.107	1.881					0.167
R steal					-7.375	7.678	-0.212	-0.961	0.349
L steal					-8.375	6.465	-0.294	-1.295	0.211
Bilateral steal					-13.804	5.869	-0.541	-2.352	0.030*
Parent Metacog									
Compared with no steal	0.643	0.413	0.315	4.224					0.020
R steal									
L steal					-0.857	7.221	-0.024	-0.119	0.907
Bilateral steal					-6.657	6.127	-0.226	-1.086	0.292
					-18.571	5.593	-0.700	-3.320	0.004*
Parent GECT									
Compared with no steal	0.612	0.375	0.276	3.799					0.027
R steal					-5.000	6.176	-0.167	-0.810	0.428
L steal					-8.200	5.769	-0.299	-1.421	0.171
Bilateral steal					-17.429	5.267	-0.708	-3.309	0.004*

Table 76 Linear regression for effect of right, left or bilateral steal vs no steal on Teacher BRIEF measures of executive function

	R	R^2	Adj R^2	F	B	SE for B	Beta	T	p
Teacher BRIS	0.511	0.261	0.137	2.115					0.134
Compared with no steal									
R Steal					-19.333	9.405	-0.463	-2.056	0.055**
L steal					-9.400	7.980	-0.275	-1.178	0.254
Bilateral steal					-15.571	7.285	-0.506	-2.137	0.047*
Teacher Metacog	0.445	0.198	0.064	1.482					0.253
Compared with no steal									
R Steal					-14.810	8.368	-0.415	-1.770	0.094*
L steal					-6.943	7.101	-0.238	-1.741	0.341
Bilateral steal					-11.286	6.482	-0.429	-0.978	0.099**
Teacher GECT	0.523	0.274	0.159	2.385					0.101
Compared with no steal									
R Steal					-19.429	7.947	-0.545	-2.445	0.024*
L steal					-6.229	7.424	-0.190	-0.839	0.412
Bilateral steal					-13.000	6.777	-0.442	-1.918	0.070**

Figure 72 Boxplots show full scale, verbal and performance Intelligence Quotient (IQ) Scores, Working Memory index and Processing Speed Index by Steal Laterality

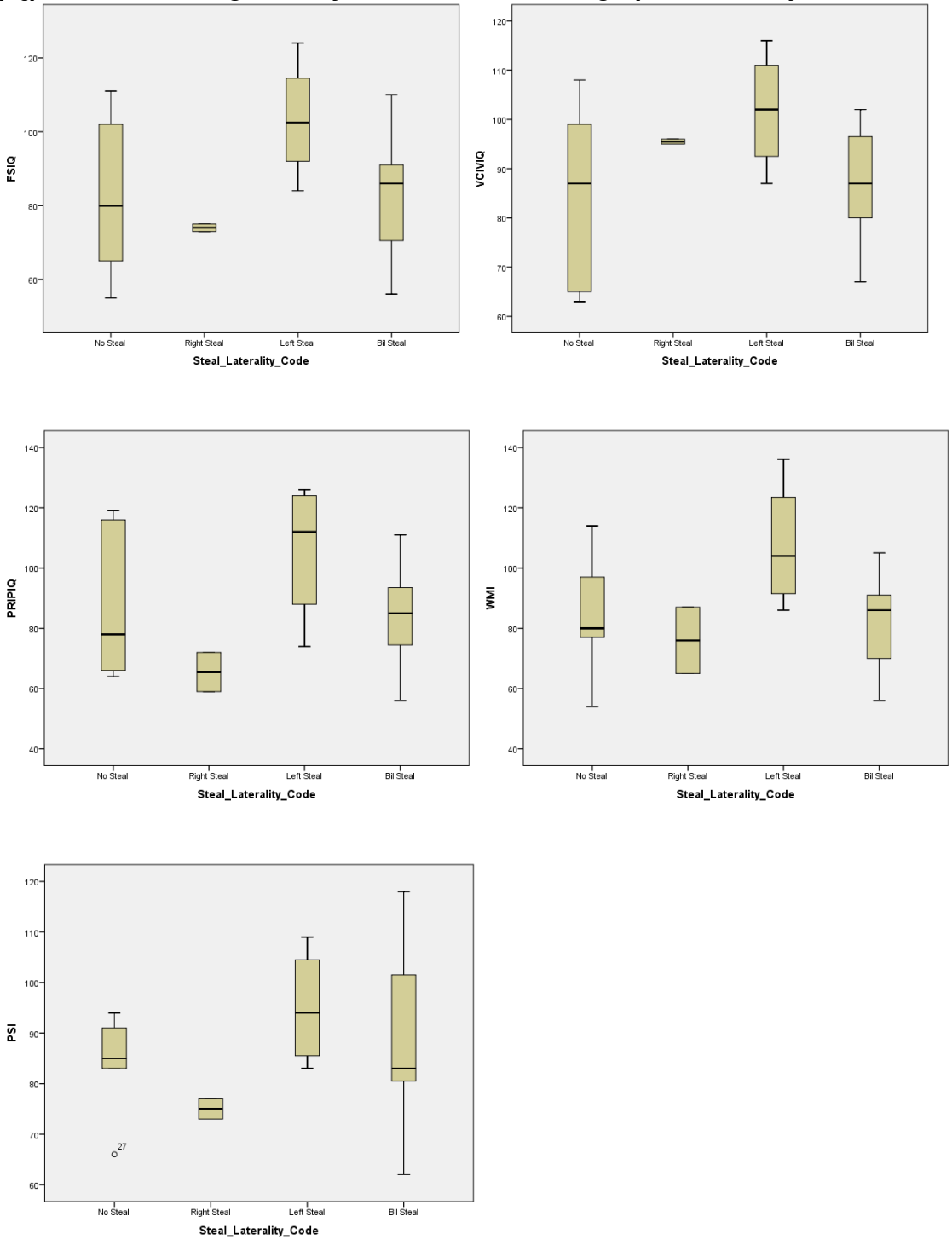
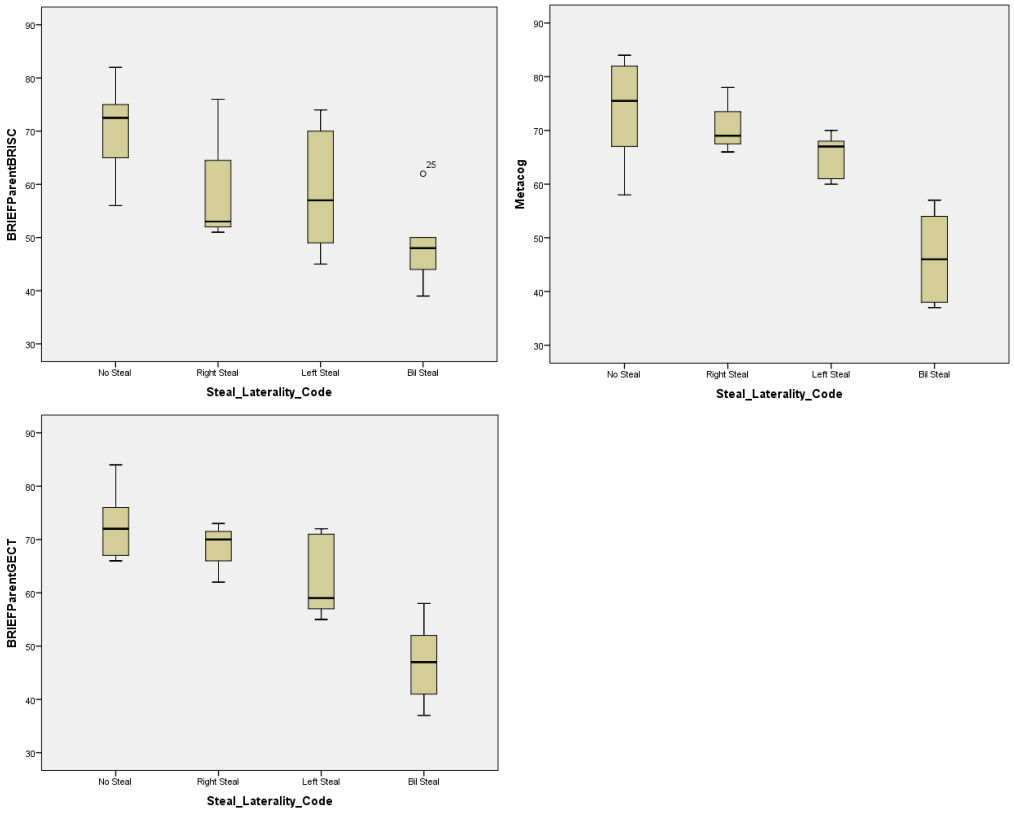
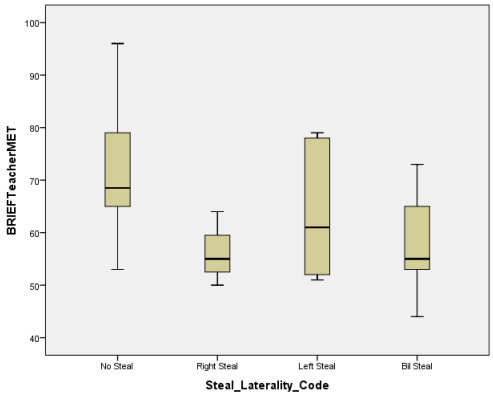


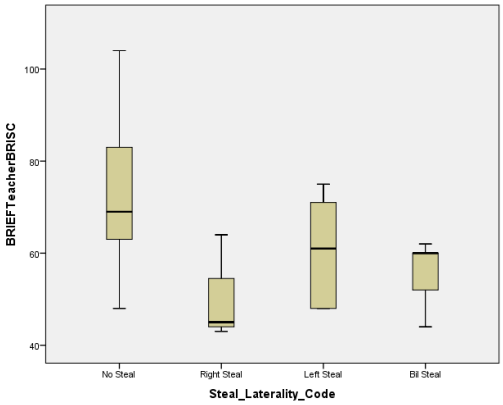
Figure 73 Boxplots showing (a) Parent and (b) Teacher Scores of Executive Function (BRISC, Metacognition and GECT) by Steal Laterality

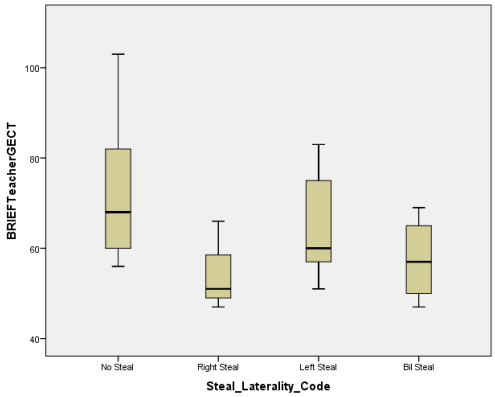
(a) Parent



(b) Teacher







8.5.2.3.2. Unilateral and Bilateral Steal and Neurocognitive Function

Groups were further collapsed into unilateral (n=3 right, n=5 left) steal and bilateral (n=10) steal. In univariable regression VCI, Parent and Teacher BRIS were significantly lower in those with unilateral steal when compared to those with no steal; Parent MET and GEC were significantly lower in those with bilateral steal when compared to those with no steal. Teacher MET and GEC scores were lower in those with bilateral steal when compared to no steal (Tables 77-79).

Table 77 Linear regression for effect of unilateral and bilateral steal vs no steal on scores of intelligence

IQ (n)	R	R^2	Adj R^2	F	B	SE for B	Beta	T	P
FSIQ (23) *Compared with no steal* Unilateral steal Bilateral steal	.340	.116	.027	1.308	 12.79 -1.29	 9.83 10.43	 .323 -.031	 1.30 -.123	.293 .208 .903
VCI (23) *Compared with no steal* Unilateral steal Bilateral steal	.454	.206	.127	2.595	 14.19 1.86	 6.91 7.33	 .483 .060	 2.054 .253	.10 .053* .803
PRI (25) *Compared with no steal* Unilateral steal Bilateral steal	.284	.081	-.003	.964	 12.68 -1.0	 11.09 11.42	 .273 -.021	 1.143 -.088	.397 .265 .931
WMI (18) *Compared with no steal* Unilateral steal Bilateral steal	.337	.114	-.004	.964	 12.6 -3.114	 12.787 12.365	 .290 -.074	 .985 -.252	.404 .340 .805
PSI (19) *Compared with no steal* Unilateral steal Bilateral steal	.223	.05	-.069	.417	 8.63 5.77	 9.50 9.50	 .273 .182	 .908 .607	.666 .377 .552

Table 78 Linear regression for effect of unilateral or bilateral steal vs no steal on Parent BRIEF measures of executive function

	R	R^2	Adj R^2	F	B	SE for B	Beta	T	P
Parent BRIS *Compared with no steal* Unilateral steal Bilateral steal	0.478	0.228	.151	2.961	 -8.0 -13.80	 5.723 5.529	 -0.541 -0.325	 -2.412 -1.447	.075 0.026* 0.163†
Parent Metacog *Compared with no steal* Unilateral steal Bilateral steal	0.628	0.394	.331	6.186	 -4.482 -18.571	 5.35 5.53	 -0.174 -0.700	 -0.837 -3.358	.009 0.413 0.003**
Parent GECT *Compared with no steal* Unilateral steal Bilateral steal	0.606	0.367	0.304	5.804	 -6.778 -17.429	 4.869 5.165	 -0.292 -0.708	 -1.392 -3.374	.01 0.179† 0.003**

***** significant at p<.05 level ** p<.01

Table 79 Linear regression for effect of unilateral or bilateral steal vs no steal on Teacher BRIEF measures of executive function

	R	R^2	Adj R^2	F	B	SE for B	Beta	T	P
Teacher BRIS *Compared with no steal* Unilateral steal Bilateral steal	.469	.220	.138	2.676	 -13.125 -15.571	 7.053 7.284	 -0.44 -0.506	 -1.861 -2.138	.095 .046* .078†
Teacher Metacog *Compared with no steal* Unilateral steal Bilateral steal	0.404	0.163	0.075	1.849	 -9.893 -11.286	 6.241 6.446	 -0.429 -0.389	 -1.585 -1.751	0.185 0.129 0.096†
Teacher GECT *Compared with no steal* Unilateral steal Bilateral steal	0.426	0.181	0.100	2.217	 -12.095 -13.000	 6.661 7.012	 -0.437 -0.442	 -1.830 -1.854	0.135 0.082† 0.079†

*significant at p<.5 level; †trend toward significance at p<0.1 level

In summary examination for an association between CVR abnmormality as measured by steal in the whole group (n=27) is suggestive of a negative association between right and left steal and VCI; left steal and WMI; and right and bilateral steal with indices of executive function.

8.5.3. Intellectual and Executive Function by Stroke, Comorbidity and Moyamoya Laterality

I sought to examine for potential confounders of the analysis for association between CVR abnormality and neurocognitive function. Namely, I sought to examine whether differences in neurocognitive outcome were explained by a history of 1) stroke 2) comorbidity or 3) moyamoya laterality. Due to the numbers in my study I used exploratory group descriptive analysis to determine this.

8.5.3.1. Effect of stroke on full scale, verbal and performance IQ, and on working memory index and processing speed index and Parent and Teacher BRIEF scores of executive function

At the time of intellectual assessment ten children had a stroke, seventeen no stroke. Children with bilateral stroke had lower scores of intelligence than children with right sided or no stroke (Fig 71). However, using Kruskal-Wallis there was no statistically significant difference in FSIQ, VCI, PIQ, WMS and PS between the clinically overt stroke and no stroke groups (Table 80 and 82; Fig 75 and 76).

There was no statistically significant difference in measures of executive function by Parent or Teacher BRIEF by stroke (Table 81, 82, 83; Fig 77 and 78).

Figure 74 Presence of Stroke and Stroke Laterality

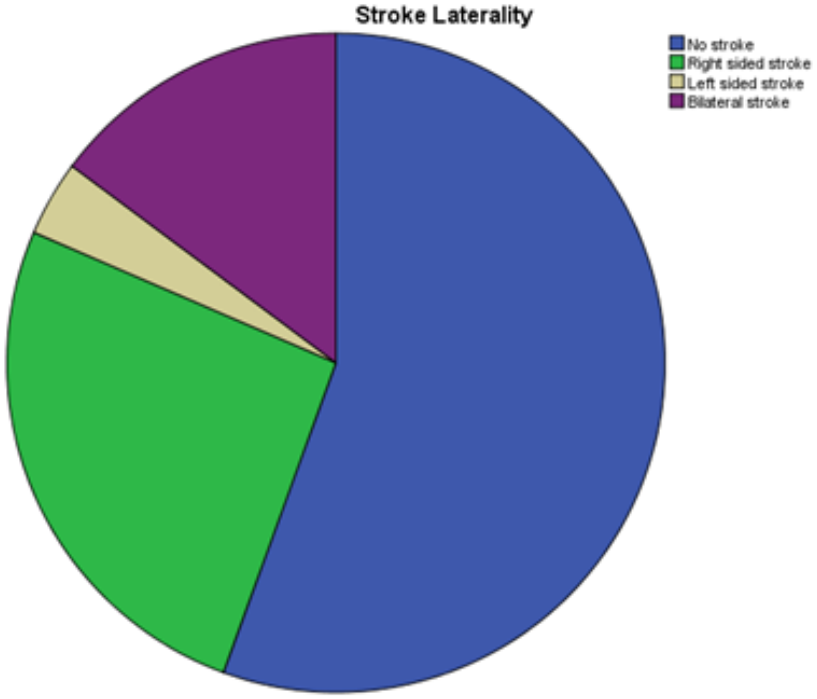


Table 80 Mean Scores of Intelligence in children with moyamoya with and without stroke

	Stroke		Mean difference (CI)	t-test p value
	Yes mean (SD) n=10	**No** mean (SD) n=15		
FSIQ	85.78 (17.22)	89.5 (21.76)	-3.72 (-21.63 – 14.18)	ns
VCI	90.89 (11.48)	91.5 (16.82)	-.611 (-13.95 – 12.73)	Ns
PRIQ	86.1 (20.9)	91.13 (24.48)	-5.033 (-24.58 – 14.51)	ns
WMS	86.43 (19.51)	88 (22.9)	-1.57 (-23.82 – 20.68)	ns
PSI	88.14 (17.92)	89.67 (15.06)	-1.52 (-17.71 – 14.66)	ns

Table 81 Mean Scores of Executive Function by Parent and Teacher BRIEF scores in children with moyamoya with and without stroke

	Parent				Teacher			
	Stroke **Yes** mean (SD)	**No** mean (SD)	mean difference (CI)	t-test p value	**Stroke** **Yes** mean (SD)	**No** mean (SD)	mean difference (CI)	t-test p value
Int	57.27 (12.93)	58.77 (8.15)	-1.5 (-10.5 – 7.5)	ns	55.64 (15.74)	56.67 (12.02)	-1.03 (-13.12–11.05)	ns
Shift	58.09 (12.97)	61.31 (14.82)	-3.22 (-15.12 – 8.69)	ns	62.91 (16.67)	61.25 (15.05)	1.66 (-12.09 – 15.41)	ns
EmC	56.45 (13.81)	59.46 (12.7)	-3.01 (-14.24 – 8.22)	ns	59.0 (16.47)	59.58 (13.52)	-.58 (-13.60 – 12.44)	ns
Initi	57.3 (12.82)	63.27 (12.65)	-5.97 (-17.61 – 5.67)	ns	65.0 (12.82)	62.5 (10.97	2.5 (-8.71 – 13.71)	ns
WMT	63.27 (13.82)	65.23 (12.98)	-1.96 (-13.32 – 9.4)	ns	65.27 (18.27)	61.0 (12.04)	4.27 (-9.03 – 17.57)	ns
Plan	62.9 (13.61)	65.38 (10.95)	-2.49 (-13.12 – 8.15)	ns	62.27 (10.51)	60.42 (10.18)	1.86 (-7.12 – 10.83)	ns
Org	54.3 (8.04)	57.18 (8.67)	-2.88 (-10.54 – 4.78)	ns	61.4 (25.84)	61.0 (14.77)	.40 (-19.37 – 20.17)	ns
Mon	59.2 (11.29)	63 (10.66)	-3.8 (-13.83 – 6.23)	ns	60.9 (15.09)	59.9 (9.91)	1.00 (-10.99 – 12.99)	ns
BRI	58.64 (12.73)	62 (11.62)	-3.36 (-13.92 – 7.19)	ns	60.09 (16.85)	60.45 (12.97)	-.36 (-13.74 – 13.01)	ns
MIT	62.7 (14.52)	65.67 (11.33)	-2.97 (-14.46 – 8.53)	ns	64.36 (15.39)	63.55 (9.64)	.82 (-10.60 – 12.24)	ns
GEC	61 (12.56)	64.62 (11.02)	-3.62 (-13.85 – 6.62)	ns	63.27 (15.98)	62.25 (12.23)	1.02 (-11.25 – 13.3)	ns
Flex n=1								

Table 82 Mean Scores of Intelligence and Parent and Teacher BRIEF by Presence of Stroke and Stroke Laterality

	No stroke	Right Stroke	Bilateral stroke	Chi squared	df	p Value
IQ						
Number	12	4	2			
FSIQ	87.75 (20.68)	90.00 (17.8)	68.00 (16.97)	1.914	2	ns
VIQ	89.33 (16)	98.25 (13.57)	82.0 (7.07)	1.493	2	ns
PIQ	91.75 (23.79)	87.75 (23.43)	67.00 (15.56)	1.920	2	ns
WMI	89.92 (22.84)	90.25 (14.22)	66.50 (14.85)	2.482	2	ns
PSI	88.25 (12.9)	90.25 (18.72)	78.00 (22.63)	.763	2	ns
BRIEF Parent						
Number	14	7	1			
BRI	63.5 (11.39)	56.43 (12.71)	52.50 (12.02)	3.120	2	ns
MET	65.43 (10.70)	63.14 (17.27)	57.00 n=1	1.245	2	ns
GEC	65.57 (10.58)	60.14 (14.19)	52.00 n=1	2.704	2	ns
BRIEF Teacher						
Number	13	6	3			
BRI	60.54 (12.98)	61.33 (22.02)	57.00 (4.36)	.438	2	ns
MET	61.85 (9.73)	64.83 (19.61)	71.33 (2.89)	2.23	2	ns
GEC	61.29 (11.65)	64.17 (21.6)	62.74 (3.22)	1.247	2	ns

Figure 75 Box plots showing Mean Scores of Intellectual Function (full scale verbal and performance IQ, working memory index, processing speed index) by Stroke

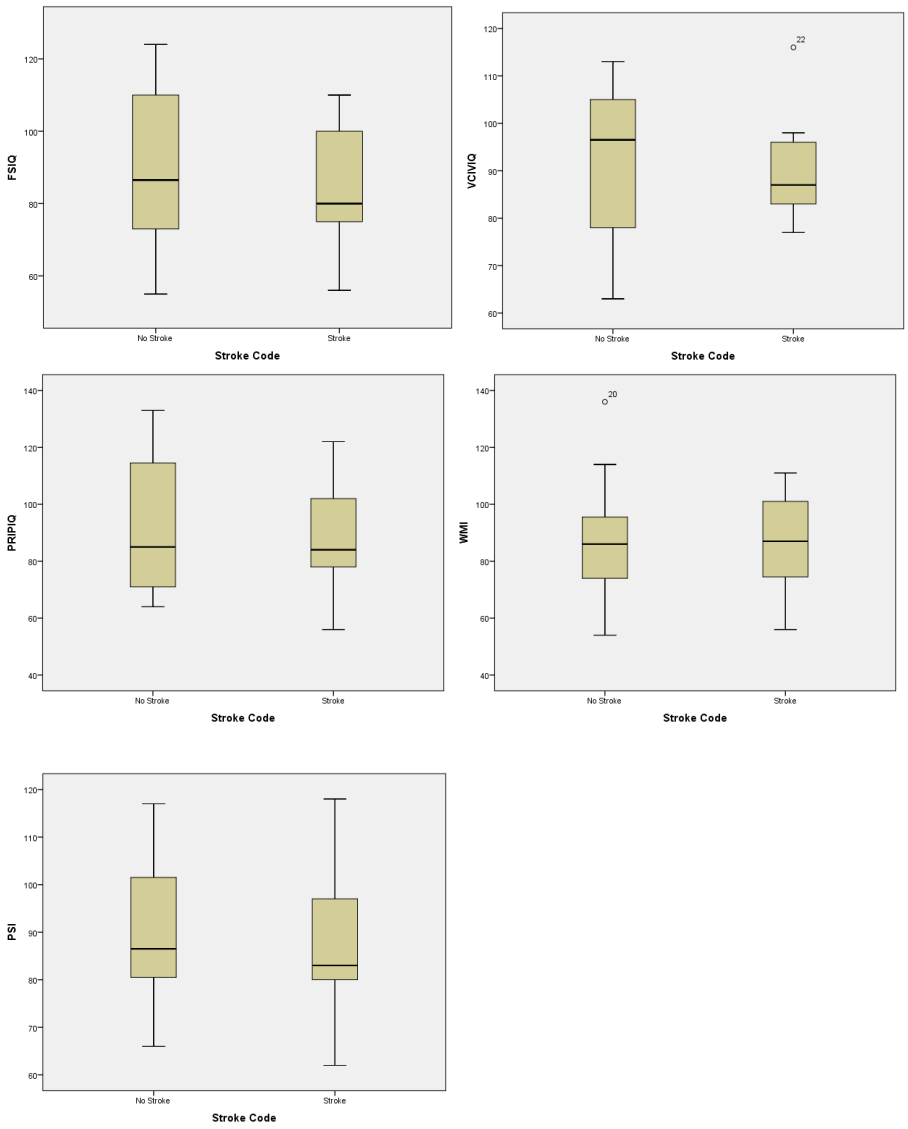


Figure 76 Box plots showing Mean Scores of Intellectual Function (full scale verbal and performance IQ, working memory index, processing speed index) by Stroke Laterality

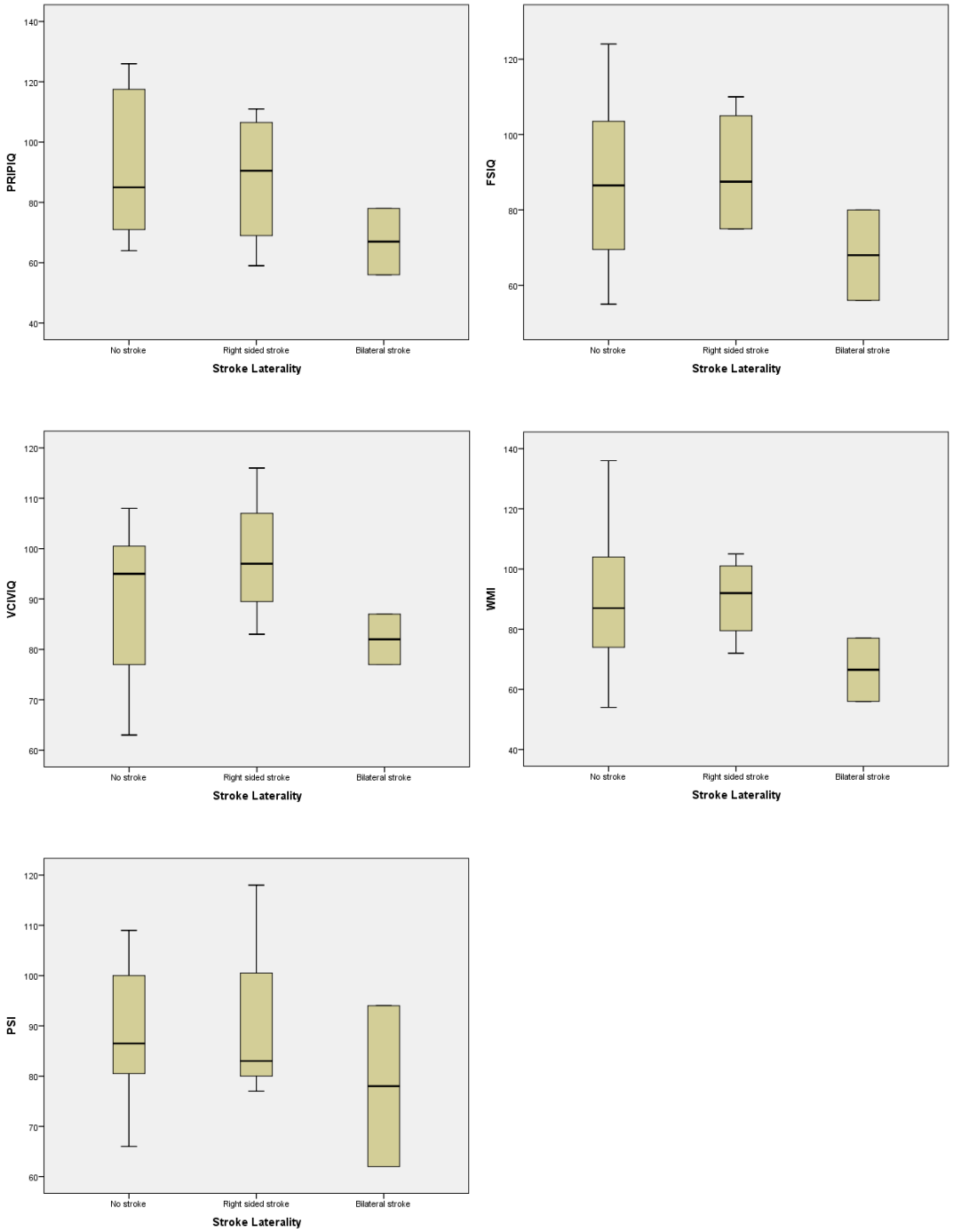


Table 83 Mean Parent and Teacher BRIEF Scores of Executive Function by Stroke

	Parent				Teacher			
	Stroke **Yes** mean (SD)	**No** mean (SD)	mean difference (CI)	t-test p value	**Stroke** **Yes** mean (SD)	**No** mean (SD)	mean difference (CI)	t-test p value
Int	57.27 (12.93)	58.77 (8.15)	-1.5 (-10.5 – 7.5)	ns	55.64 (15.74)	56.67 (12.02)	-1.03 (-13.12–11.05)	ns
Shift	58.09 (12.97)	61.31 (14.82)	-3.22 (-15.12 – 8.69)	ns	62.91 (16.67)	61.25 (15.05)	1.66 (-12.09 – 15.41)	ns
EmC	56.45 (13.81)	59.46 (12.7)	-3.01 (-14.24 – 8.22)	ns	59.0 (16.47)	59.58 (13.52)	-.58 (-13.60 – 12.44)	ns
Initi	57.3 (12.82)	63.27 (12.65)	-5.97 (-17.61 – 5.67)	ns	65.0 (12.82)	62.5 (10.97	2.5 (-8.71 – 13.71)	ns
WMT	63.27 (13.82)	65.23 (12.98)	-1.96 (-13.32 – 9.4)	ns	65.27 (18.27)	61.0 (12.04)	4.27 (-9.03 – 17.57)	ns
Plan	62.9 (13.61)	65.38 (10.95)	-2.49 (-13.12 – 8.15)	ns	62.27 (10.51)	60.42 (10.18)	1.86 (-7.12 – 10.83)	ns
Org	54.3 (8.04)	57.18 (8.67)	-2.88 (-10.54 – 4.78)	ns	61.4 (25.84)	61.0 (14.77)	.40 (-19.37 – 20.17)	ns
Mon	59.2 (11.29)	63 (10.66)	-3.8 (-13.83 – 6.23)	ns	60.9 (15.09)	59.9 (9.91)	1.00 (-10.99 – 12.99)	ns
BRI	58.64 (12.73)	62 (11.62)	-3.36 (-13.92 – 7.19)	ns	60.09 (16.85)	60.45 (12.97)	-.36 (-13.74 – 13.01)	ns
MIT	62.7 (14.52)	65.67 (11.33)	-2.97 (-14.46 – 8.53)	ns	64.36 (15.39)	63.55 (9.64)	.82 (-10.60 – 12.24)	ns
GEC	61 (12.56)	64.62 (11.02)	-3.62 (-13.85 – 6.62)	ns	63.27 (15.98)	62.25 (12.23)	1.02 (-11.25 – 13.3)	ns
Flex n=1								

Figure 77 Boxplots showing Mean Parent and Teacher BRIEF (BRISC, metacognition and GEC) Scores in Stroke and No Stroke Groups

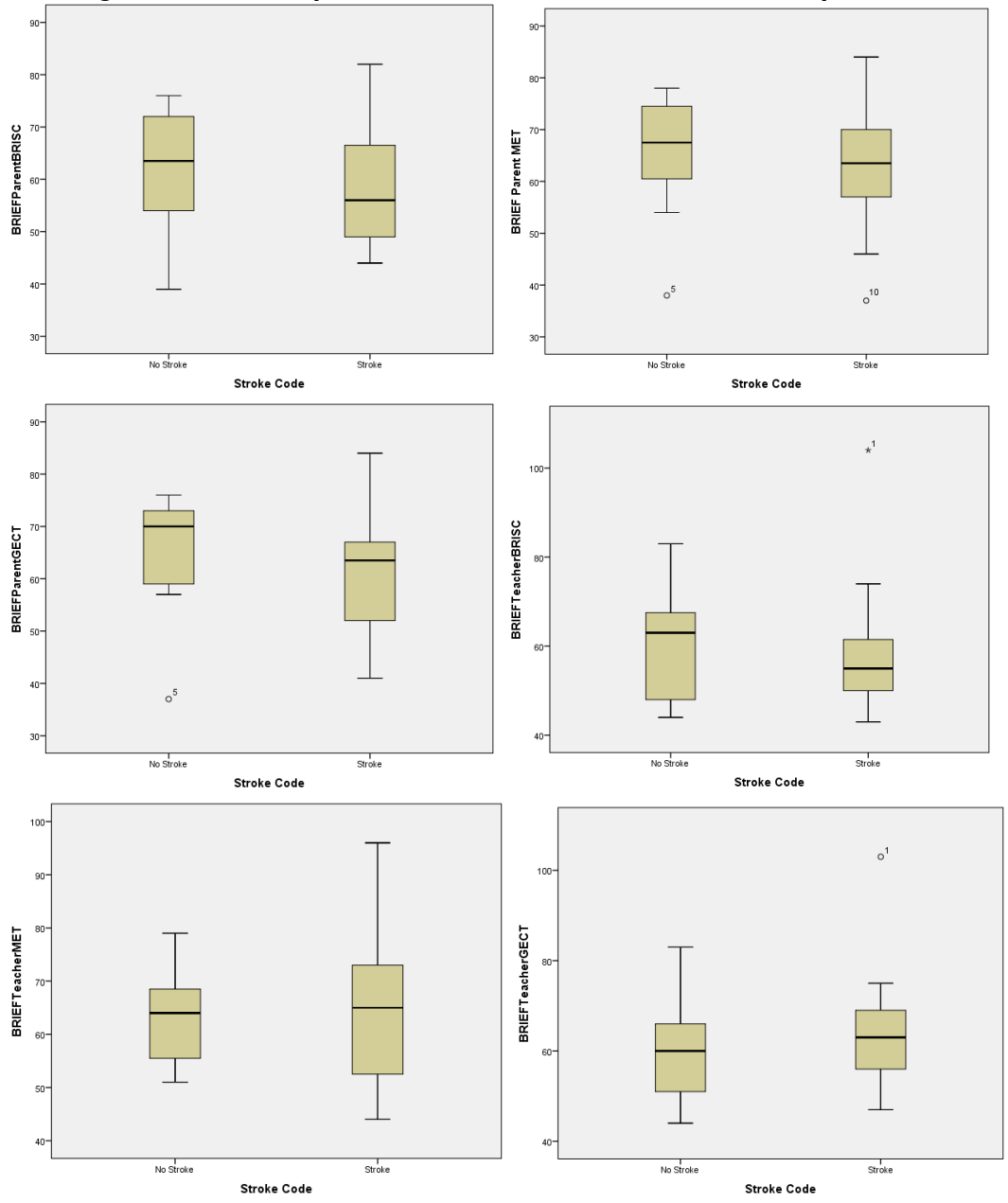
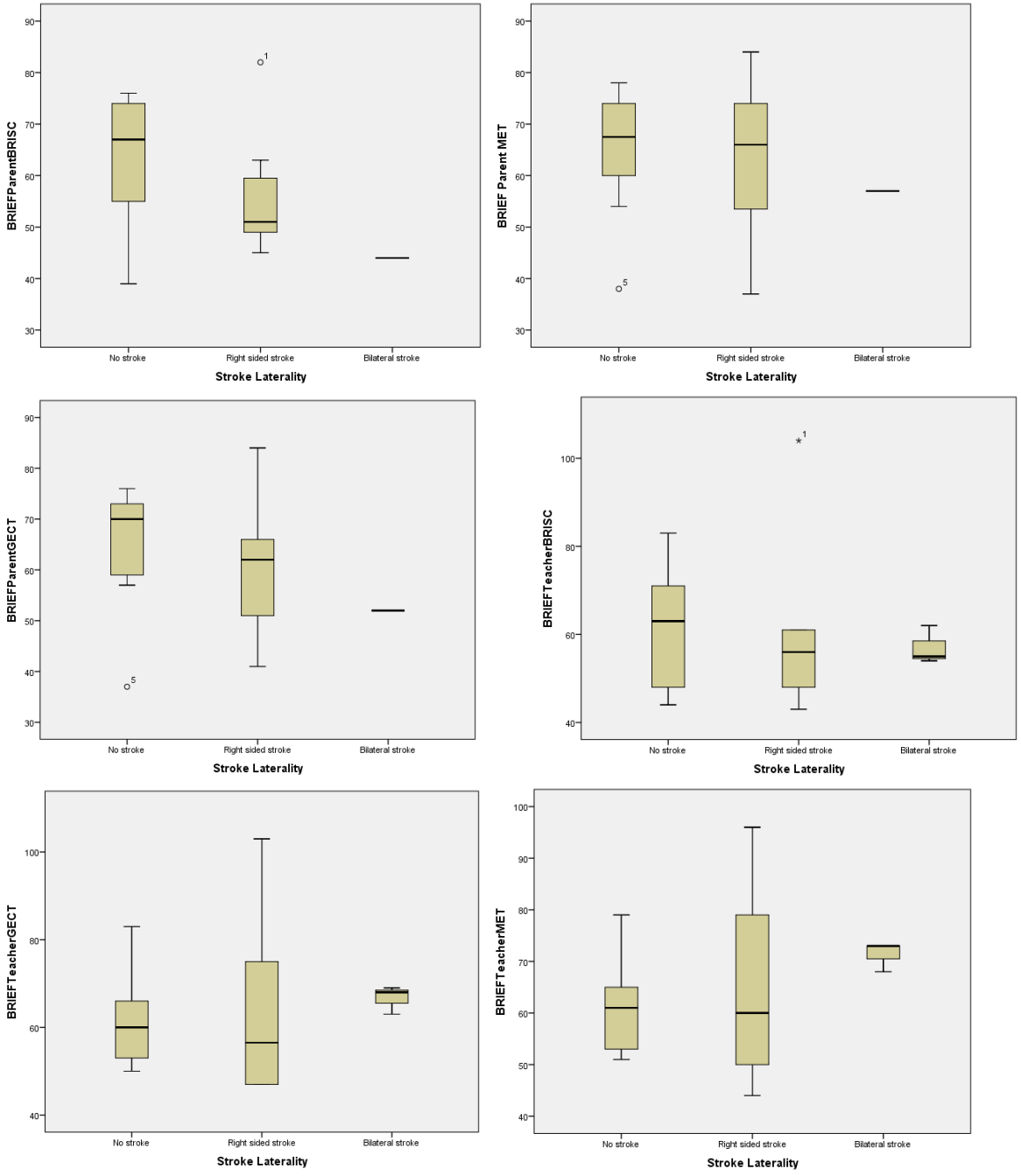


Figure 78 Boxplots showing Mean Parent and Teacher BRIEF (BRISC, metacognition and GEC) Scores of Executive Function by Stroke Laterality



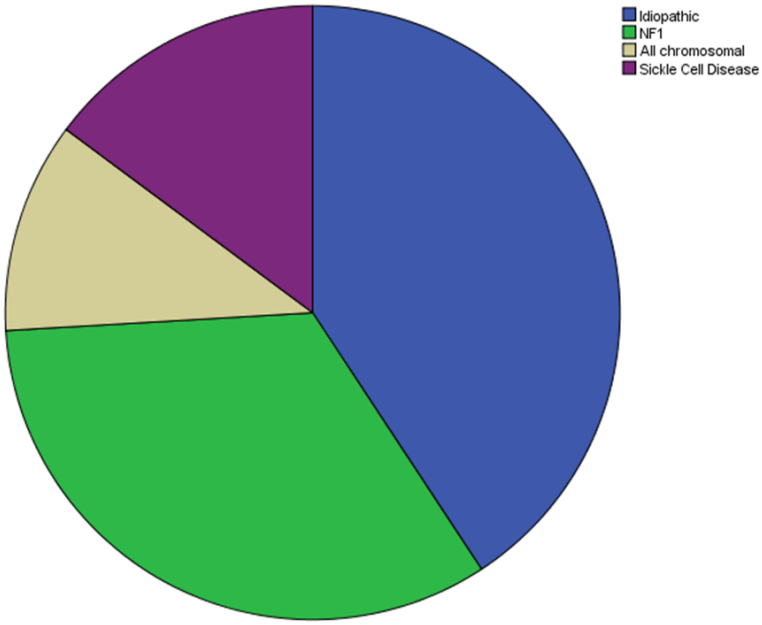
8.5.3.2. Effect of comorbidity on full scale, verbal and performance IQ, and on working memory index and processing speed index and Parent and Teacher BRIEF scores of executive function

Eleven children had MMD and 16 MMS (Table 83 and Fig 76). The comorbidity diagnoses of the MMS group were Neurofibromatosis Type 1 ([NF1] n=9), Sickle Cell Disease ([SCD] n=4) and Chromosomal disorders (n=3). Of the children with chromosomal disorders two had Trisomy 21 and one Turner's syndrome. There was no statistically significant differences in age of either moyamoya diagnosis, or intellectual assessment between the MMD and MMS groups by comorbidity (Table 84).

Table 84 Frequency, Age of Moyamoya Diagnosis and Age of Intellectual Assessment by Moyamoya Diagnosis

Moyamoya Classification	N	Percent	Mean Age Diagnosis (SD; range) years	Mean Age of Assessment (SD; range) years
Idiopathic	11	37.0	8.46 (3.18; 2.9-12.75)	10.13 (3.78; 4.08-16.17)
Neurofibromatosis Type 1	9	33.3	9.23 (3.18; 5.17-14.42)	11.13 (4.58; 5.33-16.33)
Sickle Cell Disease	4	14.8	7.69 (4.86; .83-11.83)	8.88 (2.94; 5.92-12.42)
All chromosomal	3	11.1	9.42 (5.25; 3.83-14.25)	11.56 (4.03; 7-14.67)
Total	27	100.0		

Figure 79 Frequency of Moyamoya Diagnosis by Comorbidity



8.5.3.2.1. Comorbidity and Intelligence Scores

Mean scores of intelligence were within the normal range for children with Idiopathic-MM and children with NF1-MM. Children with SCD-MM had below normal scores of intelligence in all domains. However, there was no statistically significant difference in mean scores between groups (Table 84). Only one child in the Chromosomal-MM group had usable scores of intelligence as two of the children with Chromosomal-MM had severe learning difficulties (Fig 80).

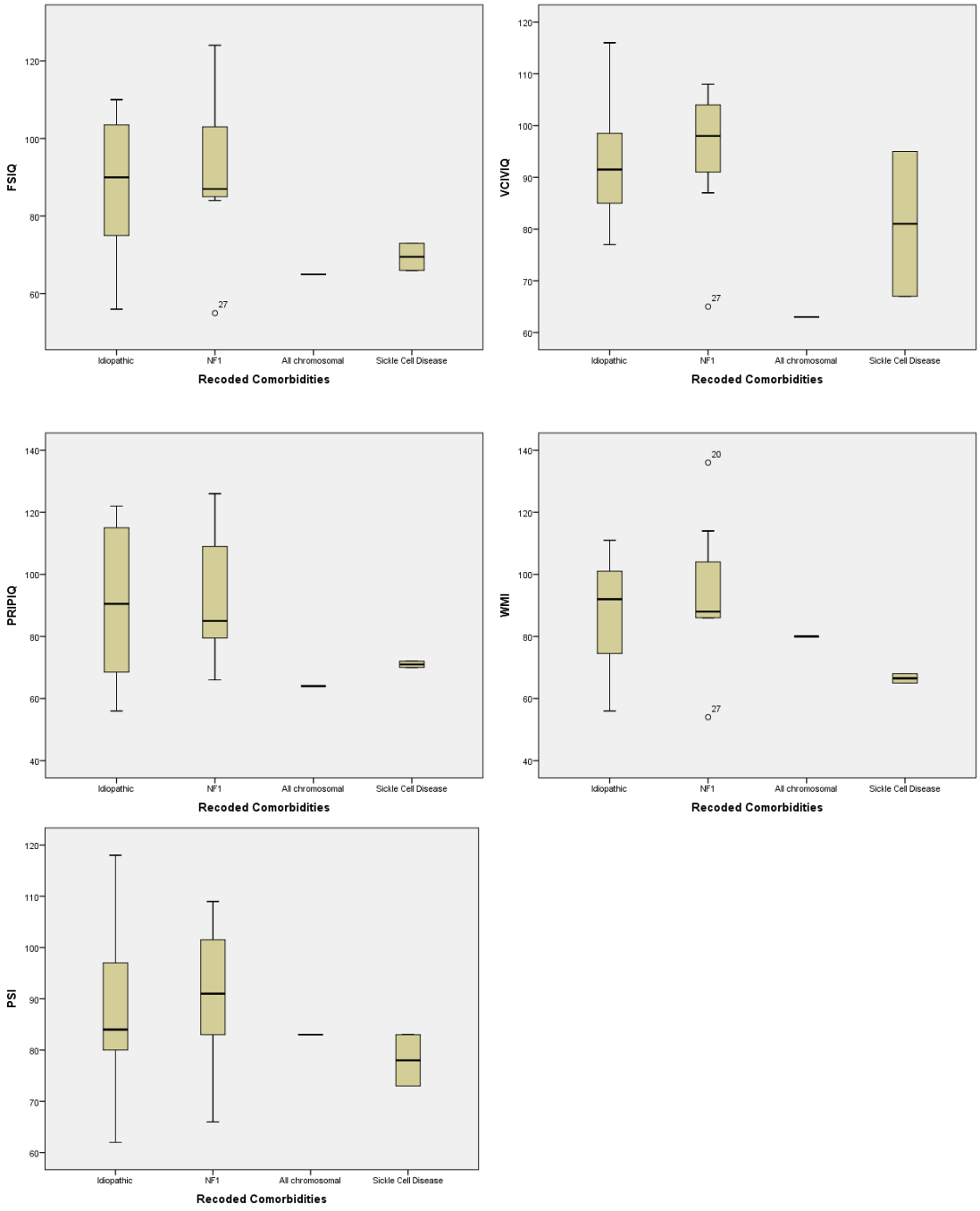
Table 85 Mean Scores of Intelligence by Comorbidity

Moyamoya Classification n (male)	MM Laterality B/R/L	Clinical Stroke Yes	FSIQ mean (SD)	VIQ mean (SD)	PRIQ mean (SD)	WM mean SD	PS mean (SD)
Idiopathic n=11 (6)	8/2/1	7	87.88 (19.05)	92.88 (12.11)	90.75 (26.26)	87.75 (18.45)	87.75 (16.63)
NF1 n=9 (6)	2/2/5	1	91.71 (21.94)	94.43 (14.77)	93.43 (22.04)	94 (25.59)	90.71 (15.01)
Sickle Cell Disease n=4 (2)	4/0/0	2	69.5 (4.95)	81 (19.8)	71 (1.41)	66.5 (2.12)	78 (7.07)
Chromosomal n=3 (0)	2/0/1	2*	66	63	64	80	83
P value One way ANOVA Kruskal Wallis			.307 .185	.114^ .186	.399 .365	.460 .345	.750 .579

^trend toward significance at p<0.1 level

*Two with severe learning disability.

Figure 80 Boxplots showing Mean Intelligence Scores (full scale, verbal and performance), Working Memory Index and Processing Speed Index by Comorbidity



8.5.3.2.2. Comorbidity and Executive Function

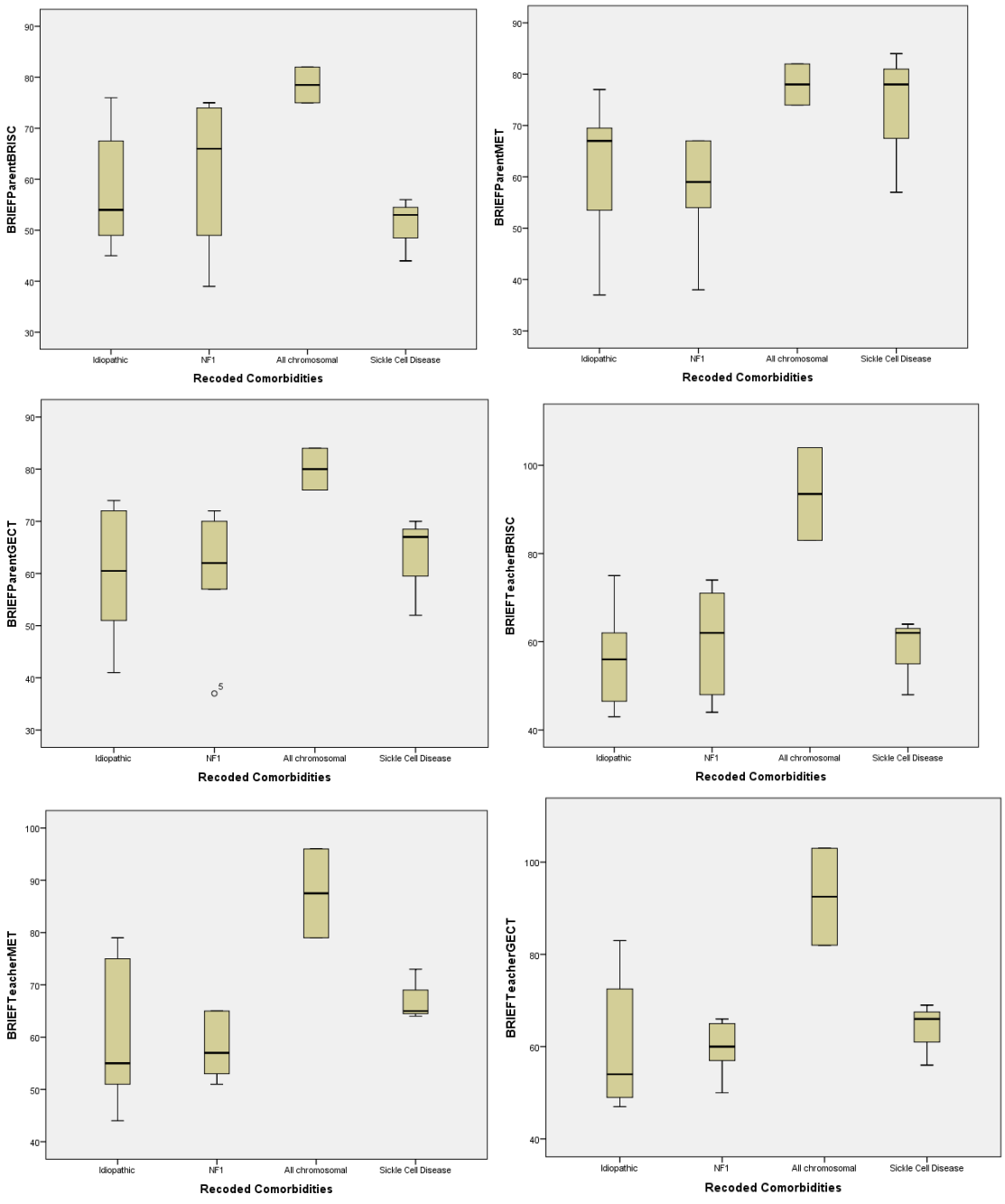
Children with Chromosomal-MM had abnormal and severely abnormal Parent and Teacher BRI, MET and GEC scores, which were significantly higher for the Teacher scores (Table 86, Fig 81).

Table 86 Parent and Teacher Mean Summary Scores of Executive Function

Moyamoya Classification	MM Laterality B/R/L	Clinical Stroke Yes	Parent BRIEF			Teacher BRIEF		
			BRI mean mean (SD) (SD)	MET	GEC mean (SD)	BRI mean mean (SD)	MET (SD)	GEC mean (SD)
Idiopathic n=11 (6)	8/2/1	7	57.75 (11.34)	61.75 (13.48)	60.25 (12.2)	55.88 (10.83)	60.63 (13.61)	60.13 (13.95)
NF1 n=9 (6)	2/2/5	1	61.5 (14.65)	57.33 (10.76)	60.0 (12.82)	60.17 (12.11)	58.0 (6.42)	59.67 (5.82)
Sickle Cell Disease n=4 (2)	4/0/0	2	51.0 (6.25)	73 (14.18)	63.0 (9.64)	58.0 (8.72)	67.33 (4.93)	63.67 (6.81)
Chromosomal n=3 (0)	2/0/1	2	78.5 (4.95)	78 (5.66)	80 (5.66)	93.5 (14.85)	87.5 (12.02)	92.5 (14.85)
p value			.118	.223	.289	.002**	.022* .	.007* .

One way ANOVA significant at the p<.05 level*; significant at p<.01 level**

Figure 81 Boxplots showing Parent and Teacher Mean Summary Scores of Executive Function (BRISC, Metacognition and GEC) by Comorbidity



8.5.3.2.3. Ischaemic Risk Profile by Comorbidity

Using history of clinic stroke, TIA or steal on CVR as indicators of ischaemic risk, children with Idiopathic-MM had high proportions of stroke prevalence, TIA and prevalence of steal. There was apparent discordance between clinical indicators of ischaemic risk (stroke and TIA) and steal in the syndromic moyamoya group. This was most evident in the Chromosomal-MM in whom there was a high proportion of stroke, but low proportion of children with steal (Table 87).

Table 87 Ischaemic Profile by Comorbidity

	Idiopathic n=11	NF1 n=9	SCD n=4	Chromosomal n=3
Steal	n (%)	n (%)	n (%)	n (%)
Yes	9 (82)	5 (56)	3 (75)	1 (33)
No	2 (18)	4 (44)	1 (25)	2 (67)
Stroke				
Yes	8 (73)	2 (22)	2 (50)	2 (67)
No	3 (27)	7 (78)	2 (50)	1 (33)
TIA				
Yes	5 (83)	1 (11)	0	0
No	6 (17)	8 (89)	4 (100)	4 (100)

8.5.3.3. Effect of moyamoya laterality on full scale, verbal and performance IQ, and on working memory index and processing speed index and Parent and Teacher BRIEF scores of executive function

Moyamoya was bilateral in 16 and unilateral in 11 (7 left, 4 right). Available scores of intelligence were analysed by moyamoya laterality. Mean scores of intelligence were highest and within normal range in all children with right sided moyamoya and overall lowest in children with bilateral moyamoya, specifically mean FSIQ, WMI and PSI were lowest in children with bilateral moyamoya. Mean VCI scores were lowest in children with left moyamoya. Apparently paradoxically, mean PRIQ was highest in children with right moyamoya but there were only 2 children in this group and there were no statistically significant differences in scores on the Wechsler scales or the BRIEF by moyamoya laterality (Table 88). Kruskal-Wallis revealed a trend toward significance by moyamoya laterality for FSIQ ($\chi^2 = 4.652$ (2), p=.096) but not for other mean scores of intelligence or Parent and Teacher BRIEF scores of executive function (p values > 0.05) (Table 88 and Fig 82).

Figure 82 Pie chart Showing Moyamoya Laterality in Group

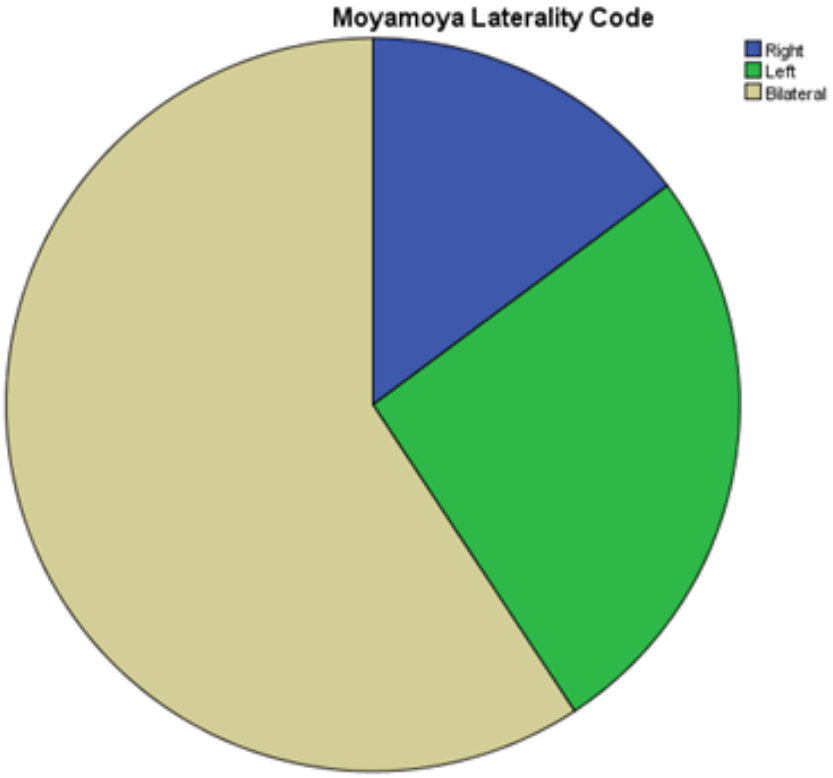


Table 88 Mean Scores of Intelligence and Summary Parent and Teacher Scores of Executive Function by Moyamoya Laterality

	Moyamoya Laterality			χ^2 (df)	p value
	Right	Left	Bilateral		
	mean (SD)	Mean (SD)	mean (SD)		
IQ n	2	6	10		
FSIQ	98.5 (17.68)	85 (24.19)	84.2 (18.57)	4.65 (2)	.096
VIQ	97.5 (14.85)	88.17 (19.09)	90.5 (13.52)	3.50 (2)	.173
PRIQ	100.5 (21.92)	86.17 (24.02)	86.8 (24.6)	4.32 (2)	.115
WMI	100 (19.8)	89.67 (26.64)	83.5 (18.59)	.917 (2)	ns
PSI	95.5 (6.36)	87.83 (15.97)	85.5 (15.41)	2.79 (2)	ns
BRIEF Parent N	2	5	12		
BRI	69 (9.9)	58.8 (15.69)	59.08 (12.77)	1.45 (2)	ns
MET	61.5 (10.6)	61.4 (14)	65.25 (14.35)	.253 (2)	ns
GEC	65.5 (10.61)	60.2 (15.3)	63.25 (12.34)	.037 (2)	ns
BRIEF Teacher N	2	5	12		
BRI	52.5 (10.61)	64.2 (17.23)	61.9 (15.88)	1.25 (2)	ns
MET	60 (7.07)	64.4 (13.41)	64 (14.58)	.082 (2)	ns
GEC	58 (9.9)	66.4 (15.14)	63.9 (15.34)	2.11 (2)	ns

In exploratory Wilcoxon post hoc analysis FSIQ, PRIPIQ were statistically different by right and bilateral moyamoya laterality (p=.020 and.046) and there was a trend toward significant difference in VCI and PSI (p=.103 and .112). There was no statistically significant difference in mean scores of WMI by right and left

moyamoya laterality. There was no statistically significant difference between scores of intelligence by left and bilateral moyamoya laterality.

In univariable linear regression, right moyamoya laterality was significantly predictive of FSIQ and PRI but not of Parent or Teacher indices of executive function (Table 90 and 91).

Figure 83 Boxplots showing effect of moyamoya laterality on full scale, verbal and performance IQ Scores and on Working Memory Index and Processing Speed Index

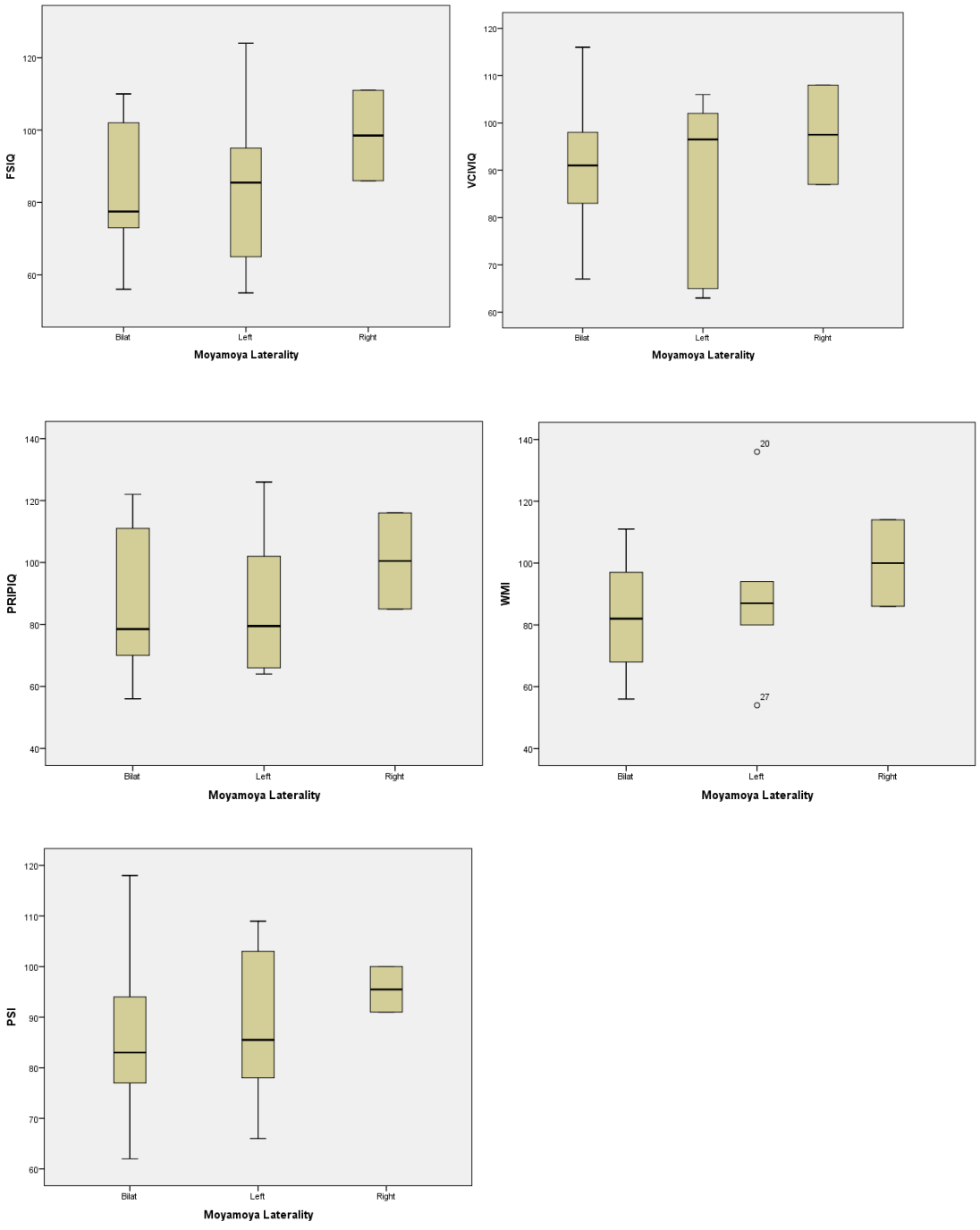


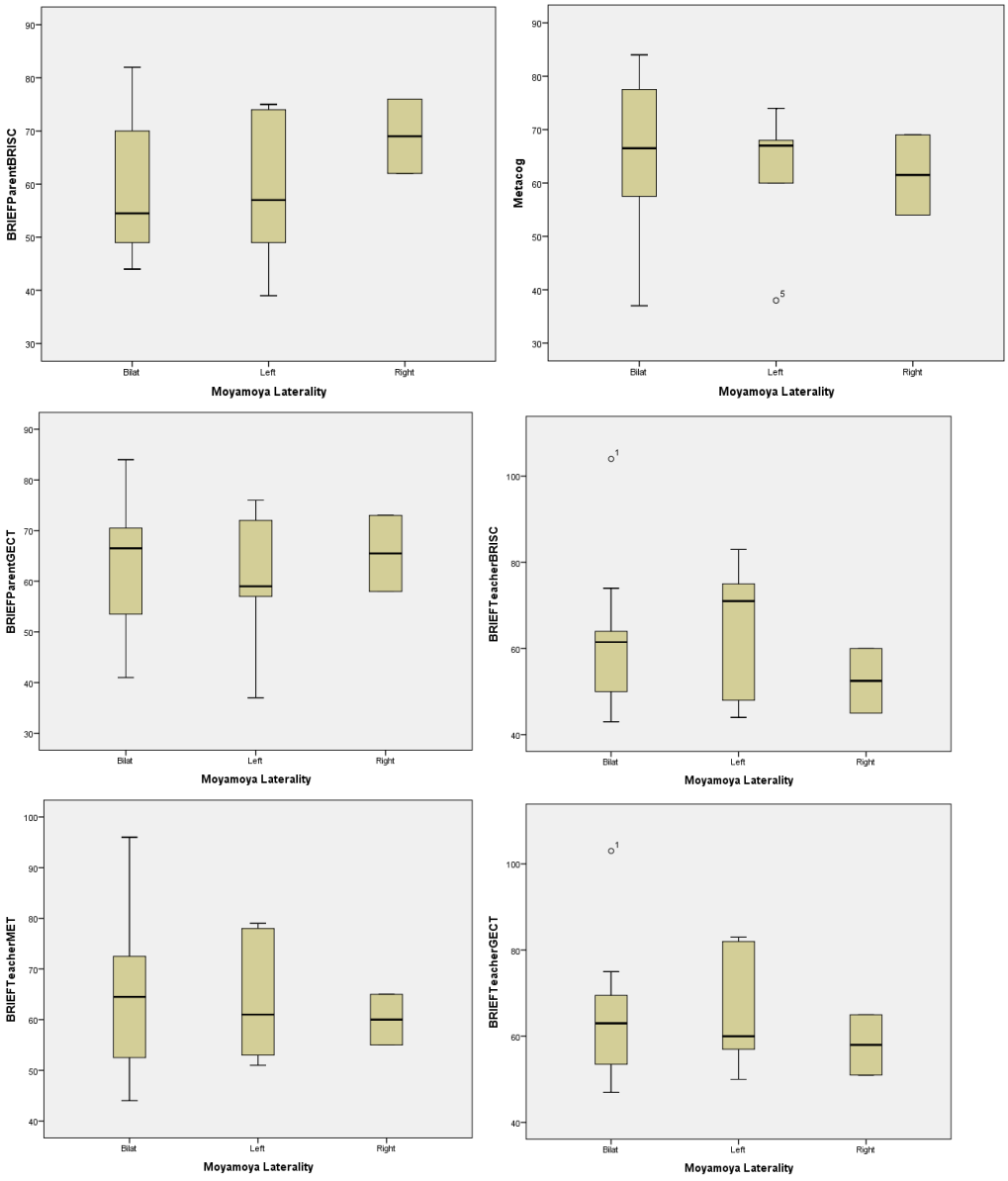
Table 89 Linear regression for effect of moyamoya laterality on Parent BRIEF measures of executive function

	R	R^2	Adj R^2	F	B	SE for B	Beta	T	p
Parent BRIS *Compared with bilateral moyamoya* R MM L MM	0.226	0.051	-0.044	0.541	 9.500 0.214	 9.269 5.676	 0.228 0.008	 1.025 0.038	0.591 0.318 0.970
Parent Metacog *Compared with bilateral moyamoya* R MM L MM	0.104	0.011	-0.093	0.103	 -3.808 -2.022	 10.044 6.200	 -0.089 -0.076	 -0.379 -0.326	0.902 0.709 0.748
Parent GEC *Compared with bilateral moyamoya* R MM L MM	0.044	0.002	-0.098	0.020	 -0.051 -1.099	 7.773 5.689	 -0.002 -0.045	 -0.007 -0.193	0.981 0.995 0.849

Table 90 Linear regression for effect of moyamoya laterality on Teacher BRIEF measures of executive function

	R	R^2	Adj R^2	F	B	SE for B	Beta	T	p
Teacher BRIS *Compared with bilat moyamoya* R MM L MM	.21	.044	-.057	.436	 -7.5 4.2	 11.36 7.79	 -.15 .123	 -.66 .539	.652 .517 .596
Teacher Metacog *Compared with bilatl moyamoya* R MM L MM	. 102	.010	-.094	.1	 -4.33 .067	 9.87 6.77	 .102 .002	 -.439 .010	 .666 .992
Teacher GECT *Compared with bilat moyamoya* R MM L MM	0.284	0.081	-0.011	0.877	 -10.067 3.000	 8.793 7.179	 - 0.251 0.092	 - 1.145 0.418	0.431 0.266 0.680

Figure 84 Boxplots showing Mean Summary Parent and Teacher Scores (BRISC, Metacognition and GEC) of Executive Function by Moyamoya Laterality



In summary stroke and comorbidity appeared to be associated with neurocognitive outcome in the children with MM. Moyamoya laterality did not seem to have a significant effect on neurocognitive outcome.

I therefore sought to further explore whether there was an association between CVR abnormality and neurocognitive outcome in a group of children without a history of stroke, and examine within this group the effect of comorbidity and moyamoya laterality. I also excluded the children with a prior history of surgery.

8.5.4. hBOLD CVR and Intellectual Outcome in Children with No History of Stroke or Surgery Prior to Assessment

8.5.4.1. Group Description

Ten children did not have a history of stroke or revascularisation surgery prior to their hBOLD CVR and intellectual study. One child without stroke had surgery five days (inadequate time to alter haemodynamic status) before their intellectual assessment, and after their hBOLD CVR study so was included in the group (n=11, 4 male, 7 female, mean age diagnosis 9.43 years, range 5.17 – 15.58 years). Mean interval from intellectual assessment to hBOLD CVR study was 0.18 years SD 0.27 years. Six of these children had NF1-MM, 3 Idiopathic-MM and two SCD-MM (Table 92).

Table 91 Group Description of Children with No Stroke and No Surgery Prior to Intellectual Assessment

	No Stroke No Presurgery n = 11
MM Classification	
Idiopathic	3
Neurofibromatosis Type 1	6
Sickle Cell Disease	2
Arteriopathy Laterality	
Bilateral	6
Right	3
Left	2
Steal	
None	5
Right	2
Left	1
Bilateral	3
Age at Diagnosis	years (SD, range) 9.4 (3.45; 5.17-15.6))
Age at Intellectual Assessment	10.36 (3.88; 5.33-16.33)
Time between hBOLD CVR and Intellectual Assessment	.18 (.27; .00 - .83)
Mean age at intellectual assessment (SD) years	10.55 (4.03, 5.33-16.33)
Radiographic Findings (Y:N)	
Not ischaemic	5
Cortical	0
Watershed	5
(Deep white matter)	(5)
(Cortical)	(0)
Cortical ischaemic and Watershed	1
Other^	4

^NF1 lesions

8.5.4.2. Effect of Steal on Full Scale, Verbal and Performance IQ, and on Working Memory Index and Processing Speed Index

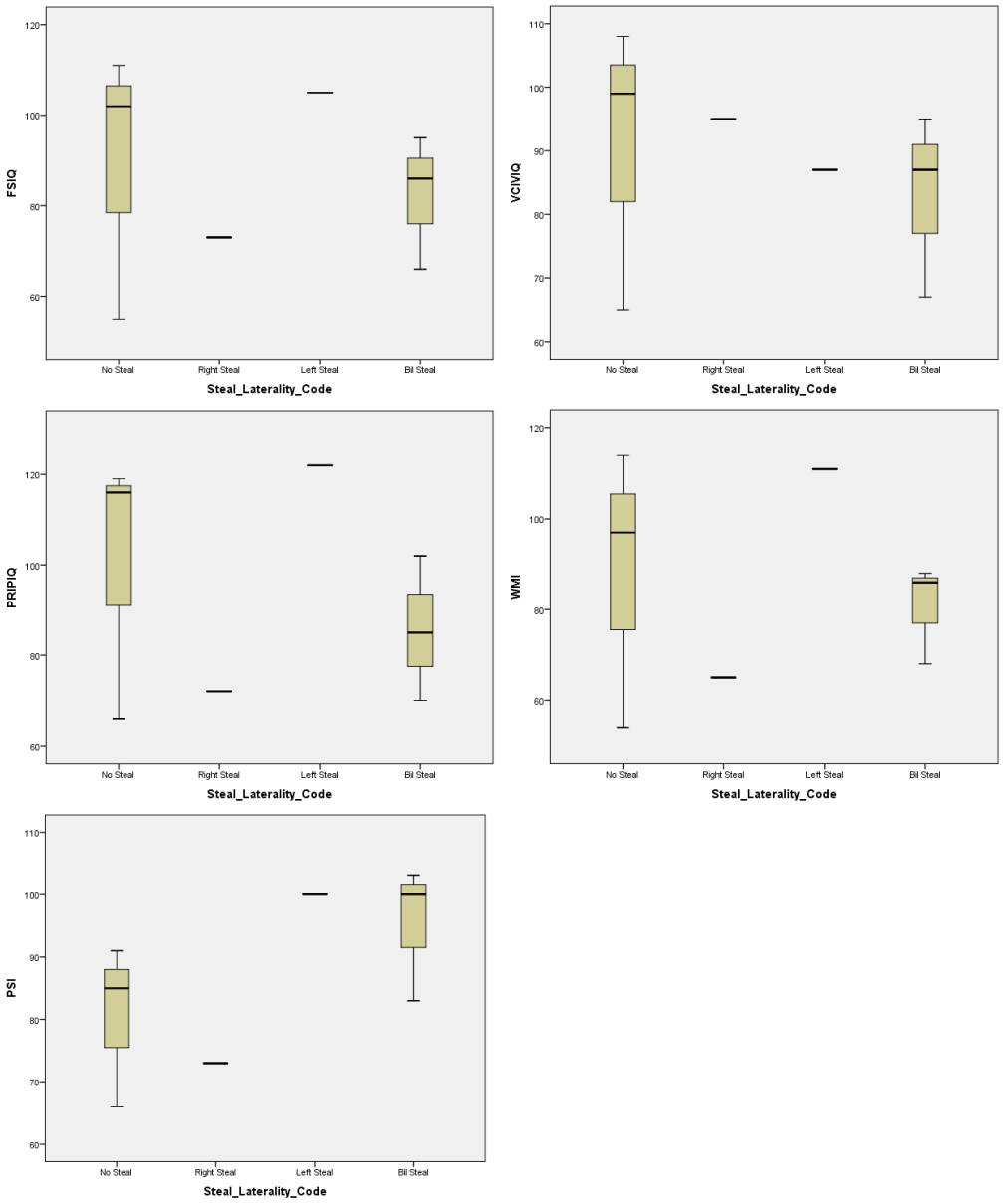
There was no statistically significant difference between scores of intellectual function by laterality of steal. There was no statistically significant difference in scores of intellectual function between children by steal or laterality of steal (Table 93 and Fig 85).

Table 92 Intelligence Scores by Laterality of Steal in No Stroke No Surgery Group

	No Steal n=3	Bilateral Steal n=3	Right Steal n=1	Left Steal n=1
FSIQ	89.33 (30.07)	82.33 (14.84)	110	105
VCI	90.67 (22.68)	83.00 (14.42)	105	87
PRIQ	100.33 (29.77)	85.67 (16.01)	113	122
WMS	88.33 (30.93)	80.67 (11.02)	N/A	111
PSI	80.67 (13.05)	95.33 (10.79)	N/A	100

All p>0.05

Figure 85 Boxplots showing Scores of Intelligence (Full scale, Verbal and Performance), Working Memory Index and Processing Speed Index by Laterality of Steal



8.5.4.3. Effect of Steal on Indices of Executive Function as Measured using the BRI, Metacognition, and General Cognitive Index from the Parent and the Teacher BRIEF questionnaires

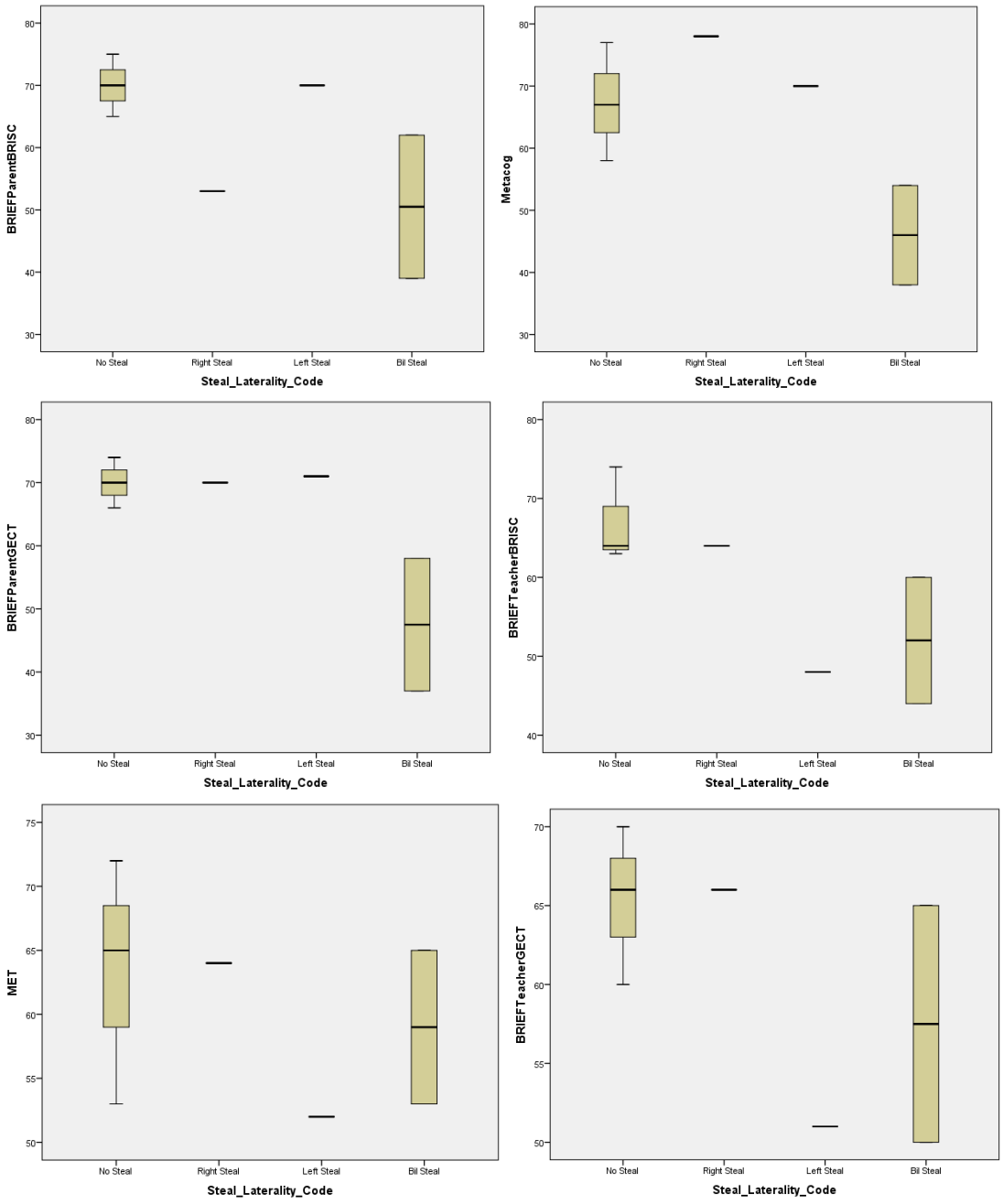
There was no statistically significant difference in scores of executive function between children with no steal, bilateral steal and unilateral steal (Table 94 and Fig 86).

Table 93 Indices of Executive Function by Laterality of Steal in No Stroke No Surgery Group

	No Steal n=3	Bilateral Steal n=2	Right Steal n=1	Left Steal n=1
Parent				
BRI	70 (5)	50.5 (16.26)	N/A	70
MET	67.33 (9.5)	46 (11.31)	N/A	70
GEC	70 (4)	47.5 (14.85)	59	71
Teacher				
BRI	67 (6.08)	52 (11.31)	N/A	48
MET	63.33 (9.61)	59 (8.49)	N/A	52
GEC	65.33 (5.03)	57.5 (10.61)	44	51

All $p > 0.05$

Figure 86 Boxplots showing Parent and Teacher Mean Scores of Executive Function (BRISC, Metacognition and GECF) by Laterality of Steal



8.5.4.4. Laterality of Steal by Comorbidity

Within the group, three patients had Idiopathic-MM, six NF1-MM and two SCD-MM. Four of the five patients with no steal had NF1-MM. (Table 95, 96 and Fig 87)

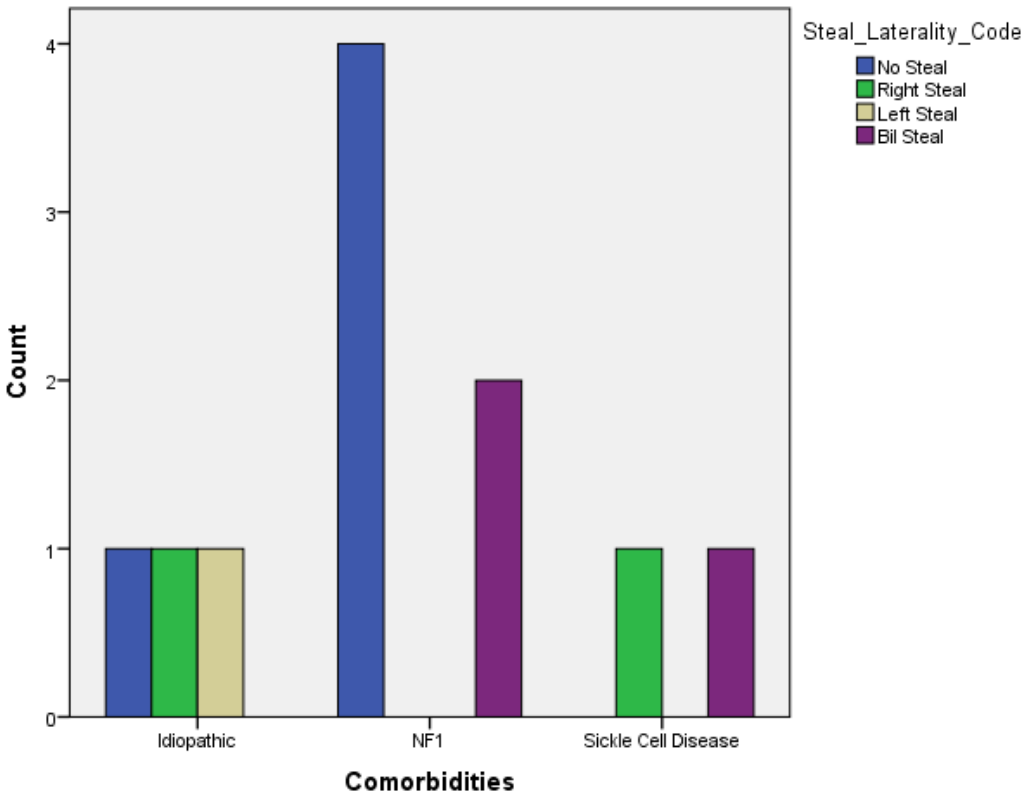
Table 94 Laterality of Steal by Comorbidity

	Steal Laterality				Total
Comorbidity	No Steal	Bilateral Steal	Right Steal	Left Steal	
Idiopathic	1	0	1	1	3
Neurofibromatosis Type 1	4	2	0	0	6
Sickle Cell Disease	0	1	1	0	2
Total	5	3	2	1	11

Table 95 Scores of Intellectual and Executive Function by Comorbidity

						Parent			Teacher		
	FSIQ	VCI	PRI	WMI	PSI	BRI	MET	GEC	BRI	MET	GEC
Idiopathic n=2	103.5 (2.12)	93.0 8.49	120.50 (2.12)	104 (9.9)	92.5 (10.61)	67.5 (3.54)	73.5 (4.95)	72.5 (2.12)	55.5 (10.61)	62 (14.14)	60.5 (13.44)
NF1 n=4	86.75 (23.56)	88.75 (18.04)	92.25 (21.61)	85.5 (24.57)	90 (16.79)	61.5 (15.93)	50.25 (12.12)	57.75 (14.71)	60.5 (12.48)	59 (6.93)	60.25 (7.32)
SCD n=2	69.50 (1.41)	81.0 (19.8)	71 (1.41)	66.5 (2.12)	78 (7.07)	65 (n=1)	77 (n=1)	74 (n=1)	63 (n=1)	72 (n=1)	70 (n=1)

Figure 87 Steal Laterality by Comorbidity



8.5.4.5. Hemispheric pixels and Intellectual Outcome

Using Pearson's bivariate correlation there was no statistically significant correlation between scores of intellectual outcome and hemispheric pixels.

Using Pearson's bivariate correlation there was a statistically significant correlation between BRIEF Teacher Initiate scores and right and left PTNP (p=.049 and p=.046) and a trend toward correlation between BRIEF Teacher Initiate scores and left NTNP (p=0.054) and BRIEF Parent Initiate scores and right and left PTPP (p=0.095 and p=0.096 respectively). There was no statistically significant

correlation between the remaining executive scores of function and hemispheric pixels (Table 97 and 98).

Table 96 Hemispheric Pixels and Scores of Intellectual Function in No Stroke No Pre Surgery Group

	Pearson Correlation				
	FSIQ	VCI	PRIPIQ	WMI	PSI
PTPP					
Right	-.394	-.268	-.461**	-.367	-.098
Left	.398	.271	.466**	.357	.110
PTNP					
Right	.317	-.008	.460**	.457	.551**
Left	.268	-.039	.416	.453	.571**
NTNP					
Right	-.062	-.048	-.187	-.286	-.036
Left	-.280	-.284	-.381	-.098	.176

Table 97 Parent and Teacher BRIEF Scores of Executive Function

	Pearson Correlation BRIEF Parent										
	Inhibit T	ShiftT	EmC onT	InitT	WM T	PlanT	Org MatT	Mon T	BRIS	MET	GECT
PTPP											
Right	-.395	.110	.010	-0.629**		-.275	-.649**	-.405	-.214	-.617**	-.240
Left	.398	-.104	-.004	.628**	.162	.277	.643**	.398	.218	.611**	.245

PTNP											
Right	.064	-.146	.149	.123	-.064	-.083	.199	-.211	.027	-.039	-0.035
Left	-.015	-.141	.131	.084	-.065	-.101	.169	-.246	-.016	-.079	-.064
NTNP											
Right	.014	-.429	-.010	-.255	-.439	-.386	-.121	-.473	-.142	-.410	-.329
Left	-.252	-.021	.108	-.235	-.168	-.294	-.028	-.491	-.111	-.397	-.219

	BRIEF Teacher										
	Inhibit T	ShiftT	EmCon T	InitT	WM T	PlanT	Org Mat T	MonT	BRIS	MET	GECT
PTPP											
Right	.231	.478	.583	.271	.384	.255	-.120	.265	.392	-.009	.524**
Left	-.237	-.454	-.568**	-.299	-.374	-.264	.143	-.206	-.369	.012	-.516**
PTNP											

Right	-.390	-.370	-.504**	-.710*	-.336	-.255	-.325	-.330	-.590**	-.536**	-.454
Left	-.339	-.327	-.461	-.716*	-.262	-.205	-.318	-.316	-.603**	-.539**	-.373
NTNP											
Right	-.249	-.499**	-.335	-.190	-.505**	-.582**	-.428	-.479	-.203	-.422	-.613**
Left	.150	-.054	.043	-.699	-.070	-.503	-.406	-.235	-.139	-.722*	-.074

*p<0.05; ** p<0.2

8.5.4.6. Hemispheric pixels, Comorbidity and Intellectual Outcome

Using Pearson's bivariate correlation there was no statistically significant correlation between hemispheric pixels and scores of intelligence by NF1-MM. There was a statistically significant negative correlation between right and left PTNP and BRIEF Parent MET, Parent GEC and Teacher BRI. There was a trend toward significant negative correlation between right and left PTNP and BRIEF Parent BRI and Teacher GEC (Table 99).

Numbers in the Idiopathic-MM and SCD-MM group were too small (2 and 2 respectively) for individual correlation.

Table 98 Bivariate Pearson Correlation of Hemispheric Pixels and Scores of Executive Function in NF1 moyamoya

	Pearson Correlation BRIEF					
	Parent			Teacher		
	BRI	MET	GEC	BRI	MET	GEC
PTPP						
Right	-.079	-.274	-.206	.054	-.902**	-.653
Left	.079	282	.210	-.054	.907**	.656
PTNP						
Right	-.826**	-.935*	-.939*	-.962*	-.354	-.807**
Left	-.834**	-.932*	-.941*	-.966*	.731	-.812**
NTNP						
Right	.107	.261	.216	.097	-.740	615
Left	-.113	-.271	.224	100	.326	-.624

*p<0.05; **p<0.2

8.5.4.7. hBOLD CVR scores and Intellectual Outcome

Childrens' hBOLD CVR scores were recategorized as normal to mildly abnormal hBOLD CVR score (total score 2-3) or moderate to severely abnormal hBOLD CVR score (total score 4-6) (Table 100).

Table 99 Frequency of Recategorized hBOLD CVR Scores

CVR Scores	Frequency	Percent
Normal to mild (2-3)	5	45.5
Moderate to severe (4-6)	6	54.5
Total	11	100.0

In the group with normal to mild abnormality of hBOLD CVR by score, median scores of intellectual function were above the average for the group (90) for FSIQ, VCI, PIQ and WMI and below the average for the group (90) for PSI. In the group with moderate to severe abnormality of hBOLD CVR by score, median scores of intellectual function were below the average for the group for FSIQ, VCI, PIQ and WMI and above the average for the group for PSI. However, none of these differences were statistically significant (Table 101 and Fig 87 and 88).

Median scores of Parent and Teacher executive function were higher in the normal to mild abnormality hBOLD CVR by recategorized score group than in the moderate to severe hBOLD CVR by recategorized score group. There was a trend toward significance in difference between the BRIEF Teacher BRI and GEC scores.

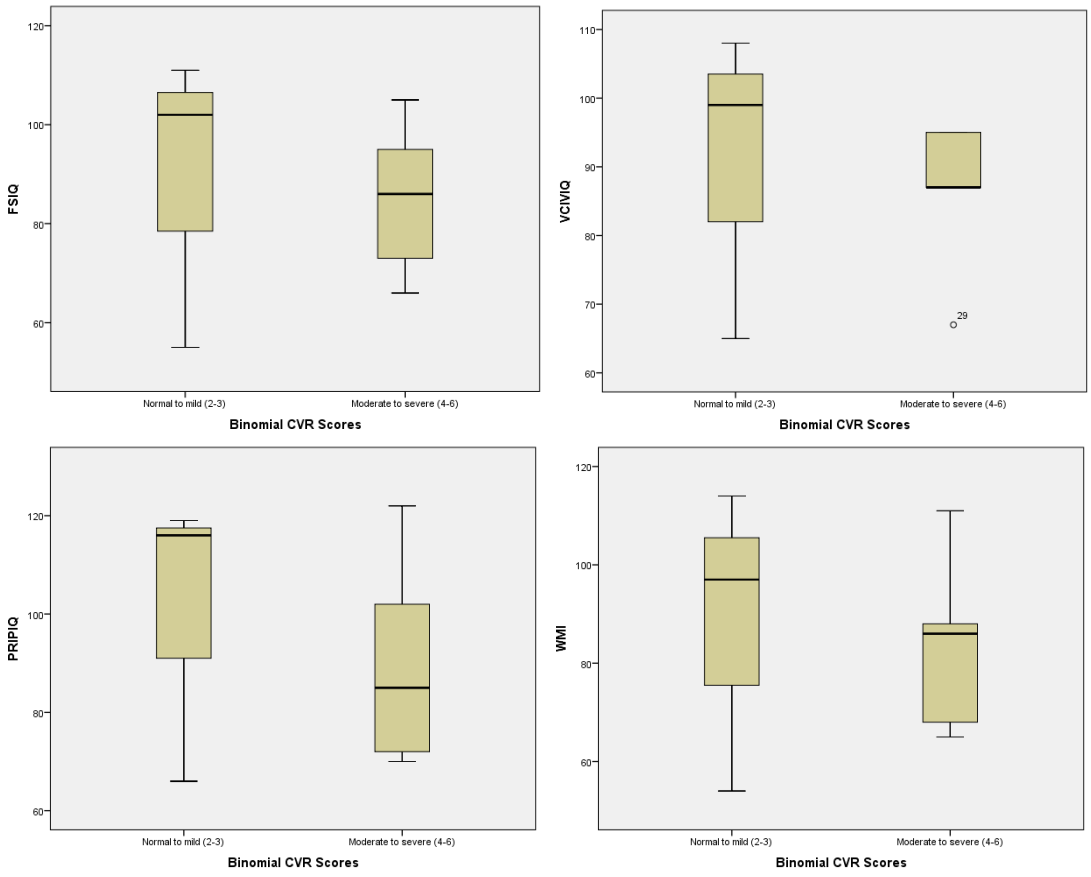
Table 100 Scores of Intellectual and Executive Function by Recategorized hBOLD CVR Scores

	hBOLD CVR Abnormality by Score		
	Normal to mild	Moderate to Severe	p value
	mean; median (SD; range)	mean; median (SD; range)	
IQ N	5	6	
FSIQ	89.3; 102 (30.07; 55-111)	85; 86 (15.86; 66-105)	ns
VCI	90.67; 99 (22.68; 65-108)	86.2; 87 (11.45; 67-95)	ns
PIQ	100.3; 116 (29.77; 66-119)	90.2; 85 (21.89; 70-122)	ns
WMI	88.3; 97 (30.93; 54-114)	83.6; 86 (18.47; 65-111)	ns
PSI	80.67; 85 (13.05; 66-91)	91.80; 100 (13.14; 73-103)	ns
BRIEF Parent N	4	4	
BRI	66.25; 67.5 (8.53; 55-75)	56; 57.5 (13.29; 39-70)	.191†
MET	65.75; 64 (8.38; 58-77)	60; 62 (17.74; 38-78)	ns
GEC	67.5; 68 (5.97; 60-74)	59; 64 (15.81; 37-71)	ns

BRIEF Teacher N	3	6	
BRI	67; 64 (6.08; 63-74)	52.8; 48 (8.67; 44-64)	.070†
MET	63.33; 65 (9.61; 53-72)	58; 56 (6.12; 52-65)	ns
GEC	65.3; 66 (5; 60 – 70)	57; 53 (7.84; 50-66)	.092†

†trend toward significance at p<0.1 level

Figure 88 Boxplots showing Scores of Intellectual Function (full scale, verbal and performance IQ, working memory index, processing speed index) by Recategorized hBOLD CVR Scores



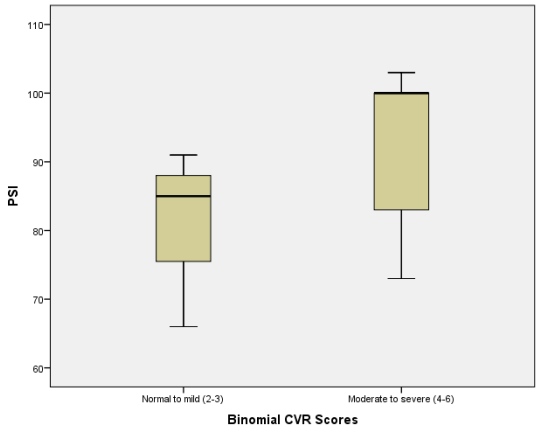
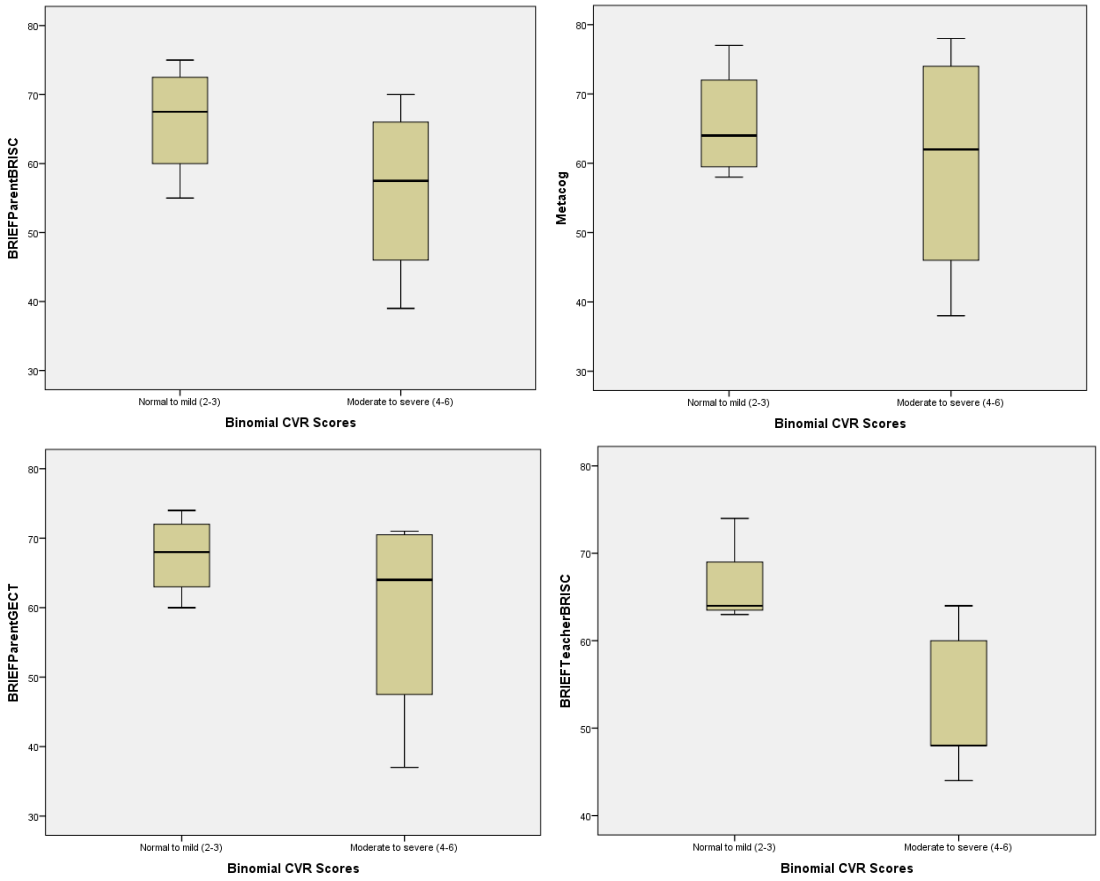
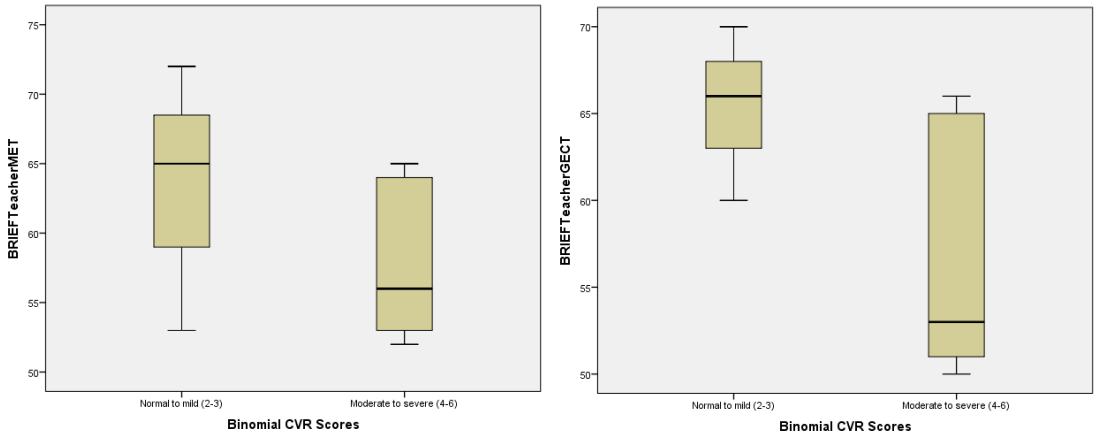


Figure 89 Boxplots showing Parent and Teacher Summary Scores (BRISC, Metacognition and GEC) of Executive Function by Recategorized hBOLD CVR Scores





8.5.4.8. Age at Diagnosis and Hemispheric Pixels

There was no statistically significant relationship between age of moyamoya diagnosis and hemispheric pixels.

8.5.4.9. Age at Diagnosis and Scores of Intellectual Function

There was no statistically significant relationship between age of moyamoya diagnosis and scores of intellectual function.

8.5.4.10. Age at Diagnosis and Scores of Executive Function by Parent and Teacher BRIEF questionnaire

There was a statistically significant correlation between age of moyamoya diagnosis and BRIEF Teacher BRIS score n=8, r= -.733, p=.038. There was a

trend toward significant correlation between age of moyamoya diagnosis and BRIEF Teacher Emotional Control n=8, r= -.641, p=.063 and BRIEF Teacher Initiation Control n=8, r= -.695, p=.056. There were no further statistically significant correlations between age of diagnosis and scores of executive function.

8.5.4.11. Age at Diagnosis – less than two years and less than 9 years

Only two children in our group were diagnosed under the age of two years. Five children were less than nine years of age, i.e. definitely prepubertal. Scores of intellectual function were higher in the children diagnosed under the age of nine years. However, there was no statistically significant difference in scores of intellectual function between children less than nine years of age and children who were age nine or above at the time of moyamoya diagnosis.

There was a statistically significant difference in scores of executive function by Teacher BRIEF for scores of Shift control, Emotional control, Initiation and Behavioural regulation Index (p=.04; .042 and .037 respectively). There was a trend toward significance difference in Teacher BRIEF scores of Monitor control, and Parent BRIEF scores of Behaviour regulation Index (Tables 102 and 103).

Table 101 Intelligence Scores by Age at MM Diagnosis

	Age at Diagnosis of Moyamoya (years) Less than 9	Greater than 9	p value
	mean (SD)	mean (SD)	
IQ N	5	6	
FSIQ	94 (11.31)	84.17 (22.7)	Ns
VIQ	93 (8.46)	86.17 (17.02)	Ns
PIQ	102 (24.04)	91.33 (25)	Ns
WMI	91.5 (7.78)	83.33 (25.14)	Ns
PSI	92.5 (10.61)	86 (14.75)	Ns

Table 102 Indices of Executive Function by Age at MM Diagnosis

	Parent			Teacher		
	Age at MM Diagnosis (years) <9 mean (SD)	>9 mean (SD)	t-test p value	Age at MM Diagnosis (years) <9 mean (SD)	>9 mean (SD)	t-test p value
Inh	64 (6.68)	53.5 (11.56)	.102	52.5 (3.87)	54.25 (13)	.582
Shift	63.5 (9.75)	55.25 (11.62)	.495	69.25 (3.59)	49.75 (6.29)	.04
EmC	68 (7.26)	53.25 (12.66)	.119	72.5 (9.68)	48.5 (5.69)	.042
Initi	58.5 (9.75)	66.5 (19)	.482	64.25 (3.59)	57.75 (3.3)	.037
WMT	62 (9.63)	61.25 (16.58)	.890	62.75 (8.66)	54.25 (7.18)	.490
Plan	67 (10.92)	62.75 (17.04)	.761	65 (11.02)	55.25 (4.03)	.357
Org	57.5 (1.29)	55 (14.07)	.736	58.75 (12.58)	56.75 (20.44)	.873

Mon	63.5 (12.18)	56 (12.06)	.415	62 (3.83)	52.5 (8.58)	.090
BRI	68 (5.72)	54.25 (12.69)	.095	66.25 (6.08)	51 (8.87)	.038
MIT	64 (10.23)	61.75 (17.29)	.830	63.75 (7.89)	56.25 (5.44)	.169
GEC	67 (6.83)	59.5 (15.8)	.473	65.25 (4.11)	55 (7.44)	.358
Flex n=1						

8.6. Discussion

8.6.1. Group Description

The 27 children within this group had MMD and MMS. It also included children with and without a history of stroke and revascularization surgery. Almost 40% of the children had MMD and another 30% children had NF1-MM. The remaining two groups had either SCD-MM or Chromosomal-MM (2 Trisomy 21 and 1 Turner's syndrome). Only a third of the children had a stroke prior to their hBOLD CVR and intellectual assessment. The majority of the strokes occurred within the right hemisphere despite the majority of the children having bilateral moyamoya. Despite only a third of children having a history of overt stroke, 74% had radiographic evidence of ischaemic injury, which was mostly within the watershed territories.

To date, most studies of intellectual outcome in children with moyamoya have been in those who have had a stroke (Matsushima *et al.* 1997, Bowen *et al.* 1998,

Imaizumi *et al.* 1998, Hogan *et al.* 2005, Lee *et al.* 2011, Williams *et al.* 2012). The lower scores of intelligence in children with bilateral stroke in our group is concordant with findings in other studies (Hogan *et al.* 2005). However, data on children who have not had a stroke is limited (Hogan *et al.* 2005, Williams *et al.* 2012).

8.6.2. CVR Abnormality and Intellectual Outcome

8.6.2.1. Whole Group

The presence of steal is suggestive of poor tissue microvascular health and represents tissue at risk of ischaemia. Hence the findings of the relationship between steal and intellectual outcome in our study were surprising. The presence of steal was associated with higher intelligence and better scores of indices of executive function. Right steal was associated with higher VCI and left steal higher scores of WMI. Children with bilateral steal had highest scores of executive function. Hemispheric steal was present on the side affected with moyamoya in all. There was the suggestion of association between laterality of moyamoya and intellectual outcome (specifically left sided MM with reduced VCI and bilateral MM PSI and WMI). Conversely children with right MM had high PIQ and FSIQ scores. However, numbers with unilateral MM and unilateral steal were small making it difficult to infer significant meaning from the results. Calviere et al demonstrated an association between cognitive dysfunction and cerebral haemodynamic

impairment using dynamic susceptibility contrast-enhanced MR perfusion imaging in adults with MMD. Specifically impaired executive function was associated with significantly lower frontal regional CVR. (Calviere *et al.* 2010) Analysis in this study looked for association between cognitive outcome and hemispheric measures of tissue level microvascular compromise. This approach was probably too simplistic, and further regional association analysis would be valuable.

The whole group was composed of children with stroke and no stroke; children with a history of revascularizaiton surgery and no surgery. It is possible to have no steal following a stroke or revascularization surgery as these two categories of children may have already experienced a significant amount of ischaemic demise (given the presentation and/or need for surgery) with effect on intellectual function. Therefore further analysis was conducted in a group with no history of stroke or revascularization surgery.

8.6.2.2. No Stroke, No Surgery Group

High hemispheric negative pixel measures were positively associated with PSI and positive pixel measures with PRI. However, there was no clear pattern observed therefore interpretation of these findings is difficult.

However, a more expected relationship between intellectual function and abnormality of CVR with higher intellectual scores demonstrated in the no steal and normal to mild CVR abnormalities score group. However, the finding of higher

(poorer) indices of executive function in children with bilateral steal, and normal to mild CVR abnormality group when compared to those with no steal, or moderate to severe CVR abnormality was surprising and in contrast to the findings of the Calviere et al study (Calviere *et al.* 2010) Another possibility is that the maintenance or preservation of executive function despite apparent tissue level microvascular dysfunction as manifest by steal, may be as a result of increased CMRO2. In this situation the metabolic demand is increased in the face of reduced cerebrovascular reserve and increased OEF resulting in – unexpected – steal However, further studies are required to explore this. The no steal and normal to mild group was however, dominated by children with NF1-MM. Interpretation of these findings is limited by the small numbers. The children with NF1 had high IQ scores. However, children with NF1 are recognized as having difficulties with executive function. This would suggest that comorbidity may be more predictive of neurocognitive outcome than the presence of MM itself. This has, however, not been clearly demonstrated in my study nor other reported studies (Williams *et al.* 2012) often limited by numbers.

8.6.3. CVR Measures, Comorbidity and Intellectual Outcome

In this study children with NF1-MM had the highest scores of intelligence across all domains when compared to children with Idiopathic-MM, SCD-MM and Chromosomal-MM. They were also the group with the lowest occurrence of stroke.

Executive function was poor across all the moyamoya groups and profoundly so in the group with Chromosomal-MM. This is concordant with the noted severely low scores of intelligence. However, in the NF1-MM group the high scores of executive function are discordant with the high levels of intelligence in this sample, but in keeping with the relative abnormalities in executive function noted in published literature. There may be a number of comorbidity specific factors that influence the MRI BOLD signal and hence the interpretation of CVR in the syndromic group. These are discussed below for the main comorbidities represented in this study, namely NF1-MM, SCD-MM and Chromosomal-MM.

8.6.3.1. Neurofibromatosis Type 1

Areas of increased T2 weighted signal intensity also known as foci of abnormal signal intenisity (FASIs) are typically seen in NF1. Research to date is conflicting regarding a link between the presence and location of T2 hyperitensities and neuropsychological function in children with NF1. T2 hyperintensities are frequently located in frontal subcortical networks through basal ganglia and thalamus (Chapman *et al.* 1996) (Joy *et al.* 1995). Frontal networks are one of the most richly connected brain structures with a protracted development with age, so they are subject to a higher risk of impairment in case of general dysfunction

(Perecman 1987). Hence the frontal sub-cortical vulnerability hypothesis. The BOLD signal in NF1 is likely a result of a combination of general and syndrome specific factors. Neurofibromin has a role in modifying synaptic plasiticity and excitation. Imaging can be used as a biomarker for synaptic activity eg.PET, SPECT and BOLD MRI. Microglia are key in this process and their activation results in upregulation of local blood flow and with a concomitant increase in energy demands (Tremblay and Majewska 2011, Siskova and Tremblay 2013, Welberg 2014, Apostolova *et al.* 2015). In NF1 microglial activation has been associated with brain tumorigenesis (Daginakatte *et al.* 2008). Juvenile NF1n31 mice show hyperperfusion bilaterally in the amygdala, which normalizes when reaching adulthood (Apostolova *et al.* 2015). This behavior resembles the typical time course of cognitive deficits in NF1. However, further studies are required to test for causal relationship. It could be hypothesised that impairment of of cognitive function in NFI is associated with increased synaptic activity, energy demands, and cerebral blood flow. This combination of factors are likely to have implictions for the interpretation of CVR in NF1-MM.

8.6.3.2. Sickle Cell Disease

Children with SCD generally obtain lower IQ scores compared with sibling controls (Watkins *et al.* 1998). Furthermore, additional factors may contribute to the cognitive burden seen in SCD such as covert stroke (Watkins *et al.* 1998, Noll *et al.*

2001, Gold *et al.* 2008) and hypoxaemia (Kirkham and Hogan 2004). Hogan et al demonstrated significantly lower VIQ and PIQ in children with SCA when compared to healthy controls and a furtherther statistically significant reduction in PIQ associated with MMS. In a study of 54 children with SCD by Steen et al, 30 with normal MRI had significant deficits in Wechsler Full-Scale IQ, Verbal IQ and Performance IQ compared to age- and gender-matched African-American controls (Steen *et al.* 2005). Despite a focus on frontal lobe MRI findings and deficits in attention, tests of executive functioning have been shown to be more sensitive indicators of cognitive difficulties within this group, which if not spectifically examined for can be missed (Hariman *et al.* 1991, DeBaun *et al.* 1998, Watkins *et al.* 1998, Brown *et al.* 2000). In Brown's study of neurocognitive function in 63 children and adolescents with SCD, almost half of the children (n=30) had no cerebrovascular accident (i.e. stroke or silent infarct). Despite the normal appearing MRI, one third of these children were in receipt of special education. Although the high proportion of educational needs in this group may reflect study participant referral bias, the observation remains unexplained (Brown *et al.* 2000). Hence, the neuropathological basis of the observed abnormalities in executive function in SCD remains unexplained in the absence of silent infarcts. Despite the children in my study not having a history of stroke or revascularization surgery, both (n=2) had white matter silent infarcts on MRI therefore my cohort does not provide a group of children with SCD and no deep white matter infarcts for further study and comparative analysis.

8.6.3.3. Chromosomal Disorders

The association between certain chromosomal disorders and neurocognitive difficulties is well recognized. Down syndrome is the most common identified genetic syndrome associated with learning difficulties, and accounts for approximately 15% of the significantly learning impaired population (Lai and Williams 1989, Pinter *et al.* 2001). Genetically mediated cortical developmental, neurophysiological and perfusion related abnormalities are reported in children with Trisomy 21 with a possible important role for microglia noted to be increased in number in fetal Trisomy 21 (Golden and Hyman 1994, Puri *et al.* 1994, Engidawork and Lubec 2003). In particular fronto-parietal-temporal abnormalities of perfusion, both hypo and hyper, right more than left, are reported in studies using PET and SPECT techniques (Nakayasu *et al.* 1991, Maruyama *et al.* 1995, Gokcora *et al.* 1999, Aydin *et al.* 2007).

In addition neuronal and neurotransmitter level abnormalities are shown to be associated with learning and memory deficits deficits in children with Trisomy 21 (Lubec *et al.* 2001, Begni *et al.* 2003, Hill *et al.* 2003). Hence, it is clear that there are a number of factors which may contribute both to the MRI BOLD signal seen in these children, as well as the neurocognitive profile a substantial proportion of which may be realted to having Trisomy 21 rather than moyamoya. Further studies are required to explore this.

8.7. Study Limitations and Future Directions

My study was limited by the small numbers and number of variables to be considered within each group. Longitudinal analysis in a similar but larger cohort would be informative. Furthermore and specifically:

1) We were unable to replicate the association reported by Matsushima et al of age of onset of MM before 2 years of age and cognitive outcome in my study because of the small numbers (Matsushima *et al.* 1990). Exploration of outcome by presentation before and after 9 years at age of diagnosis was suggestive of lower scores of intellectual outcome in the younger group. Further studies of neurocognitive outcome and hBOLD CVR could help elucidate developmental changes in CVR and their impact on outcome in the younger child with MM.

2) The absence of a non-moyamoya syndromic control group is a limitation of this study. Further hBOLD CVR studies in healthy children with comorbid diagnosis such as NF1 and SCD paired with measures of intellectual outcome would provide a useful comparison for future studies.

8.8. Conclusions

1) Paradoxically the presence of steal was associated with higher intelligence and better scores of indices of executive function.

2) Bilateral steal was associated with lower intelligence scores in all domains apart from processing speed.

3) Abnormal left hemispheric CVR was associated with reduced verbal IQ

4) Abnormal right hemispheric CVR was associated with lower intelligence scores in all domains apart from verbal IQ.

5) Comorbidity is likely to contribute significantly to the neurocognitive profile of children with MMS. Elements of the contributory factors may be measurable using hBOLD MRI and CVR. However, this is yet to be demonstrated and requires further study.

9. Cortical Thickness and hBOLD CVR

9.1. Abstract

9.1.1. Background

The mechanism of chronic neuronal injury in moyamoya is principally one of chronic hypoperfusion distal to the steno-occlusive moyamoya arteriopathy. Cortical thickness is a measure of neuronal number and density. Repeated and chronic periods of hypoperfusion may not cause immediate necrotic cell death, but may lead to apoptosis, cell involution and neuronal dropout, with consequent thinning of the cortical rim. An association between abnormality of hBOLD CVR measures and cortical thickness has been shown in adults.

9.1.2. Objective

To determine whether abnormalities in quantitative measures of hBOLD CVR are associated with reduced cortical thickness in children with moyamoya.

9.1.3. Method

Children with moyamoya, no history of stroke and normal appearing cortical rim on FLAIR sequences were included in the study. Automated measures of cortical thickness were obtained using Freesurfer and high resolution 3D T1 anatomic sequences. Association between qualitative hBOLD CVR measures, cortical thickness, and predictors of cortical thickness were explored for all.

9.1.4. Results

Twelve children (7 male, 5 female; mean age 9.04 +/- 4.29 years) were included in the study. Ten had unilateral MM. In the majority, hemispheric cortical thickness was lower in the hemisphere affected by moyamoya and with impaired quantitative and qualitative CVRs. In addition cortical thickness improved over time in those who had revascularization surgery and declined over time in those who did not. However, these findings did not reach statistical significance.

9.1.5. Conclusions

Cortical thickness may be a useful biomarker of severity of chronic hypoperfusion injury in moyamoya, particularly in those with an apparently low ischaemic burden. Recovery of cortical thickness following surgery lends further support to current management strategies. However, the mechanisms underpinning the apparent cortical thinning and cortical reconstitution, and interpretation of these findings remains challenging and requires further study.

9.2. Introduction

Brain development and neuroanatomical organization in the human fetus is a complex but well ordered process. In the early preterm period, thalamocortical afferents accumulate within the superficial subplate of the ventricular zone and grow into the cortical plate with the simultaneous development of synapses. Neuronal migration from the ventricular zone to the cortical plate is essentially complete by 26 weeks gestation (Sidman and Rakic 1973, Kostovic *et al.* 2002). In the late preterm period, the resolution of the subplate and growth of cortico-cortical fibres into the cortical plate occur simultaneously with gyration. In neonates, the long cortico-cortical pathways stop growing, and the main histogenetic events are an elaboration of intracortical circuitry and synaptogenesis (Kostovic and Jovanov-Milosevic 2006).

In a study of 45 children between 5-9 years, Sowell et al (2004) demonstrated a mean cortical thickness of 1.5 – 5.5 mm over the lateral and medial surface of the brain. Grey matter thinning and brain growth was observed to occur in the same regions (i.e., frontal and parieto-occipital regions) and at the same time (Sowell *et al.* 2004). This is thought to be secondary to a relative reduction in neuronal bodies and cortical gray matter density attributable to, in part, increased proliferation of myelin into the periphery of the cortical neuropil (Sowell *et al.* 2001, Sowell *et al.* 2003). Regional (left) cortical grey matter thinning in childhood is associated with increases in vocabulary. Changes in cortical thickness in childhood may be

associated with cognitive development. Reduction in cortical thickness in childhood is not necessarily pathological. Although age related processes such as reduction in size or number of neuronal cell bodies or their synaptic processes may play a part, this does does not entirely account for the changes (Sowell *et al.* 2003, Richards *et al.* 2009).

Lesion volume and lesion location have been shown to be predictive of outcome in paediatric stroke (Ganesan *et al.* 1999). Involvement of cortical tissue is thought to be of particular importance, with significant impact on outcome, while the prognosis of children with subcortical infarction is thought to be good (Dusser *et al.* 1986, Abram *et al.* 1996). However, in children with moyamoya, neurocognitive decline is recognised in the absence of infarction and is thought to be secondary to chronic hypoperfusion. Pre-infarctive MRI changes are not well recognized, but possibilities include the presence of the IVY sign and atrophy on MRI, although there are few longitudinal data. Therefore, biomarkers of this chronic injury are still lacking. hBOLD CVR and measures of cortical thickness are potential candidate biomarkers for use in this population.

In adults, cortical thickness measures have been used to investigate a range of neurological disorders including Alzheimer's disease, schizophrenia and autism (Hadjikhani *et al.* 2006, Du *et al.* 2007, Goghari *et al.* 2007). In childhood SCD global measures of cortical thickness and sub-cortical volumes have been shown to be diminished when compared to age-matched healthy controls (Kirk *et al.* 2009, Kawadler *et al.* 2013).

In moyamoya, cerebral perfusion pressure drops distal to the moderate-severe stenosis or occlusion as the autoregulatory response of the smooth muscle arterioles fails with uncoupling of the autoregulatory curve. In addition, the normal vasodilatory response to increased PCO2 in the unaffected vascular bed(s) results in the diversion of blood away from the affected vascular beds, i.e. the 'steal' phenomenon. which has been associated with increased ischaemic risk in adults (Reinhard *et al.* 2014). Hypoperfusion occurs maximally at the distal vascular watershed territories (Fig). Experimental animal models have demonstrated disruption of neuronal structure and hence increased neuronal loss as a consequence of chronic hypoperfusion (Sekhon *et al.* 1994, Hai *et al.* 2009).

Fierstra et al (Fierstra *et al.* 2010) demonstrated a relationship between steal, as seen on hBOLD CVR, and cortical thinning in a mostly adult population with MM (age range 12 – 75; mean 42). In addition, the same group demonstrated improvements in cortical thickness following successful revascularization surgery in the revascularized hemisphere with concomitant reversal in steal physiology as demonstrated by hBOLD CVR (Fierstra *et al.* 2011). Postulated mechanisms underpinning the change before and after surgery include loss and gain of neuronal tissue, changes in cortical blood volume or changes in extracellular fluid volume (Duning *et al.* 2005).

Selective neuronal loss has been observed in the penumbra of acute ischaemic stroke, and the chronic hypoperfusion state of moyamoya may represent a chronic penumbra-like state, which results in progressive tissue loss. Therefore it is

possible that within a region of steal physiology, the penumbra-like state may result in a higher rate of cell death and attrition, a decrease in synaptic density and dendritic arborisation, reduction in the number or volumes of glial cells and myelin loss within the cortex (Horner *et al.* 1996, Kurumatani *et al.* 1998, Tomimoto *et al.* 2003).

To my knowledge to date there are no published studies of cortical thickness and any relation to CVR in children with MM.

9.3. Hypothesis:

I hypothesized that cortical thickness

- is significantly attenuated in the hemisphere with intracranial arteriopathy and
- is associated with abnormality in CVR as demonstrated by steal in the respective hemisphere.

9.4. Method

9.4.1. Inclusion Criteria:

- Clinically acquired T1 3D TEF scans (needed for anatomical overlay during Functional MRI Study) and functional hBOLD CVR study

9.4.2. Exclusion Criteria:

- MRI evidence of ischaemic injury on fluid attenuated inversion recovery imaging (FLAIR)

9.4.3. Image Pre-processing

BOLD images were imported into the functional image-processing package, AFNI. We applied standard pre-processing steps, including time-shift correction, volume re-registration, and signal de-trending.

9.4.3.1. Time-shift correction:

Data was time-shifted to correct for difference in acquisition time from one slice to the next. For this, the signal time series in every voxel was interpolated to a common grid of time (0, 1*TR, 2*TR, 3*TR and so on). The AFNI 3dTshift was used in this step.

9.4.3.2. Volume re-registration:

We used the AFNI 3dvolreg program to perform a least square function to minimize the difference between each acquired volume and the first one.

9.4.3.3. Signal drift

System noise and head motion can contribute to low frequency BOLD signal drift over time. In this study, we de-trended our BOLD data with a 3rd order polynomial function.

9.4.4. Breath-hold reference waveform

Subsequent to pre-processing steps, the data was regressed against a reference waveform. The reference waveform represents the breath hold or hyperventilation stimulus.

In some cases, when breath hold instructions were not followed properly by the patient, it was more appropriate to use the BOLD signal from a healthy part of the brain. As the posterior circulation is usually affected late, if at all, in MM, we used the cerebellum for the reference waveform.

9.4.4.1. Regression

BOLD signal in each voxel of the brain was first scaled as a percentage before being regressed against the reference waveform. The slope of this regression was

the cerebrovascular reactivity measure expressed as a percent BOLD signal change.

9.4.4.2. Regression motion correction

Regression motion correction was used in some patients with significant motion.

9.4.5. Cortical Thickness

The high resolution T1-weighted images from the acquired anatomic volume data were used to calculate the cortical thickness using Freesurfer (http://freesurfer.net/). This is a method for automated surface reconstruction and accurate cortical thickness measurement (Sailer *et al.* 2003). An inflated 3-dimensional brain surface image is reconstructed by the software, which allows interpretation of functional MRI data across the entire cortical surface after accounting for cortical folding (Dale *et al.* 1999, Fischl *et al.* 1999, Salat *et al.* 2004). This program constructs models of the boundary between white matter and cortical gray matter as well as the pial surface. Once those surfaces are reconstructed, the cortical thickness can be measured.

9.4.5.1. ROI analysis

We used Freesurfer to visualize the inflated pial surface onto which the BOLD CVR map was projected. A region of interest (ROI) was then manually drawn on the

affected hemisphere. This ROI was restricted to the most affected area, where hBOLD CVR was negative, i.e. representative of steal physiology (score 3), or just positive reactivity was diminished (score 2) compared with normal (score 1). Cortical thickness measurements were confined to the ROI, and the results were generated by the software. This ROI was reflected on the other side to use as a control ROI. For each patient, the mean CVR and cortical thickness within those ROIs could be calculated.

9.5. Statistical Analysis

The paired t-test was used to calculate the difference between group means, and the independent samples t-test was used to compare the mean hemispheric cortical thickness in the unilateral group by steal. The independent samples t-test was also used to evaluate significant differences in cortical thickness before and after surgical revascularization.

9.5.1. Group Description

Images of all patients with moyamoya and who had an hBOLD CVR study were reviewed to identify those with no MRI evidence of ischaemic injury on Axial T2 FLAIR sequences. Fifteen out of 37 children were identified. High resolution T1 3D anatomic images were available in 12 (7 boys, 5 girls, mean age at moyamoya

diagnosis 7.93 +/- 3.47 years, mean age at first CVR 9.05 years +/- 4.29 years). The mean age at first included CVR for girls and boys was 8.95 +/- 2.07 and 9.11 +/- 1.67 years respectively.

Three had Idiopathic-MM, seven NF1-MM and two Chromosomal-MM (1 Turner syndrome). The children with NF1 had foci of abnormal signal intensity (FASIs) on MRI. Clinical descriptions are presented in Table 104.

Eight (8/12) children had revascularization surgery. Four of these had hBOLD CVR and 3D anatomic sequences prior to surgery, and four after surgery. Nine (9/12) had two time-point studies available for analysis.

Table 103 Clinical Characteristics of Group

Patient (M/F)	Moyamoya Disease or Syndromic Diagnosis	Moyamoya Laterality	Age at Moyamoya Diagnosis (years)	Moyamoya Presentation	hBOLD CVR 1 Age (years)	Age at Surgery (years)	hBOLD CVR 2 Age (years)
1 (M)	NF1	Left	11.17	Asymptomatic	11.42	11.83	12.33
2 (M)	NF1	Left	11.50	Headache	12	12	12.58
3 (F)	NF1	Right	10.83	Headache	16.33	16.42	16.92
4 (F)	Turners Syn	Left	3.83	Asymptomatic	4.08	4.17	6.58
5 (F)	Idiopathic	Right	5.67	Paroxysmal sensory episodes	5.75	5.75	7.17
6 (M)	Idiopathic	Left	2.92	Seizures	2.83	n/a	n/a
7 (M)	NF1	Left	6.33	L AIS (movement disorder)	7.25	6.42	8.08
8 (F)	NF1	Right	8.58	Asymptomatic	9.00	n/a	9.92
9 (M)	NF1	Left	14.42	Headache	15	n/a	n/a
10 (F)	Chromosomal	Right	8.00	Headache	9.08	n/a	11.75
11 (M)	Idiopathic	Bilateral	6.75	Headache	8.67	6.92	10.08

12 (M)	NF1	Bilateral	5.17	TIA	5.42	5.42	n/a

9.5.2. Cortical Thickness and Steal

Of the 12 children, 7 (58%) had steal. The laterality of steal was cross-tabulated with moyamoya laterality (Table 105). Neither of the two with bilateral MM had steal, although both had impaired qualitative measures of CVR (Pt 11: reduced right positive pixels; Pt 12: bilateral negative pixels). Steal was otherwise confined to the ipsilateral hemisphere with moyamoya (2/4 with right MM, 3/6 with left MM), was bilateral (1/4 with right MM, 1/6 with left MM) or not present (1/4 right MM, 2/6 with left MM). The 5/12 with no steal had reduced positive reactivity in the ipsilateral hemisphere with MM, and bilateral increased negative pixels in one of the patients with bilateral MM (as above).

Table 104 Steal Laterality by Moyamoya Laterality

Steal Laterality	Moyamoya Laterality			Total
	Bilateral	Right	Left	
Bilateral	0	1	1	2
Right	0	2	0	2
Left	0	0	3	3
None	2	1	2	5
Total	2	4	6	12

Mean cortical thickness was compared by presence of steal/reduced positive reactivity and no steal in the children with unilateral MM without a history of prior revascularization surgery (n=7). Using the paired t-test there was no statistically significant difference between the two groups. The biggest difference in cortical

thickness between steal/reduced reactivity and no steal hemispheres was seen in the oldest child in the group (patient 3; age 16 years) (Table 106).

In binary logistic regression there was a trend toward left hemispheric steal being predictive of left cortical thickness B (-6.66), p=.098, confidence interval .00-3.41. Right cortical thickness was not predictive of left or right cortical thickness.

Table 105 Cortical Thickness in Hemispheres with Steal and No Steal or Reduced Positive Reactivity

Patient Number (n=7)	**Age**	**Cortical Thickness (mm)**		**Thickness Difference**
		No Steal	Steal/Reduced Reactivity	
1	11	2.6995	2.684	.02
3	16	2.5656	2.3776	.19
4	4	2.4912	2.4436	.05
6	3	2.762	2.662	.10
8	9	3.0702	3.08	-.01
9	13	2.886	2.9426	-.06
10	8	3.0512	3.0483	.00
Mean +/- SD	9	2.79 +/- .225	2.75 +/- .283	.04

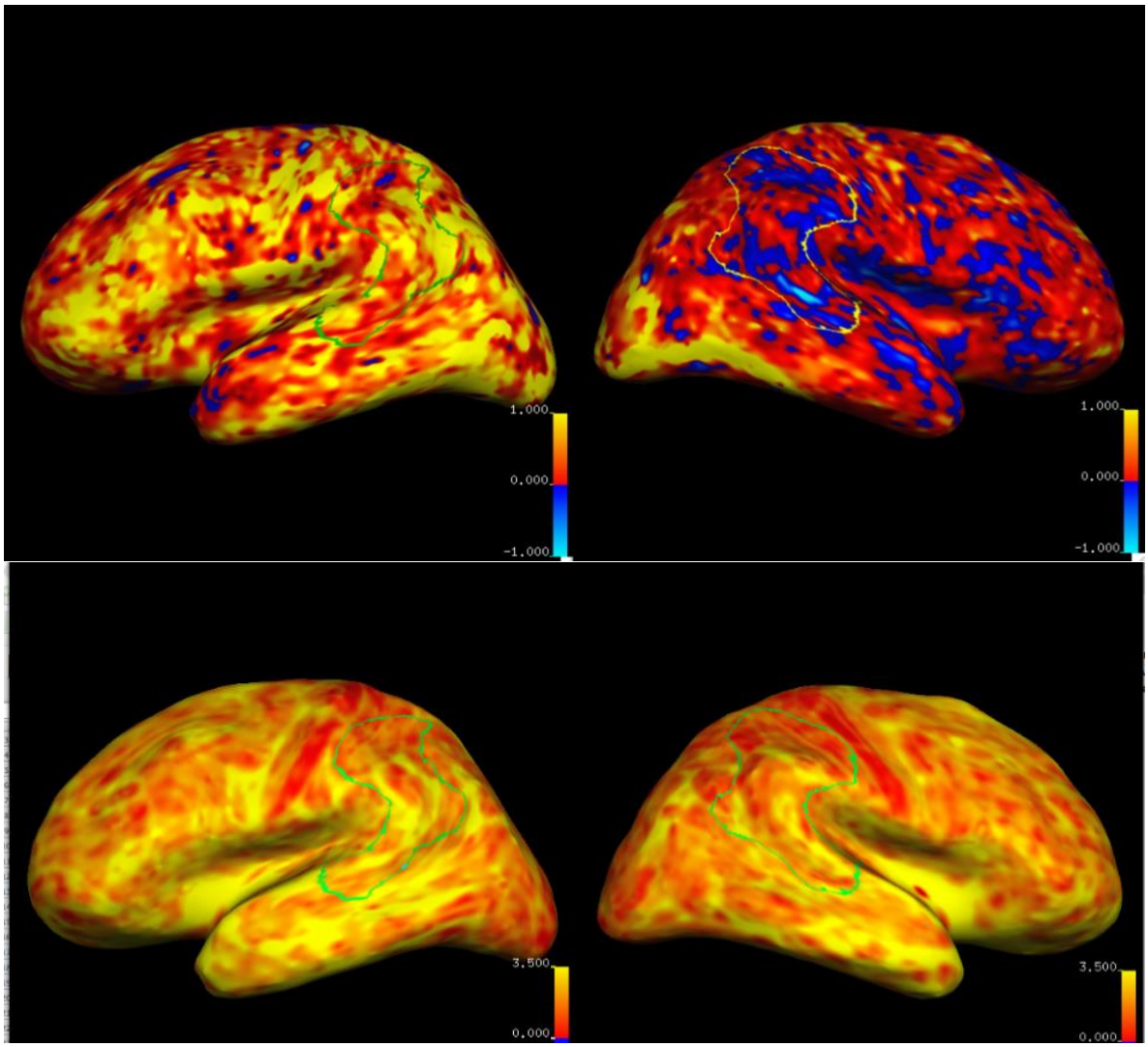
9.5.3. Cortical Thickness and Quantitative CVR

Quantitative CVR was calculable in six of the seven children with unilateral MM and no history of revascularization surgery. Hemispheric CVR was lower in the hemisphere affected by MM. Although there was no statisitically significant difference in CVR between the hemispheres, cortical thickness was lower in the hemisphere with lower CVR in four of the six children. The two children (Patient 8 and 9) with discordant cortical thickness and hemispheric CVR had NF1. Serial measures of cortical thickness in Patient 8 demonstrated a subsequent drop in right (affected) hemisphere cortical thickness, whereas left hemispheric cortical thickness remained the same. No serial measures were available for Patient 9. Linear regression analysis was conducted to examine whether hemispheric CVR was predictive of hemispheric cortical thickness. Using a one-sided t-test there was a trend toward correlation between left hemispheric cortical thickness and CVR ($r=.581$, $p=.113$) and no significant correlation between right hemispheric cortical thickness and CVR ($r=.151$, $p=.388$). Hemsipheric CVR was not predictive of cortical thickness (Table 107, Figs 90 and 91).

Table 106 Hemispheric Cortical Thickness and Quantitative CVR

Patient Number (n=6)	Moyamoya Laterality	Cortical Thickness		Hemispheric CVR	
		Right	Left	Right	Left
1	Left	2.6995	2.684	.5555	.1466
3	Right	2.3776	2.5656	.0056	.6802
4	Left	2.4912	2.4436	2.5346	.4163
6	Left	2.762	2.662	.518	.3617
8	Right	3.08	3.0702	-.4996	.7976
9	Left	2.886	2.9426	2.367	2.0577
Mean +/- SD		2.79 +/- .225	2.75 +/- .283	.913 +/- 1.253	.743 +/- .685

Figure 90 CVR Parametric Maps (top images) and Cortical Thickness (lower images) for Patient 3 right moyamoya (ROI outlined)

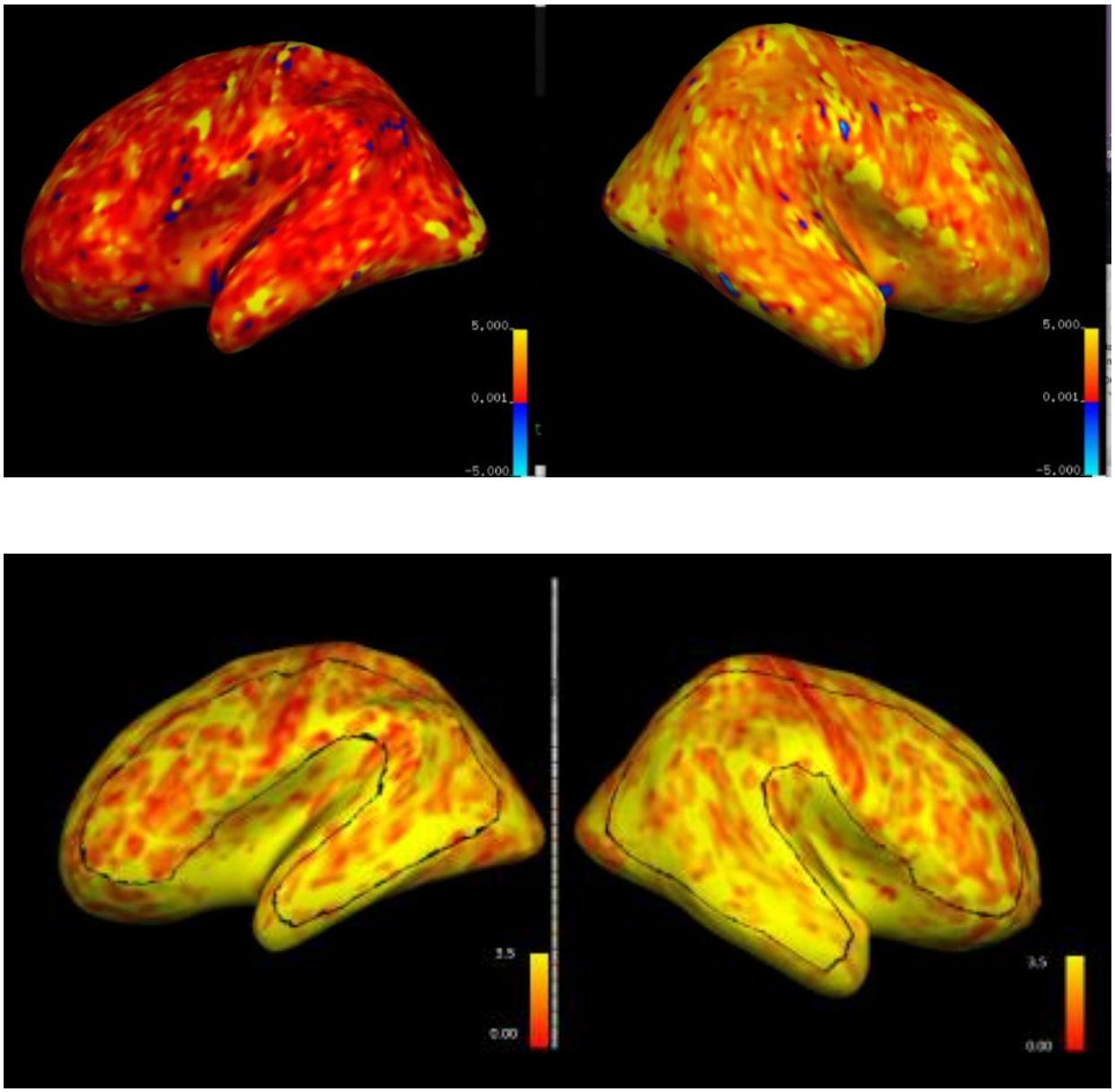


Left Hemisphere **Right Hemisphere**

Top row: CVR maps showing steal within the outlined ROI in the affected right hemisphere, and reduced reactivity in the unaffected left hemisphere.

Bottom row: Cortical thickness maps showing reduced (more red) cortical thickness in ROI compared to the unaffected (comparatively less red) ROI in the unaffected hemisphere.

Figure 91 CVR Parametric Maps (top images) and Cortical Thickness (lower images) for Patient 4, left moyamoya (ROI outlined).



Left Hemisphere **Right Hemisphere**

Top row: CVR maps showing left, more than right hemispheric impaired CVR

Bottom row: Cortical thickness maps showing reduced (more red) cortical thickness in affected left hemisphere.

Table 107 Cortical Thickness over time and following Surgery

	Operative Hemisphere	Pre Surgery/First Study		Post Surgery/Second Study		Post Surgery/Third Study		Post Surgery	
		Mean Cortical Thickness (mm)		Mean Cortical Thickness (mm)		Mean Cortical Thickness (mm)		Mean Cortical Thickness (mm)	
Patient	Operative Hemisphere	No Steal	Steal or reduced reactivity	No Steal	Steal or reduced reactivity	No Steal	Steal or reduced reactivity		
1	Left	2.6995	2.684	2.6089	2.5864				
2	Left			2.6578	2.6971				
3	Right	2.5656	2.3776	2.58	2.41				
4	Left	2.4912	2.4436	2.9563	2.78				
5	Right			3.0839	3.1884	3.099	3.2054	3.3376	3.2176
6	NS	2.762	2.662						
7	Left			2.9525	2.6723				
8	NS	3.0702	3.08	2.9786	3.0718				
9	NS	2.886	2.9426						
10	NS	3.0512	3.0483	3.0208	2.9856	2.9093	2.8526		
11	Bilateral			2.7054	2.7161	2.6969	2.6270		
12	Bilateral	2.3225	2.4492						

NS=no surgery

9.5.4. Change in Cortical Thickness over Time

Children were divided into three groups depending on whether they had revascularization surgery prior to their first included hBOLD CVR study or not (Table 108). The three groups were configured as follows:

1) No surgery at any time-point: No surgery (n=3)

2) No surgery prior to first hBOLD CVR study and surgery after first hBOLD CVR study: Pre and post surgery. (n=5)

3) Surgery prior to first hBOLD CVR study: Post surgery (n=4)

Seven children had serial studies (five studies over two time-points, two studies over three time-points). The mean interval between the first and second (T1 and T2) hBOLD CVR was 16.7 months +/- 9.78, range 7 – 31 months. The mean interval between the second and third (T2 and T3) hBOLD CVR was 10 months +/- 4.24, range 7 – 13 months.

The mean age at first CVR in the No Surgery group was 11.03 years +/- 3.44, second CVR 10.83 years +/- 1.3 and third 12.83 years. The mean age at first CVR in the Pre and post Surgery group was 8.02 years +/- 5.7 and second CVR 11.94 years +/- 5.18. The mean age at first CVR in the Post Surgery group was 8.42 years +/- 2.67, second CVR 9.48 years +/- 2.4 and third CVR 7.75 years.

The group mean cortical thickness was calculated according to the presence or absence of steal or reduced postitive reactivity on initial qualitative hBOLD CVR

assessment. Mean cortical thickness was highest in the No Surgery group at T1 and lowest in the Pre and post Surgery group at T1 (Table 108). Mean cortical thickness increased between the first and last available studies in the Pre and post Surgery group (T1 and T2) and the Post Surgery group (T1 and T3) (Fig 91). Mean cortical thickness declined over time (T1 and T3) in the No Surgery group.

It is interesting to note the greater mean difference over time in cortical thickness in the hemisphere affected by steal in the No Surgery group, and the Pre and post Surgery group (Table 109; Fig 91). Consequently apparent cortical thinning occurred at a faster annual rate in the No Surgery group, and increases in cortical thickness occurred at a slower rate in the Pre and post Surgery group. The increases in cortical thickness occurred at a slower rate in the no steal, not surgically treated hemisphere in the surgical group.

Two of the three children over 11 years had serial studies. Both had revascularization surgery between their first and second study. Cortical thickness decreased over time in one child (11.42 years old) and increased (16.3 years) in the other (Fig 92).

Table 108 Mean Cortical Thickness by Steal over Time

Mean Cortical Thickness by Patient Group (mm^3)	No Steal			Mean Difference Cortical Thickness/Time (years)	Steal/Reduced Positive Reactivity			Mean Difference Cortical Thickness
	T1	T2	T3		T1	T2	T3	
No Surgery (n=3)	3.002	3.046	2.909	-0.093/1.5= -0.062/year	3.024	2.982	2.853	-0.171/1.5= -0.114/year
Pre and post Surgery (n=5)	2.568	2.715		0.147/1.3= .113/year	2.523	2.592		.069/1.3= .053/year
Post Surgery (n=4)	2.876	2.900	3.218	0.342/3.67= .093/year	2.792	2.717	3.338	0.546/3.67= 0.149/year

T1= first time-point hBOLD CVR; T2=second time-point hBOLD CVR; T3=third time-point hBOLD CVR

Figure 92 Group Measures of Cortical Thickness by Presence of Steal

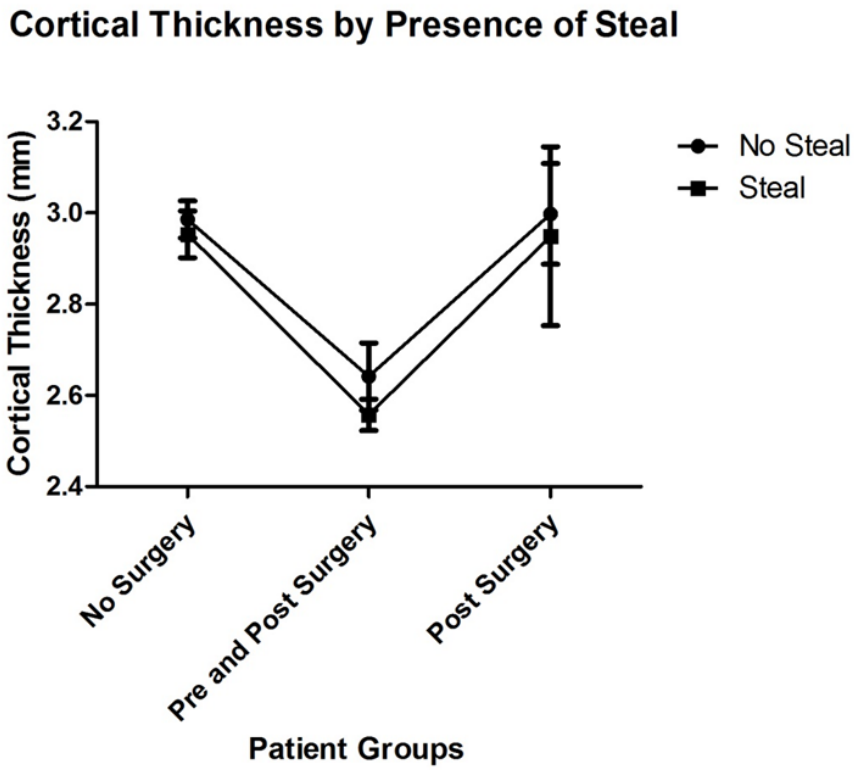


Figure 93 Changes in Cortical Thickness in Children who had Serial Measures by Steal

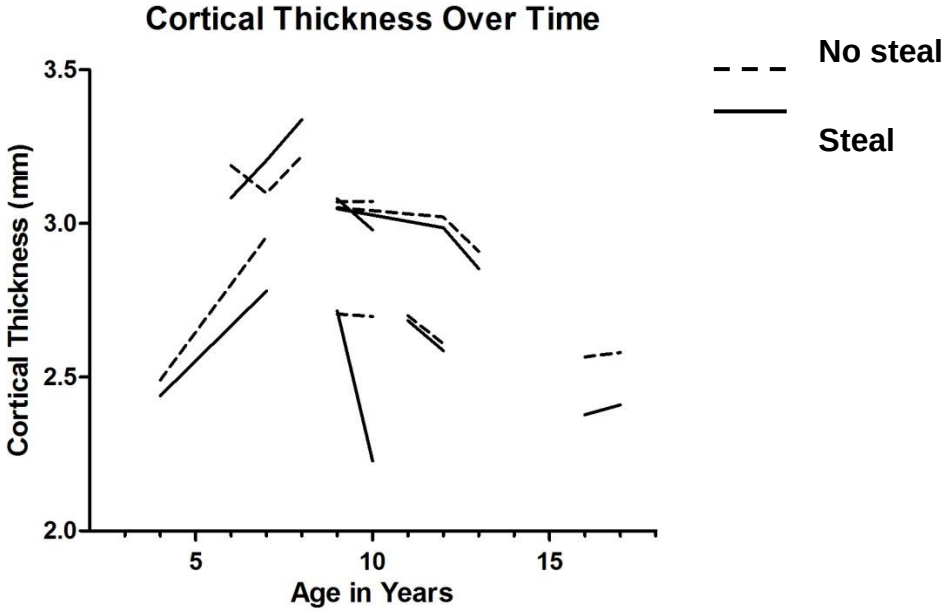
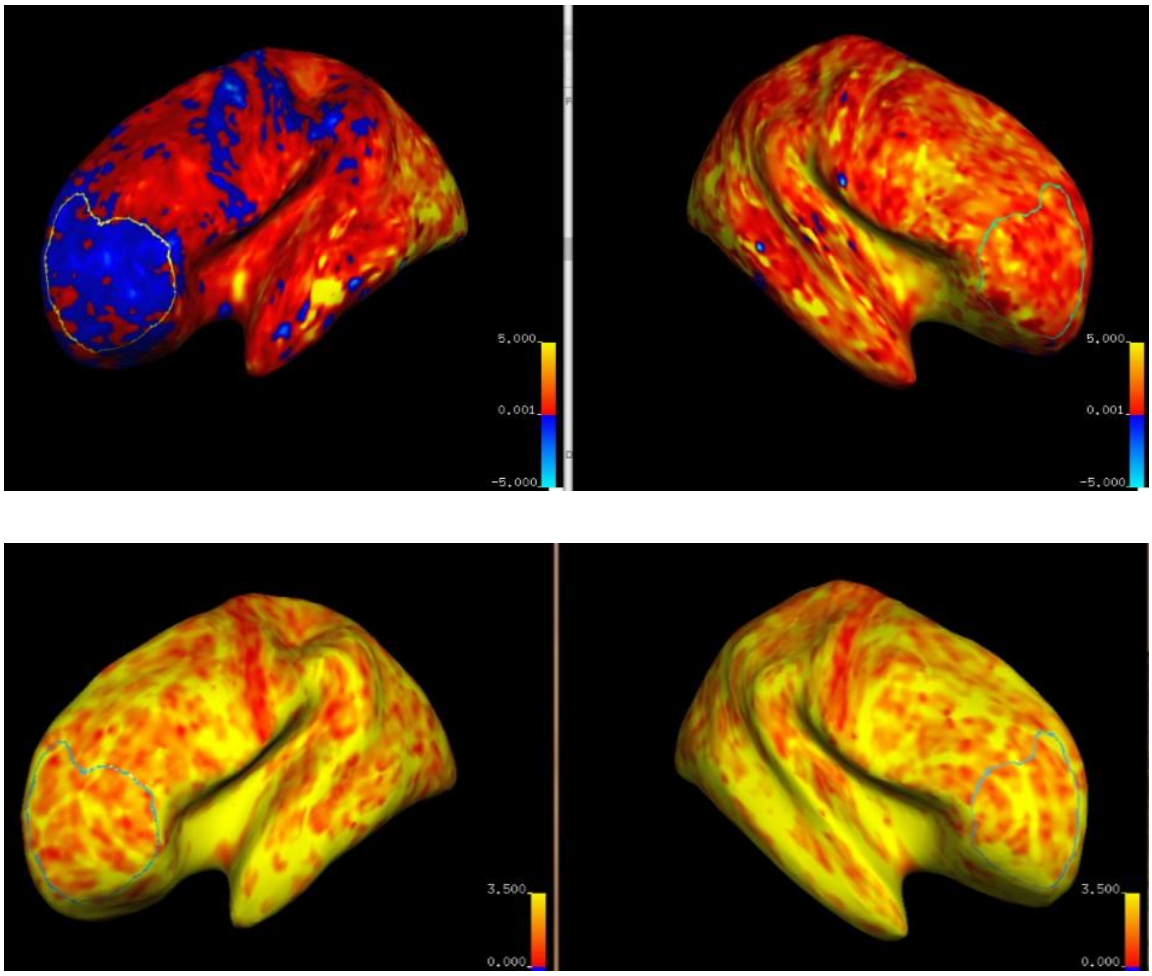


Figure 94 CVR Parametric Maps (top images) and Cortical Thickness (lower images) for Patient 7 Following Surgery (ROI outlined)



Left Hemisphere	Right Hemisphere

Top row: CVR parametric maps showing area of steal in left hemisphere (circled ROI)

Bottom row: Reduced (more red) cortical thickness in left hemisphere (circled ROI)

9.6. Discussion

Changes in cortical volume in childhood do not conform to the more linear model ascribed to adults, where over time there is an expectation of decline. Published data of changes in cortical volume in childhood suggest an age related increase in cortical volume leading to a peak just before adolescence with subsequent decline over time. This in itself is not simple as there are regional and gender specific differences in the observed changes (Lenroot *et al.* 2007, Sowell *et al.* 2007, Shaw *et al.* 2008). In Lenroot et al's longitudinal study of 829 scans from 387 subjects, ages 3 to 27 years, mapping brain development during childhood, total cerebral volume was noted to peak at 10.5 years in females and 14.5 years in males, with total grey matter peaks of 8.5 years in females and 10.5 years in males. Trajectories followed an inverted U shape with onset of gradual decline occurring earlier in females (Lenroot *et al.* 2007).

Kirk et al (Kirk *et al.* 2009) demonstrated a significant association between areas of cortical thinning and watershed (ACA/PCA) regions in older (over 12 years) children with SCD, no stroke and no cerebral vasculopathy, providing a link between cerebral hypoperfusion and cortical thickness in children.

9.6.1. Group Description

The majority of the children in this study were asymptomatic or had headache. Hence this group is representative of children with a low ischaemic symptom

burden. The majority had revascularization surgery at some time-point. Only three did not. The mean cortical thickness in our group was within normal described ranges in childhood (Sowell *et al.* 2004).

The two children over 11 years of age with serial studies of cortical thickness had different post-operative trajectories of cortical thickness. The younger child had continued thinning of the cortex despite surgery in contrast to the older (16 year old) that had an increase in cortical thickness post-operatively. The latter is more in keeping with the findings in the study by Fierstra et al, in which successful revascularization surgery and reversal of steal resulted in restoration of cortical thickness (Fierstra *et al.* 2011).

9.6.2. Cortical Thickness and Steal

The lack of a significant difference in group mean cortical thickness by steal/reduced reactivity in this study may be as a result of a number of confounding factors. Aside from the small numbers, the gender and age differences within the group limit the ability to make meaningful group comparisions. However, in all but two (Patient 8 and 9), the cortical thickness was lower on the side of the moyamoya vessels with impaired CVR. Cortical thickness on the side wih impaired CVR in Patient 8 dropped in the subsequent study eleven months later. The observation of reduced cortical thickness in the three (3/7) with left moyamoya may be more significant than immediately apparent, given the occurrence of the

difference in the typically thicker left hemisphere (Jones *et al.* 2000, Luders *et al.* 2006). Furthermore, my study (Chapters 4 and 5) demonstrated the presence of abnormalities of CVR in the contralateral, unaffected hemisphere, in some children with unilateral moyamoya. Therefore it is possible that the contralateral cortex is also thinned, albeit not to the same extent as the primarily affected hemisphere. This could reduce the magnitude of difference in cortical thickness between hemispheres. This, however, requires exploration in further studies and comparison of findings with age and sex matched healthy controls.

The presence of steal also appeared to have a negative effect on the rate of increase in cortical thickness in the ipsilateral hemisphere of the no surgery and pre and post-surgical group, with a faster rate of cortical thinning in the group not treated with surgery, and slower rate of increase in the operated/steal hemisphere of the pre post-surgery group. This is not seen in the group with surgery at first hBOLD CVR and hence with the longest time post revascularization. In this group the annual rate of increase in cortical thickness was lower in the non-operative/no steal hemisphere. This may be suggestive of the benefits of surgery persisting and increasing relative to the operation naïve hemisphere over time.

9.6.3. Cortical Thickness and Quantitative CVR

Despite small numbers, there was a trend toward correlation between left hemispheric quantitative CVR and cortical thickness.

9.6.4. Change in Cortical Thickness over Time

The mean age of CVR and cortical thickness calculation in the no surgery group was higher than both surgical groups and around 11 years of age. However, the two (2/3) with serial studies within this group were average age 8 years. Hence, older age with expectant decline in cortical thickness would not be an adequate explanation for the decline in cortical thickness in this untreated group.

The greatest increase in cortical thickness was noted in the Post Surgery group, who represent those already revascularized at first included hBOLD CVR and therefore with the longest period of revascularization. These preliminary observations would suggest longer duration of successful revascularization surgery result in greater reconstitution of cortical thickness.

9.6.4.1. Interpretation of Findings and Proposed Mechanisms of Change in Cortical Thickness

Although we were unable to examine for the association between neurodevelopmental outcome and cortical thickness in our study, it has been shown that adverse neurocognitive outcomes may just as well be associated with larger cortical thickness measures as smaller measures, for example in the development of vocabulary (Sowell *et al.* 2004). Specifically grey matter cortical thinning occurs in normal children with a bilateral posterior and right lateral frontal predominance at a rate of 0.1-0.33 mm/year. In addition bilateral perisylvian and

left inferior frontal increases in cortical thickness are both developmentally appropriate and occur at a rate of 0.05-0.2 mm/year (Sowell *et al.* 2001, Sowell *et al.* 2003, Sowell *et al.* 2004). The rate of cortical thinning was lower (.062/year) in the no steal hemisphere of the no surgery group in my study compared to the reported norms. Other rates of thinning and thickening in this study were in keeping with reported norms.

The results of this study have to be interpreted in the context of a chronic injury in a developing brain. Impaired CVR, and in particular the presence of steal physiology, is likely to result in chronic and/or intermittent episodes of hypoxic-ischaemic injury. Cell death may not be the result of this injury, hence the absence of diffusion restriction and normal looking FLAIR sequence on MRI. However, injury in the form of apoptosis or involution of cells may result in selective neuronal loss and consequent cortical thinning (Yamauchi *et al.* 2005, Saur *et al.* 2006, Guadagno *et al.* 2008). This has been observed in the penumbra of acute ischaemic stroke. In addition, intermittent hypoxemia are also likely to result in neuroinflammation, degeneration and diminution of the induction of long-term potentiation, all of which are mechanisms likely to play a role in cortical thinning (Payne *et al.* 2004, Pena and Ramirez 2005).

The mechanisms underlying the apparent recovery of the cortex post revascularization are not clear, and could potentially be explained by loss or gain

or neuroglial tissue, reversal of cortical myelin loss or post-operative increase in cortical blood volume (Horner *et al.* 1996, Kurumatani *et al.* 1998, Derdeyn *et al.* 2002, Tomimoto *et al.* 2003). This requires further exploration.

9.7. Study Limitations and Future Directions

The main limiting factor to this study was the limited numbers. Given the aforementioned important variables and determinants of cortical thickness future studies should be powered to at least account for differnces in gender and age. Matching a larger group to healthy age and gender controls would be ideal.

Larger numbers would also allow for further exploration of the association between cortical thickness and neurocognitive outcome.

9.8. Conclusions

To my knowledge this is the first study to investigate the association of impaired vascular reactivity in childhood moyamoya and cortical thickness, and the change in cortical thickness measures over time, with and without intervening surgery.

Improvements in cortical thickness following revascularization surgery lends support to revascularization surgery being an appropriate intervention for this group of children even when they appear to be mildly affected. Correlation with

neurocognitive outcomes in a larger cohort would help explore the implications of increased post-operative cortical thickness.

Transient Cerebral Arteriopathy as a model for Chronic Non-progressive Arterial Stenosis and hCVR

9.9. Abstract

9.9.1. Background

In adults, cerebral arterial stenosis is frequently treated by endovascular or surgical revascularization to restore perfusion and reduce ongoing and recurrent ischaemia. Hypercapnic-challenge blood oxygen level-dependent MRI cerebrovascular reactivity (hBOLD CVR) measures the regional perfusion response to altered CO2. hBOLD CVR correlates with the risk of ischaemia, informing revascularization strategies. Among children with arterial ischaemic stroke (AIS), transient cerebral arteriopathy (TCA) is a frequent and non-progressive unilateral intracranial arteriopathy with basal ganglia infarction and chronic cerebral artery stenosis. Therefore TCA provides a model for studying cortical perfusion distal to the stenotic artery in a chronic non-progressive arteriopathy. Altered hBOLD CVR perfusion and secondary cognitive decline are unstudied in TCA but, if impaired during chronic stenosis, may suggest a role for reperfusion strategies.

9.9.2. Objective

To describe, in children with TCA, whether chronic non-progressive intracranial stenosis is associated with impaired cortical hBOLD CVR or cognitive decline.

9.9.3. Methods

Children with a prior diagnosis of unilateral TCA having 1) significant (>50% diameter) residual stenosis of the distal internal carotid (dICA), proximal anterior (pACA) or middle cerebral artery (pMCA) and 2) AIS within basal ganglia sparing overlying cortex were enrolled in this thesis study. We quantified infarct volumes, the degree of arterial stenosis, hBOLD CVR in the cortex, and intellectual function.

9.9.4. Results

hBOLD CVR studies were conducted in six children. One child had a normal initial MRI and TOF MRA and was removed from further analysis. The five remaining children had hBOLD CVR studies at a mean age of 8.96 years (3.33 – 14.58 years) post-stroke. Impaired CVR was limited to the infarct zone and adjacent white matter. Overlying cortex demonstrated normal CVR on inspection of parametric maps. Neuropsychological assessment demonstrated intellectual deficits in four children, all referable to the initial sub-cortical infarct.

9.9.5. Conclusion

In children with TCA and sub-cortical infarction, ipsilateral cortical CVR and ~~cortical~~ intellectual function referable to the ipsilateral cortex are preserved despite persistent arterial stenosis. These findings suggest that reperfusion strategies in TCA are unlikely to be of benefit.

9.10.Introduction

Arteriopathy occurs in 63% of children with AIS. These arteriopathies are usually intracranial, characteristically involving the circle of Willis arteries, in particular the distal internal carotid (dICA), proximal middle cerebral (MCA) and anterior cerebral (ACA) arteries. Childhood arteriopathies are associated with recurrent stroke in up to 66% (Fullerton *et al.* 2007). The most frequent intracranial arteriopathies are moyamoya in 22% of these patients, which is bilateral and progressive, and TCA in 25% of these patients which is unilateral and, in the chronic phase, non-progressive (Amlie-Lefond *et al.* 2009).

Moyamoya is characterized by bilateral involvement, progressive arterial stenosis, chronic hypoperfusion and ongoing recurrent strokes. Both recurrent strokes and chronic hypoperfusion are well-recognized and each is posited to contribute to the observed chronic progressive cognitive decline (Ishii *et al.* 1984, Ikezaki *et al.* 1994, Hogan *et al.* 2005).

In contrast, TCA is characterized by unilateral arteriopathy involving the circle of Willis arteries (dICA, pMCA, pACA) with infarction in the territory of perforator arteries arising from these arteries (Chabrier *et al.* 1998, Sebire 2006). TCA is the most frequent cause for chronic non-progressive intracranial arteriopathy associated with childhood stroke (Braun *et al.* 2009). Stenosis can increase in the initial 3-6 months post-stroke, however beyond 6 months the course is static or resolving. Therefore TCA is a useful model for studying whether static arterial

stenosis is associated with chronic perfusion deficits in ipsilateral cortex and if so, whether these deficits are associated with cognitive decline referable to the uninfarcted ipsilateral cortex. This in turn can elucidate whether reperfusion strategies are indicated in children with TCA.

As a biomarker of neurological injury neuropsychological evaluation can be used to measure cognitive deficits referable to either cortical or sub-cortical injury. Deficits in verbal and visual reasoning are typically associated with cortical injury, and deficits in information processing and working memory with sub-cortical injury.

In this chapter I explored whether the chronic stenosis seen in TCA results in impaired hBOLD CVR in the region beyond the stenosis and/or cognitive decline.

9.11.Hypothesis

The hypotheses were that:

1. In children with TCA, despite persistent moderate-severe stenosis, CVR abnormalities as an indicator of perfusion abnormalities would be limited to the infarct location and spare the overlying cortex.
2. Intellectual deficits would be limited to those referable to the sub-cortical stroke lesion (including information processing and working memory) and spare those referable to the cortex in the distal perfusion zone of brain supplied by the stenosed artery (such as verbal and visual reasoning).

9.12.Method

9.12.1. Patient Population

Clinical and radiographic data of all children enrolled in our institutional Stroke Registry with TCA diagnosed per published criteria (Chabrier *et al.* 1998) between 1990-2007 were screened. Children with persistent moderate to severe stenosis on conventional angiography (defined below) of pMCA, pACA and/or dICA, plus infarcts limited to sub-cortical structures with spared cortex at the time of their follow-up imaging were selected and consent sought.

Formal neuropsychological assessments and follow-up clinical information were obtained as part of routine standardized follow-up in our Stroke Clinic. In addition all children underwent neuropsychological assessments at school entry and these data were utilized for the current study (Westmacott *et al.* 2009).

9.12.2. Patient Definition

9.12.2.1. Inclusion criteria were:

i) acute AIS limited to sub-cortical structures (basal ganglia and adjacent white matter)

ii) conventional angiography performed within three months of the initial stroke, demonstrating unilateral stenosis or occlusion involving dICA, pMCA or pACA.

iii) MRI/MRA ≥6 months from index stroke demonstrating non-progressive vascular disease

iv) MRI/MRA ≥12 months from index stroke demonstrating stable moderate to severe stenosis (defined below) in dICA, pMCA or pACA.

v) MRIs showing no recurrent AIS after 6 months post-initial stroke

vi) able to co-operate with awake research MRI and breathe with a face mask in place for minimum 20 minutes

9.12.3. Magnetic Resonance Imaging Including BOLD CVR

MR imaging was performed on a 3.0T MRI system using an 8-channel head coil (Philips Healthcare, Best, the Netherlands). Anatomical imaging included standard FLAIR, DWI, 3D time-of-flight (TOF), and a high-resolution T1-weighted sequence. BOLD-CVR data were acquired using a T2*-weighted single-shot EPI gradient echo sequence (TR/TE=2000/30 ms, FA=90°, FOV=220×220 mm^2, matrix=64×64, slices=25, slice thickness=5 mm, dynamics=270). During BOLD-CVR imaging, precise and independent manipulation of subject partial pressures of end-tidal CO_2 ($P_{ET}CO_2$) and O_2 ($P_{ET}O_2$) was achieved by introducing a CO_2 breathing challenge via a rebreathing mask and computer-controlled gas sequencer (RespirAct; Thornhill Research Inc., Toronto, Canada) (Slessarev *et al.* 2007). The challenge involved alternating 60 second blocks of targeted normocapnia ($P_{ET}CO_2$=40

mmHg) and hypercapnia ($P_{ET}CO_2$=45 mmHg) while $P_{ET}O_2$ was maintained at 100 mmHg.

9.12.4. Grading of stenosis

Grading of arterial stenosis was performed by the study neuroradiologist (DA), through visual inspection of initial and follow-up 3D TOF MRAs and conventional angiograms. Stenosis was graded as a percentage of the normal arterial diameter. If more than one vessel was involved all were graded and the vessel with maximal stenosis was chosen for the purpose of study analysis:

1 = 0-25%; 2 = 26-49%; 3=50-74%; 4=75-100%.

9.12.5. BOLD CVR analysis

MRI and end-tidal data were analysed offline using FSL v4.1.1 (FMRIB Software Library; FMRIB Analysis Group, Oxford, UK) (Smith *et al.* 2004). BOLD-CVR images were first corrected for motion then filtered to remove signal drift. The $P_{ET}CO_2$ waveform for each subject was temporally aligned with the BOLD-CVR data to adjust for sampling delays. The linear correlation between the BOLD-CVR values of each voxel and the subject's $P_{ET}CO_2$ waveform was calculated and then normalized to generate a CVR map expressed as a percent signal change per

mmHg of $P_{ET}CO_2$. Each map was co-registered to the associated high-resolution anatomical images to allow for qualitative clinical assessment.

9.12.6. Measurement of Lesion Volumes

The ischaemic regions of interest were outlined using semi-automated planimetry on axial FLAIR research MRIs employing Analyze 8.0 software (Analyze Direct, KS). Total brain volume (all supra-tentorial brain parenchyma excluding the brainstem, cerebellum, ventricles and CSF) was also measured and infarct volumes were expressed as a proportion of total brain volume to account for the variable brain size across childhood.

9.12.7. Neuropsychological Assessments

All children had a full neuropsychological assessment conducted at 3-9 years following their clinical stroke. Assessment included appropriate standardized Wechsler Intelligence Scales for Children (WISC-IV).for overall intellectual ability (Full-scale IQ), language, and verbal ability (Verbal Comprehension Index), visual–spatial ability (Perceptual Reasoning ⁄Organization Index), auditory attention and mental manipulation (Working Memory ⁄ Freedom from Distractibility Index), and visual–motor speed (Processing Speed Index). Participants' scores were compared with the normative sample of the test, where each index score has a

mean of 100 and a standard deviation of 15. An abnormal score was defined as equal to or less than 85.

9.13.Results

9.13.1. Patient Population

Forty-five children diagnosed with TCA at our institution between 1990 and 2007 were screened for inclusion. We excluded 10 children with infarcts involving overlying cortex and 24 children with normal artery calibre or only mild residual arterial stenosis. Among the remaining 11 children, two patients refused participation and three could not be contacted. The remaining six children with sub-cortical infarcts and moderately severe arterial stenosis comprised our study sample (Table 109). At the time of the study CVRs, review of the initial MRI and TOF MRA revealed a normal MRI and no abnormality on MRA in one child. Therefore this child was removed from further analysis. One child (Patient 4) had a recurrent stroke 2 months following the initial stroke while the remainder had no symptomatic or silent recurrent strokes after the initial stroke. All were managed with anticoagulant or aspirin therapy during the course of the follow-up.

9.13.2. Parenchymal and vascular findings

hBOLD CVRs were conducted in 2011 at a group mean age of 8.96 years (3.33 – 14.58 years) following initial stroke diagnosis. Parenchymal MRI demonstrated unilateral infarcts limited to the basal ganglia and adjacent sub-cortical structures (Table 110; Fig 95). MRA showed moderate to severe arterial stenosis. Infarcts were all confined to the vascular territory of the stenotic cerebral artery. Infarct volumes were mean 4436 mm^3 (range 802 – 8107), and mean 0.4% (range 0.06-0.7%) of total brain volume. There appeared to be a relationship between the degree of arterial occlusion and infarct volume as the patient with the smallest degree of occlusion had the smallest volume infarct (Patient 5; 25-50% occlusion; 0.06%) and the patients with the largest degree of arterial occlusion had the largest infarct volumes (Patients 1 and 2; 100% occlusion; 0.7%).

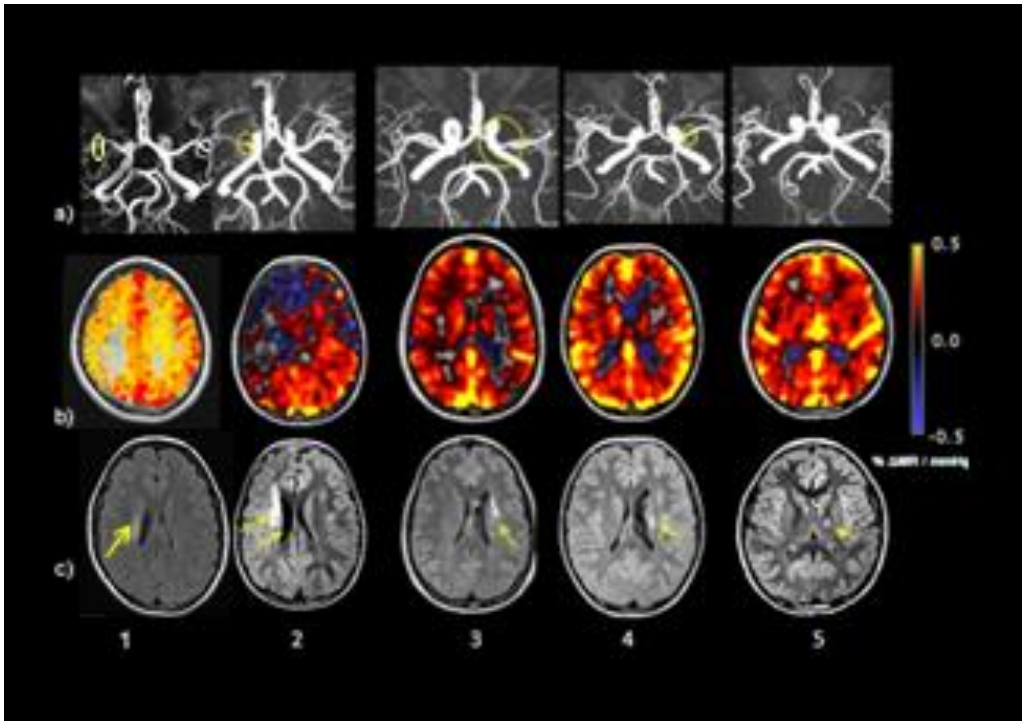
Compared to the MRAs performed in the initial 3 months post-stroke, the research MRAs showed stable, moderate to severe, unilateral stenosis involving pMCA alone (Patients 1 and 4); pMCA and dICA (Patient 3); pMCA, ACA and dICA (Patient 2) and pACA alone (Patient 5).

Table 109 Patient Clinical and Imaging Characteristics

Patient No (Age)	Age at Time of Stroke (years)	Time to CVR (years)	Parenchymal Lesions	Vessel/ %Occlusion	Vascular Stenosis	CVR Impaired reactivity stroke area (Yes/No)	CVR impaired in deep WM (Yes/No)	CVR Impaired cortex Y/N (Yes/No)
1). 15, M	0.5	14	Right caudate, putamen, corona radiata	M1/100	Right M1	Yes	No	No
2). 7, M	4.0	3	Right deep WM, caudate, putamen	M1/100	Right M1, A1, dICA	Yes	No	No
3). 17, F	7.9	10	Left corona radiata	M1/100	Left M1, dICA	Yes	No	No
4). 10, M	3.2	7	Left deep WM, Left putamen	M2/51-75	Left distal M1/M2 junction	Yes	No	No
5). 9, M	1.9	7	Left internal capsule	A1/25-50	Left M1, A1, dICA	Yes	No	No

M=male; F=female; WM= white matter; M1=proximal middle cerebral artery; A1=proximal anterior cerebral artery; M2= second branch of middle cerebral artery; dICA= distal internal carotid artery; Vessel = worst vessel affected; occlusion = percentage occlusion at time of diagnosis

Figure 95 MR Angiography, CVR Parametric Maps and Axial FLAIR Images of Patients (1-5) with Transient Cerebral Arteriopathy



MRA: yellow circled area demonstrating area of stenosis on

CVR parametric maps demonstrating intact positive reactivity in stroke and non-stroke hemispheres except in the area of infarction

MRI: arrows showing region of ischaemic injury

9.13.3. BOLD CVR Results

hBOLD CVR studies were done at a mean age of 11.6 years (range 7-15 years) and at a mean of 7.7 years from the acute stroke (range 3.3-14.5 years). The fit of the BOLD-CVR data with the $PetCO_2$ waveform was excellent in four of the five patients. (Fig 96) In the fifth child (Patient 2) CVR study was sub-optimal due to

excessive patient motion and a small (poor) magnitude of CO_2 change (Fig 97). Within the infarct territory CVR in all children during hypercapnic challenge demonstrated either no increase or paradoxical decrease in BOLD signal. Within the intact cortex overlying the deep infarct, the CVR was normal and symmetric to the non-lesioned hemisphere in the four children with adequate studies. (Fig 94)

Figure 96 BOLD fit Curves in Patient 3 Showing Good Fit

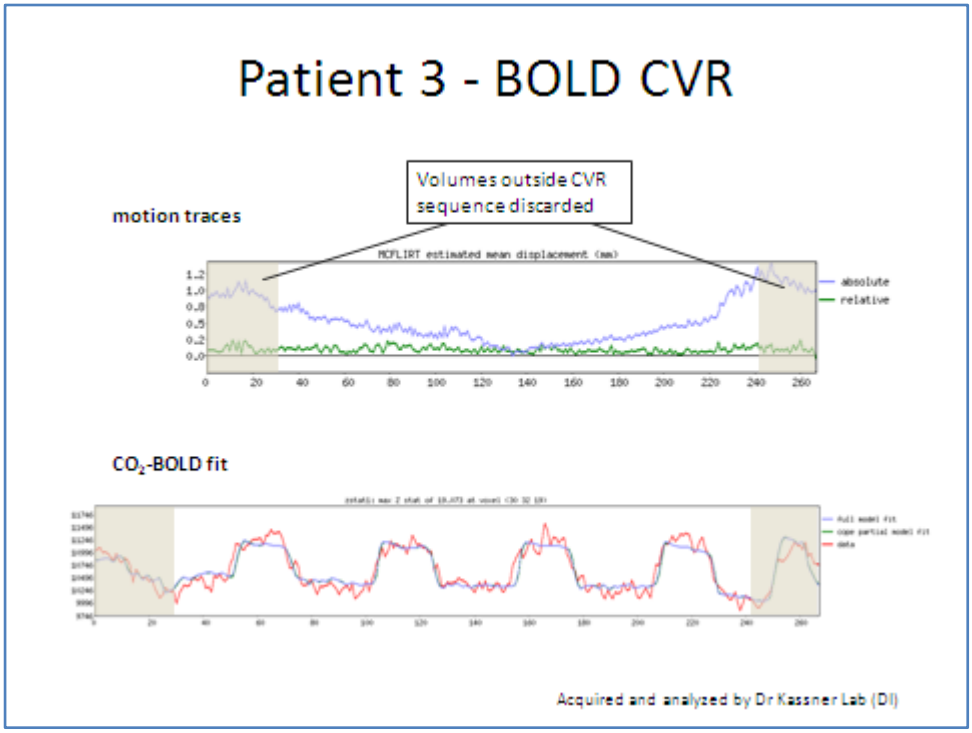
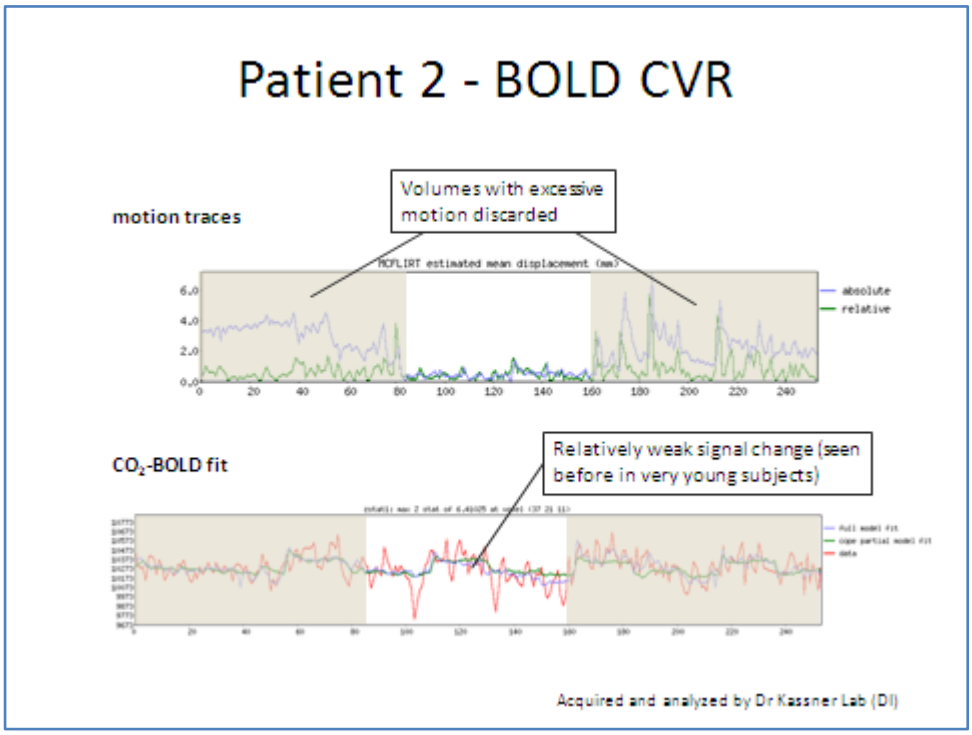


Figure 97 BOLD fit Curves in Youngest Patient (Patient 2) Showing Poor Fit



9.13.4. Neuropsychological Outcomes

Neuropsychological assessments were conducted in all children at mean age 6.9 years (3.2-14.3 years) at intervals ranging from 3 to 9 years post stroke. The mean interval between the neuropsychological assessments and hBOLD CVR was 3.1 years (range 0.2-5.7 years). Group mean intelligence scores were normal in all domains (Table 110). However, scores in sub-domains fell into the abnormal range in three of the six patients. Three children (Patients 2, 3 and 4) demonstrated lower scores in their processing speed and working memory (predominantly sub-cortical

functions) compared with higher scores in verbal-reasoning (predominantly cortical function) (Table 111). Patient 1 had the lowest scores in all domains (all abnormal): FSIQ (74), VCI (75), PRI (76) and PSI (80) and also had the largest volume stroke. In the five patients, the calculated mean neuropsychological scores in each domain were highest in Patients 4 (FSIQ 95) and 5 (FSIQ 107) who had the two smallest lesion volumes compared to lower scores in Patients 1, 2 and 3 (FSIQ 74, 87 and 89 respectively) who had the largest lesion volumes (Table 111).

Table 110 Volume of Infarct and Neuropsychological Findings

Patient Number, age, sex	Age at time of stroke (years)	Age at time of NP (years)	Parenchymal lesions (%Occlusion)	Volume of Infarct (mm3)/ %Total Brain	Weschler Scales of Intelligence Scores FSIQ VCI PRI WI PSI				
1). 15, M	0.5	9.8	Right caudate, putamen, corona radiata (100)	8109/0.7	74	75	76	86	80
2). 7, M	4.0	7.3	Right deep WM, caudate, putamen (100)	7910/0.7	87	95	91	91	83

3). 17, F	7.9	13.5	Left corona radiata (100)	2822/0.2	89	91	100	88	88
4). 10, M	3.2	7.3	Left deep WM, left putamen (50-75)	2537/0.2	95	109	82	98	87
5). 9, M	1.9	9.2	Left internal capsule (25-50)	802/0.06	107	104	114	103	110

Group Findings									
Patient Number	Age at Study (mean; range) years	Age at Stroke (mean; range) years	Age at NP (mean; range) years	Infarct volume (mean; range) mm^3	FSIQ VCI PRI WMI PSI (mean;range)				
n=5	9.4; 7.3-13.5	3.5; 0.5-7.9	6.9; 3.2-14.3	4436; 802-8109	90.4; 74-107	95; 75-109	93; 76-114	93; 86-103	90; 80-110

FSIQ=full scale intelligence quotient; VCI=verbal comprehension index; PRI=perceptual reasoning index; WI=working memory; PSI=processing speed index

9.14.Discussion

We studied five children with TCA to determine the association between persistent moderate to severe arterial stenosis, distal CVR, and neuropsychological outcome. We found CVR abnormalities and intellectual deficits limited only to the zone of the sub-cortical infarct and not in the overlying cortex. Together with the lack of late

recurrent visible infarcts on the follow-up MRIs, our findings support the absence of significant ongoing ischaemia or chronic hypoperfusion in the cortex supplied by a stenotic artery, at least in the developing brain. To our knowledge, these are the first data looking at cerebrovascular reactivity in a non-progressive arteriopathy in childhood although there are some studies of perfusion using arterial spin labelling (Chen *et al.* 2009).

9.14.1. TCA and CVR

TCA is common in childhood stroke. In its chronic phase (beyond 3-6 months post-stroke), TCA provides an excellent model of non-progressive arteriopathy. Among 79 children with TCA longitudinal vascular imaging demonstrated that beyond 6 months, arterial stenosis stabilized in 32% and improved in the remainder (Sebire *et al.* 2004, Braun *et al.* 2009) In a single center study we were able to enrol a group with childhood TCA, stable significant stenosis and infarcts limited to sub-cortical structures. This enabled us to explore whether tissue level microvascular dysfunction and under-perfusion of the non-infarcted cortex distal to the stenotic artery was present (using CVR) and clinically significant (neuropsychological testing).

The results of hBOLD CVR we observed showed that CVR abnormalities were limited to the region of the previous stroke and were not present in the cortex. hBOLD CVR measures cerebrovascular reactivity in response to changes in CO_2.

Prior studies in adults with cerebrovascular disease have demonstrated that hBOLD CVR is a valid indicator of tissue at risk of ischemia (Mikulis *et al.* 2005). Cortical thinning in non-infarcted brain distal and secondary to chronic stenosis is seen in the presence of paradoxical 'steal' reactivity (Fierstra *et al.* 2010) supporting subtle ongoing tissue damage. Normal cerebral vasculature responds with vasodilation to the CO_2 stimulis. However, in regions with pre-existing poor perfusion, the vasculature is already maximally vasodilated, and therefore such regions show either failure of augmentation or a paradoxical reaction consisting of decreased perfusion. Alternative methods of hBOLD CVR are available for use in younger children including general anaesthetic (with anaesthetic manipulation of CO_2) and awake breath holding (Thomason *et al.* 2005). In our study five of the six children had technically excellent CVRs consistent with prior reports on the reproducibility and reliability of this technique in children (Han *et al.* 2011). The only child with a sub-optimal study was the youngest child studied (Patient 2, age 7 years). This child did not tolerate the prolonged positioning of the re-breathing mask. The limitations of this method of hypercapnic challenge have been discussed in previous chapters (Chapter 2 and 5).

In the study subjects, there was an absence of CVR response within the previously infarcted tissue. Within the cortex overlying the deep infarct CVR was normal and symmetric when compared to the non-lesioned hemisphere in the five technically adequate studies. This indicates normal perfusion of the cerebral cortex even under a stressed condition of hypercapnia. Based on this finding suggestive of

intact tissue level microvascular function, we would suggest that the risk of ischaemic demise beyond the chronic moderate to severe stenosis is minimal. The theoretical concern for neuronal loss, which cannot be visualized on parenchymal MRI, appeared not to be supported by our findings.

These findings contrast those in moyamoya. BOLD CVR studies in moyamoya have demonstrated cortical perfusion deficits consistent with, and resulting from, the vascular steno-occlusive disease (Tzika *et al.* 1997) (Mikulis *et al.* 2005, Han *et al.* 2011) Abnormalities in CVR concordant with moyamoya have been demonstrated in prior chapters in this thesis. Thus in moyamoya ongoing ischemia exists and is deleterious to brain tissue. Early PET studies in children with moyamoya demonstrated disrupted cerebral haemodynamics with loss of vascular reserve in brain tissue and 'misery perfusion' even in the absence of cerebral infarcts (Ohtaki *et al.* 1998) (Kuwabara *et al.* 1997) (Kuwabara *et al.* 1997). Silent or overt cerebral infarcts may develop, and these appear to be reduced by revacularization surgery (Kuwabara *et al.* 1997) (Scott *et al.* 2004) (Ng *et al.* 2012).

9.14.2. Neuropsychological Tests

Neuropsychological test results were employed in this study to assess for the presence of clinical deficits referable to the cortex distal to arterial stenosis. Processing speed typically recruits both cortical and sub-cortical networks whereas verbal reasoning is classically considered a cortically based cognitive function. The

results summarized in Table 110, demonstrated age appropriate cognitive deficits that were accounted for by the location and size of the initial sub-cortical infarct. Patient 5, with the smallest sub-cortical stroke had a normal IQ. Patient 1, the youngest at stroke (6 months age) had abnormal scores across all domains and the worst scores of the group. This non- lateralized pattern of deficits is consistent with research showing that brain injury prior to age four or five years results in widespread cognitive deficits (Vargha-Khadem *et al.* 1992, Anderson *et al.* 2000, Anderson and Catroppa 2007, Westmacott *et al.* 2009).

Among these subjects, the group mean score for FSIQ was normal (90; range 74-107) indicating relative sparing of intellectual ability. Although the small numbers did not allow for statistical analysis our observations are in keeping with the previously published findings of specific cognitive vulnerability in FSIQ, WMI and PSI for unilateral focal ischaemic lesions acquired in early childhood (Westmacott *et al.* 2009) and white matter and basal ganglia injury association with slowed perceptual reasoning (O'Dougherty *et al.* 1985, Caine and Watson 2000, Maneru *et al.* 2001, Banwell and Anderson 2005, Raman *et al.* 2006). Deficits are expected to be associated with weaker overall intellectual ability (Westmacott *et al.* 2009). The combined findings of normal CVR within the cortex overlying the stroke and relative sparing of FSIQ are reassuring and suggest the risk of tissue ischaemia and neuronal dropout (Saver 2008) are low in this group.

This *s*tudy does not support a need for revascularization surgery, endovascular stenting or other delayed reperfusion techniques in children with unilateral non-

progressive arteriopathy. The dangers associated with catheter-related interventions have been highlighted in a recent communications from the NIH, National Institute of Neurological Disorders and Stroke (NINDS) ((NIH) and (CDC) 2011). Persistent arterial stenosis still presents a risk for recurrent thrombotic stroke (albeit rare beyond the initial 6 to 12 months post-stroke), for which medical antithrombotic treatment is indicated and apparently effective.

9.15. Study Limitations and Future Directions

This is a study of a small number of children, thus the results may only be presented in a descriptive manner. Confidence in the findings remains relatively limited by this small number. However, the children formed a homogenous group that were suitable for the purpose of the study.

In addition, the time interval between the most recent neuropsychological testing and CVR testing was disparate in most children and additional neuropsychological deficits could have emerged in the interval to CVR. However, since both studies were performed on average several years from stroke, there should have been time for evidence of neuropsychological deficits in the cortical tissue to emerge. Future larger cross-sectional BOLD CVR studies or longitudinal studies of serial BOLD CVRs paired with repeated neuropsychological assessments would enable more robust statistical findings regarding cortical CVR and neuropsychological deficits in cortical or sub-cortical patterns.

9.16.Conclusion

This study demonstrates in children with moderately severe non-progressive stenosing arteriopathy, that distal perfusion appears to be adequate, in contrast to the ongoing perfusion deficits that characterize the progressive childhood arteriopathies.

This study of TCA as a model of chronic non-progressive arteriopathies adds important new information to our understanding of the consequences on intellectual function, post-stenotic occlusion and CVR. This study demonstrates normal CVR responses outside of the infarct zone and intellectual deficits referable to the age and location of stroke. The risk of recurrent stroke, neuronal dropout and cognitive decline in this non-progressive arteriopathy group is therefor likely to be low and the need for revascularization minimal.

10. Discussion

Moyamoya disease is a lifelong, progressive condition which results in ischaemic demise as evidenced by neurocognitive decline, transient ischaemic attacks and death in childhood and adulthood. There is no treatment that halts the progression of the vasculopathy, therefore medical and surgical strategies to improve cerebral blood flow distal to the steno-occusive disease are the mainstay of treatment. The ability to identify tissue level poor perfusion and quantify ischaemic risk would potentially allow for primary preventative strategies to be employed to prevent stroke and ischaemic demise. The aim of this thesis was to validate the use of qualitiative methods of hypercapnic challenge BOLD cerebrovascular reactivity and assess their utility in the evaluation of ischaemic risk in children with moyamoya arteriopathy.

10.1. Moyamoya Disease and Syndrome: challenges in management

In Chapters 1-3 of this thesis I highlighted the differences in natural history and outcome of children with progressive and non-progressive arteriopathies. A unique challenge in the management of children with moyamoya is the increasingly early age of diagnosis due to improved access and use of MRI imaging and disease

specific screening protocols. This has resulted in asymptomatic diagnosis of moyamoya in children of all age groups. Detailed reports of natural history in moyamoya are mostly derived from Asian (principally Japanese) populations. There remains a substantial gap in our understanding of the natural history of moyamoya in Caucasian and non-Sickle Afro-Caribbean moyamoya disease. Furthermore our understanding of the natural history of the syndromic moyamoyas is confined to case reports. However, this study has demonstrated variability in natural history between the moyamoya disease and syndrome groups, as well as within the moyamoya disease and within the moyamoya syndromic groups. Of note the children with NF1 had a more indolent clinical course and mostly left sided arteriopathy. Smith et al suggest that given the radiologic and clinical progression seen in asymptomatic children with moyamoya, revascularization surgery should be considered in all (Lin *et al.* 2011). However, operative technical constraints, higher complication rate and anaesthetic risk in the very young countered by the risk of cognitive decline and stroke over time highlights the need for a clinically usable biomarker of ischaemic risk to inform the decision to proceed to surgery, operative procedure choice – including whether to operate on one or both hemispheres - and optimal timing of surgery.

10.2. Qualitative hBOLD CVR: feasibility and reproducibility of measures and interobserver reliability

Having described the clinical need and risk profile of the population studies in Chapters 1-3, I sought to demonstrate that it was feasible to conduct qualitative hBOLD CVR studies in all age groups, including the very young in Chapter 5. I also sought to ascertain whether the methodology was reliable and repeatable in Chapter 4 and to demonstrate good interobserver reliability in scoring of the studies if they were to be clinically useful.

i) ***Feasibility*** - to my knowledge to date, this is the largest group of children who have had clinically qualitative hBOLD CVR studies. The limited utility of quantitative controlled CO2 delivery techniques for the measurement of CVR in childhood has been discussed. Our experience of qualitative hBOLD CVR is in keeping with that of adult centers using breath-hold studies for clinical practice and research (Pillai and Mikulis 2015). The ability to regionally assess and compare CVR in our patients - sequentially if necessary - is hence served by this tool.

ii) ***Reproducibility*** - Reproducibility of measures was mostly good for the hemispheric pixels apart from the negative pixels generated at the threshold set to generate 200 positive pixels. The poor reproducibility indices for these (PTNP) pixels would suggest that they are not reliably useful for application in clinical practice. Repeat measures demonstrated good reliability of the methods of analysis for both GA and BH studies which was improved by selection of good

quality studies for analysis. In practice the technically better study is often apparent, and can be judged by the child's ability to perform the breath-hold task successfully or the adequacy of CO_2 change achieved by ventilator manipulation under GA. It may be possible to improve repeatability by measuring CO_2 before and after the breath-hold in the younger children, either with an end-tidal CO2 or a transcutaneous method as described in a number of adult studies ((Bright and Murphy 2013, Pillai and Mikulis 2015).

The noting of technical and/or patient factors which may compromise the quality of the studies is important to ensure accurate interpretation of qualitative hBOLD CVR using these techniques.

iii) ***Scoring*** - we developed objective and subjective methods of CVR scoring as described in Chapter 2. The pre-setting of a total number of target pixels however, did not allow for a full assessment of individual magnitude of CVR response. The presence of true negative reactivity should be a more robust indicator of impaired CVR and tissue at risk of ischaemic damage. Analysis of the presence and extent of negative reactivity or steal is hence essential in both our subjective and objective scoring methods for the determination of normal and abnormal CVR, allowing us to make some intra and inter patient comparisons of CVR response.

There was excellent agreement between the two neurologist's independently obtained scores. This method is simpler and less time consuming than the pixel

method and may be an adequate scoring tool. The scoring tool was good at discriminating between normal and abnormal studies, but less good at discriminating between the two abnormal scores (two and three). Hence, further revisioin of these scores is required.

Therefore, in Chapters 1 -5 of the thesis I demonstrated the clinical need for a tool to measure ischaemic risk, proposed that qualitative hBOLD CVR would be a suitable tool, and demonstrated that it is feasible to use in children with good reproductibilty of measures and interobserver reliability in scoring.

10.3. Qualitative hBOLD CVR and clinical application:

i) hBOLD CVR and angiographic measures –

Chapter 6 demonstrated that in this study hBOLD CVR abnormality was concordant with laterality of moyamoya. An interesting observation was the bilateral CVR abnormality seen in patients with unilateral moyamoya. This has been described in adult studies of CVR in moyamoya (Heyn et al 2010). The observation of a relationship between heart rate, hematocrit and CVR is also reported in adult studies of CVR. However, I did not demonstrate an association between CVR abnormality and severity of moyamoya as measured by Modified Suzukii Staging but discussed the limitations of the staging applied and plan to revise this in future studies.

ii) hBOLD CVR pre and post surgery -

In Chapter 7 abnormality in CVR in asymptomatic children prior to surgery appeared to be premonitory of clinical deterioration. Post-surgical change in CVR was variable but appeared to be predictive of clinical course in the study. Children with bilateral moyamoya sometimes appeared to have deterioration of hBOLD CVR measures in the operation naïve hemisphere with clinical deterioration and symptoms referrable to that hemisphere potentially requiring further revascularization surgery. Hence, the reported findings would suggest that qualitative hBOLD CVR might usefully be employed in the presurgical work-up and follow-up of children with moyamoya.

iii) hBOLD CVR and cognitive testing –

The results in Chapter 8 were significantly confounded by the small study numbers and the different moyamoya arteriopathy groups. As in other published studies children with bilateral stroke had poorer neurocognitive outcomes than children with unilateral stroke. In addition this study demonstrated that moyamoya arteriopathy alone, in the absence of stroke, was associated with specific cognitive difficulties for example, left moyamoya and reduced VIQ. Hence there is a suggestion that the effects seen may be explained by chronic hypoperfusion, resulting in sub-clinical ischaemia and neuronal damage, and in some children possibly representing children at increased risk of acute AIS. The relationship between abnormal CVR and indices of executive function was unexpected (ie poor

indices of executive function associated with good CVR). Whether this can be explained by the effect of disease specific pathology eg NF1 and chromosomal disorders on CVR and cognitive outcome was not fully elucidated by this study and requires further exploration.

iv) hBOLD CVR and cortical thickness –

In adults with moyamoya and significant steal, and without cortical infarction cortical thinning is demonstrated on the side of the arterial disease, lending support to the suggestion that chronic hypoperfusion in the context of moyamoya may have significant effects detectable on measurement of the cortical rim, even in the absence of ischaemic parenchymal changes and/or clinical stroke.(Fierstra *et al.* 2010). The cortical rim in childhood thickens and thins in a regional, hemispheric and temporally specific manner. In Chapter 9, our study of hBOLD CVR and cortical thicknesss in children with moyamoya, it was interesting to note the association between the presence of hemispheric steal and reduced (within patient) comparative cortical thickness. Change in cortical thickness over time varied according to whether there was a history of revascularization surgery and also seemed to be influenced by age. Maximal increase in the cortical rim occurred in those with a history prior to the first included CVR, and thinning appeared to occur in those without intervention. The study was not powered to explore the relationship between these findings and cognitive outcomes. Further study of the changes in cortical thickness over time in children with moyamoya and

examination of the association between these findings and cognitive outcome would inform us of the impact and import of chronic hypoperfusion in moyamoya.

v) hBOLD CVR and Transient Cerebral Arteriopathy, as a model for Unilateral Non-progressive arteriopathy -

In Chapter 10 I demonstrated that there was no significant abnormality in CVR outside the region of infarction in children with a moderate-severe chronic unilateral non-progressive stenosis. Hence, there was no suggestion of chronic hypoperfusion as seen in moyamoya, in this group. This was further supported by the finding of deficits in cognitive outcome that were mostly explained by age of stroke and lesion volume. These findings are reassuring and do not support the need for revascularization surgery in this group.

10.4.Conclusion

The results of the thesis studies highlight the clinical need for a tool for assessment of ischaemic risk in this chronic, lifelong condition that is associated with recurrent strokes and poor outcome. The thesis has demonstrated that qualitative methods of hypercapnic challenge BOLD CVR are feasible, repeatable and the method of analysing the scans reliable. The latter in turn implied that the repeat parametric maps looked the same. Interobserver reliability of scoring was high, supporting it's utility in clinical practice. In this qualitative study we did not demonstrate repeatability or reliability of the BOLD CVR measures themselves. Qualitative

hBOLD CVR abnormalities were concordant with moyamoya laterality, and in unilateral moyamoya demonstrated tissue level microvascular dysfunction in the contralateral unaffected hemisphere. Qualitative hBOLD CVR abnormalities demonstrated concordance with clinically important manifestations of ischaemia including stroke, transient ischaemic attacks, cortical thinning and IQ. The lack of concordance with indices of executive function may have been related to underlying disease specifc pathology. In addition the lack of association between moderate to severe stenosis in Transient Cerebral Arteriopathy as a model for the Non-progressive, unilateral arteriopathies is reassuring.

There were however, a number of study limitations. This study was an unfunded clinical research study with prospective enrolment conducted over a period of 13 years. During this period the MRI scanner used changed from a 1.5T scanner to a 3.0T scanner. Despite this prolonged period of recruitment, the numbers were still small and few of the results were statistically significant. What the study was able to demonstrate was also limited by the challenge of obtaining reliable MR perfusion imaging or CO2 values in the group. Therefore I was unable to quantitatively validate the tool.

Although further longitudinal studies, in a larger cohort are required to confirm many of the findings, the results of this study of the use of qualitative hypercapnic challenge blood oxygen level dependent (BOLD) CVR brings us closer to answering the question asked by parents and carers of children with moyamoya, “When is my child going to have a stroke?” Increased ability to use this tool to

identify children on the ischaemic precipice may allow us to intervene prior to an event, and maybe be able to answer, “Never”.

11.5 Future Directions

1) Validation of BH hBOLD CVR technique – I plan to do this first by conducting BH CVR studies with end-tidal CO2 monitoring, controlled CO2 delivery studies, and MR perfusion studies (ASL) in healthy children (age 7 – 17 years).

Following this I plan to repeat the above CVR and perfusion studies in children with unilateral/probable moyamoya disease and unilateral NF1-moyamoya.

Protocol development with respect to the use of different methods of capnic challenge such as paced breathing; hyperventialation; pre and post-processing methodology such as semi-automated and manual motion corredtion; use of processing pipelines; reporting and integration of studies into clinical practice will be explored as part of this study.

2) GA hBOLD CVR studies – collaboration with anaesthetic and respiratory colleagues toward standardization of a CVR protocol with integrated CO2 and other physiologic monitoring is planned. MRI measures of CBF and perfusion are also to be added. This is essential to address the need to assess ischaemic risk in the developmentally non-compliant child, typically under the age of 7 years.

3) Future longitudinal studies – these are required to demonstrate whether qualitative and/or semi-quantiative assessment of ishaemic risk can be achieved using hBOLD MRI CVR. This will require funding.

References

(1988). "National Institutes of Health Consensus Development Conference Statement: neurofibromatosis. Bethesda, Md., USA, July 13-15, 1987." Neurofibromatosis **1**(3): 172-178.

(NIH), N. I. o. H. and C. f. D. C. a. P. (CDC). (2011). "CDC and NIH Update Guidelines to Protect Patients from Bloodstream Infections. Health care providers offered new recommendations for preventing the most deadly and costly healthcare-associated infections."

Abram, H. S., L. E. Knepper, V. S. Warty and M. J. Painter (1996). "Natural history, prognosis, and lipid abnormalities of idiopathic ischemic childhood stroke." J Child Neurol **11**(4): 276-282.

Adams, R. J., V. C. McKie, L. Hsu, B. Files, E. Vichinsky, C. Pegelow, M. Abboud, D. Gallagher, A. Kutlar, F. T. Nichols, D. R. Bonds and D. Brambilla (1998). "Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography." N Engl J Med **339**(1): 5-11.

Al-Yassin, A., D. E. Saunders, M. T. Mackay and V. Ganesan (2015). "Early-onset bilateral cerebral arteriopathies: Cohort study of phenotype and disease course." Neurology **85**(13): 1146-1153.

Amlie-Lefond, C., T. J. Bernard, G. Sebire, N. R. Friedman, G. L. Heyer, N. B. Lerner, G. deVeber and H. J. Fullerton (2009). "Predictors of cerebral arteriopathy in children with arterial ischemic stroke: results of the International Pediatric Stroke Study." Circulation **119**(10): 1417-1423.

Anderson, V. and C. Catroppa (2007). "Memory outcome at 5 years post-childhood traumatic brain injury." Brain Inj **21**(13-14): 1399-1409.

Anderson, V., C. Catroppa, S. Morse, F. Haritou and J. Rosenfeld (2000). "Recovery of intellectual ability following traumatic brain injury in childhood: impact of injury severity and age at injury." Pediatr Neurosurg **32**(6): 282-290.

Antachopoulos, C., T. Liakopoulou, F. Palamidou, D. Papathanassiou and S. Youroukos (2002). "Posterior cerebral artery occlusion associated with Mycoplasma pneumoniae infection." J Child Neurol **17**(1): 55-57.

Apostolova, I., D. Niedzielska, T. Derlin, E. J. Koziolek, H. Amthauer, B. Salmen, J. Pahnke, W. Brenner, V. F. Mautner and R. Buchert (2015). "Perfusion single photon emission computed tomography in a mouse model of neurofibromatosis type 1: towards a biomarker of neurologic deficits." J Cereb Blood Flow Metab **35**(8): 1304-1312.

Askalan, R., S. Laughlin, S. Mayank, A. Chan, D. MacGregor, M. Andrew, R. Curtis, B. Meaney and G. deVeber (2001). "Chickenpox and stroke in childhood: a study of frequency and causation." Stroke **32**(6): 1257-1262.

Attwell, D., A. M. Buchan, S. Charpak, M. Lauritzen, B. A. Macvicar and E. A. Newman (2010). "Glial and neuronal control of brain blood flow." Nature **468**(7321): 232-243.

Aviv, R. I., S. M. Benseler, G. DeVeber, E. D. Silverman, P. N. Tyrrell, L. M. Tsang and D. Armstrong (2007). "Angiography of primary central nervous system angiitis of childhood: conventional angiography versus magnetic resonance angiography at presentation." AJNR Am J Neuroradiol **28**(1): 9-15.

Aviv, R. I., S. M. Benseler, E. D. Silverman, P. N. Tyrrell, G. Deveber, L. M. Tsang and D. Armstrong (2006). "MR imaging and angiography of primary CNS vasculitis of childhood." AJNR Am J Neuroradiol **27**(1): 192-199.

Aydin, M., N. Kabakus, T. A. Balci and A. Ayar (2007). "Correlative study of the cognitive impairment, regional cerebral blood flow, and electroencephalogram abnormalities in children with Down's syndrome." Int J Neurosci **117**(3): 327-336.

Azevedo, E., B. Rosengarten, R. Santos, J. Freitas and M. Kaps (2007). "Interplay of cerebral autoregulation and neurovascular coupling evaluated by functional TCD in different orthostatic conditions." J Neurol **254**(2): 236-241.

Baba, T., K. Houkin and S. Kuroda (2008). "Novel epidemiological features of moyamoya disease." J. Neurol. Neurosurg. Psychiatry **79**(8): 900-904.

Balestri, P., G. Lucignani, A. Fois, L. Magliani, L. Calistri, C. Grana, R. M. Di Bartolo, D. Perani and F. Fazio (1994). "Cerebral glucose metabolism in neurofibromatosis type 1 assessed with [18F]-2-fluoro-2-deoxy-D-glucose and PET." J Neurol Neurosurg Psychiatry **57**(12): 1479-1483.

Banwell, B. L. and P. E. Anderson (2005). "The cognitive burden of multiple sclerosis in children." Neurology **64**(5): 891-894.

Baron, J. C., M. G. Bousser, A. Rey, A. Guillard, D. Comar and P. Castaigne (1981). "Reversal of focal "misery-perfusion syndrome" by extra-intracranial arterial bypass in hemodynamic cerebral ischemia. A case study with 15O positron emission tomography." Stroke **12**(4): 454-459.

Bartlett, J. W. and C. Frost (2008). "Reliability, repeatability and reproducibility: analysis of measurement errors in continuous variables." Ultrasound Obstet Gynecol **31**(4): 466-475.

Begni, B., L. Brighina, L. Fumagalli, S. Andreoni, E. Castelli, C. Francesconi, R. Del Bo, N. Bresolin and C. Ferrarese (2003). "Altered glutamate uptake in peripheral tissues from Down syndrome patients." Neurosci Lett **343**(2): 73-76.

Benseler, S. M., E. Silverman, R. I. Aviv, R. Schneider, D. Armstrong, P. N. Tyrrell and G. deVeber (2006). "Primary central nervous system vasculitis in children." Arthritis Rheum **54**(4): 1291-1297.

Bertolino, A., G. Blasi, G. Caforio, V. Latorre, M. De Candia, V. Rubino, J. H. Callicott, V. S. Mattay, A. Bellomo, T. Scarabino, D. R. Weinberger and M. Nardini (2004). "Functional lateralization of the sensorimotor cortex in patients with schizophrenia: effects of treatment with olanzapine." Biol Psychiatry **56**(3): 190-197.

Beslow, L. A. and L. C. Jordan (2010). "Pediatric stroke: the importance of cerebral arteriopathy and vascular malformations." Childs Nerv Syst **26**(10): 1263-1273.

Biagi, L., A. Abbruzzese, M. C. Bianchi, D. C. Alsop, G. A. Del and M. Tosetti (2007). "Age dependence of cerebral perfusion assessed by magnetic resonance continuous arterial spin labeling." J Magn Reson. Imaging **25**(4): 696-702.

Birn, R. M., Z. S. Saad and P. A. Bandettini (2001). "Spatial heterogeneity of the nonlinear dynamics in the FMRI BOLD response." Neuroimage **14**(4): 817-826.

Bland, J. M. and D. G. Altman (1986). "Statistical methods for assessing agreement between two methods of clinical measurement." Lancet **1**(8476): 307-310.

Blinder, P., A. Y. Shih, C. Rafie and D. Kleinfeld (2010). "Topological basis for the robust distribution of blood to rodent neocortex." Proc Natl Acad Sci U S A **107**(28): 12670-12675.

Blinder, P., P. S. Tsai, J. P. Kaufhold, P. M. Knutsen, H. Suhl and D. Kleinfeld (2013). "The cortical angiome: an interconnected vascular network with noncolumnar patterns of blood flow." Nat Neurosci **16**(7): 889-897.

Blockley, N. P., I. D. Driver, S. T. Francis, J. A. Fisher and P. A. Gowland (2011). "An improved method for acquiring cerebrovascular reactivity maps." Magn Reson Med **65**(5): 1278-1286.

Bowen, M., M. P. Marks and G. K. Steinberg (1998). "Neuropsychological recovery from childhood moyamoya disease." Brain Dev **20**(2): 119-123.

Bowers, D. C., Y. Liu, W. Leisenring, E. McNeil, M. Stovall, J. G. Gurney, L. L. Robison, R. J. Packer and K. C. Oeffinger (2006). "Late-occurring stroke among long-term survivors of childhood leukemia and brain tumors: a report from the Childhood Cancer Survivor Study." J Clin Oncol **24**(33): 5277-5282.

Bowers, D. C., D. E. McNeil, Y. Liu, Y. Yasui, M. Stovall, J. G. Gurney, M. M. Hudson, S. S. Donaldson, R. J. Packer, P. A. Mitby, C. E. Kasper, L. L. Robison and K. C. Oeffinger (2005). "Stroke as a late treatment effect of Hodgkin's Disease: a report from the Childhood Cancer Survivor Study." J Clin Oncol **23**(27): 6508-6515.

Boxerman, J. L., P. A. Bandettini, K. K. Kwong, J. R. Baker, T. L. Davis, B. R. Rosen and R. M. Weisskoff (1995). "The intravascular contribution to fMRI signal change: Monte Carlo modeling and diffusion-weighted studies in vivo." Magn Reson Med **34**(1): 4-10.

Braun, K. P., M. M. Bulder, S. Chabrier, F. J. Kirkham, C. S. Uiterwaal, M. Tardieu and G. Sebire (2009). "The course and outcome of unilateral intracranial arteriopathy in 79 children with ischaemic stroke." Brain **132**(Pt 2): 544-557.

Brierley, J. B. and B. J. Excell (1966). "The effects of profound systemic hypotension upon the brain of M. rhesus: physiological and pathological observations." Brain **89**(2): 269-298.

Bright, M. G. and K. Murphy (2013). "Reliable quantification of BOLD fMRI cerebrovascular reactivity despite poor breath-hold performance." Neuroimage **83**: 559-568.

Brown, R. T., P. C. Davis, R. Lambert, L. Hsu, K. Hopkins and J. Eckman (2000). "Neurocognitive functioning and magnetic resonance imaging in children with sickle cell disease." J Pediatr Psychol **25**(7): 503-513.

Buchert, R., D. von Borczyskowski, F. Wilke, M. Gronowsky, R. E. Friedrich, W. Brenner, J. Mester, M. Clausen and V. F. Mautner (2008). "Reduced thalamic 18F-flurodeoxyglucose retention in adults with neurofibromatosis type 1." Nucl Med Commun **29**(1): 17-26.

Burrows, P. E., R. L. Robertson, J. B. Mulliken, D. S. Beardsley, J. C. Chaloupka, R. A. Ezekowitz and R. M. Scott (1998). "Cerebral vasculopathy and neurologic sequelae in infants with cervicofacial hemangioma: report of eight patients." Radiology **207**(3): 601-607.

Buxton, R. B., K. Uludag, D. J. Dubowitz and T. T. Liu (2004). "Modeling the hemodynamic response to brain activation." Neuroimage **23 Suppl 1**: S220-233.

Caine, D. and J. D. Watson (2000). "Neuropsychological and neuropathological sequelae of cerebral anoxia: a critical review." J Int Neuropsychol Soc **6**(1): 86-99.

Calabrese, L. H. and J. A. Mallek (1988). "Primary angiitis of the central nervous system. Report of 8 new cases, review of the literature, and proposal for diagnostic criteria." Medicine (Baltimore) **67**(1): 20-39.

Calamante, F., V. Ganesan, F. J. Kirkham, W. Jan, W. K. Chong, D. G. Gadian and A. Connelly (2001). "MR Perfusion Imaging in Moyamoya Syndrome: Potential Implications for Clinical Evaluation of Occlusive Cerebrovascular Disease." Stroke **32**(12): 2810-2816.

Calviere, L., I. Catalaa, F. Marlats, A. Viguier, F. Bonneville, C. Cognard and V. Larrue (2010). "Correlation between cognitive impairment and cerebral hemodynamic disturbances on perfusion magnetic resonance imaging in European adults with moyamoya disease. Clinical article." J Neurosurg **113**(4): 753-759.

Caplan, L. R. and M. Hennerici (1998). "Impaired clearance of emboli (washout) is an important link between hypoperfusion, embolism, and ischemic stroke." Arch. Neurol **55**(11): 1475-1482.

Cappelli-Bigazzi, M., G. Santoro, C. Battaglia, M. T. Palladino, M. Carrozza, M. G. Russo, G. Pacileo and R. Calabro (2004). "Endothelial cell function in patients with Down's syndrome." Am J Cardiol **94**(3): 392-395.

Cellucci, T. and S. M. Benseler (2010). "Central nervous system vasculitis in children." Curr Opin Rheumatol **22**(5): 590-597.

Chabbert, V., J. P. Ranjeva, A. Sevely, S. Boetto, I. Berry and C. Manelfe (1998). "Diffusion- and magnetisation transfer-weighted MRI in childhood moya-moya." Neuroradiology **40**(4): 267-271.

Chabrier, S., G. Rodesch, P. Lasjaunias, M. Tardieu, P. Landrieu and G. Sebire (1998). "Transient cerebral arteriopathy: a disorder recognized by serial angiograms in children with stroke." J Child Neurol **13**(1): 27-32.

Chamoun, R. B., M. E. Mawad, W. E. Whitehead, T. G. Luerssen and A. Jea (2008). "Extracranial traumatic carotid artery dissections in children: a review of current diagnosis and treatment options." J Neurosurg Pediatr **2**(2): 101-108.

Chapman, C. A., D. P. Waber, N. Bassett, D. K. Urion and B. R. Korf (1996). "Neurobehavioral profiles of children with neurofibromatosis 1 referred for learning disabilities are sex-specific." Am J Med Genet **67**(2): 127-132.

Chen, J., D. J. Licht, S. E. Smith, S. C. Agner, S. Mason, S. Wang, D. W. Silvestre, J. A. Detre, R. A. Zimmerman, R. N. Ichord and J. Wang (2009). "Arterial spin labeling perfusion MRI in pediatric arterial ischemic stroke: initial experiences." J Magn Reson Imaging **29**(2): 282-290.

Chen, P. C., S. H. Yang, K. L. Chien, I. J. Tsai and M. F. Kuo (2014). "Epidemiology of moyamoya disease in Taiwan: a nationwide population-based study." Stroke **45**(5): 1258-1263.

Chi, T. P. L. K. J. (1975). "The pulmonary vascular bed in children with Down syndrome." J Pediatr **86**(4): 533-538.

Chiu, D., P. Shedden, P. Bratina and J. C. Grotta (1998). "Clinical features of moyamoya disease in the United States." Stroke **29**(7): 1347-1351.

Choi, J. U., D. S. Kim, E. Y. Kim and K. C. Lee (1997). "Natural history of moyamoya disease: comparison of activity of daily living in surgery and non surgery groups." Clin. Neurol Neurosurg **99 Suppl 2**: S11-S18.

Chugani, H. T., M. E. Phelps and J. C. Mazziotta (1987). "Positron emission tomography study of human brain functional development." Ann Neurol **22**(4): 487-497.

Chung, B. and V. Wong (2004). "Pediatric stroke among Hong Kong Chinese subjects." Pediatrics **114**(2): e206-e212.

Clifton, G. L., H. T. Haden, J. R. Taylor and M. Sobel (1988). "Cerebrovascular CO2 reactivity after carotid artery occlusion." J. Neurosurg **69**(1): 24-28.

Costa, R. M. and A. J. Silva (2002). "Molecular and cellular mechanisms underlying the cognitive deficits associated with neurofibromatosis 1." J Child Neurol **17**(8): 622-626; discussion 627-629, 646-651.

Cravioto, H. and I. Feigin (1959). "Noninfectious granulomatous angiitis with a predilection for the nervous system." Neurology **9**: 599-609.

Cushing, K. E., V. Ramesh, D. Gardner-Medwin, N. V. Todd, A. Gholkar, P. Baxter and P. D. Griffiths (2001). "Tethering of the vertebral artery in the congenital arcuate foramen of the atlas vertebra: a possible cause of vertebral artery dissection in children." Dev Med Child Neurol **43**(7): 491-496.

D'Esposito, M., L. Y. Deouell and A. Gazzaley (2003). "Alterations in the BOLD fMRI signal with ageing and disease: a challenge for neuroimaging." Nat. Rev. Neurosci **4**(11): 863-872.

Daginakatte, G. C., S. M. Gianino, N. W. Zhao, A. S. Parsadanian and D. H. Gutmann (2008). "Increased c-Jun-NH2-kinase signaling in neurofibromatosis-1 heterozygous microglia drives microglia activation and promotes optic glioma proliferation." Cancer Res **68**(24): 10358-10366.

Dahl, A., D. Russell, K. Rootwelt, R. Nyberg-Hansen and E. Kerty (1995). "Cerebral vasoreactivity assessed with transcranial Doppler and regional cerebral blood flow measurements. Dose, serum concentration, and time course of the response to acetazolamide." Stroke **26**(12): 2302-2306.

Dai, A. I., Z. A. Shaikh and M. E. Cohen (2000). "Early-onset Moyamoya syndrome in a patient with Down syndrome: case report and review of the literature." J. Child Neurol **15**(10): 696-699.

Dale, A. M., B. Fischl and M. I. Sereno (1999). "Cortical surface-based analysis. I. Segmentation and surface reconstruction." Neuroimage **9**(2): 179-194.

Danchaivijitr, N., T. C. Cox, D. E. Saunders and V. Ganesan (2006). "Evolution of cerebral arteriopathies in childhood arterial ischemic stroke." Ann. Neurol **59**(4): 620-626.

Dasgupta, B. and D. H. Gutmann (2003). "Neurofibromatosis 1: closing the GAP between mice and men." Curr Opin Genet Dev **13**(1): 20-27.

Dashdorj, N., et al. (2013). "Effects of subanesthetic dose of nitrous oxide on cerebral blood flow and metabolism: a multimodal magnetic resonance imaging study in healthy volunteers." Anesthesiology 118(3): 577-586.

Davis, S. M., R. H. Ackerman, J. A. Correia, N. M. Alpert, J. Chang, F. Buonanno, R. E. Kelley, B. Rosner and J. M. Taveras (1983). "Cerebral blood flow and cerebrovascular CO2 reactivity in stroke-age normal controls." Neurology **33**(4): 391-399.

Davis, T. L., K. K. Kwong, R. M. Weisskoff and B. R. Rosen (1998). "Calibrated functional MRI: mapping the dynamics of oxidative metabolism." Proc Natl. Acad. Sci U S A **95**(4): 1834-1839.

de Haas, E. C., S. F. Oosting, J. D. Lefrandt, B. H. Wolffenbuttel, D. T. Sleijfer and J. A. Gietema (2010). "The metabolic syndrome in cancer survivors." Lancet Oncol **11**(2): 193-203.

DeBaun, M. R., J. Schatz, M. J. Siegel, M. Koby, S. Craft, L. Resar, J. Y. Chu, G. Launius, M. Dadash-Zadeh, R. B. Lee and M. Noetzel (1998). "Cognitive screening examinations for silent cerebral infarcts in sickle cell disease." Neurology **50**(6): 1678-1682.

Degterev, A., Z. Huang, M. Boyce, Y. Li, P. Jagtap, N. Mizushima, G. D. Cuny, T. J. Mitchison, M. A. Moskowitz and J. Yuan (2005). "Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury." Nat Chem Biol **1**(2): 112-119.

del Zoppo, G. J. and J. M. Hallenbeck (2000). "Advances in the vascular pathophysiology of ischemic stroke." Thromb Res **98**(3): 73-81.

Derdeyn, C. P., A. Shaibani, C. J. Moran, D. T. Cross, 3rd, R. L. Grubb, Jr. and W. J. Powers (1999). "Lack of correlation between pattern of collateralization and misery perfusion in patients with carotid occlusion." Stroke **30**(5): 1025-1032.

Derdeyn, C. P., T. O. Videen, K. D. Yundt, S. M. Fritsch, D. A. Carpenter, R. L. Grubb and W. J. Powers (2002). "Variability of cerebral blood volume and oxygen extraction: stages of cerebral haemodynamic impairment revisited." Brain **125**(Pt 3): 595-607.

Detre, J. A. and J. Wang (2002). "Technical aspects and utility of fMRI using BOLD and ASL." Clin Neurophysiol **113**(5): 621-634.

Dlamini, N., J. L. Freeman, M. T. Mackay, C. Hawkins, M. Shroff, H. J. Fullerton and G. A. Deveber (2011). "Intracranial dissection mimicking transient cerebral arteriopathy in childhood arterial ischemic stroke." J Child Neurol **26**(9): 1203-1206.

Dobson, S. R., K. R. Holden, P. J. Nietert, J. K. Cure, J. H. Laver, D. Disco and M. R. Abboud (2002). "Moyamoya syndrome in childhood sickle cell disease: a predictive factor for recurrent cerebrovascular events." Blood **99**(9): 3144-3150.

Donahue, M. J., M. Ayad, R. Moore, O. M. van, R. Singer, P. Clemmons and M. Strother (2013). "Relationships between hypercarbic reactivity, cerebral blood flow, and arterial circulation times in patients with moyamoya disease." J. Magn Reson. Imaging **38**(5): 1129-1139.

Donnan, G., J. C. Baron, S. Davis and F. R. Sharp (2007). The Ischaemic Penumbra. New York, Informa Healthcare.

Drew, J. M., J. A. Scott and G. T. Chua (1993). "General case of the day. Moyamoya syndrome in a child with sickle cell disease." Radiographics **13**(2): 483-484.

Driver, I. D., J. Andoh, N. P. Blockley, S. T. Francis, P. A. Gowland and T. Paus (2015). "Hemispheric asymmetry in cerebrovascular reactivity of the human primary motor cortex: an in vivo study at 7 T." NMR Biomed **28**(5): 538-545.

Drolet, B. A., M. Dohil, M. R. Golomb, R. Wells, L. Murowski, J. Tamburro, J. Sty and S. F. Friedlander (2006). "Early stroke and cerebral vasculopathy in children with facial hemangiomas and PHACE association." Pediatrics **117**(3): 959-964.

Du, A. T., N. Schuff, J. H. Kramer, H. J. Rosen, M. L. Gorno-Tempini, K. Rankin, B. L. Miller and M. W. Weiner (2007). "Different regional patterns of cortical thinning in Alzheimer's disease and frontotemporal dementia." Brain **130**(Pt 4): 1159-1166.

Duat-Rodriguez, A., L. F. Carceller, M. A. Lopez Pino, F. C. Rodriguez and L. Gonzalez-Gutierrez-Solana (2014). "Neurofibromatosis type 1 associated with moyamoya syndrome in children." Pediatr. Neurol **50**(1): 96-98.

Duboc, V., P. Dufourcq, P. Blader and M. Roussigne (2015). "Asymmetry of the Brain: Development and Implications." Annu Rev Genet.

Duling, B. R. and R. M. Berne (1970). "Longitudinal gradients in periarteriolar oxygen tension. A possible mechanism for the participation of oxygen in local regulation of blood flow." Circ Res 27(5): 669-678.

Duning, T., S. Kloska, O. Steinstrater, H. Kugel, W. Heindel and S. Knecht (2005). "Dehydration confounds the assessment of brain atrophy." Neurology **64**(3): 548-550.

Dusser, A., F. Goutieres and J. Aicardi (1986). "Ischemic strokes in children." J Child Neurol **1**(2): 131-136.

Earley, C. J., S. J. Kittner, B. R. Feeser, J. Gardner, A. Epstein, M. A. Wozniak, R. Wityk, B. J. Stern, T. R. Price, R. F. Macko, C. Johnson, M. A. Sloan and D. Buchholz (1998). "Stroke in children and sickle-cell disease: Baltimore-Washington Cooperative Young Stroke Study." Neurology **51**(1): 169-176.

Elbers, J. and S. M. Benseler (2008). "Central nervous system vasculitis in children." Curr Opin Rheumatol **20**(1): 47-54.

Engidawork, E. and G. Lubec (2003). "Molecular changes in fetal Down syndrome brain." J Neurochem **84**(5): 895-904.

Erguven, M., M. Deveci and T. Turgut (2005). "Moyamoya disease and Down syndrome." Indian J. Pediatr **72**(8): 697-699.

Everts, R., K. Lidzba, M. Wilke, C. Kiefer, M. Mordasini, G. Schroth, W. Perrig and M. Steinlin (2009). "Strengthening of laterality of verbal and visuospatial functions during childhood and adolescence." Hum Brain Mapp **30**(2): 473-483.

Everts, R., K. Lidzba, M. Wilke, C. Kiefer, K. Wingeier, G. Schroth, W. Perrig and M. Steinlin (2010). "Lateralization of cognitive functions after stroke in childhood." Brain Inj **24**(6): 859-870.

Ezura, M., T. Yoshimoto, S. Fujiwara, A. Takahashi, R. Shirane and K. Mizoi (1995). "Clinical and angiographic follow-up of childhood-onset moyamoya disease." Childs Nerv. Syst **11**(10): 591-594.

Fairgrieve, R., D. A. Rowney, C. Karsli and B. Bissonnette (2003). "The effect of sevoflurane on cerebral blood flow velocity in children." Acta Anaesthesiol. Scand **47**(10): 1226-1230.

Fajardo, L. F. (2005). "The pathology of ionizing radiation as defined by morphologic patterns." Acta Oncol **44**(1): 13-22.

Fierstra, J., D. B. Maclean, J. A. Fisher, J. S. Han, D. M. Mandell, J. Conklin, J. Poublanc, A. P. Crawley, L. Regli, D. J. Mikulis and M. Tymianski (2011). "Surgical revascularization reverses cerebral cortical thinning in patients with severe cerebrovascular steno-occlusive disease." Stroke **42**(6): 1631-1637.

Fierstra, J., J. Poublanc, J. S. Han, F. Silver, M. Tymianski, A. P. Crawley, J. A. Fisher and D. J. Mikulis (2010). "Steal physiology is spatially associated with cortical thinning." J. Neurol. Neurosurg. Psychiatry **81**(3): 290-293.

Fischl, B., M. I. Sereno and A. M. Dale (1999). "Cortical surface-based analysis. II: Inflation, flattening, and a surface-based coordinate system." Neuroimage **9**(2): 195-207.

Fisher, M. (1997). "Characterizing the Target of Acute Stroke Therapy." Stroke **28**(4): 866-872.

Fleisher, G. R., B. E. Buck and D. Cornfeld (1978). "Primary intimal fibroplasia in a child with Down's syndrome." Am J Dis Child **132**(7): 700-703.

Foster, P. S., V. Drago, B. J. Ferguson and D. W. Harrison (2008). "Cerebral moderation of cardiovascular functioning: a functional cerebral systems perspective." Clin Neurophysiol **119**(12): 2846-2854.

Foster, P. S., T. Hubbard, R. C. Yung, B. J. Ferguson, V. Drago and D. W. Harrison (2013). "Cerebral asymmetry in the control of cardiovascular functioning: evidence from lateral vibrotactile stimulation." Laterality **18**(1): 108-119.

Fox, P. T. and M. E. Raichle (1986). "Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects." Proc Natl. Acad. Sci U S A **83**(4): 1140-1144.

Friston, K. J. (1998). "Imaging neuroscience: principles or maps?" Proc Natl Acad Sci U S A **95**(3): 796-802.

Frush, D. P., L. F. Donnelly and N. S. Rosen (2003). "Computed tomography and radiation risks: what pediatric health care providers should know." Pediatrics **112**(4): 951-957.

Fujimura, M., T. Kaneta, H. Shimizu and T. Tominaga (2009). "Cerebral ischemia owing to compression of the brain by swollen temporal muscle used for encephalo-myo-synangiosis in moyamoya disease." Neurosurg Rev **32**(2): 245-249; discussion 249.

Fujiwara, H., S. Momoshima and S. Kuribayashi (2005). "Leptomeningeal high signal intensity (ivy sign) on fluid-attenuated inversion-recovery (FLAIR) MR images in moyamoya disease." Eur. J. Radiol **55**(2): 224-230.

Fukuda, M. and T. S. Kuroda (2002). "Slac2-c (synaptotagmin-like protein homologue lacking C2 domains-c), a novel linker protein that interacts with Rab27, myosin Va/VIIa, and actin." J Biol Chem **277**(45): 43096-43103.

Fukui, M. (1997). "Guidelines for the diagnosis and treatment of spontaneous occlusion of the circle of Willis ('moyamoya' disease). Research Committee on Spontaneous Occlusion of the Circle of Willis (Moyamoya Disease) of the Ministry of Health and Welfare, Japan." Clin. Neurol. Neurosurg **99 Suppl 2**: S238-S240.

Fullerton, H. J., S. C. Johnston and W. S. Smith (2001). "Arterial dissection and stroke in children." Neurology **57**(7): 1155-1160.

Fullerton, H. J., Y. W. Wu, S. Sidney and S. C. Johnston (2007). "Risk of recurrent childhood arterial ischemic stroke in a population-based cohort: the importance of cerebrovascular imaging." Pediatrics **119**(3): 495-501.

Fullerton, H. J., Y. W. Wu, S. Zhao and S. C. Johnston (2003). "Risk of stroke in children: ethnic and gender disparities." Neurology **61**(2): 189-194.

Fung, L. W., D. Thompson and V. Ganesan (2005). "Revascularisation surgery for paediatric moyamoya: a review of the literature." Childs Nerv. Syst **21**(5): 358-364.

Furuya, K., N. Kawahara, A. Morita, T. Momose, S. Aoki and T. Kirino (2004). "Focal hyperperfusion after superficial temporal artery-middle cerebral artery anastomosis in a patient with moyamoya disease. Case report." J. Neurosurg **100**(1): 128-132.

Gadoth, N. and M. Hirsch (1980). "Primary and acquired forms of moyamoya syndrome. A review and three case reports." Isr. J. Med. Sci **16**(5): 370-377.

Ganesan, V., W. K. Chong, T. C. Cox, S. J. Chawda, M. Prengler and F. J. Kirkham (2002). "Posterior circulation stroke in childhood: risk factors and recurrence." Neurology **59**(10): 1552-1556.

Ganesan, V. and F. J. Kirkham (1997). "Noonan syndrome and moyamoya." Pediatr. Neurol **16**(3): 256-258.

Ganesan, V., V. Ng, W. K. Chong, F. J. Kirkham and A. Connelly (1999). "Lesion volume, lesion location, and outcome after middle cerebral artery territory stroke." Arch Dis Child **81**(4): 295-300.

Ganesan, V., M. Prengler, M. A. McShane, A. M. Wade and F. J. Kirkham (2003). "Investigation of risk factors in children with arterial ischemic stroke." Ann Neurol **53**(2): 167-173.

Ganesan, V., M. Prengler, A. Wade and F. J. Kirkham (2006). "Clinical and radiological recurrence after childhood arterial ischemic stroke." Circulation **114**(20): 2170-2177.

Ganesan, V., L. Savvy, W. K. Chong and F. J. Kirkham (1999). "Conventional cerebral angiography in children with ischemic stroke." Pediatr. Neurol **20**(1): 38-42.

Geranmayeh, F., R. J. Wise, R. Leech and K. Murphy (2015). "Measuring vascular reactivity with breath-holds after stroke: A method to aid interpretation of group-level BOLD signal changes in longitudinal fMRI studies." Hum. Brain Mapp **36**(5): 1755-1771.

Gibbs, J. M., R. J. Wise, K. L. Leenders and T. Jones (1984). "Evaluation of cerebral perfusion reserve in patients with carotid-artery occlusion." Lancet **1**(8372): 310-314.

Gioia, G. A., P. K. Isquith, S. C. Guy and L. Kenworthy (2000). "Behavior rating inventory of executive function." Child Neuropsychol **6**(3): 235-238.

Giroud, M., M. Lemesle, G. Madinier, E. Manceau, G. V. Osseby and R. Dumas (1997). "Stroke in children under 16 years of age. Clinical and etiological difference with adults." Acta Neurol. Scand **96**(6): 401-406.

Goghari, V. M., K. Rehm, C. S. Carter and A. W. MacDonald, 3rd (2007). "Regionally specific cortical thinning and gray matter abnormalities in the healthy relatives of schizophrenia patients." Cereb Cortex **17**(2): 415-424.

Gokcora, N., T. Atasever, N. I. Karabacak, G. Vural and K. Gucuyener (1999). "Tc-99m HMPAO brain perfusion imaging in young Down's syndrome patients." Brain Dev **21**(2): 107-112.

Golay, X., E. T. Petersen, I. Zimine and T. C. Lim (2007). "Arterial Spin Labeling: a one-stop-shop for measurement of brain perfusion in the clinical settings." Conf Proc IEEE Eng Med Biol Soc **2007**: 4320-4323.

Gold, J. I., C. B. Johnson, M. J. Treadwell, N. Hans and E. Vichinsky (2008). "Detection and assessment of stroke in patients with sickle cell disease: neuropsychological functioning and magnetic resonance imaging." Pediatr Hematol Oncol **25**(5): 409-421.

Golden, J. A. and B. T. Hyman (1994). "Development of the superior temporal neocortex is anomalous in trisomy 21." J Neuropathol Exp Neurol **53**(5): 513-520.

Goldenberg, N. A., T. J. Bernard, H. J. Fullerton, A. Gordon and G. deVeber (2009). "Antithrombotic treatments, outcomes, and prognostic factors in acute childhood-onset arterial ischaemic stroke: a multicentre, observational, cohort study." Lancet Neurol **8**(12): 1120-1127.

Goto, Y. and Y. Yonekawa (1992). "Worldwide distribution of moyamoya disease." Neurol Med Chir (Tokyo) **32**(12): 883-886.

Goto, Y. and Y. Yonekawa (1992). "Worldwide distribution of moyamoya disease." Neurol. Med. Chir (Tokyo) **32**(12): 883-886.

Graham, G. D., J. Zhong, O. A. Petroff, R. T. Constable, J. W. Prichard and J. C. Gore (1994). "BOLD MRI monitoring of changes in cerebral perfusion induced by acetazolamide and hypercarbia in the rat." Magn Reson. Med **31**(5): 557-560.

Graham, J. F. and A. Matoba (1997). "A survey of moyamoya disease in Hawaii." Clin. Neurol. Neurosurg **99 Suppl 2**: S31-S35.

Grant, D. A., C. Franzini, J. Wild, K. J. Eede and A. M. Walker (2005). "Autoregulation of the cerebral circulation during sleep in newborn lambs." J Physiol **564**(Pt 3): 923-930.

Green, D. M., C. L. Cox, L. Zhu, K. R. Krull, D. K. Srivastava, M. Stovall, V. G. Nolan, K. K. Ness, S. S. Donaldson, K. C. Oeffinger, L. R. Meacham, C. A. Sklar, G. T. Armstrong and L. L. Robison (2012). "Risk factors for obesity in adult survivors of childhood cancer: a report from the Childhood Cancer Survivor Study." J Clin Oncol **30**(3): 246-255.

Group, P. S. W. (2004). "Stroke in childhood: Clinical guidelines for diagnosis, management and rehabilitation." Royal College of Physicians of London.

Guadagno, J. V., P. S. Jones, F. I. Aigbirhio, D. Wang, T. D. Fryer, D. J. Day, N. Antoun, I. Nimmo-Smith, E. A. Warburton and J. C. Baron (2008). "Selective neuronal loss in rescued penumbra relates to initial hypoperfusion." Brain **131**(Pt 10): 2666-2678.

Guey, S., E. Tournier-Lasserve, D. Herve and M. Kossorotoff (2015). "Moyamoya disease and syndromes: from genetics to clinical management." Appl. Clin. Genet **8**: 49-68.

Guo, D. C., C. L. Papke, V. Tran-Fadulu, E. S. Regalado, N. Avidan, R. J. Johnson, D. H. Kim, H. Pannu, M. C. Willing, E. Sparks, R. E. Pyeritz, M. N. Singh, R. L. Dalman, J. C. Grotta, A. J. Marian, E. A. Boerwinkle, L. Q. Frazier, S. A. LeMaire, J. S. Coselli, A. L. Estrera, H. J. Safi, S. Veeraraghavan, D. M. Muzny, D. A. Wheeler, J. T. Willerson, R. K. Yu, S. S. Shete, S. E. Scherer, C. S. Raman, L. M. Buja and D. M. Milewicz (2009). "Mutations in smooth muscle alpha-actin (ACTA2) cause coronary artery disease, stroke, and Moyamoya disease, along with thoracic aortic disease." Am. J. Hum. Genet **84**(5): 617-627.

Gupta, A., J. L. Chazen, M. Hartman, D. Delgado, N. Anumula, H. Shao, M. Mazumdar, A. Z. Segal, H. Kamel, D. Leifer and P. C. Sanelli (2012). "Cerebrovascular reserve and stroke risk in patients with carotid stenosis or occlusion: a systematic review and meta-analysis." Stroke **43**(11): 2884-2891.

Gut, M., A. Urbanik, L. Forsberg, M. Binder, K. Rymarczyk, B. Sobiecka, J. Kozub and A. Grabowska (2007). "Brain correlates of right-handedness." Acta Neurobiol Exp (Wars) **67**(1): 43-51.

Guzman, R., M. Lee, A. Achrol, T. Bell-Stephens, M. Kelly, H. M. Do, M. P. Marks and G. K. Steinberg (2009). "Clinical outcome after 450 revascularization procedures for moyamoya disease. Clinical article." J Neurosurg **111**(5): 927-935.

Haaland, K. Y., C. L. Elsinger, A. R. Mayer, S. Durgerian and S. M. Rao (2004). "Motor sequence complexity and performing hand produce differential patterns of hemispheric lateralization." J Cogn Neurosci **16**(4): 621-636.

Hadjikhani, N., R. M. Joseph, J. Snyder and H. Tager-Flusberg (2006). "Anatomical differences in the mirror neuron system and social cognition network in autism." Cereb Cortex **16**(9): 1276-1282.

Hai, J., J. F. Wan, Q. Lin, F. Wang, L. Zhang, H. Li, L. Zhang, Y. Y. Chen and Y. Lu (2009). "Cognitive dysfunction induced by chronic cerebral hypoperfusion in a rat model associated with arteriovenous malformations." Brain Res **1301**: 80-88.

Halevy, A., O. Konen, R. Straussberg, S. D. Michowitz and A. Shuper (2008). "Vertebral artery dissection and posterior stroke in a child." J Child Neurol **23**(5): 568-571.

Hallemeier, C. L., K. M. Rich, R. L. Grubb, Jr., M. R. Chicoine, C. J. Moran, D. T. Cross, III, G. J. Zipfel, R. G. Dacey, Jr. and C. P. Derdeyn (2006). "Clinical features and outcome in North American adults with moyamoya phenomenon." Stroke **37**(6): 1490-1496.

Hamel, E. (2006). "Perivascular nerves and the regulation of cerebrovascular tone." J Appl Physiol (1985) **100**(3): 1059-1064.

Hamilton, S. J. and J. M. Friedman (2000). "Insights into the pathogenesis of neurofibromatosis 1 vasculopathy." Clin Genet **58**(5): 341-344.

Han, D. H., O. K. Kwon, B. J. Byun, B. Y. Choi, C. W. Choi, J. U. Choi, S. G. Choi, J. O. Doh, J. W. Han, S. Jung, S. D. Kang, D. J. Kim, H. I. Kim, H. D. Kim, M. C. Kim, S. C. Kim, S. C. Kim, Y. Kim, B. D. Kwun, B. G. Lee, Y. J. Lim, J. G. Moon, H. S. Park, M. S. Shin, J. H. Song, J. S. Suk and M. B. Yim (2000). "A co-operative study: clinical characteristics of 334 Korean patients with moyamoya disease treated at neurosurgical institutes (1976-1994). The Korean Society for Cerebrovascular Disease." Acta Neurochir. (Wien.) **142**(11): 1263-1273.

Han, D. H., D. H. Nam and C. W. Oh (1997). "Moyamoya disease in adults: characteristics of clinical presentation and outcome after encephalo-duro-arterio-synangiosis." Clin. Neurol. Neurosurg **99 Suppl 2**: S151-S155.

Han, J. S., D. J. Mikulis, A. Mardimae, A. Kassner, J. Poublanc, A. P. Crawley, G. A. deVeber, J. A. Fisher and W. J. Logan (2011). "Measurement of cerebrovascular reactivity in pediatric patients with cerebral vasculopathy using blood oxygen level-dependent MRI." Stroke **42**(5): 1261-1269.

Hariman, L. M., E. R. Griffith, A. L. Hurtig and M. T. Keehn (1991). "Functional outcomes of children with sickle-cell disease affected by stroke." Arch Phys Med Rehabil **72**(7): 498-502.

Harper, A. M. and H. I. Glass (1965). "Effect of alterations in the arterial carbon dioxide tension on the blood flow through the cerebral cortex at normal and low arterial blood pressures." J Neurol Neurosurg. Psychiatry **28**(5): 449-452.

Harrison, E. G., Jr. and L. J. McCormack (1971). "Pathologic classification of renal arterial disease in renovascular hypertension." Mayo Clin Proc **46**(3): 161-167.

Hayashi, K., N. Horie, K. Suyama and I. Nagata (2013). "An epidemiological survey of moyamoya disease, unilateral moyamoya disease and quasi-moyamoya disease in Japan." Clin Neurol Neurosurg **115**(7): 930-933.

Hayashi, T., R. Shirane, M. Fujimura and T. Tominaga (2010). "Postoperative neurological deterioration in pediatric moyamoya disease: watershed shift and hyperperfusion." J Neurosurg Pediatr **6**(1): 73-81.

He, X. and D. A. Yablonskiy (2007). "Quantitative BOLD: mapping of human cerebral deoxygenated blood volume and oxygen extraction fraction: default state." Magn Reson Med **57**(1): 115-126.

Herve, D., P. Touraine, A. Verloes, S. Miskinyte, V. Krivosic, D. Logeart, N. Alili, J. D. Laredo, A. Gaudric, E. Houdart, J. P. Metzger, E. Tournier-Lasserve and F. Woimant (2010). "A hereditary moyamoya syndrome with multisystemic manifestations." Neurology **75**(3): 259-264.

Heyer, G. L., M. M. Dowling, D. J. Licht, S. K. Tay, K. Morel, M. C. Garzon and P. Meyers (2008). "The cerebral vasculopathy of PHACES syndrome." Stroke **39**(2): 308-316.

Heyer, G. L., W. S. Millar, S. Ghatan and M. C. Garzon (2006). "The neurologic aspects of PHACE: case report and review of the literature." Pediatr. Neurol **35**(6): 419-424.

Heyn, C., J. Poublanc, A. Crawley, D. Mandell, J. S. Han, M. Tymianski, K. terBrugge, J. A. Fisher and D. J. Mikulis (2010). "Quantification of cerebrovascular reactivity by blood oxygen level-dependent MR imaging and correlation with conventional angiography in patients with Moyamoya disease." AJNR Am. J Neuroradiol **31**(5): 862-867.

Hill, J. M., A. M. Ades, S. K. McCune, N. Sahir, E. M. Moody, D. T. Abebe, L. S. Crnic and D. E. Brenneman (2003). "Vasoactive intestinal peptide in the brain of a mouse model for Down syndrome." Exp Neurol **183**(1): 56-65.

Hilz, M. J., M. Dutsch, K. Perrine, P. K. Nelson, U. Rauhut and O. Devinsky (2001). "Hemispheric influence on autonomic modulation and baroreflex sensitivity." Ann Neurol **49**(5): 575-584.

Hirotsune, N., T. Meguro, S. Kawada, H. Nakashima and T. Ohmoto (1997). "Long-term follow-up study of patients with unilateral moyamoya disease." Clin. Neurol. Neurosurg **99 Suppl 2**: S178-S181.

Hirsch, S., J. Reichold, M. Schneider, G. Szekely and B. Weber (2012). "Topology and hemodynamics of the cortical cerebrovascular system." J Cereb Blood Flow Metab **32**(6): 952-967.

Hoffman, H. J. (1997). "Moyamoya disease and syndrome." Clin. Neurol. Neurosurg **99 Suppl 2**: S39-S44.

Hogan, A. M., F. J. Kirkham, E. B. Isaacs, A. M. Wade and F. Vargha-Khadem (2005). "Intellectual decline in children with moyamoya and sickle cell anaemia." Dev Med Child Neurol **47**(12): 824-829.

Hoge, R. D., J. Atkinson, B. Gill, G. R. Crelier, S. Marrett and G. B. Pike (1999). "Investigation of BOLD signal dependence on cerebral blood flow and oxygen consumption: the deoxyhemoglobin dilution model." Magn Reson Med **42**(5): 849-863.

Holland, S. K., E. Plante, A. Weber Byars, R. H. Strawsburg, V. J. Schmithorst and W. S. Ball, Jr. (2001). "Normal fMRI brain activation patterns in children performing a verb generation task." Neuroimage **14**(4): 837-843.

Horner, C. H., H. A. Davies, J. Brown and M. G. Stewart (1996). "Reduction in numerical synapse density in chick (Gallus domesticus) dorsal hippocampus following transient cerebral ischaemia." Brain Res **735**(2): 354-359.

Horowitz, M., H. Yonas and A. L. Albright (1995). "Evaluation of cerebral blood flow and hemodynamic reserve in symptomatic moyamoya disease using stable Xenon-CT blood flow." Surg. Neurol **44**(3): 251-261.

Hossain, M. D. and A. S. Evers (1994). "Volatile anesthetic-induced efflux of calcium from IP3-gated stores in clonal (GH3) pituitary cells." Anesthesiology **80**(6): 1379-1389; discussion 1327A-1328A.

Hou, B. L., M. Bradbury, K. K. Peck, N. M. Petrovich, P. H. Gutin and A. I. Holodny (2006). "Effect of brain tumor neovasculature defined by rCBV on BOLD fMRI activation volume in the primary motor cortex." Neuroimage **32**(2): 489-497.

Houkin, K., H. Abe, T. Yoshimoto and A. Takahashi (1996). "Is "unilateral" moyamoya disease different from moyamoya disease?" J. Neurosurg **85**(5): 772-776.

Howard, R., P. Trend and R. W. Russell (1987). "Clinical features of ischemia in cerebral arterial border zones after periods of reduced cerebral blood flow." Arch Neurol **44**(9): 934-940.

Ikeda, E. and Y. Hosoda (1992). "Spontaneous occlusion of the circle of Willis (cerebrovascular moyamoya disease): with special reference to its clinicopathological identity." Brain Dev **14**(4): 251-253.

Ikezaki, K., T. Matsushima, Y. Kuwabara, S. O. Suzuki, T. Nomura and M. Fukui (1994). "Cerebral circulation and oxygen metabolism in childhood moyamoya disease: a perioperative positron emission tomography study." J Neurosurg **81**(6): 843-850.

Imaizumi, C., T. Imaizumi, M. Osawa, Y. Fukuyama and M. Takeshita (1999). "Serial intelligence test scores in pediatric moyamoya disease." Neuropediatrics **30**(6): 294-299.

Imaizumi, T., K. Hayashi, K. Saito, M. Osawa and Y. Fukuyama (1998). "Long-term outcomes of pediatric moyamoya disease monitored to adulthood." Pediatr Neurol **18**(4): 321-325.

Inzitari, D., M. Eliasziw, P. Gates, B. L. Sharpe, R. K. Chan, H. E. Meldrum and H. J. Barnett (2000). "The causes and risk of stroke in patients with asymptomatic internal-carotid-artery stenosis. North American Symptomatic Carotid Endarterectomy Trial Collaborators." N Engl J Med **342**(23): 1693-1700.

Ishii, R., S. Takeuchi, K. Ibayashi and R. Tanaka (1984). "Intelligence in children with moyamoya disease: evaluation after surgical treatments with special reference to changes in cerebral blood flow." Stroke **15**(5): 873-877.

Ishikawa, T., K. Houkin, H. Kamiyama and H. Abe (1997). "Effects of surgical revascularization on outcome of patients with pediatric moyamoya disease." Stroke **28**(6): 1170-1173.

Ishikawa, T., N. Tanaka, K. Houkin, S. Kuroda, H. Abe and K. Mitsumori (1998). "Regional cerebral blood flow in pediatric moyamoya disease: age-dependent decline in specific regions." Childs Nerv. Syst **14**(8): 366-371.

Ito, H., I. Kanno and H. Fukuda (2005). "Human cerebral circulation: positron emission tomography studies." Ann Nucl Med **19**(2): 65-74.

Ito, H., I. Yokoyama, H. Iida, T. Kinoshita, J. Hatazawa, E. Shimosegawa, T. Okudera and I. Kanno (2000). "Regional differences in cerebral vascular response to PaCO2 changes in humans measured by positron emission tomography." J Cereb Blood Flow Metab **20**(8): 1264-1270.

Ito, S., A. Mardimae, J. Han, J. Duffin, G. Wells, L. Fedorko, L. Minkovich, R. Katznelson, M. Meineri, T. Arenovich, C. Kessler and J. A. Fisher (2008). "Non-invasive prospective targeting of arterial P(CO2) in subjects at rest." J Physiol **586**(Pt 15): 3675-3682.

Jackman, K. and C. Iadecola (2015). "Neurovascular regulation in the ischemic brain." Antioxid Redox Signal **22**(2): 149-160.

Jackson, E. M., N. Lin, S. Manjila, R. M. Scott and E. R. Smith (2014). "Pial synangiosis in patients with moyamoya younger than 2 years of age." J. Neurosurg. Pediatr **13**(4): 420-425.

Jea, A., E. R. Smith, R. Robertson and R. M. Scott (2005). "Moyamoya syndrome associated with Down syndrome: outcome after surgical revascularization." Pediatrics **116**(5): e694-e701.

Jones, S. E., B. R. Buchbinder and I. Aharon (2000). "Three-dimensional mapping of cortical thickness using Laplace's equation." Hum Brain Mapp **11**(1): 12-32.

Joy, P., C. Roberts, K. North and M. de Silva (1995). "Neuropsychological function and MRI abnormalities in neurofibromatosis type 1." Dev Med Child Neurol **37**(10): 906-914.

Kainth, D. S., S. A. Chaudhry, H. S. Kainth, F. K. Suri and A. I. Qureshi (2013). "Prevalence and characteristics of concurrent down syndrome in patients with moyamoya disease." Neurosurgery **72**(2): 210-215.

Kaku, Y., K. Iihara, N. Nakajima, H. Kataoka, K. Fukuda, J. Masuoka, K. Fukushima, H. Iida and N. Hashimoto (2012). "Cerebral blood flow and metabolism of hyperperfusion after cerebral revascularization in patients with moyamoya disease." J. Cereb. Blood Flow Metab **32**(11): 2066-2075.

Kanno, I., K. Uemura, S. Higano, M. Murakami, H. Iida, S. Miura, F. Shishido, A. Inugami and I. Sayama (1988). "Oxygen extraction fraction at maximally vasodilated tissue in the ischemic brain estimated from the regional CO2 responsiveness measured by positron emission tomography." J. Cereb. Blood Flow Metab **8**(2): 227-235.

Kaplan, A. M., K. Chen, M. A. Lawson, D. L. Wodrich, C. T. Bonstelle and E. M. Reiman (1997). "Positron emission tomography in children with neurofibromatosis-1." J Child Neurol **12**(8): 499-506.

Karsli, C., I. Luginbuehl, M. Farrar and B. Bissonnette (2003). "Cerebrovascular carbon dioxide reactivity in children anaesthetized with propofol." Paediatr. Anaesth **13**(1): 26-31.

Karsli, C., E. Wilson-Smith, I. Luginbuehl and B. Bissonnette (2003). "The effect of nitrous oxide on cerebrovascular reactivity to carbon dioxide in children during propofol anesthesia." Anesth. Analg **97**(3): 694-698.

Kasner, S. E., M. I. Chimowitz, M. J. Lynn, H. Howlett-Smith, B. J. Stern, V. S. Hertzberg, M. R. Frankel, S. R. Levine, S. Chaturvedi, C. G. Benesch, C. A. Sila, T. G. Jovin, J. G. Romano, H. J. Cloft and I. Warfarin Aspirin Symptomatic Intracranial Disease Trial (2006). "Predictors of ischemic stroke in the territory of a symptomatic intracranial arterial stenosis." Circulation **113**(4): 555-563.

Kassner, A., J. D. Winter, J. Poublanc, D. J. Mikulis and A. P. Crawley (2010). "Blood-oxygen level dependent MRI measures of cerebrovascular reactivity using a controlled respiratory challenge: reproducibility and gender differences." J. Magn Reson. Imaging **31**(2): 298-304.

Kastrup, A., T. Q. Li, A. Takahashi, G. H. Glover and M. E. Moseley (1998). "Functional magnetic resonance imaging of regional cerebral blood oxygenation changes during breath holding." Stroke **29**(12): 2641-2645.

Kawadler, J. M., J. D. Clayden, F. J. Kirkham, T. C. Cox, D. E. Saunders and C. A. Clark (2013). "Subcortical and cerebellar volumetric deficits in paediatric sickle cell anaemia." Br J Haematol **163**(3): 373-376.

Kawano, T., M. Fukui, N. Hashimoto and Y. Yonekawa (1994). "Follow-up study of patients with "unilateral" moyamoya disease." Neurol. Med. Chir (Tokyo) **34**(11): 744-747.

Kelly, M. E., T. E. Bell-Stephens, M. P. Marks, H. M. Do and G. K. Steinberg (2006). "Progression of unilateral moyamoya disease: A clinical series." Cerebrovasc. Dis **22**(2-3): 109-115.

Kennedy, C. and L. Sokoloff (1957). "An adaptation of the nitrous oxide method to the study of the cerebral circulation in children; normal values for cerebral blood flow and cerebral metabolic rate in childhood." J Clin Invest **36**(7): 1130-1137.

Kety, S. S. And C. F. Schmidt (1948a). "The Effects Of Altered Arterial Tensions of Carbon Dioxide and Oxygen on Cerebral Blood Flow and Cerebral Oxygen Consumption Of Normal Young Men." J Clin Invest **27**(4): 484-492.

Kety, S. S. and C. F. Schmidt (1948b). "The nitrous oxide method for the quantitative determination of cerebral blood flow in man: theory, procedure and normal values." J Clin Invest 27(4): 476-483.

Khan, N., B. Schuknecht, E. Boltshauser, A. Capone, A. Buck, H. G. Imhof and Y. Yonekawa (2003). "Moyamoya disease and Moyamoya syndrome: experience in Europe; choice of revascularisation procedures." Acta Neurochir (Wien) **145**(12): 1061-1071; discussion 1071.

Kim, J. E., C. W. Oh, O. K. Kwon, S. Q. Park, S. E. Kim and Y. K. Kim (2008). "Transient hyperperfusion after superficial temporal artery/middle cerebral artery bypass surgery as a possible cause of postoperative transient neurological deterioration." Cerebrovasc. Dis **25**(6): 580-586.

Kim, S. H., J. U. Choi, K. H. Yang, T. G. Kim and D. S. Kim (2005). "Risk factors for postoperative ischemic complications in patients with moyamoya disease." J. Neurosurg **103**(5 Suppl): 433-438.

Kim, S. K., H. J. Seol, B. K. Cho, Y. S. Hwang, D. S. Lee and K. C. Wang (2004). "Moyamoya disease among young patients: its aggressive clinical course and the role of active surgical treatment." Neurosurgery **54**(4): 840-844.

King, J. A., D. Armstrong, S. Vachhrajani and P. B. Dirks (2010). "Relative contributions of the middle meningeal artery and superficial temporal artery in revascularization surgery for moyamoya syndrome in children: the results of superselective angiography." J. Neurosurg. Pediatr **5**(2): 184-189.

Kirk, G. R., M. R. Haynes, S. Palasis, C. Brown, T. G. Burns, M. McCormick and R. A. Jones (2009). "Regionally specific cortical thinning in children with sickle cell disease." Cereb Cortex **19**(7): 1549-1556.

Kirkham, F. J., F. Calamante, M. Bynevelt, D. G. Gadian, J. P. Evans, T. C. Cox and A. Connelly (2001). "Perfusion magnetic resonance abnormalities in patients with sickle cell disease." Ann Neurol **49**(4): 477-485.

Kirkham, F. J. and M. R. DeBaun (2004). "Stroke in Children with Sickle Cell Disease." Curr. Treat. Options. Neurol **6**(5): 357-375.

Kirkham, F. J. and A. M. Hogan (2004). "Risk factors for arterial ischemic stroke in childhood." CNS. Spectr **9**(6): 451-464.

Kirkham, F. J., T. S. Padayachee, S. Parsons, L. S. Seargeant, F. R. House and R. G. Gosling (1986). "Transcranial measurement of blood velocities in the basal cerebral arteries using pulsed Doppler ultrasound: velocity as an index of flow." Ultrasound Med Biol **12**(1): 15-21.

Kirton, A., M. Crone, S. Benseler, A. Mineyko, D. Armstrong, A. Wade, G. Sebire, A. M. Crous-Tsanaclis and G. deVeber (2013). "Fibromuscular dysplasia and childhood stroke." Brain **136**(Pt 6): 1846-1856.

Kirton, A., M. Tan, D. Mikulis, J. Fisher, J. Han, G. deVeber and B. Rubin (2008). "Recurrent reversible coma in an adolescent." Lancet Neurol **7**(1): 110-112.

Kitchen, L., R. Westmacott, S. Friefeld, D. MacGregor, R. Curtis, A. Allen, I. Yau, R. Askalan, M. Moharir, T. Domi and G. deVeber (2012). "The pediatric stroke outcome measure: a validation and reliability study." Stroke **43**(6): 1602-1608.

Kleinloog, R., L. Regli, G. J. Rinkel and C. J. Klijn (2012). "Regional differences in incidence and patient characteristics of moyamoya disease: a systematic review." J. Neurol. Neurosurg. Psychiatry **83**(5): 531-536.

Klingebiel, R., G. Benndorf, M. Schmitt, A. von Moers and R. Lehmann (2002). "Large cerebral vessel occlusive disease in Lyme neuroborreliosis." Neuropediatrics **33**(1): 37-40.

Koehler, R. C., R. J. Roman and D. R. Harder (2009). "Astrocytes and the regulation of cerebral blood flow." Trends Neurosci **32**(3): 160-169.

Kontras, S. B. and J. G. Bodenbender (1966). "Abnormal capillary morphology in congenital heart disease." Pediatrics **37**(2): 316-322.

Koss, M., R. M. Scott, M. B. Irons, E. R. Smith and N. J. Ullrich (2013). "Moyamoya syndrome associated with neurofibromatosis Type 1: perioperative and long-term outcome after surgical revascularization." J. Neurosurg. Pediatr **11**(4): 417-425.

Kossorotoff, M., D. Herve, F. Toulgoat, C. Renaud, E. Presles, H. Chabriat and S. Chabrier (2012). "Paediatric moyamoya in mainland France: a comprehensive survey of academic neuropaediatric centres." Cerebrovasc. Dis **33**(1): 76-79.

Kostovic, I. and N. Jovanov-Milosevic (2006). "The development of cerebral connections during the first 20-45 weeks' gestation." Semin Fetal Neonatal Med **11**(6): 415-422.

Kostovic, I., M. Judas, M. Rados and P. Hrabac (2002). "Laminar organization of the human fetal cerebrum revealed by histochemical markers and magnetic resonance imaging." Cereb Cortex **12**(5): 536-544.

Kraemer, M. and P. Berlit (2010). "Primary central nervous system vasculitis and moyamoya disease: similarities and differences." J. Neurol **257**(5): 816-819.

Kraemer, M., W. Heienbrok and P. Berlit (2008). "Moyamoya disease in Europeans." Stroke **39**(12): 3193-3200.

Krapf, H., B. Widder and M. Skalej (1998). "Small rosarylike infarctions in the centrum ovale suggest hemodynamic failure." AJNR Am J Neuroradiol **19**(8): 1479-1484.

Krischek, B., H. Kasuya, N. Khan, M. Tatagiba, C. Roder and M. Kraemer (2011). "Genetic and clinical characteristics of Moyamoya disease in Europeans." Acta Neurochir. Suppl **112**: 31-34.

Kronenburg, A., K. P. Braun, A. van der Zwan and C. J. Klijn (2014). "Recent advances in moyamoya disease: pathophysiology and treatment." Curr. Neurol. Neurosci. Rep **14**(1): 423.

Kuriyama, S., Y. Kusaka, M. Fujimura, K. Wakai, A. Tamakoshi, S. Hashimoto, I. Tsuji, Y. Inaba and T. Yoshimoto (2008). "Prevalence and clinicoepidemiological features of moyamoya disease in Japan: findings from a nationwide epidemiological survey." Stroke **39**(1): 42-47.

Kuroda, S., N. Hashimoto, T. Yoshimoto and Y. Iwasaki (2007). "Radiological findings, clinical course, and outcome in asymptomatic moyamoya disease: results of multicenter survey in Japan." Stroke **38**(5): 1430-1435.

Kuroda, S. and K. Houkin (2008). "Moyamoya disease: current concepts and future perspectives." Lancet Neurol **7**(11): 1056-1066.

Kuroda, S. and K. Houkin (2012). "Bypass surgery for moyamoya disease: concept and essence of sugical techniques." Neurol. Med. Chir (Tokyo) **52**(5): 287-294.

Kuroda, S., K. Houkin, H. Kamiyama, H. Abe and K. Mitsumori (1995). "Regional cerebral hemodynamics in childhood moyamoya disease." Childs Nerv. Syst **11**(10): 584-590.

Kuroda, S., K. Houkin, M. Nunomura and H. Abe (2000). "Frontal lobe infarction due to hemodynamic change after surgical revascularization in moyamoya disease--two case reports." Neurol Med Chir (Tokyo) **40**(6): 315-320.

Kuroda, S., T. Ishikawa, K. Houkin, R. Nanba, M. Hokari and Y. Iwasaki (2005). "Incidence and clinical features of disease progression in adult moyamoya disease." Stroke **36**(10): 2148-2153.

Kuroda, S., H. Kamiyama, M. Isobe, K. Houkin, H. Abe and K. Mitsumori (1995). "Cerebral hemodynamics and "re-build-up" phenomenon on electroencephalogram in children with moyamoya disease." Childs Nerv. Syst **11**(4): 214-219.

Kurokawa, T., S. Tomita, K. Ueda, O. Narazaki, T. Hanai, K. Hasuo, T. Matsushima and K. Kitamura (1985). "Prognosis of occlusive disease of the circle of Willis (moyamoya disease) in children." Pediatr Neurol **1**(5): 274-277.

Kurumatani, T., T. Kudo, Y. Ikura and M. Takeda (1998). "White matter changes in the gerbil brain under chronic cerebral hypoperfusion." Stroke **29**(5): 1058-1062.

Kuwabara, Y., Y. Ichiya, M. Otsuka, T. Tahara, R. Gunasekera, K. Hasuo, K. Masuda, T. Matsushima and M. Fukui (1990). "Cerebral hemodynamic change in the child and the adult with moyamoya disease." Stroke **21**(2): 272-277.

Kuwabara, Y., Y. Ichiya, M. Sasaki, T. Yoshida, K. Masuda, K. Ikezaki, T. Matsushima and M. Fukui (1997). "Cerebral hemodynamics and metabolism in moyamoya disease--a positron emission tomography study." Clin. Neurol Neurosurg **99 Suppl 2**: S74-S78.

Kuwabara, Y., Y. Ichiya, M. Sasaki, T. Yoshida, K. Masuda, T. Matsushima and M. Fukui (1997). "Response to hypercapnia in moyamoya disease. Cerebrovascular response to hypercapnia in pediatric and adult patients with moyamoya disease." Stroke **28**(4): 701-707.

Lai, F. and R. S. Williams (1989). "A prospective study of Alzheimer disease in Down syndrome." Arch Neurol **46**(8): 849-853.

Landis, J. R. and G. G. Koch (1977). "The measurement of observer agreement for categorical data." Biometrics **33**(1): 159-174.

Lanthier, S., A. Lortie, J. Michaud, R. Laxer, V. Jay and G. deVeber (2001). "Isolated angiitis of the CNS in children." Neurology **56**(7): 837-842.

Lee, J. Y., K. S. Kim, S. K. Song, S. H. Ahn, H. S. Nam and J. H. Heo (2005). "Atypical territorial infarction in moyamoya disease." Neurology **65**(12): E28.

Lee, J. Y., J. H. Phi, K. C. Wang, B. K. Cho, M. S. Shin and S. K. Kim (2011). "Neurocognitive profiles of children with moyamoya disease before and after surgical intervention." Cerebrovasc. Dis **31**(3): 230-237.

Lee, M., G. Zaharchuk, R. Guzman, A. Achrol, T. Bell-Stephens and G. K. Steinberg (2009). "Quantitative hemodynamic studies in moyamoya disease: a review." Neurosurg. Focus **26**(4): E5.

Lenneberg, E. H. (1967). Biological Foundations of Language. New York, Wiley.

Lenroot, R. K., N. Gogtay, D. K. Greenstein, E. M. Wells, G. L. Wallace, L. S. Clasen, J. D. Blumenthal, J. Lerch, A. P. Zijdenbos, A. C. Evans, P. M. Thompson and J. N. Giedd (2007). "Sexual dimorphism of brain developmental trajectories during childhood and adolescence." Neuroimage **36**(4): 1065-1073.

Leon, J. E. and B. Bissonnette (1991). "Cerebrovascular responses to carbon dioxide in children anaesthetized with halothane and isoflurane." Can. J. Anaesth **38**(7): 817-825.

Leung, J., J. A. Kim and A. Kassner (2015). "Reproducibility of cerebrovascular reactivity measures in children using BOLD MRI." J Magn Reson Imaging.

Lev, M. H., J. Farkas, J. J. Gemmete, S. T. Hossain, G. J. Hunter, W. J. Koroshetz and R. G. Gonzalez (1999). "Acute stroke: improved nonenhanced CT detection--benefits of soft-copy interpretation by using variable window width and center level settings." Radiology **213**(1): 150-155.

Liebeskind, D. S., G. A. Cotsonis, J. L. Saver, M. J. Lynn, T. N. Turan, H. J. Cloft, M. I. Chimowitz and I. Warfarin-Aspirin Symptomatic Intracranial Disease (2011). "Collaterals dramatically alter stroke risk in intracranial atherosclerosis." Ann Neurol **69**(6): 963-974.

Lin, N., L. Baird, M. Koss, K. E. Kopecky, E. Gone, N. J. Ullrich, R. M. Scott and E. R. Smith (2011). "Discovery of asymptomatic moyamoya arteriopathy in pediatric syndromic populations: radiographic and clinical progression." Neurosurg Focus **31**(6): E6.

Lin, N., L. Baird, M. Koss, K. E. Kopecky, E. Gone, N. J. Ullrich, R. M. Scott and E. R. Smith (2011). "Discovery of asymptomatic moyamoya arteriopathy in pediatric syndromic populations: radiographic and clinical progression." Neurosurg. Focus **31**(6): E6.

Liu, M. and L. Zhou (2014). "Cerebrovascular reserve may be a more accurate predictor of stroke than degree of ICA or MCA stenosis." Med Sci Monit **20**: 2082-2087.

Liu, W., H. Hashikata, K. Inoue, N. Matsuura, Y. Mineharu, H. Kobayashi, K. Kikuta, Y. Takagi, T. Hitomi, B. Krischek, L. P. Zou, F. Fang, R. Herzig, J. E. Kim, H. S. Kang, C. W. Oh, D. A. Tregouet, N. Hashimoto and A. Koizumi (2010). "A rare Asian founder polymorphism of Raptor may explain the high prevalence of Moyamoya disease among East Asians and its low prevalence among Caucasians." Environ. Health Prev. Med **15**(2): 94-104.

Liu, W., D. Morito, S. Takashima, Y. Mineharu, H. Kobayashi, T. Hitomi, H. Hashikata, N. Matsuura, S. Yamazaki, A. Toyoda, K. Kikuta, Y. Takagi, K. H. Harada, A. Fujiyama, R. Herzig, B. Krischek, L. Zou,

J. E. Kim, M. Kitakaze, S. Miyamoto, K. Nagata, N. Hashimoto and A. Koizumi (2011). "Identification of RNF213 as a susceptibility gene for moyamoya disease and its possible role in vascular development." PLoS. One **6**(7): e22542.

Liu, W., S. T. Senevirathna, T. Hitomi, H. Kobayashi, C. Roder, R. Herzig, M. Kraemer, M. H. Voormolen, P. Cahova, B. Krischek and A. Koizumi (2013). "Genomewide association study identifies no major founder variant in Caucasian moyamoya disease." J. Genet **92**(3): 605-609.

Lo, W., K. Zamel, K. Ponnappa, A. Allen, D. Chisolm, M. Tang, B. Kerlin and K. O. Yeates (2008). "The cost of pediatric stroke care and rehabilitation." Stroke **39**(1): 161-165.

Logothetis, N. K. and B. A. Wandell (2004). "Interpreting the BOLD signal." Annu. Rev. Physiol **66**: 735-769.

Lok, J., P. Gupta, S. Guo, W. J. Kim, M. J. Whalen, K. van Leyen and E. H. Lo (2007). "Cell-cell signaling in the neurovascular unit." Neurochem Res **32**(12): 2032-2045.

Lubec, B., B. C. Yoo, M. Dierssen, N. Balic and G. Lubec (2001). "Down syndrome patients start early prenatal life with normal cholinergic, monoaminergic and serotoninergic innervation." J Neural Transm Suppl(61): 303-310.

Luders, E., K. L. Narr, P. M. Thompson, D. E. Rex, L. Jancke and A. W. Toga (2006). "Hemispheric asymmetries in cortical thickness." Cereb Cortex **16**(8): 1232-1238.

Lutterman, J., M. Scott, R. Nass and T. Geva (1998). "Moyamoya syndrome associated with congenital heart disease." Pediatrics **101**(1 Pt 1): 57-60.

Lynch, J. K., D. G. Hirtz, G. DeVeber and K. B. Nelson (2002). "Report of the National Institute of Neurological Disorders and Stroke workshop on perinatal and childhood stroke." Pediatrics **109**(1): 116-123.

Mackay, M. T., M. Wiznitzer, S. L. Benedict, K. J. Lee, G. A. Deveber, V. Ganesan and G. International Pediatric Stroke Study (2011). "Arterial ischemic stroke risk factors: the International Pediatric Stroke Study." Ann Neurol **69**(1): 130-140.

Maeda, M. and C. Tsuchida (1999). ""Ivy sign" on fluid-attenuated inversion-recovery images in childhood moyamoya disease." AJNR Am. J. Neuroradiol **20**(10): 1836-1838.

Magon, S., G. Basso, P. Farace, G. K. Ricciardi, A. Beltramello and A. Sbarbati (2009). "Reproducibility of BOLD signal change induced by breath holding." Neuroimage **45**(3): 702-712.

Maki, Y., Y. Nakada, T. Nose and Y. Yoshii (1976). "Clinical and radioisotopic follow-up study of 'Moyamoya'." Childs Brain **2**(4): 257-271.

Mandell, D. M., J. S. Han, J. Poublanc, A. P. Crawley, J. Fierstra, M. Tymianski, J. A. Fisher and D. J. Mikulis (2011). "Quantitative measurement of cerebrovascular reactivity by blood oxygen level-dependent MR imaging in patients with intracranial stenosis: preoperative cerebrovascular reactivity predicts the effect of extracranial-intracranial bypass surgery." AJNR Am. J. Neuroradiol **32**(4): 721-727.

Mandell, D. M., J. S. Han, J. Poublanc, A. P. Crawley, J. A. Stainsby, J. A. Fisher and D. J. Mikulis (2008). "Mapping cerebrovascular reactivity using blood oxygen level-dependent MRI in Patients

with arterial steno-occlusive disease: comparison with arterial spin labeling MRI." Stroke **39**(7): 2021-2028.

Mandeville, J. B., J. J. Marota, C. Ayata, G. Zaharchuk, M. A. Moskowitz, B. R. Rosen and R. M. Weisskoff (1999). "Evidence of a cerebrovascular postarteriole windkessel with delayed compliance." J Cereb Blood Flow Metab **19**(6): 679-689.

Maneru, C., C. Junque, F. Botet, M. Tallada and J. Guardia (2001). "Neuropsychological long-term sequelae of perinatal asphyxia." Brain Inj **15**(12): 1029-1039.

Markus, H. and M. Cullinane (2001). "Severely impaired cerebrovascular reactivity predicts stroke and TIA risk in patients with carotid artery stenosis and occlusion." Brain **124**(Pt 3): 457-467.

Markus, H. S. and M. J. Harrison (1992). "Estimation of cerebrovascular reactivity using transcranial Doppler, including the use of breath-holding as the vasodilatory stimulus." Stroke **23**(5): 668-673.

Marshall, R. S. (2012). "Effects of altered cerebral hemodynamics on cognitive function." J Alzheimers Dis **32**(3): 633-642.

Marshall, R. S., J. R. Festa, Y. K. Cheung, R. Chen, M. A. Pavol, C. P. Derdeyn, W. R. Clarke, T. O. Videen, R. L. Grubb, H. P. Adams, W. J. Powers and R. M. Lazar (2012). "Cerebral hemodynamics and cognitive impairment: baseline data from the RECON trial." Neurology **78**(4): 250-255.

Maruyama, K., S. Ikeda and N. Yanagisawa (1995). "[Correlative study of the brain CT and clinical features of patients with Down's syndrome in three clinical stages of Alzheimer type dementia]." Rinsho Shinkeigaku **35**(7): 775-780.

Masuda, J., C. Yutani, J. Ogata, Y. Kuriyama and T. Yamaguchi (1994). "Atheromatous embolism in the brain: a clinicopathologic analysis of 15 autopsy cases." Neurology **44**(7): 1231-1237.

Matsuki, Y., M. Kawakami, T. Ishizuka, Y. Kawaguchi, T. Hidaka, K. Suzuki and H. Nakamura (1997). "SLE and Sjogren's syndrome associated with unilateral moyamoya vessels in cerebral arteries." Scand. J. Rheumatol **26**(5): 392-394.

Matsushima, T., M. Fukui, K. Kitamura, K. Hasuo, Y. Kuwabara and T. Kurokawa (1990). "Encephalo-duro-arterio-synangiosis in children with moyamoya disease." Acta Neurochir. (Wien.) **104**(3-4): 96-102.

Matsushima, T., T. Inoue, S. O. Suzuki, K. Fujii, M. Fukui and K. Hasuo (1992). "Surgical treatment of moyamoya disease in pediatric patients--comparison between the results of indirect and direct revascularization procedures." Neurosurgery **31**(3): 401-405.

Matsushima, Y., M. Aoyagi, H. Masaoka, R. Suzuki and K. Ohno (1990). "Mental outcome following encephaloduroarteriosynangiosis in children with moyamoya disease with the onset earlier than 5 years of age." Childs Nerv. Syst **6**(8): 440-443.

Matsushima, Y., M. Aoyagi, T. Nariai, Y. Takada and K. Hirakawa (1997). "Long-term intelligence outcome of post-encephalo-duro-arterio-synangiosis childhood moyamoya patients." Clin. Neurol. Neurosurg **99 Suppl 2**: S147-S150.

Matsushima, Y., M. Aoyagi, Y. Niimi, H. Masaoka and K. Ohno (1990). "Symptoms and their pattern of progression in childhood moyamoya disease." Brain Dev **12**(6): 784-789.

Matta, B. F., K. J. Heath, K. Tipping and A. C. Summors (1999). "Direct cerebral vasodilatory effects of sevoflurane and isoflurane." Anesthesiology **91**(3): 677-680.

McAuley, D. J., K. Poskitt and P. Steinbok (2004). "Predicting stroke risk in pediatric moyamoya disease with xenon-enhanced computed tomography." Neurosurgery **55**(2): 327-332.

Mikulis, D. J., G. Krolczyk, H. Desal, W. Logan, G. DeVeber, P. Dirks, M. Tymianski, A. Crawley, A. Vesely, A. Kassner, D. Preiss, R. Somogyi and J. A. Fisher (2005). "Preoperative and postoperative mapping of cerebrovascular reactivity in moyamoya disease by using blood oxygen level-dependent magnetic resonance imaging." J Neurosurg **103**(2): 347-355.

Milewicz, D. M., C. S. Kwartler, C. L. Papke, E. S. Regalado, J. Cao and A. J. Reid (2010). "Genetic variants promoting smooth muscle cell proliferation can result in diffuse and diverse vascular diseases: evidence for a hyperplastic vasculomyopathy." Genet. Med **12**(4): 196-203.

Milewicz, D. M., J. R. Ostergaard, L. M. Ala-Kokko, N. Khan, D. K. Grange, R. Mendoza-Londono, T. J. Bradley, A. H. Olney, L. Ades, J. F. Maher, D. Guo, L. M. Buja, D. Kim, J. C. Hyland and E. S. Regalado (2010). "De novo ACTA2 mutation causes a novel syndrome of multisystemic smooth muscle dysfunction." Am J Med Genet A **152A**(10): 2437-2443.

Mineyko, A. and A. Kirton (2013). "Mechanisms of pediatric cerebral arteriopathy: an inflammatory debate." Pediatr Neurol **48**(1): 14-23.

Miskinyte, S., M. G. Butler, D. Herve, C. Sarret, M. Nicolino, J. D. Petralia, F. Bergametti, M. Arnould, V. N. Pham, A. V. Gore, K. Spengos, S. Gazal, F. Woimant, G. K. Steinberg, B. M. Weinstein and E. Tournier-Lasserve (2011). "Loss of BRCC3 deubiquitinating enzyme leads to abnormal angiogenesis and is associated with syndromic moyamoya." Am. J. Hum. Genet **88**(6): 718-728.

Mitra, S., M. Czosnyka, P. Smielewski, H. O'Reilly, K. Brady and T. Austin (2014). "Heart rate passivity of cerebral tissue oxygenation is associated with predictors of poor outcome in preterm infants." Acta Paediatr.

Mitra, S., M. Czosnyka, P. Smielewski, H. O'Reilly, K. Brady and T. Austin (2014). "Heart rate passivity of cerebral tissue oxygenation is associated with predictors of poor outcome in preterm infants." Acta Paediatr **103**(9): e374-382.

Monagle, P., E. Chalmers, A. Chan, G. DeVeber, F. Kirkham, P. Massicotte, A. D. Michelson and P. American College of Chest (2008). "Antithrombotic therapy in neonates and children: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition)." Chest **133**(6 Suppl): 887S-968S.

Moretti, J. L., M. Caglar and P. Weinmann (1995). "Cerebral perfusion imaging tracers for SPECT: which one to choose?" J Nucl Med **36**(3): 359-363.

Mori, N., S. Mugikura, S. Higano, T. Kaneta, M. Fujimura, A. Umetsu, T. Murata and S. Takahashi (2009). "The leptomeningeal "ivy sign" on fluid-attenuated inversion recovery MR imaging in Moyamoya disease: a sign of decreased cerebral vascular reserve?" AJNR Am. J. Neuroradiol **30**(5): 930-935.

Moritake, K., H. Handa, Y. Yonekawa, W. Taki and T. Okuno (1986). "[Follow-up study on the relationship between age at onset of illness and outcome in patients with moyamoya disease]." No Shinkei Geka **14**(8): 957-963.

Moriwaki, H., M. Matsumoto, K. Hashikawa, N. Oku, M. Ishida, Y. Seike, Y. Watanabe, H. Hougaku, N. Handa and T. Nishimura (1997). "Hemodynamic aspect of cerebral watershed infarction: assessment of perfusion reserve using iodine-123-iodoamphetamine SPECT." J Nucl Med **38**(10): 1556-1562.

Munot, P., Y. J. Crow and V. Ganesan (2011). "Paediatric stroke: genetic insights into disease mechanisms and treatment targets." Lancet Neurol **10**(3): 264-274.

Munot, P., D. E. Saunders, D. M. Milewicz, E. S. Regalado, J. R. Ostergaard, K. P. Braun, T. Kerr, K. D. Lichtenbelt, S. Philip, C. Rittey, T. S. Jacques, T. C. Cox and V. Ganesan (2012). "A novel distinctive cerebrovascular phenotype is associated with heterozygous Arg179 ACTA2 mutations." Brain **135**(Pt 8): 2506-2514.

Nakagawara, J., R. Takeda, K. Suematsu and J. Nakamura (1997). "Quantification of regional cerebral blood flow and vascular reserve in childhood moyamoya disease using [123I]IMP-ARG method." Clin. Neurol. Neurosurg **99 Suppl 2**: S96-S99.

Nakayasu, H., S. Araga, K. Takahashi, K. Otsuki and M. Murata (1991). "[Two cases of adult Down's syndrome presenting parietal low uptake in 123I-IMP-SPECT]." Rinsho Shinkeigaku **31**(5): 557-560.

Nambu, K., R. Suzuki and K. Hirakawa (1995). "Cerebral blood flow: measurement with xenon-enhanced dynamic helical CT." Radiology **195**(1): 53-57.

Nariai, T., Y. Matsushima, S. Imae, Y. Tanaka, K. Ishii, M. Senda and K. Ohno (2005). "Severe haemodynamic stress in selected subtypes of patients with moyamoya disease: a positron emission tomography study." J. Neurol. Neurosurg. Psychiatry **76**(5): 663-669.

Ng, J., D. Thompson, J. P. Lumley, D. E. Saunders and V. Ganesan (2012). "Surgical revascularisation for childhood moyamoya." Childs Nerv. Syst **28**(7): 1041-1048.

Nishiyama, T., T. Matsukawa, T. Yokoyama and K. Hanaoka (1999). "Cerebrovascular carbon dioxide reactivity during general anesthesia: a comparison between sevoflurane and isoflurane." Anesth. Analg **89**(6): 1437-1441.

Noll, R. B., L. Stith, M. A. Gartstein, M. D. Ris, R. Grueneich, K. Vannatta and K. Kalinyak (2001). "Neuropsychological functioning of youths with sickle cell disease: comparison with non-chronically ill peers." J Pediatr Psychol **26**(2): 69-78.

O'Dougherty, M., F. S. Wright, R. B. Loewenson and F. Torres (1985). "Cerebral dysfunction after chronic hypoxia in children." Neurology **35**(1): 42-46.

Oeffinger, K. C., A. C. Mertens, C. A. Sklar, T. Kawashima, M. M. Hudson, A. T. Meadows, D. L. Friedman, N. Marina, W. Hobbie, N. S. Kadan-Lottick, C. L. Schwartz, W. Leisenring, L. L. Robison and S. Childhood Cancer Survivor (2006). "Chronic health conditions in adult survivors of childhood cancer." N Engl J Med **355**(15): 1572-1582.

Ogasawara, K., N. Komoribayashi, M. Kobayashi, T. Fukuda, T. Inoue, K. Yamadate and A. Ogawa (2005). "Neural damage caused by cerebral hyperperfusion after arterial bypass surgery in a patient with moyamoya disease: case report." Neurosurgery **56**(6): E1380; discussion E1380.

Ogasawara, K., N. Komoribayashi, M. Kobayashi, T. Fukuda, T. Inoue, K. Yamadate and A. Ogawa (2005). "Neural damage caused by cerebral hyperperfusion after arterial bypass surgery in a patient with moyamoya disease: case report." Neurosurgery **56**(6): E1380.

Ogawa, A., N. Nakamura, T. Yoshimoto and J. Suzuki (1990). "Cerebral blood flow in moyamoya disease. Part 2: Autoregulation and CO2 response." Acta Neurochir. (Wien.) **105**(3-4): 107-111.

Ogawa, S., T. M. Lee and B. Barrere (1993). "The sensitivity of magnetic resonance image signals of a rat brain to changes in the cerebral venous blood oxygenation." Magn Reson Med **29**(2): 205-210.

Ogawa, S., T. M. Lee, A. R. Kay and D. W. Tank (1990). "Brain magnetic resonance imaging with contrast dependent on blood oxygenation." Proc Natl. Acad. Sci U S A **87**(24): 9868-9872.

Ohta, T., H. Tanaka and T. Kuroiwa (1995). "Diffuse leptomeningeal enhancement, "ivy sign," in magnetic resonance images of moyamoya disease in childhood: case report." Neurosurgery **37**(5): 1009-1012.

Ohtaki, M., T. Uede, S. Morimoto, T. Nonaka, S. Tanabe and K. Hashi (1998). "Intellectual functions and regional cerebral haemodynamics after extensive omental transplantation spread over both frontal lobes in childhood moyamoya disease." Acta Neurochir. (Wien.) **140**(10): 1043-1053.

Ohue, S., Y. Kumon, K. Kohno, H. Watanabe, S. Iwata and T. Ohnishi (2008). "Postoperative temporary neurological deficits in adults with moyamoya disease." Surg Neurol **69**(3): 281-286; discussion 286-287.

Okazawa, H., H. Yamauchi, H. Toyoda, K. Sugimoto, Y. Fujibayashi and Y. Yonekura (2003). "Relationship between vasodilatation and cerebral blood flow increase in impaired hemodynamics: a PET study with the acetazolamide test in cerebrovascular disease." J Nucl Med **44**(12): 1875-1883.

Outwater, E. K., R. C. Platenberg and S. M. Wolpert (1989). "Moyamoya disease in Down syndrome." AJNR Am. J. Neuroradiol **10**(5 Suppl): S23-S24.

Pantano, P., J. C. Baron, P. Lebrun-Grandie, N. Duquesnoy, M. G. Bousser and D. Comar (1984). "Regional cerebral blood flow and oxygen consumption in human aging." Stroke **15**(4): 635-641.

Payne, R. S., A. Goldbart, D. Gozal and A. Schurr (2004). "Effect of intermittent hypoxia on long-term potentiation in rat hippocampal slices." Brain Res **1029**(2): 195-199.

Pearson, E., N. J. Lenn and W. S. Cail (1985). "Moyamoya and other causes of stroke in patients with Down syndrome." Pediatr. Neurol **1**(3): 174-179.

Pena, F. and J. M. Ramirez (2005). "Hypoxia-induced changes in neuronal network properties." Mol Neurobiol **32**(3): 251-283.

Perecman, E. (1987). The Frontal lobes revisited. New York, IRBN Press.

Petersen, E. T., I. Zimine, Y. C. Ho and X. Golay (2006). "Non-invasive measurement of perfusion: a critical review of arterial spin labelling techniques." Br J Radiol **79**(944): 688-701.

Philip, S., Y. Udomphorn, F. J. Kirkham and M. S. Vavilala (2009). "Cerebrovascular pathophysiology in pediatric traumatic brain injury." J Trauma **67**(2 Suppl): S128-S134.

Piepgras, D. G., M. K. Morgan, T. M. Sundt, Jr., T. Yanagihara and L. M. Mussman (1988). "Intracerebral hemorrhage after carotid endarterectomy." J Neurosurg **68**(4): 532-536.

Pillai, J. J. and D. J. Mikulis (2015). "Cerebrovascular reactivity mapping: an evolving standard for clinical functional imaging." AJNR Am. J. Neuroradiol **36**(1): 7-13.

Pillai, J. J. and D. Zaca (2011). "Clinical utility of cerebrovascular reactivity mapping in patients with low grade gliomas." World J. Clin. Oncol **2**(12): 397-403.

Pindzola, R. R., J. R. Balzer, E. M. Nemoto, S. Goldstein and H. Yonas (2001). "Cerebrovascular reserve in patients with carotid occlusive disease assessed by stable xenon-enhanced ct cerebral blood flow and transcranial Doppler." Stroke **32**(8): 1811-1817.

Pinter, J. D., W. E. Brown, S. Eliez, J. E. Schmitt, G. T. Capone and A. L. Reiss (2001). "Amygdala and hippocampal volumes in children with Down syndrome: a high-resolution MRI study." Neurology **56**(7): 972-974.

Pittman, R. N. (2005). "Oxygen transport and exchange in the microcirculation." Microcirculation 12(1): 59-70.

Pittman, R. N. (2011). Regulation of Tissue Oxygenation. San Rafael CA, 2011 by Morgan & Claypool Life Sciences.

Pollanen, M. S. and J. H. Deck (1990). "The mechanism of embolic watershed infarction: experimental studies." Can J Neurol Sci **17**(4): 395-398.

Prengler, M., S. G. Pavlakis, I. Prohovnik and R. J. Adams (2002). "Sickle cell disease: the neurological complications." Ann Neurol **51**(5): 543-552.

Prisman, E., M. Slessarev, J. Han, J. Poublanc, A. Mardimae, A. Crawley, J. Fisher and D. Mikulis (2008). "Comparison of the effects of independently-controlled end-tidal PCO(2) and PO(2) on blood oxygen level-dependent (BOLD) MRI." J Magn Reson. Imaging **27**(1): 185-191.

Prohovnik, I., A. Hurlet-Jensen, R. Adams, V. D. De and S. G. Pavlakis (2009). "Hemodynamic etiology of elevated flow velocity and stroke in sickle-cell disease." J Cereb. Blood Flow Metab **29**(4): 803-810.

Puri, B. K., Z. Zhang and I. Singh (1994). "SPECT in adult mosaic Down's syndrome with early dementia." Clin Nucl Med **19**(11): 989-991.

Rachmel, A., A. Zeharia, M. Neuman-Levin, R. Weitz, R. Shamir and G. Dinari (1989). "Alagille syndrome associated with moyamoya disease." Am. J. Med. Genet **33**(1): 89-91.

Rafay, M. F., D. Armstrong, G. Deveber, T. Domi, A. Chan and D. L. MacGregor (2006). "Craniocervical arterial dissection in children: clinical and radiographic presentation and outcome." J Child Neurol **21**(1): 8-16.

Rafay, M. F., D. Armstrong, P. Dirks, D. L. MacGregor and G. DeVeber (2015). "Patterns of cerebral ischemia in children with moyamoya." Pediatr. Neurol **52**(1): 65-72.

Rajakulasingam, K., L. J. Cerullo and A. J. Raimondi (1979). "Childhood moyamoya syndrome. Postradiation pathogenesis." Childs Brain **5**(5): 467-475.

Raman, L., M. K. Georgieff and R. Rao (2006). "The role of chronic hypoxia in the development of neurocognitive abnormalities in preterm infants with bronchopulmonary dysplasia." Dev Sci **9**(4): 359-367.

Rea, D., J. F. Brandsema, D. Armstrong, P. C. Parkin, G. DeVeber, D. MacGregor, W. J. Logan and R. Askalan (2009). "Cerebral arteriopathy in children with neurofibromatosis type 1." Pediatrics **124**(3): e476-e483.

Reinhard, M., G. Schwarzer, M. Briel, C. Altamura, P. Palazzo, A. King, N. M. Bornstein, N. Petersen, E. Motschall, A. Hetzel, R. S. Marshall, C. J. Klijn, M. Silvestrini, H. S. Markus and F. Vernieri (2014). "Cerebrovascular reactivity predicts stroke in high-grade carotid artery disease." Neurology **83**(16): 1424-1431.

Research Committee on the, P., W. Treatment of Spontaneous Occlusion of the Circle of and D. Health Labour Sciences Research Grant for Research on Measures for Infractable (2012). "Guidelines for diagnosis and treatment of moyamoya disease (spontaneous occlusion of the circle of Willis)." Neurol Med Chir (Tokyo) **52**(5): 245-266.

Ressel, V., M. Wilke, K. Lidzba, W. Lutzenberger and I. Krageloh-Mann (2008). "Increases in language lateralization in normal children as observed using magnetoencephalography." Brain Lang **106**(3): 167-176.

Richards, B. A., H. Chertkow, V. Singh, A. Robillard, F. Massoud, A. C. Evans and N. J. Kabani (2009). "Patterns of cortical thinning in Alzheimer's disease and frontotemporal dementia." Neurobiol Aging **30**(10): 1626-1636.

Ringelstein, E. B., C. Weiller, M. Weckesser and S. Weckesser (1994). "Cerebral vasomotor reactivity is significantly reduced in low-flow as compared to thromboembolic infarctions: the key role of the circle of Willis." J. Neurol. Sci **121**(1): 103-109.

Roach, E. S., M. R. Golomb, R. Adams, J. Biller, S. Daniels, G. deVeber, D. Ferriero, B. V. Jones, F. J. Kirkham, R. M. Scott and E. R. Smith (2008). "Management of stroke in infants and children: a scientific statement from a Special Writing Group of the American Heart Association Stroke Council and the Council on Cardiovascular Disease in the Young." Stroke **39**(9): 2644-2691.

Romanul, F. C. and A. Abramowicz (1964). "CHANGES IN BRAIN AND PIAL VESSELS IN ARTERIAL BORDER ZONES; A STUDY OF 13 CASES." Arch Neurol **11**: 40-65.

Rothman, S. M., K. H. Fulling and J. S. Nelson (1986). "Sickle cell anemia and central nervous system infarction: a neuropathological study." Ann Neurol **20**(6): 684-690.

Rowney, D. A., R. Fairgrieve and B. Bissonnette (2002). "Cerebrovascular carbon dioxide reactivity in children anaesthetized with sevoflurane." Br. J. Anaesth **88**(3): 357-361.

Sacco, R. L., S. E. Kasner, J. P. Broderick, L. R. Caplan, J. J. Connors, A. Culebras, M. S. V. Elkind, M. G. George, A. D. Hamdan, R. T. Higashida, B. L. Hoh, L. S. Janis, C. S. Kase, D. O. Kleindorfer, J.-M. Lee, M. E. Moseley, E. D. Peterson, T. N. Turan, A. L. Valderrama and H. V. Vinters (2013). "An Updated Definition of Stroke for the 21st Century." Stroke **44**(7): 2064.

Saeki, N., M. N. Silva, M. Kubota, J. Takanashi, K. Sugita, S. Nakazaki and A. Yamaura (2000). "Comparative performance of magnetic resonance angiography and conventional angiography in moyamoya disease." J. Clin. Neurosci **7**(2): 112-115.

Sailer, M., B. Fischl, D. Salat, C. Tempelmann, M. A. Schonfeld, E. Busa, N. Bodammer, H. J. Heinze and A. Dale (2003). "Focal thinning of the cerebral cortex in multiple sclerosis." Brain **126**(Pt 8): 1734-1744.

Saito, H., K. Ogasawara, T. Suzuki, H. Kuroda, M. Kobayashi, K. Yoshida, Y. Kubo and A. Ogawa (2011). "Adverse effects of intravenous acetazolamide administration for evaluation of cerebrovascular reactivity using brain perfusion single-photon emission computed tomography in patients with major cerebral artery steno-occlusive diseases." Neurol. Med. Chir (Tokyo) **51**(7): 479-483.

Salat, D. H., R. L. Buckner, A. Z. Snyder, D. N. Greve, R. S. Desikan, E. Busa, J. C. Morris, A. M. Dale and B. Fischl (2004). "Thinning of the cerebral cortex in aging." Cereb Cortex **14**(7): 721-730.

Saur, D., R. Buchert, R. Knab, C. Weiller and J. Rother (2006). "Iomazenil-single-photon emission computed tomography reveals selective neuronal loss in magnetic resonance-defined mismatch areas." Stroke **37**(11): 2713-2719.

Saver, J. L. (2008). "Proposal for a universal definition of cerebral infarction." Stroke **39**(11): 3110-3115.

Schmidt, R. and G. Thews (1983). Human Physiology. Berlin, Springer-Verlag.

Scott, R. M. and E. R. Smith (2009). "Moyamoya disease and moyamoya syndrome." N. Engl. J Med **360**(12): 1226-1237.

Scott, R. M., J. L. Smith, R. L. Robertson, J. R. Madsen, S. G. Soriano and M. A. Rockoff (2004). "Long-term outcome in children with moyamoya syndrome after cranial revascularization by pial synangiosis." J Neurosurg **100**(2 Suppl Pediatrics): 142-149.

Scouten, A. and R. T. Constable (2008). "VASO-based calculations of CBV change: accounting for the dynamic CSF volume." Magn Reson. Med **59**(2): 308-315.

Sebire, G. (2006). "Transient cerebral arteriopathy in childhood." Lancet **368**(9529): 8-10.

Sebire, G., H. Fullerton, E. Riou and G. DeVeber (2004). "Toward the definition of cerebral arteriopathies of childhood." Curr. Opin. Pediatr **16**(6): 617-622.

Sebire, G., L. Meyer and S. Chabrier (1999). "Varicella as a risk factor for cerebral infarction in childhood: a case-control study." Ann Neurol **45**(5): 679-680.

See, A. P., A. E. Ropper, D. L. Underberg, R. L. Robertson, R. M. Scott and E. R. Smith (2015). "Down syndrome and moyamoya: clinical presentation and surgical management." J Neurosurg Pediatr **16**(1): 58-63.

See, A. P., A. E. Ropper, D. L. Underberg, R. L. Robertson, R. M. Scott and E. R. Smith (2015). "Down syndrome and moyamoya: clinical presentation and surgical management." J. Neurosurg. Pediatr: 1-6.

Sekhon, L. H., M. K. Morgan, I. Spence and N. C. Weber (1994). "Chronic cerebral hypoperfusion and impaired neuronal function in rats." Stroke **25**(5): 1022-1027.

Sen, E. S., V. Leone, M. Abinun, R. Forsyth, V. Ramesh, M. Friswell, F. O'Callaghan and A. V. Ramanan (2010). "Treatment of primary angiitis of the central nervous system in childhood with mycophenolate mofetil." Rheumatology (Oxford) **49**(4): 806-811.

Settakis, G., D. Pall, C. Molnar, D. Bereczki, L. Csiba and B. Fulesdi (2003). "Cerebrovascular reactivity in hypertensive and healthy adolescents: TCD with vasodilatory challenge." J. Neuroimaging **13**(2): 106-112.

Settakis, G., D. Pall, C. Molnar, E. Katona, D. Bereczki and B. Fulesdi (2006). "Hyperventilation-induced cerebrovascular reactivity among hypertensive and healthy adolescents." Kidney Blood Press Res **29**(5): 306-311.

Shaw, P., N. J. Kabani, J. P. Lerch, K. Eckstrand, R. Lenroot, N. Gogtay, D. Greenstein, L. Clasen, A. Evans, J. L. Rapoport, J. N. Giedd and S. P. Wise (2008). "Neurodevelopmental trajectories of the human cerebral cortex." J Neurosci **28**(14): 3586-3594.

Shrout, P. E. and J. L. Fleiss (1979). "Intraclass correlations: uses in assessing rater reliability." Psychol Bull **86**(2): 420-428.

Sidman, R. L. and P. Rakic (1973). "Neuronal migration, with special reference to developing human brain: a review." Brain Res **62**(1): 1-35.

Silvestrini, M., F. Vernieri, P. Pasqualetti, M. Matteis, F. Passarelli, E. Troisi and C. Caltagirone (2000). "Impaired cerebral vasoreactivity and risk of stroke in patients with asymptomatic carotid artery stenosis." JAMA **283**(16): 2122-2127.

Siriussawakul, A., D. Sharma, P. Sookplung, W. Armstead and M. S. Vavilala (2011). "Gender differences in cerebrovascular reactivity to carbon dioxide during sevoflurane anesthesia in children: preliminary findings." Paediatr. Anaesth **21**(2): 141-147.

Siskova, Z. and M. E. Tremblay (2013). "Microglia and synapse: interactions in health and neurodegeneration." Neural Plast **2013**: 425845.

Skeik, N., K. K. Rumery, P. D. Udayakumar, B. M. Crandall, K. J. Warrington and T. M. Sullivan (2013). "Concurrent Takayasu arteritis with common variable immunodeficiency and moyamoya disease." Ann. Vasc. Surg **27**(2): 240-248.

Slessarev, M., J. Han, A. Mardimae, E. Prisman, D. Preiss, G. Volgyesi, C. Ansel, J. Duffin and J. A. Fisher (2007). "Prospective targeting and control of end-tidal CO2 and O2 concentrations." J Physiol **581**(Pt 3): 1207-1219.

Slovut, D. P. and J. W. Olin (2004). "Fibromuscular dysplasia." N Engl J Med **350**(18): 1862-1871.

Smith, E. R. and R. M. Scott (2012). "Spontaneous occlusion of the circle of Willis in children: pediatric moyamoya summary with proposed evidence-based practice guidelines. A review." J. Neurosurg. Pediatr **9**(4): 353-360.

Smith, S. M., M. Jenkinson, M. W. Woolrich, C. F. Beckmann, T. E. Behrens, H. Johansen-Berg, P. R. Bannister, M. De Luca, I. Drobnjak, D. E. Flitney, R. K. Niazy, J. Saunders, J. Vickers, Y. Zhang, N. De Stefano, J. M. Brady and P. M. Matthews (2004). "Advances in functional and structural MR image analysis and implementation as FSL." Neuroimage **23 Suppl 1**: S208-219.

Soriano, S. G., N. F. Sethna and R. M. Scott (1993). "Anesthetic management of children with moyamoya syndrome." Anesth. Analg **77**(5): 1066-1070.

Sowell, E. R., B. S. Peterson, E. Kan, R. P. Woods, J. Yoshii, R. Bansal, D. Xu, H. Zhu, P. M. Thompson and A. W. Toga (2007). "Sex differences in cortical thickness mapped in 176 healthy individuals between 7 and 87 years of age." Cereb Cortex **17**(7): 1550-1560.

Sowell, E. R., B. S. Peterson, P. M. Thompson, S. E. Welcome, A. L. Henkenius and A. W. Toga (2003). "Mapping cortical change across the human life span." Nat Neurosci **6**(3): 309-315.

Sowell, E. R., P. M. Thompson, C. M. Leonard, S. E. Welcome, E. Kan and A. W. Toga (2004). "Longitudinal mapping of cortical thickness and brain growth in normal children." J Neurosci **24**(38): 8223-8231.

Sowell, E. R., P. M. Thompson, K. D. Tessner and A. W. Toga (2001). "Mapping continued brain growth and gray matter density reduction in dorsal frontal cortex: Inverse relationships during postadolescent brain maturation." J Neurosci **21**(22): 8819-8829.

Srinivasan, A., M. Goyal, F. Al Azri and C. Lum (2006). "State-of-the-art imaging of acute stroke." Radiographics **26 Suppl 1**: S75-95.

Steen, R. G., C. Fineberg-Buchner, G. Hankins, L. Weiss, A. Prifitera and R. K. Mulhern (2005). "Cognitive deficits in children with sickle cell disease." J Child Neurol **20**(2): 102-107.

Stewart, F. A., S. Hoving and N. S. Russell (2010). "Vascular damage as an underlying mechanism of cardiac and cerebral toxicity in irradiated cancer patients." Radiat Res **174**(6): 865-869.

Strater, R., S. Becker, A. von Eckardstein, A. Heinecke, S. Gutsche, R. Junker, K. Kurnik, R. Schobess and U. Nowak-Gottl (2002). "Prospective assessment of risk factors for recurrent stroke during childhood--a 5-year follow-up study." Lancet **360**(9345): 1540-1545.

Sultan, S. M., L. A. Beslow, A. Vossough, M. S. Elkind, S. E. Kasner, D. M. Mirsky, D. J. Licht and R. N. Ichord (2015). "Predictive validity of severity grading for cerebral steno-occlusive arteriopathy in recurrent childhood ischemic stroke." Int. J. Stroke **10**(2): 213-218.

Sundt, T. M., Jr., F. W. Sharbrough, D. G. Piepgras, T. P. Kearns, J. M. Messick, Jr. and W. M. O'Fallon (1981). "Correlation of cerebral blood flow and electroencephalographic changes during carotid endarterectomy: with results of surgery and hemodynamics of cerebral ischemia." Mayo Clin Proc **56**(9): 533-543.

Suzuki, J. and A. Takaku (1969). "Cerebrovascular "moyamoya" disease. Disease showing abnormal net-like vessels in base of brain." Arch Neurol **20**(3): 288-299.

Suzuki, R., T. Nariai, Y. Matsushima and K. Hirakawa (1996). "Xe-CT in cerebrovascular disease and moyamoya disease." Acta Neurol. Scand. Suppl **166**: 69-71.

Switzer, J. A., D. C. Hess, F. T. Nichols and R. J. Adams (2006). "Pathophysiology and treatment of stroke in sickle-cell disease: present and future." Lancet Neurol **5**(6): 501-512.

Symon, L., G. Donnan, J. C. Baron, S. Davis and F. R. Sharp (2007). The ischemic penumbra: the beginning; in. New York, Informa Healthcare.

Tamaki, T. and N. Yoji (2010). "Occlusion of Internal Carotid Artery in Kimura's Disease." Case. Rep. Med **2010**: 407538.

Tan, M. A., D. Armstrong, D. L. MacGregor and A. Kirton (2009). "Late complications of vertebral artery dissection in children: pseudoaneurysm, thrombosis, and recurrent stroke." J Child Neurol **24**(3): 354-360.

Tan, M. A., G. DeVeber, A. Kirton, L. Vidarsson, D. MacGregor and M. Shroff (2009). "Low detection rate of craniocervical arterial dissection in children using time-of-flight magnetic resonance angiography: causes and strategies to improve diagnosis." J Child Neurol **24**(10): 1250-1257.

Tancredi, F. B. and R. D. Hoge (2013). "Comparison of cerebral vascular reactivity measures obtained using breath-holding and CO2 inhalation." J. Cereb. Blood Flow Metab **33**(7): 1066-1074.

Thatcher, R. W., M. Camacho, A. Salazar, C. Linden, C. Biver and L. Clarke (1997). "Quantitative MRI of the gray-white matter distribution in traumatic brain injury." J Neurotrauma **14**(1): 1-14.

Thomas, B., W. Logan, E. J. Donner and M. Shroff (2013). "Assessment of cerebrovascular reactivity using real-time BOLD fMRI in children with moyamoya disease: a pilot study." Childs Nerv. Syst **29**(3): 457-463.

Thomas, B. P., P. Liu, D. C. Park, M. J. van Osch and H. Lu (2014). "Cerebrovascular reactivity in the brain white matter: magnitude, temporal characteristics, and age effects." J Cereb Blood Flow Metab **34**(2): 242-247.

Thomas, D. L., M. F. Lythgoe, F. Calamante, D. G. Gadian and R. J. Ordidge (2001). "Simultaneous noninvasive measurement of CBF and CBV using double-echo FAIR (DEFAIR)." Magn Reson. Med **45**(5): 853-863.

Thomason, M. E., B. E. Burrows, J. D. Gabrieli and G. H. Glover (2005). "Breath holding reveals differences in fMRI BOLD signal in children and adults." Neuroimage **25**(3): 824-837.

Thomason, M. E. and G. H. Glover (2008). "Controlled inspiration depth reduces variance in breath-holding-induced BOLD signal." Neuroimage **39**(1): 206-214.

Tolani, A. T., K. W. Yeom and J. Elbers (2015). "Focal Cerebral Arteriopathy: The Face With Many Names." Pediatr Neurol **53**(3): 247-252.

Tomimoto, H., M. Ihara, H. Wakita, R. Ohtani, J. X. Lin, I. Akiguchi, M. Kinoshita and H. Shibasaki (2003). "Chronic cerebral hypoperfusion induces white matter lesions and loss of oligodendroglia with DNA fragmentation in the rat." Acta Neuropathol **106**(6): 527-534.

Touze, E., C. Oppenheim, D. Trystram, G. Nokam, M. Pasquini, S. Alamowitch, D. Herve, P. Garnier, E. Mousseaux and P. F. Plouin (2010). "Fibromuscular dysplasia of cervical and intracranial arteries." Int J Stroke **5**(4): 296-305.

Tremblay, M. E. and A. K. Majewska (2011). "A role for microglia in synaptic plasticity?" Commun Integr Biol **4**(2): 220-222.

Tzika, A. A., R. L. Robertson, P. D. Barnes, S. Vajapeyam, P. E. Burrows, S. T. Treves and R. M. Scott (1997). "Childhood moyamoya disease: hemodynamic MRI." Pediatr Radiol **27**(9): 727-735.

Uchino, K., S. C. Johnston, K. J. Becker and D. L. Tirschwell (2005). "Moyamoya disease in Washington State and California." Neurology **65**(6): 956-958.

Uchino, K., S. C. Johnston, K. J. Becker and D. L. Tirschwell (2005). "Moyamoya disease in Washington State and California." Neurology **65**(6): 956-958.

Uno, M., S. Ueda, H. Hondo, K. Matsumoto and M. Harada (1996). "Effectiveness of revascularization surgery evaluated by proton magnetic resonance spectroscopy and single photon emission computed tomography." Neurol Med Chir (Tokyo) **36**(8): 560-566; discussion 566-567.

Vandesteene, A., V. Trempont, E. Engelman, T. Deloof, M. Focroul, A. Schoutens and M. de Rood (1988). "Effect of propofol on cerebral blood flow and metabolism in man." Anaesthesia **43 Suppl**: 42-43.

Vargha-Khadem, F., E. Isaacs, S. van der Werf, S. Robb and J. Wilson (1992). "Development of intelligence and memory in children with hemiplegic cerebral palsy. The deleterious consequences of early seizures." Brain **115 Pt 1**: 315-329.

Verlhac, S., S. Balandra, I. Cussenot, F. Kasbi, M. Vasile, A. Kheniche, M. Elmaleh-Berges, G. Ithier, M. Benkerrou, F. Bernaudin and G. Sebag (2014). "Extracranial carotid arteriopathy in stroke-free children with sickle cell anemia: detection by submandibular Doppler sonography." Pediatr Radiol **44**(5): 587-596.

Vesely, A., H. Sasano, G. Volgyesi, R. Somogyi, J. Tesler, L. Fedorko, J. Grynspan, A. Crawley, J. A. Fisher and D. Mikulis (2001). "MRI mapping of cerebrovascular reactivity using square wave changes in end-tidal PCO2." Magn Reson. Med **45**(6): 1011-1013.

Vorstrup, S., L. Henriksen and O. B. Paulson (1984). "Effect of acetazolamide on cerebral blood flow and cerebral metabolic rate for oxygen." J Clin Invest **74**(5): 1634-1639.

Wakai, K., A. Tamakoshi, K. Ikezaki, M. Fukui, T. Kawamura, R. Aoki, M. Kojima, Y. Lin and Y. Ohno (1997). "Epidemiological features of moyamoya disease in Japan: findings from a nationwide survey." Clin. Neurol. Neurosurg **99 Suppl 2**: S1-S5.

Wang, Z. J., Y. J. Zhou, Y. Y. Liu, M. Yu, D. M. Shi, Y. X. Zhao, Y. H. Guo, W. J. Cheng, d. A. Jia, Z. Cao, B. Nie, H. L. Ge, S. W. Yang and Z. X. Yan (2009). "Impact of clopidogrel resistance on thrombotic events after percutaneous coronary intervention with drug-eluting stent." Thromb. Res **124**(1): 46-51.

Watkins, K. E., D. K. Hewes, A. Connelly, B. E. Kendall, D. P. Kingsley, J. E. Evans, D. G. Gadian, F. Vargha-Khadem and F. J. Kirkham (1998). "Cognitive deficits associated with frontal-lobe infarction in children with sickle cell disease." Dev Med Child Neurol **40**(8): 536-543.

Welberg, L. (2014). "Synaptic plasticity: a synaptic role for microglia." Nat Rev Neurosci **15**(2): 69.

Westmacott, R., D. MacGregor, R. Askalan and G. deVeber (2009). "Late emergence of cognitive deficits after unilateral neonatal stroke." Stroke **40**(6): 2012-2019.

WHO (1971). Cerebrovascular diseases: Prevention, treatment, and rehabilitation. World Health Organization Technical Report Series. Geneva. **469**.

Williams, T. S., R. Westmacott, N. Dlamini, L. Granite, P. Dirks, R. Askalan, D. MacGregor, M. Moharir and G. DeVeber (2012). "Intellectual ability and executive function in pediatric moyamoya vasculopathy." Dev. Med. Child Neurol **54**(1): 30-37.

Wilson-Smith, E., C. Karsli, I. Luginbuehl and B. Bissonnette (2003). "Effect of nitrous oxide on cerebrovascular reactivity to carbon dioxide in children during sevoflurane anaesthesia." Br. J. Anaesth **91**(2): 190-195.

Wilson, C. M., M. W. Gaber, O. M. Sabek, J. A. Zawaski and T. E. Merchant (2009). "Radiation-induced astrogliosis and blood-brain barrier damage can be abrogated using anti-TNF treatment." Int J Radiat Oncol Biol Phys **74**(3): 934-941.

Winter, J. D., J. Fierstra, S. Dorner, J. A. Fisher, K. S. St Lawrence and A. Kassner (2010). "Feasibility and precision of cerebral blood flow and cerebrovascular reactivity MRI measurements using a computer-controlled gas delivery system in an anesthetised juvenile animal model." J. Magn Reson. Imaging **32**(5): 1068-1075.

Wintermark, M., N. K. Hills, G. A. deVeber, A. J. Barkovich, M. S. Elkind, K. Sear, G. Zhu, C. Leiva-Salinas, Q. Hou, M. M. Dowling, T. J. Bernard, N. R. Friedman, R. N. Ichord, H. J. Fullerton and V. Investigators (2014). "Arteriopathy diagnosis in childhood arterial ischemic stroke: results of the vascular effects of infection in pediatric stroke study." Stroke **45**(12): 3597-3605.

Wong, L. J., J. C. Kupferman, I. Prohovnik, F. J. Kirkham, S. Goodman, K. Paterno, M. Sharma, Y. Brosgol and S. G. Pavlakis (2011). "Hypertension impairs vascular reactivity in the pediatric brain." Stroke **42**(7): 1834-1838.

Wu, M., Z. Huang, D. Zhang, L. Wang, J. Sun, S. Wang, Y. Zhao and J. Zhao (2011). "Color doppler hemodynamic study of the superficial temporal arteries in superficial temporal artery-middle cerebral artery (STA-MCA) bypass surgery for Moyamoya disease." World Neurosurg **75**(2): 258-263.

Xu, M. and H. L. Zhang (2011). "Death and survival of neuronal and astrocytic cells in ischemic brain injury: a role of autophagy." Acta Pharmacol Sin **32**(9): 1089-1099.

Yamada, I., Y. Matsushima and S. Suzuki (1992). "Moyamoya disease: diagnosis with three-dimensional time-of-flight MR angiography." Radiology **184**(3): 773-778.

Yamashita, T., S. Kashiwagi, K. Nakashima, H. Ishihara, T. Kitahara, S. Nakano and H. Ito (1996). "Modulation of cerebral hemodynamics by surgical revascularization in patients with moyamoya disease." Acta Neurol. Scand. Suppl **166**: 82-84.

Yamauchi, H., T. Kudoh, Y. Kishibe, J. Iwasaki and S. Kagawa (2005). "Selective neuronal damage and borderzone infarction in carotid artery occlusive disease: a 11C-flumazenil PET study." J Nucl Med **46**(12): 1973-1979.

Yang, J., J. C. Hong, C. W. Oh, O. K. Kwon, G. Hwang, J. E. Kim, H. S. Kang, W. S. Cho, T. Kim, J. U. Moon, S. Y. Ahn, J. H. Kim and J. S. Bang (2014). "Clinicoepidemiological features of asymptomatic moyamoya disease in adult patients." J Cerebrovasc Endovasc Neurosurg **16**(3): 241-246.

Yeom, K. W., R. M. Lober, P. D. Barnes and C. J. Campen (2013). "Reduced cerebral arterial spin-labeled perfusion in children with neurofibromatosis type 1." AJNR Am J Neuroradiol **34**(9): 1823-1828.

Yeon, J. Y., H. J. Shin, H. J. Seol, J. S. Kim and S. C. Hong (2014). "Unilateral intracranial arteriopathy in pediatric stroke: course, outcome, and prediction of reversible arteriopathy." Stroke **45**(4): 1173-1176.

Yezhuvath, U. S., K. Lewis-Amezcua, R. Varghese, G. Xiao and H. Lu (2009). "On the assessment of cerebrovascular reactivity using hypercapnia BOLD MRI." NMR Biomed **22**(7): 779-786.

Yonekawa, Y., N. Ogata, Y. Kaku, E. Taub and H. G. Imhof (1997). "Moyamoya disease in Europe, past and present status." Clin Neurol Neurosurg **99 Suppl 2**: S58-60.

Yonekawa, Y., N. Ogata, Y. Kaku, E. Taub and H. G. Imhof (1997). "Moyamoya disease in Europe, past and present status." Clin. Neurol. Neurosurg **99 Suppl 2**: S58-S60.

Yuan, H., M. W. Gaber, K. Boyd, C. M. Wilson, M. F. Kiani and T. E. Merchant (2006). "Effects of fractionated radiation on the brain vasculature in a murine model: blood-brain barrier permeability, astrocyte proliferation, and ultrastructural changes." Int J Radiat Oncol Biol Phys **66**(3): 860-866.

Zaca, D., J. Jovicich, S. R. Nadar, J. T. Voyvodic and J. J. Pillai (2014). "Cerebrovascular reactivity mapping in patients with low grade gliomas undergoing presurgical sensorimotor mapping with BOLD fMRI." J. Magn Reson. Imaging **40**(2): 383-390.

Zeng, L., H. Chen, L. Ouyang, D. Yao and J. H. Gao (2007). "Quantitative analysis of asymmetrical cortical activity in motor areas during sequential finger movement." Magn Reson Imaging **25**(10): 1370-1375.

Zlokovic, B. V. (2008). "The blood-brain barrier in health and chronic neurodegenerative disorders." Neuron **57**(2): 178-201.