AN INVESTIGATION OF THE INFANT CEREBRODURAL VENOUS SYSTEM IN RELATION TO THE SOURCE OF SUBDURAL HAEMORRHAGE IN PAEDIATRIC HEAD INJURY

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Abstract

‘An Investigation of the Infant Cerebrodual Venous System in Relation to the Source of Subdural Haemorrhage in Paediatric Head Injury’

Emma C. Cheshire

A common finding in babies who suffer a head injury is subdural haemorrhage (SDH). When an infant presents with SDH, alongside other signs of injury (e.g. retinal haemorrhages, encephalopathy, bone fractures and bruising), the majority of relevant medical professionals would be concerned that the baby could have been subjected to abusive head trauma (AHT). However, the events that lead to a SDH and the source of bleeding have been debated. The most widely assumed source of bleeding is the rupture of bridging veins which extend from the surface of the brain to the dural membrane.

There is limited knowledge in the current literature of the infant’s cerebrodural venous system. Autopsy observations at the beginning of this project have subjectively indicated that infant bridging veins are relatively small and delicate. The aim of this project was to attempt to overcome some of the practical and organisational difficulties related to research in the area of paediatric head injury, and to fill the identified gap in the current medical literature.

A novel approach to the post-mortem removal of the infant calvarial bones was developed. This made it possible to document and anatomically map the locations, size and numbers of these vessels and also enhanced the ability to observe pathological features of head injury, free from post-mortem artefact. Optical clearing techniques were used alongside optical coherence tomography to image the dural membrane and within the dural venous sinuses.

Infant bridging veins were found to be more numerous than previously described, and in locations which have not previously been reported. A proportion of bridging veins were also relatively small and delicate (< 0.3mm). These findings may be important in building a more accurate picture of the mechanisms and forces that result in SDH.

The new techniques described in this thesis have expanded the scope for future autopsy-based research into AHT.

Dedicated to the parents who gave consent
for their babies to be part of this study

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Lastly, I would like to extend my heartfelt appreciation to the parents who supported this work and consented for their babies to be a part of the study. Without their contribution this PhD would not have been possible. To them I dedicate this thesis.

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List of Abbreviations

AHT	abusive head trauma
β-APP	beta amyloid precursor protein
CSF	cerebrospinal fluid
CT	computed tomography
CTV	computed tomography venography
DAI	diffuse axonal injury
DSA	digital subtraction angiography
EDH	extradural haemorrhage
EMFPU	East Midlands Forensic Pathology Unit
FEM	finite element models
GFAP	glial fibrillary acidic protein
H&E	haematoxylin and eosin
HM	Her Majesty's
Hu	hounsfield units
IRAS	Integrated research approval system
MRI	magnetic resonance imaging
NRES	National Research Ethics Service
NAHI	non-accidental head Injury
OCA	optical clearing agent
OCT	optical coherence tomography
PAA	primary anastomotic arteries
RF	radiofrequency
R&D	research and development

RHs	retinal haemorrhages
SAA	secondary anastomotic arteries
SAH	subarachnoid haemorrhage
SBS	shaken baby syndrome
SD	standard deviation
SDH	subdural haemorrhage
SSS	superior sagittal sinus
ICC	intraclass correlation coefficient
3D	three dimensions
2D	two dimensions
UHL	University Hospitals of Leicester
UoL	University of Leicester

Chapter 1 : Introduction

1.1 Overview of Paediatric Head Injury

Head injury in children can occur through accidental and non-accidental mechanisms, as well as from birth-related trauma. These injuries occur when an external force is applied to the head, and include penetrating, compression and impact forces, which cause damage to the scalp, skull or brain (Minns & Lo, 2009). One proposed mechanism of injury involves rapid accelerations and/or decelerations, resulting in sudden movement of the head which can produce traumatic brain injury due to both shearing and tensile forces (DiMaio & DiMaio, 2001). These inertial forces can cause movement of the structures inside the skull, resulting in deformation of intracranial tissues (Rorke-Adams *et al.,* 2009).

Head trauma is the leading abusive injury resulting in death and morbidity seen in infants. Infants are babies that are under the age of one year, with babies younger than 28 days being described as neonates (Office for National Statistics, 2016). Further causes of head trauma include accidental mechanisms such as motor vehicle crashes and falls, with one study showing head injuries in children under the age of 3 resulting from accidents and definite abuse in 73.8% and 26.2% respectively. However, outcome severity and mortality rates were considerably lower in the accidental group (Reece & Sege, 2000).

1.2 Abusive Head Trauma

Although the term infantile abusive head trauma (AHT) implies an injury that may result from several different mechanisms, this term is often used to describe a constellation of findings that are suggestive of a baby being subjected to repetitive rotational and acceleration/deceleration forces within the head, with or without an additional impact force. The official definition of AHT created by the Centres for Disease Control and Prevention of the United States is “an injury to the skull or intracranial contents of an infant or young child (less than five years of age) due to inflicted blunt impact and/or violent shaking” (Parks *et al.,* 2012). Penetrating injuries, including gunshot and stab wounds are excluded from this definition. The injuries seen in AHT are often due to the

actions of an adult caregiver who may have been frustrated or have become angry by the infant's crying (Adamsbaum *et al.,* 2010). It is proposed that the perpetrator typically grasps the infant by the torso or extremities and vigorously shakes the victim, resulting in the head moving in both a backwards and forwards 'whiplash-like' action and also a rotational movement. An impact force may occur to the head after the shaking event if the baby is then thrown onto a nearby surface, such as a sofa or bed, against a wall or to the ground.

This mechanism of injury often results in bleeding between the protective membranes of the brain (known as meninges). One type of bleeding is known as a subdural haematoma (or subdural haemorrhage) (SDH) because it is found between the outer most membrane, the dura mater, and the leptomeningeal coverings of the brain (arachnoid and pia mater). The majority of SDHs in children under two years of age are caused by abuse (Jayawant *et al.,* 1998) and are present in almost 90% of patients suffering from AHT (Minns & Lo, 2009). Bleeding in the eyes (retinal haemorrhages) and damage to the brain (encephalopathy) are also commonly seen in AHT. These three injuries are often referred to as 'the triad' (Gerber & Coffman, 2007). Associated injuries can include damage to the neck and spinal cord, and fracturing of ribs and long bones (Kemp, 2011; Piteau *et al.,* 2012; Choudhary *et al.,* 2014).

During much of the 1990's, an infant with the triad and other associated injuries would have been diagnosed with 'Shaken Baby Syndrome' (SBS), and this term is one of the more widely known terms to describe the cause of these injuries within the lay public today. In 2009, the American Academy of Paediatricians, followed by the UK Crown Prosecution service in 2011, came to the conclusion that the use of the term SBS should be dropped as it suggests a definitive, often unwitnessed mechanism for the triad injuries, and is therefore difficult to prove during court proceedings. It also implies that shaking alone is the only cause of all infantile AHT.

A further term that is often used to describe AHT that does not infer a mechanism of injury is Non-Accidental Head Injury (NAHI). Currently, both terms are used in the medical literature (Stoodley, 2014; Roach *et al.,* 2014) and in the courts (Re JS (2012) EWHC 1370; A Local Authority v DB, RB and SM (2013) EWHC 4066 (Fam)) but for the

purposes of this thesis AHT will be used throughout to describe an infant who has suffered traumatic head injury with features that are suggestive of abuse.

1.3 Abusive Head Trauma and the Courts

Establishing a diagnosis of AHT is a very challenging, complex, controversial and emotive process, often deliberated over at length in the criminal and family justice systems. Without accompanying evidence of extracranial inflicted trauma, such as unexplained bruises or fractures, convictions based on the triad alone have been called into question, resulting in cases being overturned on appeal. There have been several high profile cases in the UK (R v Sally Clark (2003) EWCA Crim 1020) and USA (Commonwealth v Louise Woodward (1998) 427 Mass. 659) involving the sudden death of a previously well infant where the sentenced caregiver has been released from a life sentence for murder, or a prosecution has failed because of disputed medical evidence and a lack of quality research in the area of AHT (Jaspan, 2008). Media involvement in courtroom proceedings also produces false controversies, particularly centred on conflicting opinions of expert witnesses (Levin & Donnelly, 2014; McDonald, 2015) resulting in the public becoming misinformed about the facts of these cases.

The existence of injuries that are caused by harmful shaking has been debated within the courtroom setting by a few individual expert witnesses, and within the published literature (Squier, 2011; Rorke-Adams, 2011), despite a number of cases where caregivers have admitted to shaking their babies out of anger and frustration (Starling *et al.,* 1995; Starling *et al.,* 2004; Biron & Shelton, 2005). Unproven hypotheses which are supported by scanty scientific evidence have been used during court proceedings by a group of physicians who testify frequently and convincingly for the defence in AHT cases to suggest alternative, non-abusive causes for the injuries seen in these cases, even though many of their opinions lie outwith the consensus of the wider medical community (A Local Authority v S (2009) EWHC 2115 (Fam)). It is the responsibly of mainstream professionals to face the challenge of presenting complex medical information to judges and jurors, free from bias and based on factual scientifically sound evidence, to enable a properly informed verdict to be reached.

1.4 The Source of Subdural Haemorrhage

Questions about the events that lead to a SDH and the source of bleeding below the dural membrane have been strongly debated throughout the medico-legal professions. However, few professionals involved in cases of alleged AHT question the likelihood that a violent act has led to the injuries seen in these types of abusive trauma. The most accepted theory for the source of subdural bleeding is that the forces associated with shaking an infant lead to the rupture of blood vessels which bridge from the surface of the brain to the dural membrane. These vessels are known as “bridging veins”.

1.5 Importance of Research in Paediatric Head Injury

Inaccurate diagnosis of abuse may lead to an unjustified loss of parental rights or imprisonment, and under-diagnosis may create a significant risk for a child that survives and returns to an abusive environment. Similarly, there may be risks to any other children in the care of the accused.

Experience derived from the caseload of the past few years shows the need for more scientific research in the area of AHT to aid health, law enforcement and legal professionals in accurate diagnosis. One of the key areas of importance is the determination of the source of subdural bleeding in these cases as this will help in securely determining the mechanism of injury.

Previous research on head injuries has centred around poorly comparable animal and unsuitable biomechanical models, and these studies have been used to suggest that the amount of force required to tear what some assume are ‘robust’ bridging veins is far greater than that induced by inertial forces (such as those produced by vigorous shaking). Direct evidence of ruptured bridging veins from neuroimaging of patients, during surgery or at post-mortem is also limited for obvious practical reasons.

It is important to note that there has been minimal research on the characteristics of infant bridging veins, which are smaller than adult vessels. There is, in fact, limited information in anatomy text books or peer-viewed papers on the infant vascular anatomy within and surrounding the dural membrane. Although SDH is not usually part of the mechanism of death in AHT as it is typically of relatively low volume, it is still an important marker of trauma. In the face of unproven, non-abusive theories for the cause

of subdural bleeding and alternative suggestions for the source of subdural bleeding, objective research in this area will be crucial to provide evidence that will undermine or disprove such hypotheses.

1.6 Difficulties of Research in Abusive Head Trauma

One of the main challenges to research in the field of infant AHT in the UK is to ensure appropriate ethical approvals are in place. To undertake autopsy-based research in this age group also requires access to a suitable number of cases and new methods of autopsy practice to gain access to the infant brain and protective membranes without damaging structures of interest, including the bridging veins. Conventional neuroimaging systems such as computed tomography (CT) and magnetic resonance imaging (MRI) are unlikely to have the capability to image smaller bridging veins, and therefore novel ways of assessing smaller vessels are required. The dimensions and delicacy of infant bridging veins also presents practical problems in relation to their dissection and handling for the purposes of biomechanical testing and microscopic examination.

1.7 Aims/Objectives

The aim of this project is to attempt to overcome some of the practical and organisational difficulties related to research in the area of infant head injury. This will include the production of an appropriate ethical application, in order to allow development of novel techniques during infant autopsies and to enable detailed investigation of the infant cerebrodural venous system. This will help to fill the identified gap in the current medical literature on infant blood vessels associated with the dural membrane.

The main focus of this thesis will be on the blood vessels that travel from the brain surface to the dural membrane (the cerebral bridging veins) and ultimately drain into the dural venous sinuses. The size, location and number of these veins will be documented.

Ultimately, it is hoped that this area of research will aid health, law enforcement and legal professionals in the correct recognition of cases of AHT, based on detailed scientific study, rather than be subject to the vagaries and distractions of unproven theory.

1.8 Brief Outline of the Equipment and Techniques Used Within this Thesis

1.8.1 Neurosurgical Equipment

One of the challenges of investigating the infant cerebrodural system is accessing the intracranial contents without damaging the dura and brain, and disrupting the bridging veins. The infant calvarial bones are considerably thinner than those forming the adult skull cap, and the suture lines are un-ossified and in fibrous continuity with the underlying dura and the fontanelles (Collins, 1995). For these reasons, the standard method for autopsy removal of the infant brain is to use a scalpel and scissors to incise within the edges of the bone (Okazaki & Campbell, 1979; Riezzo, 2010). This procedure results in incisions being made through the bone, dura and bridging veins.

Chapter four describes the translation of a clinical neurosurgical technique into autopsy practice to facilitate the removal of the infant calvarium without damage to the dura and underlying brain and bridging veins. This allows for the visualisation and photographical documentation of undisrupted bridging veins for subsequent anatomical mapping, and analysis of the distribution of these vessels, described in chapter seven.

1.8.2 Optical Clearing

Optical clearing agents (OCAs) increase the transparency of biological tissues by decreasing the amount of source light. They are often used in conjunction with light-based imaging systems to increase the depth of imaging.

The initial intention of finding a suitable OCA to use on the dura *in situ* was to use this technique alongside optical coherence tomography (OCT), an imaging system, briefly described below and in more detail in chapter five. An additional advantage of increasing the transparency of the dura is the enhanced post-mortem assessment of head injuries, particularly SDHs. This improved visualisation of pathologies through the dura is detailed in chapter five, throughout a series of autopsies of infants with and without head injury.

1.8.3 Optical Coherence Tomography

Previous imaging studies of the bridging veins, mainly undertaken in adults, using modalities such as MRI, CT and X-ray, have provided scans that show the larger, better

known blood vessels, which enter the sinuses located in the dural membrane, particularly the superior sagittal sinus (SSS) and transverse sinuses (Brockmann *et al.,* 2012; Han *et al.,* 2007; Han *et al.,* 2008). These systems do not have the capability to produce the high resolution images that would be required to observe the smaller infant bridging veins that we observe during post-mortem examination.

Optical coherence tomography is a light-based imaging modality that uses fiber-optic technology to provide high resolution (micron-scale), cross-sectional, subsurface images of biological tissues (Huang *et al.,* 1991; Fujimoto *et al.,* 2000).

In clinical practice, OCT is most widely used in ophthalmic diagnostics (Izatt *et al.,* 1993). There has also been an increased interest in the application of OCT in endoscopy, resulting in the development of specialised catheters to accommodate different internal organs (Tearney *et al.,* 1997). Combining the use of OCT with endoscopy enables various intravascular applications, such as imaging of atherosclerotic plaques in the coronary arteries (Dresel *et al.,* 1992; Dubois *et al.,* 2002), which can be difficult to detect by conventional radiologic techniques, particularly when trying to discern their microstructural features (Podoleanu, 2005).

The application of two different types of OCT are explored in chapter six to aid in the investigation of the anatomy of both the bridging veins and the dural venous sinuses. A benchtop-type system, used in clinical practice for imaging of the eye, provides a non-invasive scan of the blood vessels within and below a section of the dural membrane in several preliminary experiments. The second OCT system is an endovascular catheter-based device, consisting of an optic fiber encased in a hollow rotating torque cable and will be referred to as ‘rotational OCT’. The catheter will be inserted into some of the main dural sinuses to elicit the number of bridging veins which directly join these structures.

1.9 Summary of Thesis

1.9.1 Introduction

The first chapter of this thesis has given an overview of paediatric head injury and the involvement of the criminal justice system in these cases. Some of the difficulties of research in the area of AHT are listed, with the justification of why it is so important to overcome these problems and produce detailed studies related to infantile head injury. The aims and objectives of the thesis are stated, along with a broad outline of the approaches to be used.

1.9.2 Literature Review

Chapter two of this thesis provides a detailed literature review to include a more detailed overview of paediatric head injury, anatomical information on the infant head, and a discussion of the theories of the cause of SDH in this age group.

1.9.3 Ethical Approval and the Consenting Process

The application process for ethics and the subsequent approval, and thereafter, the challenges of the consenting process are discussed in chapter three.

1.9.4 Post-Mortem Removal of the Calvarial Bones using Neurosurgical Equipment

Chapter four will detail a new method for post-mortem removal of infant skull bones using neurosurgical equipment. This minimally disruptive process will allow the assessment of head injuries and various pathologies whilst the dura mater is still intact and will allow careful visualisation of bridging veins.

1.9.5 Optical Clearance of the Dura Mater

The process of optical clearing is discussed in chapter five. This technique increases the transparency of the dura to aid both OCT imaging and observation/photographic documentation of patterns of subdural haemorrhage before the introduction of post-mortem artefactual blood extravasation.

1.9.6 Optical Coherence Tomography

Rotational OCT and benchtop OCT will be investigated as potential imaging systems in chapter six, for the purposes of visualisation of blood vessels within and below the dura, and also the bridging veins directly joining the dural venous sinuses.

1.9.7 Anatomical Mapping and Measurement of Bridging Veins

Chapter seven will show a method for post-mortem removal of the brain whilst photographically documenting bridging veins which are then mapped onto a 3D infant brain model created from a MRI scan. This will show the distribution of these vessels in both head injured and non-head injured infants. Digital microscopy will also be used to measure the diameter of the blood vessels *in situ.*

1.9.8 Removal of Infant Bridging Veins

Chapter eight discusses the feasibility of removing infant bridging veins during autopsy to enable mechanical testing of these vessels using a tensile testing machine.

1.9.9 Discussion, Future Work & Conclusions

Chapter nine will provide an overall discussion of the work and the future studies that are possible as a result of the new methods developed within this project. Lastly, the overall findings and conclusions drawn from the project are stated at the end of this chapter.

Chapter 2 : Literature Review

2.1 Chapter Overview

The following non-systematic literature review was carried out using various search engines, including but not limited to PubMed, Google Scholar, and Science Direct. Numerous books and journal articles were sourced from outside the University of Leicester by using the interlibrary loans service, mainly sourcing documents from the British Library. Ebrary was used as an online digital library to source scholarly e-books. Key search phrases and key words, used singularly and in combination included; non-accidental head injury, shaken baby syndrome, subdural haematoma, subdural bleeding, triad of injuries, retinal haemorrhage, encephalopathy, bridging veins, anatomy, dura mater, subdural space, biomechanical model, vascular corrosion casting, child abuse, traumatic brain injury, magnetic resonance imaging, computed tomography, cranial meninges, pachymenix, dural venous sinuses, superior sagittal sinus, animal model, whiplash injury, anthropomorphic test device, unified hypothesis, dural vascular plexus, and birth-related haematoma.

The first section of this literature review focuses on AHT, providing a brief history of AHT, an overview of the epidemiology, the clinical findings and injuries and the role of radiological imaging in AHT. Various biomechanical models of AHT are also discussed.

The second section provides an introduction and detailed review of the current knowledge on the infant anatomy in relation to the cerebrodural vascular system.

The third part of the literature review discusses the main causative theory for SDH in infancy (damage to bridging veins) as well as discussing differential diagnoses and alternative theories for the cause of SDH.

Part I: Abusive Head trauma

2.2 Overview of Abusive Head Trauma

Over the years, AHT has been referred to by many different terms, including; whiplash shaken infant syndrome (Caffey, 1972), inflicted childhood neurotrauma (Reece & Nicholson, 2003), non-accidental head injury (Al-Holou *et al.,* 2009) and shaken baby syndrome (Ludwig & Warman, 1984). The current term, abusive head trauma (AHT), has been employed by the American Academy of Paediatrics as it does not imply a specific mechanism of injury.

The incidence of AHT varies between studies, with a range of values including 14 per 100,000 live births in a study based in Switzerland (Fanconi & Lips, 2010), 29.7 per 100,000 infants less than one year in a USA study (Keenan *et al.,* 2003) and 24.6 per 100,000 infants less than one year and 10.1 children less than two years in two UK studies (Barlow & Minns, 2000; Jayawant *et al.,* 1998). There is a higher incidence in those under one year and this is thought to coincide with peak levels of inconsolable and unpredictable crying (around 5-6 weeks) that typically occurs in this age group (Catherine *et al.,* 2008; Barr *et al.,* 2009; Barr *et al.,* 2006). It is important to note that the reported incidence rates are likely to be under estimations as many cases may go unreported or unrecognised (Ettaro *et al.,* 2004). Victims are usually under two years of age and the peak incidence is typically around four to six months (Frasier, 2008; King *et al.,* 2003), however a few isolated AHT cases of children older than two years have been described in the literature (Salehi-Had *et al.,* 2006).

The outcome for AHT victims is poor. Mortality rates are approximately 20-25% (Paul & Adamo, 2014) and the majority of survivors suffer significant life-long disability and neurologic impairment (Duhaime *et al.,* 1996; Minns *et al.,* 2005).

Various risk factors and situations have been suggested to increase the probability of the occurrence of AHT. These include; single-parent families, young parents with low educational attainment, families with low socioeconomic status, young infants, male gender and premature or low birth weight (Hennes *et al.,* 2001). Other factors include a past history of child abuse, history of domestic violence, drug or alcohol abuse and a

parental background of mental health problems (Minns & Brown, 2005). Assumptions related to the likelihood of abuse should be made with caution as not every perpetrator of inflicted infantile head injury will fit into a profile composed of these risk factors (Narang & Clarke, 2014).

2.3 Historical Review of Child Abuse in Relation to Subdural Haematoma

An article produced in 2005 by Roche et al provides a translation of a paper written in 1860 by Ambroise Tardieu, a French forensic physician (Roche *et al.,* 2005). The original paper details 32 cases of cruelty and ill-treatment of children, providing the first important description of battered child syndrome. In the late 19th and early 20th century reports started to appear of children presenting with multiple SDHs, ophthalmic haemorrhages, skeletal fractures, and multiple soft tissue swellings (Wiglesworth, 1892; Sutherland *et al.,* 1894; Herter, 1898; Cushings, 1905). At the time the aetiology of these clinical features was widely debated. In 1856 Rudolf Carl Virchow suggested that SDH was caused by infection and named the disorder "pachymeningitis haemorrhagic interna", by which it became known for almost 100 years (Lazoritz & Bier, 2001). It was not until 1914 that Wilfred Trotter, a British neurosurgeon, stated the cause of SDHs to be traumatic haemorrhage coming from cerebral veins as they pass from the brain to the tributaries of the superior sagittal sinus (Trotter, 1914).

A study by a paediatric radiologist reported an association of SDH and multiple fractures in six infants, particularly in the long bones (Caffey, 1946). These types of lesions were finally linked to trauma in the early 20th century by neurosurgeons and radiologists. However, there were still significant numbers of physicians that failed to recognise these injuries as inflicted rather than accidental (Al-Holou *et al.,* 2009). A comprehensive, scientific paper on child abuse was published in the 1960's and the topic was now being widely discussed and accepted in hospitals nationwide in the US (Kempe *et al.,* 1962).

As a consequence of Kempe's paper, child abuse become an accepted diagnosis in the medical community and further case reports continued to be published on the presence of SDHs, retinal haemorrhages (RHs) and bony lesions, often without external markers of trauma (Al-Holou *et al.,* 2009). Based on these papers and experimental

biomechanical work on animals (see biomechanical models in section 2.7), a paediatric neurosurgeon published an article in the British Medical Journal reporting a series of infants with SDHs where the caregivers admitted to violently shaking their baby (Guthkelch, 1971). This paper is the first reference in the medical literature, to what the authors termed 'whiplash injury', a mechanism of violent shaking that can cause traumatic neurotrauma.

2.4 Clinical Presentation

Infants with AHT often present to the emergency department with a sudden onset of unconsciousness and apnoea or seizures (Boos, 2006). The respiratory centres are relatively poorly developed in small babies, resulting in an increased susceptibility to apnoeic episodes. Apnoea at presentation is strongly associated with death or severe disability (Kemp *et al.,* 2003).

A history of minor accidental trauma is sometimes given for AHT, with a fall from a low height being one of the most common explanations given by caregivers (Narang & Clarke, 2014). Often no history of trauma is given for the sudden deterioration in mental state and clinical findings in AHT cases (Di Maio & Di Maio, 2001).

Mild to moderate symptoms of AHT can include irritability, vomiting, feeding difficulties, lethargy, drowsiness, increased or decreased muscle tone, increasing head size, failure to thrive, facial bruising and excessive crying. Alongside decreased levels of consciousness, apnoea, and seizures, further more severe symptoms include; respiratory distress, hypothermia, bradycardia, bulging fontanelle, cyanosis, coma and death (Pfeil, 2006).

2.5 The Injuries Seen in Abusive Head Trauma

2.5.1 Head Trauma

2.5.1.1 Subdural Haemorrhage

Subdural haemorrhage (SDH) is an abnormal collection of blood that accumulates below the dura mater (Fig. 2.1). Around 90% of infants with AHT suffer SDHs. These haemorrhages are often described as a thin film of blood, are commonly bilateral and can occur over the cerebral convexities, within the interhemispheric fissure,

subtemporally, suboccipitally or in the posterior fossa (Minns & Lo, 2009). As the haematoma is often a thin film of blood and not space-occupying, there is not usually a significant mass effect on the underlying brain (Kemp *et al.,* 2003). Although SDH may be seen as a marker of trauma, it is not the pathology responsible for the severe clinical outcome of AHT, which is more likely related to hypoxic-ischaemic damage to the brain (Kemp *et al.,* 2003). Although there are many potential causes of SDHs, several studies indicate that trauma is by far the most common (Hobbs *et al.,* 2005; Jayawant *et al.,* 1998).

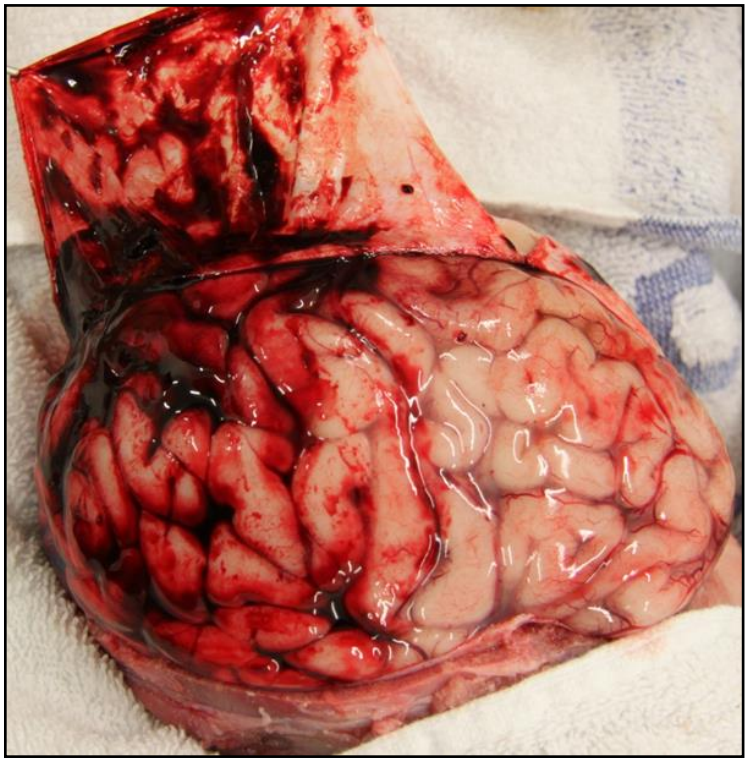


Figure 2.1 Thin film subdural haemorrhage over the right parietal and occipital lobes

2.5.1.2 Subarachnoid Haemorrhage

Subarachnoid bleeding is the second most common haemorrhage in AHT and is often seen in the parasagittal region (Rorke-Adams *et al.,* 2009). The majority of studies report the prevalence of subarachnoid haemorrhage (SAH) in AHT cases to be in the region of 20-37% (Roach *et al.,* 2014; Ewing-Cobbs *et al.,* 2000; King *et al.,* 2003).

2.5.1.3 Extradural Haemorrhage

Extradural haemorrhage (EDH) is not often seen in AHT and is usually seen when an impact injury has occurred (Rorke-Adams *et al.,* 2009). This type of haemorrhage results

in bleeding between the skull and dura and the source of EDH is often due to a skull fracture which transverses the course of a meningeal artery inside the inner surface of the skull (Rorke-Adams *et al.,* 2009).

2.5.1.4 Primary Parenchymal Injuries

Primary parenchymal injuries occur as a direct result of the traumatic forces in the abusive event. These types of injury include shear-type injuries from inertial forces and contusions which are seen as a result of impact (Caré, 2006). Previously, studies suggested that diffuse axonal injury (DAI) was one of the main pathological findings in AHT (Gleckman *et al.,* 1999). However, more recent studies have shown that DAI is rare in infants, only occurring in a small number of cases (less than 6%) (Geddes *et al.,* 2001a; Matschke *et al.,* 2015). Focal but significant traumatic axonal injury has however been shown in the brainstem of AHT victims (Matschke *et al.,* 2015).

2.5.1.5 Secondary Parenchymal Injuries

Diffuse brain damage with potentially fatal brain swelling (oedema) are often present in AHT infants, and it is these secondary injuries that are likely to be responsible for the severe clinical outcomes in these cases, however the exact pathogenesis remains unclear. Hypoxic-ischaemic injury with hypoxic-ischaemic encephalopathy rather than the primary traumatic brain injury is thought to be the principle cause of diffuse brain damage in AHT (Matschke *et al.,* 2015).

2.5.2 Associated Injuries

2.5.2.1 Retinal Haemorrhage

Retinal haemorrhages (RHs) are an important feature of AHT, with studies suggesting prevalence rates of 78-85% (Maguire *et al.,* 2013, Morad *et al.,* 2002, Kivlin *et al.,* 2000). Retinal haemorrhages associated with AHT often involve all the layers of the retina, extending to the ora serrata, are too numerous to count, are often bilateral (Maguire *et al.,* 2013) and are often described as flame-shaped or dot/blot haemorrhages (Morad *et al.,* 2010).

2.5.2.1.1 Pathogenesis of retinal haemorrhages in abusive head trauma

The exact pathogenesis of RHs in AHT cases is unknown; however, several mechanisms have been postulated. The main proposed mechanism is vitreoretinal traction. The vitreoretinal attachments in babies are extremely firm (much more so than in adults), and the repeated acceleration-deceleration movement of the eye during a shaking event is thought to induce shearing forces at the vitreoretinal interface and inside the orbit (Morad *et al.,* 2010). These mechanical forces may directly damage blood vessels or produce enough shearing force to induce disruption of vascular autoregulation, resulting in 'leaky' vessels (Morad *et al.,* 2010).

Further suggested mechanisms for the occular findings seen in AHT have included raised intracranial pressure due to intracranial haemorrhage and brain oedema, or increased intrathoracic pressure due to chest compression (Morad *et al.,* 2010). However, findings suggestive of raised intracranial pressure, including central retinal vein occlusion and papilloedema are rarely seen in infants with AHT (Forbes, 2008). Retinal haemorrhages due to cardiopulmonary resuscitation with chest compression are rare, and if this intervention does result in RHs, they are very limited in number and confined to the posterior pole (Odom *et al.,* 1997; Gilliland & Luckenbach; 1993; Goetting & Sowa, 1990).

2.5.2.1.2 Other Ocular Findings in Abusive Head Trauma

A fairly specific sign of AHT is retinoschisis (Morad *et al.,* 2010). This is a splitting of the retina between the layers, most commonly just the internal limiting membrane. This results in formation of a cystic cavity that may be partially or completely filled with blood. A pleating of the retina may also be observed, these deformations are known as paramacular folds and are highly specific for AHT (Morad *et al.,* 2010).

Further ocular findings include schisis of the macula, optic atrophy, and less commonly, retinal detachment. Optic nerve sheath haemorrhage is also very common (Wygnanski-Jaffe *et al.,* 2006).

2.5.2.1.3 Differential Diagnosis of Retinal Haemorrhages

As caregivers commonly present a history of a fall in an attempt to account for the injuries seen in cases of AHT (Narang & Clarke, 2014), the occurrence of RHs as a result of accidental trauma requires discussion. It is also prudent at this juncture to discuss RHs in relation to birth-related trauma. (For a more extensive list of the differential diagnosis for RHs in infants see Appendix 1a).

Several studies have investigated the prevalence of RHs in children suffering accidental trauma to the head. These accidents include motor vehicle accidents and falls. In one prospective study of 154 children two years or younger admitted to hospital with head trauma as a result of a fall, 16 had evidence of intracranial injuries. Only three patients had RHs (prevalence 1.9%) which were unilateral and few in number (Trenchs *et al.,* 2008). In a further study, 140 children (52 less than two years) were evaluated by an ophthalmologist for RHs after sustaining accidental head injuries with forces sufficient to cause a skull fracture and/or intracranial haemorrhage. Only two children had RHs, both had been involved in side-impact car accidents and had severe head injury. These findings led the authors to conclude that RHs rarely occur in accidental injury and that when they do, they are associated with extraordinary force (Johnson *et al.,* 1993). Retinal haemorrhages that result from accidental trauma are also suggested to be predominantly unilateral, few in number and confined to the posterior pole (Maguire *et al.,* 2013).

Birth-related RHs are not uncommon with up to 50% of newborn babies having haemorrhages (Emerson *et al.,* 2001), most often seen following vacuum extraction (Hughes *et al.,* 2006). Most birth-related haemorrhages resolve by four weeks of age (Emerson *et al.,* 2001).

2.5.2.2 Spinal Injuries

Trauma in the cervicomedually junction and spinal cord are stated as “underappreciated markers of shaking injury” (Judkins *et al.,* 2004). Injuries to the spine associated with AHT may be infrequently documented or missed clinically due to a lack of thoracolumbar spinal imaging. There may also be technical challenges related to imaging, including motion, streak artefacts on CT images, small isointense or isoattenuating haemorrhages,

as well as clinical difficulty recognising spinal injuries in extremely ill infants with severe traumatic brain injury (Choudhary *et al.,* 2012).

Spinal injuries can include direct spinal cord injuries, nerve root injuries or avulsions, epidural and subdural haemorrhages, spinal ligamentous injuries, bone oedema and fractures (Choudhary *et al.,* 2012). In one AHT study, 71% of fatal cases had documented evidence of spinal column, spinal cord and/or cervical root injury (Brennan *et al.,* 2009). In a further study, spinal SDH was shown to be present in 44% of AHT cases when imaging using MRI (Koumellis *et al.,* 2009).

Suggested mechanisms for the presence of spinal SDH in AHT include tracking of blood into the spinal compartment from an intracranial bleed and direct injury to vessels in or around the spinal cord but within the dural compartment (Koumellis *et al.,* 2009; Choudhary *et al.,* 2012).

2.5.2.3 Fractures

When fractures occur in the first four months of life they are nearly always as a result of abuse (Skellern *et al.,* 2000). Rib fractures are highly specific indicators of child abuse in this young age group, often seen posteriorly, near the costo-vertebral junction, especially the fourth to the seventh ribs. However, accidents, birth trauma, resuscitation and bone fragility need to be considered in the differential diagnosis (Skellern *et al.,* 2000). In one study of 364 AHT cases, skull fractures were seen in 95 (26%), fractures of the extremities were seen in 82 (23%) and rib fractures were seen in 80 (22%). In a further study of 106 AHT infants, 51 had a fracture, with a total number of 188 fractures over all the cases. Twenty-five (13%) of these fractures were skull fractures, 91 (48%) were rib fractures and 72 (38%) were long bone fractures (Hobbs *et al.,* 2005).

2.5.2.4 Bruising

It may be expected that an infant subjected to violent shaking would have physical evidence of gripping from the assault. However, external evidence such as bruising may not always be present in all AHT cases. A post-mortem study of bruising prevalence and distribution in 24 AHT cases showed that five (21%) subjects had no external bruising at all, and seven (29%) subjects had no fresh external bruising (Atwal *et al.,* 1998). All of the subjects without fresh external bruising had internal bruising, highlighting the

importance of a careful and layered subcuticular dissection. The commonest sites of bruising included the forehead and buttocks in this study. Sites at which one would expect to see bruising from gripping, such as the limbs, chest and abdomen were surprisingly uncommon. Suggestions for the lack of external bruising in some AHT cases included: clothing acting as a barrier between the fingertips and underlying skin, an indefinable length of time for bruising to develop, or that bruising may develop and disappear in a relatively short time (Atwal *et al.,* 1998). Further studies have shown the incidence of bruising to be around 40-50% (King *et al.,* 2003; Hobbs *et al.,* 2005).

2.6 The Role of Imaging in Abusive Head Trauma

Diagnostic imaging is a critical modality in the assessment of an infant with suspected AHT and may be used to guide medical management and potential forensic investigations (Jaspan *et al.,* 2003). Several imaging tools are used across the various institutions where cases of AHT may present. These include conventional radiography, ultrasound, computed tomography (CT) and magnetic resonance imaging (MRI).

It has been suggested that radiological evaluation of AHT cases is often incomplete or of insufficient quality, which can result in diagnostic error and subsequent difficulties in appropriating suitable child care or pursuing criminal proceedings. This led to publication of a paper in 2003 outlining a proposed protocol for the neuroimaging of AHT cases (Jaspan *et al.,* 2003). In 2008, The Royal College of Radiologists and Royal College of Paediatrics and Child Health produced an intercollegiate report of standards for radiological investigations of suspected non-accidental injury (The Royal College of Radiologists and Royal College of Paediatrics and Child Health, 2008).

2.6.1 Conventional Radiography

Conventional radiography (also known as X-rays; plain films), a form of electromagnetic radiation, is part of the skeletal survey and in cases of suspected AHT a full skull series including left and right lateral views is required. This plain film type of imaging is the most appropriate system for evaluating skull vault fractures and should be performed within the first day or two of admission (Jaspan *et al.,* 2003). Conventional radiography is also important for imaging the rest of the skeleton as a full skeletal survey can also

help rule out any possible underlying bone diseases, or may show additional injuries suspicious for child abuse (Sieswerda-Hoogendoorn *et al.,* 2012a).

2.6.2 Ultrasound

Ultrasound imaging uses ultra-high frequency sound waves to produce cross-sectional images of the body (Lisle, 2007). The role of cranial ultrasound is limited in the diagnosis of AHT. However, one suggested potential use for ultrasound is monitoring the resolution of SDH diagnosed on CT/MRI (Datta *et al.,* 2005).

2.6.3 Computed Tomography

Computed tomography allows for the acquisition of cross-sectional images obtained from X-rays which are transmitted through the area of interest whilst the patient passes through a rotating gantry. A CT image is comprised of picture elements (pixels). All of the tissues contained within the pixel attenuate the X-ray projections and result in a mean attenuation value for the pixel. This value is compared with the attenuation value for water and is displayed on a scale (the Hounsfield scale). Water is said to have a value of 0 Hounsfield units (Hu); air typically has an Hu number of -1000; fat is approximately -100; soft tissues are in the range +20 to +70 Hu; and bone is usually greater than +400 Hu (Weir, 2011). This technique uses computer analysis, therefore a much greater array of densities can be observed than with conventional plain films (Lisle, 2007).

On the day an infant presents with possible AHT, the first imaging to take priority is a cranial CT. In the acute phase, CT is useful because it can detect traumatic injury both in the form of fractures and intracranial pathology (Sieswerda-Hoogendoorn *et al.,* 2012a). The advantages of CT include, short scan times, compatibility with life-support and traction/stabilisation devices and widespread availability (Murray *et al.,* 1996). If performed at suboptimal protocols, small subdural bleeds may be missed (Datta *et al.,* 2005), and fractures orientated parallel to the scan plane can also be missed (Jaspan *et al.,* 2003). Axial scanning must be undertaken from the base of the brain to the vertex (Jaspan *et al.,* 2003). Recommended slice thickness is 5mm or less, and both soft tissue and bone window images should be acquired. Intermediate settings may also be required in the presence of equivocal extra-axial haematomas over the brain surface

(The Royal College of Radiologists and Royal College of Paediatrics and Child Health, 2008).

2.6.4 Magnetic Resonance Imaging

Magnetic resonance imaging uses the magnetic properties of the hydrogen atom to produce images. The nucleus of the hydrogen atom is a single proton (Lisle, 2007). The patient is magnetised in the bore of a powerful magnet and short pulses of radiofrequency (RF) energy resonate protons which are present in fat, protein and water. The protons produce RF echoes when their resonant energy is released and their density and location can be exactly correlated into an image matrix (Weir, 2011). In general, MRI is water imaging. T1 and T2 are the basic MR imaging sequences. Within a T1 sequence water has no signal and is black, whereas in T2 sequences water has a high signal and is white. Similar to CT, MRI also produces image slices of specified thicknesses through the body (Benseler & Ebrary, 2006).

Three to four days after admission, an AHT infant may have a MRI scan which should include at least the cervical spine, and preferably the whole spine (Jaspan *et al.,* 2003). MRI has multi-planar and multi-sequence capabilities which improve the sensitivity to most expected pathologies, including haemorrhage and ischaemic changes (Jaspan *et al.,* 2003). MRI can facilitate identification of small SDHs (Sato *et al.,* 1989) and haematomas in areas difficult to image with CT (posterior fossa, anterior part of the middle fossa and close to the inner table of the skull) (Jaspan *et al.,* 2003). One of the disadvantages of performing MRI is the long scan times which result in infants requiring general anaesthesia (Sieswerda-Hoogendoorn *et al.,* 2012a).

2.6.5 Subdural Haemorrhages

Subdural haemorrhage has been shown in imaging studies in up to 92% of AHT cases (Bradford *et al.,* 2013). On imaging, SDHs in AHT are often relatively shallow, occurring at multiple sites over the cerebral hemispheres, often in the posterior fossa (Stoodley, 2006) and in the interhemisphereic fissure (Datta *et al.,* 2005).

2.6.6 Fractures

In a radiological study of AHT, a sub-group of 29 out of 49 (59%) infants less than six months had fractures. Rib fractures were most common (14 patients, 48%), with limb

fractures (11 infants, 38%) and skull fractures (11 patients, 38%) also observed. Multiple fractures were recorded in 21 patients (72%). Evidence of impact to the head was seen in 16 cases (11 with skull fractures and an additional five with no skull fractures but showing scalp swelling) (Datta *et al.,* 2005). A further study of 104 cases of AHT, showed very similar results, with 56 (57%) infants having one or more fractures, with a total across all cases of 254 fractures. Most common were rib fractures, followed by limb fractures (Sieswerda-Hoogendoorn *et al.,* 2014).

2.6.7 Other Injuries

Neuroimaging has shown not only SDHs to be significantly associated with AHT, but also hypoxic-ischaemic injury and cerebral oedema (Kemp *et al.,* 2011). Epidural haemorrhages and cortical contusion are rarely seen in child abuse. The most common intraaxial manifestation of child abuse is diffuse cerebral hyperemia and oedema. This is seen on CT as global effacement of the subarachnoid and ventricular spaces with an inability to distinguish grey and white matter because of diffusely decreased parenchymal attenuation (Murray *et al.,* 1996).

2.7 Biomechanical Models of Inertial Mechanisms of Head Injury

There is no human data on the magnitude of inertial forces required to injure biological tissue which result in SDHs. The definitive experiment of shaking an infant to determine injury thresholds for biological tissues such as the bridging veins, subdural membrane and cervical spine cannot be performed for obvious ethical reasons. Biomechanical engineers therefore turn to various models to provide values which can only be viewed as approximations. Before discussing these models in more detail it is important to emphasise that the biomechanical literature does not yet provide a definitive answer on the dynamic forces required when shaking an infant to the point of causation of SDH (Narang *et al.,* 2013).

Numerous biomechanical studies have been carried out to investigate the likelihood that a shaking event would lead to the cranial injuries seen in abusive head trauma. These studies have included animal models (Gennarelli & Thibault. 1982), anthropormorphic test devices (Duhaime *et al.,* 1987) and finite element modelling (Roth *et al.,* 2007).

2.7.1 Animal Models

One of the earliest animal studies for what was called 'whiplash injury' during the time of the investigation was a rhesus monkey subjected to rapid acceleration along a 12 foot track after a piston delivered an impacted to the carriage containing the animal. The experiment produced a rapid linear acceleration of the head which was shown to result in concussion in some of the animals and also visible brain injury, including SDHs and cortical contusions. (Ommaya *et al.,* 1968). After publication of Ommaya's paper on whiplash injury in rhesus monkeys, consideration was given to the theory that shaking could be the cause of SDH in abused infants (Caffey, 1974; Caffey, 1972; Guthkelch, 1971).

In a further experiment using rhesus monkeys, a singular acceleration/deceleration pulse of 5-25 ms over a 60° arc was delivered at a force between 100 to 30000G. The authors demonstrated that SDH could be produced without an impact to the head and suggested the cause of this bleeding to be bridging veins which could rupture depending on the rate of acceleration/deceleration of the head. They stated that bridging veins have an ultimate strain rate which if exceeded would lead to tearing of the vessels. The idea was put forward that the force used in this study caused a sagittal movement of the head, causing a rapid acceleration which exceeded the strain rate of the bridging veins, causing them to rupture (Gennarelli & Thibault, 1982).

A more recent study examined the effects of shaking living animals (Finnie *et al.,* 2012). Nine anaesthetised lambs were chosen due to their relatively large gyrencephalic brain, large head and weak neck muscles. The animals were vigorously shaken with sufficient force to impact the head back and forth onto the chest. The authors noted a considerable amount of lateral and rotational head movement as well as the acceleration/deceleration of the head. Each lamb was shaken 10 times for 30 seconds over a 30 minute period. No head impact occurred. Three lambs died after being shaken, the remaining lambs were maintained under anesthesia and killed by perfusion fixation of the brain with 4% paraformaldehyde containing 0.02% heparin. Three lambs had macroscopic focal SDH, one from the group that died after being shaken and the other two from the perfusion group. The same study that produced the neuropathological findings in this group of lambs was also used for publication of a biomechanical paper

which characterised the kinematics of lamb's heads during shaking episodes, and general characterisation of the relative motion of the head to the body (Anderson *et al.,* 2014). Accelerations of 30-70 G were recorded, typically recorded during interactions of the head with the torso.

2.7.2 Anthropormorphic Test Device

In 1987 a study was published which implied that shaking alone could not cause fatal brain injuries and that an impact would be required (Duhaime *et al.,* 1987). This study was performed using models of one month-old infants fitted with accelerometer transducers, with various neck and skull parameters to determine the angular acceleration values that could be generated from shaking and impact onto a padded and unpadded surface (Duhaime *et al.,* 1987). The force produced by shaking alone fell below the injury thresholds established by primate 'whiplash' models of concussion, SDH and diffuse axonal injury, (which were scaled by mass to the weight of an infant brain). It is unclear which primate studies were utilised to obtain these values (Cory & Jones, 2003), but Gennarelli et al. (1982) and Thibault and Gennarelli (1985) were both referenced in the study.

There have been many criticisms of the 1987 Duhaime study, and a biomechanical assessment study of the paper was produced in 2003 (Cory & Jones, 2003). In this paper the authors found that minimal variation of the materials and construction of the model produced significant enough differences in the angular acceleration to exceed the injury thresholds stated in Duhaime's paper in eight out of 10 trials. The fidelity of the model to a real infant has also been challenged, including arguments that the necks of the model did not mechanically resemble an infant's neck. The fact that the injury threshold values were derived from adult primates has also been heavily criticised, as it has been suggested that simple mass scaling is ineffective when comparing infant and toddler piglets (Ibrahim *et al.,* 2010), let alone when scaling between different species and ages. The primates were also only subjected to a single angular acceleration-deceleration force in one plane of movement. This mechanism of injury fails to represent the damage caused by repetitive shaking (Cory & Jones, 2003). Both adult and infant brains are increasingly susceptible to repetitive injury (Prins *et al.,* 2013), implying a potential cumulatively damaging effect in shaking events.

2.7.3 Finite Element Modelling

Relatively recently biomechanics has moved into the direction of computational finite element modelling (Roth *et al.,* 2007). These models are useful in investigating the effect of anatomical variances, biomechanical parameters, and injury mechanisms on ensuing deformation and stress in the brain and skull. Finite element models require detailed geometry, biofidelic material property data and realistic boundary conditions and applied loads (Margulies & Coats, 2010).

To model a shaking event reliable data is required for the failure properties of bridging veins, however tissues from children are difficult to obtain (Margulies & Coats, 2010). Computational biomechanical modelling has been limited by an insufficient amount of quality paediatric biomechanical data and current computational models have been created as platforms on which enhancements can be made as the quality of paediatric data improves (Jones *et al.,* 2014). Current finite element models suggest that a shaking event without impact is enough to generate vitreo-retinal traction leading to RHs and to cause rupture of bridging veins leading to SDH (Nadarasa *et al.,* 2014) but more accurate models using better material property data are needed to confirm these statements.

Part II: Cerebrodural Anatomy of the Infant

The second part of this literature review provides an overview of the anatomy of the infant head, including a brief description of the skull and brain, and a more detailed discussion related to the structures associated with subdural haematoma; the dural membrane and venous system. The anatomical differences between an infant and an adult that predispose the young to injury from shaking are also described.

2.8 Predisposing Anatomical Features to Shaking Injury in the Infant

According to Newton's first law, an object at rest or in a uniform state of motion will remain this way, unless an external force is applied. An object's resistance to change in motion is its inertia and the amount of inertia is proportional to the objects mass (i.e the larger the mass, the greater the force required to get it moving) (Kieser *et al.,* 2013). In a shaking event, the brain moves within the cranial cavity to try and keep pace with the accelerating skull (Case *et al.,* 2011), and there are a number of anatomical differences in the infant head compared to the older child and adult that are thought to predispose this age group to injuries sustained by shaking.

The infant has a relatively large head in relation to body size (approximately 10-25% of total body weight) (Minns & Brown, 2005; Sanders *et al.,* 2012). The neck muscles are relatively weak (Huelke, 1998) and therefore the infant is unable to support the weight of its own head during the early stages of post-natal development. These factors would result in a tendency to a greater degree of movement of the head during a shaking event. The skull base of the infant is also relatively flat and shallow, making the brain more susceptible to rotational movements inside the cranial cavity.

At one month of age, the brain weighs approximately 482g and this value almost doubles to around 967g by the end of the first year of life (Fracasso *et al.,* 2009). By the end of the sixth year the weight of the brain will have tripled to approximately 90% of its adult weight (Augustine, 2008), with a large German study of 8000 autopsies suggesting a mean weight for the male and female adult of 1336g and 1198g, respectively (Hartmann *et al.,* 1994). The nerve fibers of the brain are largely unmyelinated at birth and the brain

has a larger amount of fluid which results in the brain being much softer than an adult brain (Minns & Brown, 2005). Furthermore, an infant's ventricles and subarachnoid spaces are proportionally larger than an adults with less cerebrospinal fluid which provides additional space for the brain to move within the cranial cavity and reduces the buffering capacity of the CSF between the rigid skull and the soft brain (Minn & Lo, 2009; Smith, 2006).

The infant brain is more susceptible to the forces involved in a shaking event because the smaller mass of an infant brain and increased ventricular and subarachnoid spaces, reduces the brain's inertia compared to that of an adult's brain. Inertial brain motion occurs due to the differences in rigidities between different tissues (i.e skull and brain) (Case, 2014) therefore greater differences (e.g. the softer infant brain) would also reduce the inertia.

With the brain relatively free to move within the cranial vault, tissues of different consistencies distort and move at different rates. Strains may occur at junctions between different tissue types which can result in shearing injuries. This may occur in the parenchyma of the brain but could also occur along the bridging veins as the brain tries to keep up with the accelerating skull which is attached to the dura (Case 2014; Case *et al.,* 2001).

2.9 The Infant Head

2.9.1 The Skull

The skull consists of the neurocranium which surrounds and encloses the brain, and the viscerocranium which forms the facial skeleton. The neurocranium can then be separated further into the cranial base and cranial vault (or membranous cranium/calvarium) (Tubbs *et al.,* 2012). The infant skull is relatively large when compared to the rest of the skeleton. At this age, there is a considerable disproportion between the facial part of the skull and the cranial vault (McMinn, 1994; Warwick & Williams, 1973), with neonates having a face-to-cranium ratio of 1:8, compared with an adult ratio of 1:2.5 (Huelke, 1998).

The thickness of infantile skull bones is relatively thin compared to that in later life with one study demonstrating a median parietal bone thickness of 1037 µm between 0 and 90 days, and 1455 µm between 91 to 180 days (Breisch *et al.,* 2010). However growth of the calvarium is very rapid during the first year of life. In comparison, a study of 64 adults (age range 16-90 years) showed a mean measurement at the right and left euryon (point marking greatest diameter of the skull) of the parietal bones to be 5.3 mm with a range of 2.7-8.6 mm (Lynnerup, 2001). This demonstrates not only the significant difference in thickness between infants and adults but also the extensive variation that occurs in the adult population.

The cranium consists of two frontal bones, two parietal bones, and the upper parts of the occipital, temporal and sphenoid bones (Fig. 2.2). In the young, complete ossification of the skull is yet to occur and the cranial bones are not fused at the sutures as in the adult skull. Between the frontal bones lies the metopic suture, the coronal sutures lie between the frontal and parietal bones and the sagittal suture separates the parietal bones. The lambdoid sutures separate the parietals from the occipital bones (Fig. 2.2). The sutures function to enable compression of the vault during labour, which facilitates delivery of the baby. The birthing process may result in a change of shape of the cranium (moulding) as the bones move and may overlap each other slightly (Daftary & Chakravarti, 2011). The cranial base is formed from the lower parts of the occipital and temporal bones, the ethmoid bone and the majority of the sphenoid bone (Scarr, 2008). There are three subdivisions in this region, known as the anterior, middle and posterior cranial fossae (Warwick & Williams, 1973) (Fig. 2.3). The anterior cranial fossa constitutes the roof of the orbits, the middle contains the pituitary gland in the sella turcica and the posterior section contains an opening (the foramen magnum) where the brain is continuous with the spinal cord (Warwick & Williams, 1973).

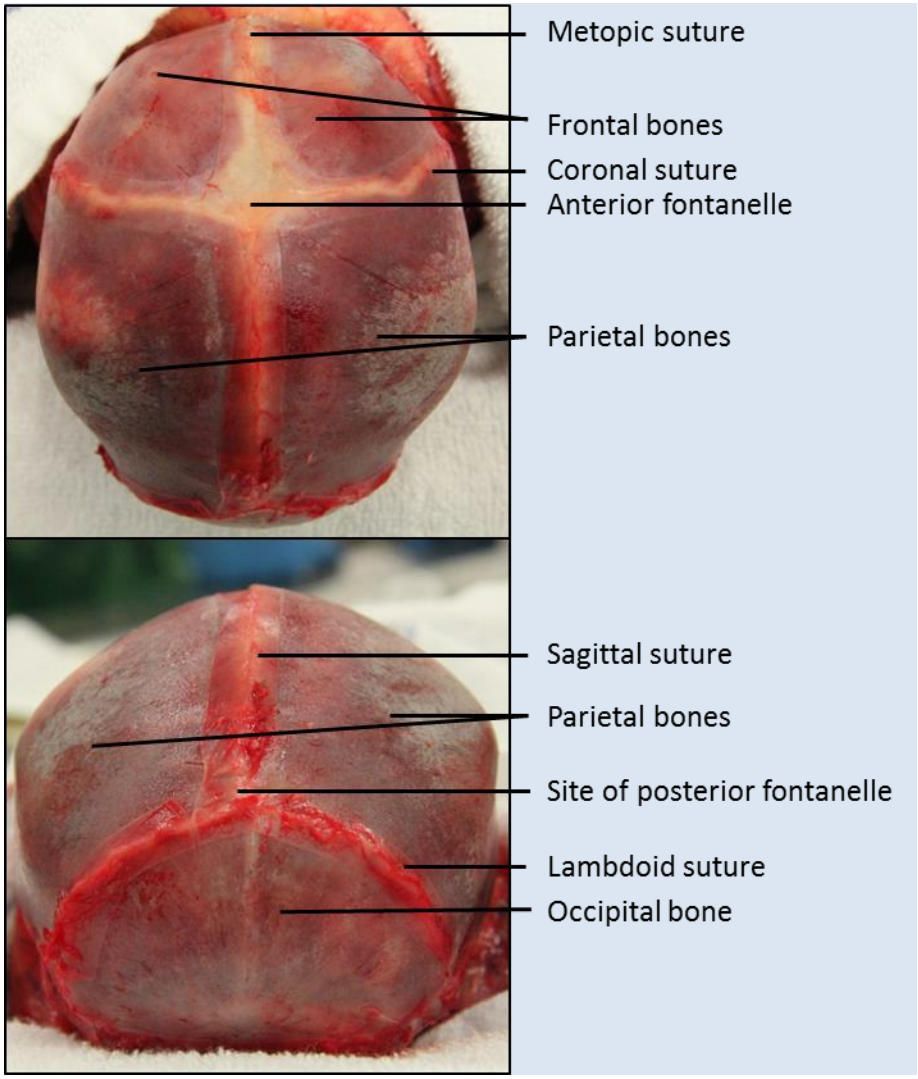


Figure 2.2 The calvarial bones of an infant showing sutures and fontanelles

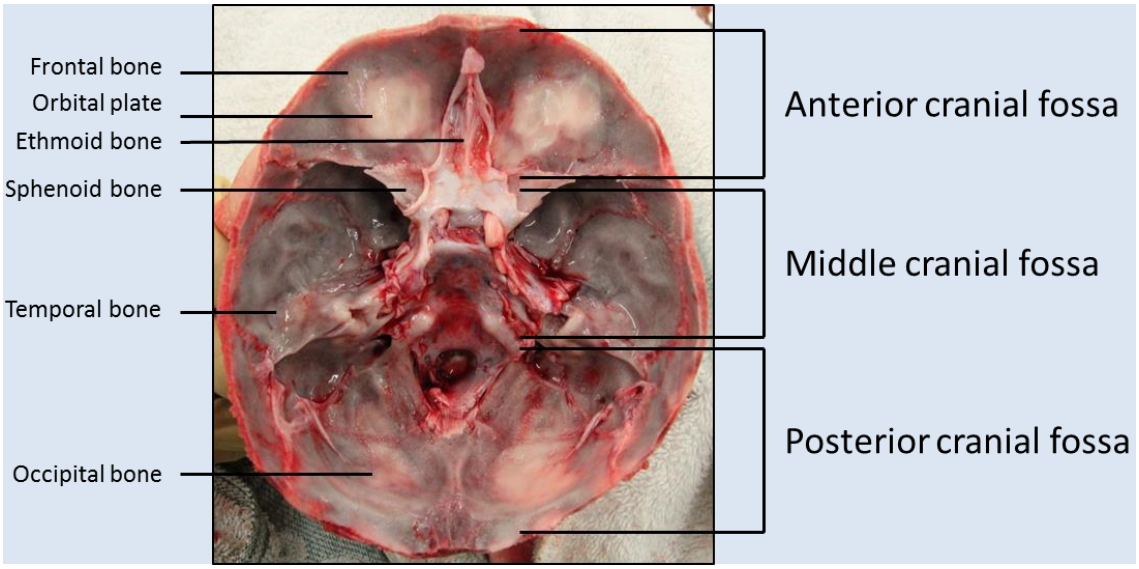


Figure 2.3 The cranial base of an infant

There are usually a total of six unossified membraneous apertures called fontanelles which are made of fibrous tissue (Snell, 2010) the anterior and posterior fontanelles (Fig. 2.2) and two sphenoidal and two mastoid fontanelles. The anterior fontanelle if formed at the junction of the metopic, coronal and sagittal sutures, and is diamond-shaped. The posterior fontanelle is situated at the junction of the sagittal suture with the lamdoid sutures and is triangular in shape (Warwick & Williams, 1973). The sphenoidal and mastoid fontanelles are on the lateral side of the cranium, with the mastoid located beneath the parietal bone and between the front of the occipital bone and the lower rear edge of the temporal bone and the sphenoidal located between the sphenoid, parietal, temporal and frontal bones. The sphenoidal and posterior fontanelles are normally obliterated by the age of two or three months, the mastoid by the end of the first year of life and the anterior usually by the middle of the second year (Warwick & Williams, 1973).

2.9.2 The Brain

The human brain consists of the following structures; brain stem, cerebellum, diencephalon and the cerebral hemispheres. The brain stem is made up of the medulla oblongata, the pons and the midbrain and has an important role in the control of respiration and the cardiovascular system. The cerebellum has three main lobes; the anterior, middle and flocculonodular lobes. This part of the brain functions to control posture and voluntary movements. The diencephalon is the central core of the brain and consists of the third ventricle and the structures in close association to the ventricle, such as the thalamus, subthalamus, epithalamus and the hypothalamus (Augustine, 2008; Snell, 2010).

The cerebral hemispheres are the largest part of the brain, separated by a midline sagittal fissure known as the longitudinal cerebral fissure. Within the depths of the fissure a white matter structure called the corpus callosum connects the two hemispheres. The surface of the hemispheres is convoluted forming folds known as cortical gyri which are separated by fissures (sulci). Several large sulci separate the hemispheres into lobes which are named from the cranial bones under which they lie; the frontal, parietal, occipital and temporal lobes (Snell, 2010).

2.9.3 Cranial Meninges

Three membranes, known collectively as the "meninges" surround the brain and spinal cord. Within the cranium the outermost membrane, the dura mater, (a.k.a. the pachymenix (from the Greek word pachy meaning "thick")), has two layers; a superficial outer layer adhering to the cranial bones and acting as the skull's inner periosteum, and an inner meningeal layer lying in close association to the middle membrane, the arachnoid mater. The innermost membrane is the pia mater and is often referred to in combination with the arachnoid mater as the leptomeninges (from the Greek word lepto meaning "thin" or "fine") as they are a lot thinner and more delicate than the dura mater.

There is a subarachnoid space between the arachnoid and pia mater which is filled with cerebrospinal fluid (CSF). If sudden acceleration or deceleration forces are applied to the head the meninges act as an anchor against any sudden movements (England & Wakely, 1991) and CSF provides a fluid cushion to protect the brain from the rigid skull (Heimer, 1995).

2.9.3.1 The Cranial Dura Mater

The periosteal layer of the dura (also referred to as the endosteal layer) is not continuous with the dura mater of the spinal cord. The dense, strong, fibrous meningeal layer is the dura mater proper, and this layer is continuous with that covering the spinal cord via the foramen magnum (Snell, 2010). At certain points the dural layers separate to form the venous sinuses and the dural projections or 'folds'.

2.9.3.1.1 Dural Projections

There are four dural projections; the diaphragma sellae, the falx cerebelli, the falx cerebri and the tentorium cerebelli (Sinnatamby, 2011). The falx cerebri is suspended vertically between the cerebral hemispheres, and the tentorium cerebelli separates the cerebellum from the cereb.rum (Jacobson *et al.,* 2008) (Fig. 2.4a). The falx cerebella is a small, incomplete midline fold between the cerebellar hemispheres (Jacobson *et al.,* 2008) and the diaphragma sellae is a small circular fold which bridges over the sella turcica covering the pituitary gland (Heimer, 1995). There is an opening in the centre of the diaphragma sellae through which passes the infundibulum (the pituitary stalk),

connecting the pituitary gland with the base of the brain (Drake *et al.,* 2005). The dural folds help to stabilise the brain, limiting displacement from rotary movement (McMinn, 1994).

2.9.3.1.2 Dural Venous Sinuses

The dural sinuses are venous channels that run between the periosteal and meningeal layers of the dura (Adeeb *et al.,* 2012), with the exception of the inferior sagittal sinus which lies between the folds of the free margin of the falx cerebri and the straight sinus occupying the folds of dura at the junction of the falx cerebri and tentorum cerebelli (Sinnatamby, 2011). They are required for the venous drainage of the brain. Drainage begins in networks of small venous channels, which lead into larger cerebral and cerebellar veins and also veins which drain the brainstem (Drake *et al.,* 2005). These larger veins, including the great cerebral vein (of Galen), eventually empty into the venous sinuses which are endothelium-lined spaces. When the veins leave the surface of the brain to enter the sinuses they are said to 'bridge' from one location to the other, therefore this section of the vein is referred to as a 'bridging vein'. Finally, the blood is transported through these sinuses to the internal jugular vein (Heimer, 1995). The dural venous sinuses include the superior sagittal, inferior sagittal, straight, transverse, sigmoid, and occipital sinuses, the confluence of sinuses, and the cavernous, sphenoparietal, superior petrosal, inferior petrosal and basilar sinuses (Drake *et al.,* 2005) (Fig. 2.4a-b).

The superior sagittal sinus (SSS) lies between the two dural layers of the falx cerebri at its superior border (Agur & Dalley, 2009). Along its course the SSS receives the superior cerebral veins, diploic and meningeal veins through the lateral lacunae (blood lakes) and pericranial veins passing through parietal foramina (Standring, 2008). Two or three lateral lacunae are connected to the SSS and lie on both sides, parallel to the sinus. These lacunae have irregular, plexiform structures and receive most of the diploic and meningeal veins as well as arachnoid granulations which return cerebrospinal fluid to the bloodstream (Standring, 2008; Tubbs *et al.,* 2008; Sinnatamby, 2011).

The inferior sagittal sinus receives veins from the falx and a few veins from the medial surfaces of the cerebral hemispheres (Standring *et al.,* 2008). It runs along the falx

cerebri at the level of the free margin of the tentorium cerebelli and joins the great cerebral vein (of Galen) to form the straight sinus (Snell, 2010).

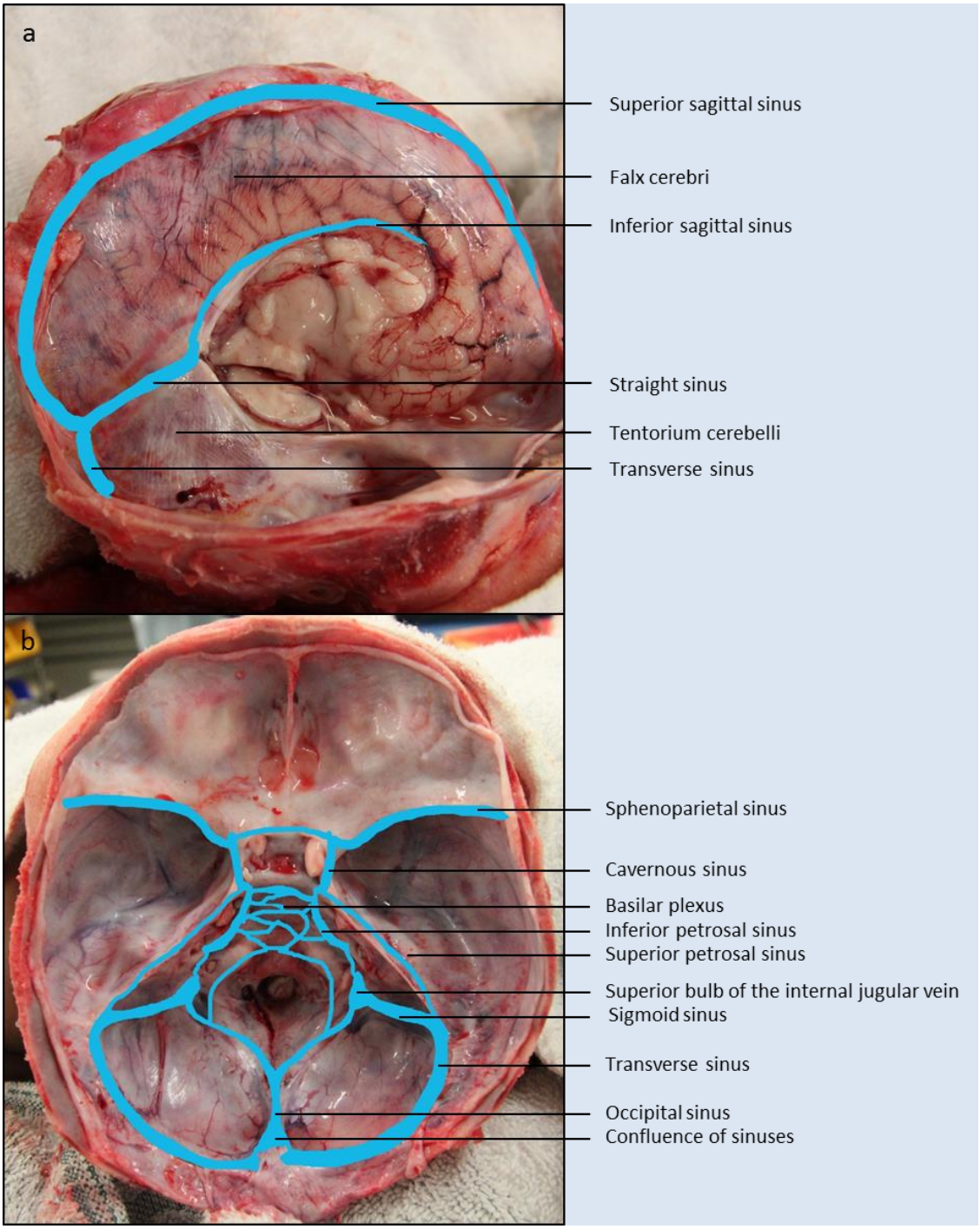


Figure 2.4 The dural venous sinuses a) shown within the falx and tentorium after right hemispherectomy and b) cranial base showing superior view of the sinuses

The straight sinus slopes down towards the internal occipital protuberance (Sinnatamby, 2011), receiving some superior cerebellar veins along its course (Standring, 2008; Snell, 2010). The occipital protuberance is the site of the confluence of sinuses (torcular

herophili, or torcula) which is the connecting point of the superior sagittal sinus, straight sinus, and occipital sinus.

There are two transverse sinuses, one of which (usually the right) is a continuation of the SSS (and therefore normally the larger of the two) and the other (usually left) is a continuation of the straight sinus. They run laterally between the two layers of the attached margin of the tentorium cerebelli, where they end to form the sigmoid sinuses (Standring, 2008; Snell, 2010). Transverse sinuses receive inferior cerebral, inferior cerebellar, diploic and inferior anastomotic veins (Standring, 2008).

Sigmoid sinuses are S-shaped sinuses which leave the tentorium to enter the mastoid groove on the mastoid part of the temporal bone. The sinuses turn anteriorly and then inferiorly, where they enter the posterior section of the jugular foramen, to become continuous with the jugular vein at the superior jugular bulb (Standring, 2008; Snell, 2010).

The superior petrosal sinus also joins the sigmoid sinus at the junction where the transverse sinus terminates. The inferior petrosal sinus joins the internal jugular vein at its first (highest) tributary. The basilar plexus is a network of veins which connects the two inferior petrosal sinuses and receives veins from the lower part of the pons and from the anterior medulla (Sinnatamby, 2011).

The occipital sinus is the smallest of all the sinuses, lying in the midline near the attachment of the falx cerebella. It communicates with the vertebral venous plexus and ends cranially, mainly at the confluence of sinuses (Kobayashi *et al.,* 2006).

The cavernous sinus lies within the middle cranial fossa. This sinus contains the internal carotid artery, transmits some cranial nerves and receives blood from the orbits, vault bones and cerebral hemispheres. It also receives the sphenoparietal sinus which runs beneath the edge of the lesser wing of the sphenoid bone. Drainage of the cavernous sinus occurs via the superior and inferior petrosal sinuses (Sinnatamby, 2011).

The dural venous sinuses also play a crucial role in cerebrospinal fluid (CSF) reabsorption (Varatharaj *et al.,* 2012), through the arachnoid granulations.

2.9.3.1.3 Arachnoid Granulations

At certain points along the arachnoid membrane are herniations known as “arachnoid granulations” which protrude into the dural venous sinuses (Grzybowski *et al.,* 2007). Cerebrospinal fluid is suggested to ‘ooze’ through the walls of the arachnoid granulations back into the blood (Sinnatamby, 2011). Arachnoid granulations begin as arachnoid villi in the young, which are discrete, forming the more visible clumps, the arachnoid granulations (a.k.a pacchionian bodies) with increasing age. At the age of six months arachnoid granulations are not visible but by 18 months they are quite obvious on close inspection (Le Gros Clark, 1920). The arachnoid granulations are most numerous within the parasagittal planes of the superior surface of the cerebral cortex (Grzybowski *et al.,* 2007).

2.9.3.1.4 Historical Studies on the Vasculature of the Dura

In the past the dura mater has been referred to as a metabolically inert, avascular fibrous covering of the brain, except in its periosteal layer (Adeeb *et al.,* 2012). Although not often alluded to in the current literature, the dura mater has been suggested in past studies to be a highly vascular tissue (Kerber and Newton, 1973; Key & Retzius, 1875; Hannah, 1936), and these previous studies have led some authors to suggest the presence of complex networks of both arterial and venous plexii (Mack *et al.,* 2009).

There are several historical texts that provide detailed descriptions of the vasculature of the dura mater. These texts provide accounts of supposed lymphatic vessels, capillary networks, and strands of connective tissue that course through the dura including ‘threads’ crossing from the dura to the arachnoid over the potential subdural space.

Key & Retzius (1875) mention blood vessels and nerves travelling through what they perceived as a subdural space, with strands of connective tissue that would ‘jump’ between the arachnoid and dura. Studying these vessels was reported as a difficult procedure as upon incision of the meninges they were easily stretched or ripped out, making them appear longer than they would be in reality. Several others in the past believed they saw lymphatic vessels in the brain, including Paolo Mascagni, whose main scientific interest was the lymphatic system (Lukic *et al.,* 2003), but these vessels are suggested to be ampullary extensions of the capillary and venous blood vessels (Key &

Retzuis, 1875). However, the exact routes of CSF circulation still present unresolved questions today and, while the current literature does not advocate lymphatic drainage systems in the brain, there are those that argue against this (Kapoor *et al.,* 2008). In past studies of histological examination of dura mater, vessels have been shown to be numerous, densely packed and relatively broad in the falx and tentorium cerebelli (Key & Retzuis, 1875).

Several historical texts have reported on the presence of extremely rich capillary networks on the inner dural surface of the dura mater, which are connected to veins (Key & Retzuis, 1875; Hannah, 1936; Robertson, 1900). Robertson comments on their remarkably large size, being three or four times the diameter of an ordinary capillary vessel. These vessels are said to lie in grooves which run for the most part in a direction parallel with the connective fibres of the membrane. These grooves, which vary considerably in size and depth, were suggested to form a system of perivascular canals (Robertson, 1900). It was even mentioned at this time that rupture of these extensive capillary networks could be the source of SDH (Hannah, 1936).

2.9.3.1.5 Arterial Blood Supply

Major blood vessels that supply the dura run in its outer (periosteal) surface and can be divided according to different areas of the skull. The largest and most important artery is the middle meningeal artery, which arises from the maxillary artery branch of the external carotid artery (Adeeb *et al.,* 2012). This artery is the main route for blood transported to the dura. To a lesser extent, other branches that carry blood to the dura include the ascending pharyngeal, accessory meningeal, vertebral and occipital arteries. A further important blood supply to the dura comes from the internal carotid artery which leads into its own small meningeal branches and from its ophthalmic branch (Rowbotham & Little, 1965).

The main meningeal arteries, often lying on the periosteal surface are approximately 400-800 microns in inner diameter (Kerber & Newton, 1973). Also in the outer dural layer, branching from the main arteries is a rich layer of vessels, named by Kerber as “primary anastomotic arteries” (PAA) (Kerber & Newton, 1973). This branching network of arteries is suggested to cross the superior sagittal sinus often, creating a single

vascular unit over the two dural hemispheres. The inner diameter of the PAA is measured at 100-300 microns (Kerber & Newton, 1973). It has been shown that the PAA have a straight or spiral arrangement, and a characteristic of the spiral-shaped arteries is that they are surrounded by loose connective tissue. Arteries near the walls of sinuses are reported as having a thin intima, while arteries near the periosteal layer have thicker more defined walls. Independent of location, the spiral arteries do not seem to exhibit thick, muscular walls (Roland *et al.,* 1987).

The PAA then branch further to give rise to four smaller arterial units; the arteries to the skull, the secondary anastomotic arteries (SAA), penetrating vessels and arteriovenous shunts. Arteries to the skull may be difficult to visualise as they are often torn as the dura is stripped from the skull. The SAA are also found upon the periosteal surface and measure approximately 20 to 40 microns in diameter. Arteriovenous shunts lie in the middle of the dura and have been suggested to be plentiful, but are difficult to demonstrate (Kerber & Newton, 1973). The shunts are around 50 to 90 microns in diameter. The penetrating vessels, which are likely to be arterioles, extend deep into the dura to within 5 to 15 microns of the inner arachnoid surface of the dura. The authors state that the arterioles end in a capillary network which is unexpected and extremely rich (Kerber & Newton, 1973). These capillaries, measuring 8 to 12 microns are present throughout the dura and are most abundant parasagittally (Kerber & Newton, 1973).

2.9.3.1.6 Dura Histology, Embryology and the Subdural Space

The dura is made up of fibroblasts and a large amount of extracellular collagen (Haines, 1991). The periosteal layer of the dura has fibroblasts that are less elongated than in the meningeal layer. These cells also have a moderately clear cytoplasm containing organelles and large, oval nuclei. The meningeal cells have a slightly darker cytoplasm and oval-shaped nuclei with chromatin that is slightly more condensed than in the periosteal layer (Haines, 1991). A third partition known as the dural border cells layer is described in several microscopy studies which consists of flat cells with elongated nuclei, often having thin, long, branching and interdigitating processes (Schachenmayr & Friede, 1978).

Throughout the literature there is mention of a potential subdural space between the dura and the arachnoid mater (Burgener, 2002; Krstic, 1991; Drake *et al.,* 2005). These numerous references suggest that a SDH is a collection of blood located in a pre-existing space. It has now been shown that there is no space but there is a distinct soft tissue layer at the interface named the "dural border cell layer" (Nabeshima *et al.,* 1975). This layer is suggested to be structurally weak when compared with the external portion of the dura and the bordering arachnoid layer, due to an absence of extracellular collagen and the presence of large extracellular spaces with few cell junctions (Haines *et al.,* 1993). Blood may create a fissure or cleft through this weak plane of cells when trauma to the head occurs (Jaspan, 2008).

Studies into the embryology of the dura also make it clear that there is no pre-formed space between the dura and the arachnoid mater. Both membranes are formed from a common origin and remain continuous, although there is variation in the structure of each membrane due to differentiation and condensation (Mack *et al.,* 2009). The meningeal coverings originate from a network of connective tissue-forming cells of mesodermal origin (Angelov & Vasilev, 1989), which are derivatives of a population of pluripotent cells known as the "cranial neural crest" (Gagan *et al.,* 2007). The tissue that the meninges originate from is known as "mesenchymal tissue". This collection of cells creates a sheath that surrounds the neural tube and was termed the "meninx primitiva" by Salvi in 1898 (Adeeb *et al.,* 2012). The meninx primitiva consists of TWO sub layers; the endomeninx and the ectomeninx. The endomeninx is responsible for the formation of the leptomeninges, while the ectomeninx forms the dura (Adeeb *et al.,* 2012) and it is the previously mentioned process of cellular condensation of the peripheral mesenchyme that creates the division of these two layers.

Part III: The Source of Subdural Haemorrhage in Abusive Head Trauma

2.10 Bridging Veins and the Origin of Subdural Haemorrhage

Bridging veins are vessels that leave the surface of the brain to cross the potential subdural space before entering the dural membrane en route to the venous sinuses (Fig. 2.5). Historically, damage to the bridging veins has been the most commonly suggested source of subdural bleeding in AHT (Leary, 1934; Cabot & Mallory, 1940), with Wilfred Trotter being the first to advocate this theory (Trotter, 1914). The alternative theories for the source of bleeding are discussed in sections 2.11.3 and 2.11.4 of this literature review. The arguments against ruptured bridging veins have included: the limited demonstration of ruptured vessels during surgery, post-mortem examinations and imaging; and the suggestion that bridging veins are too 'robust' to be damaged by the forces involved in a shaking event.

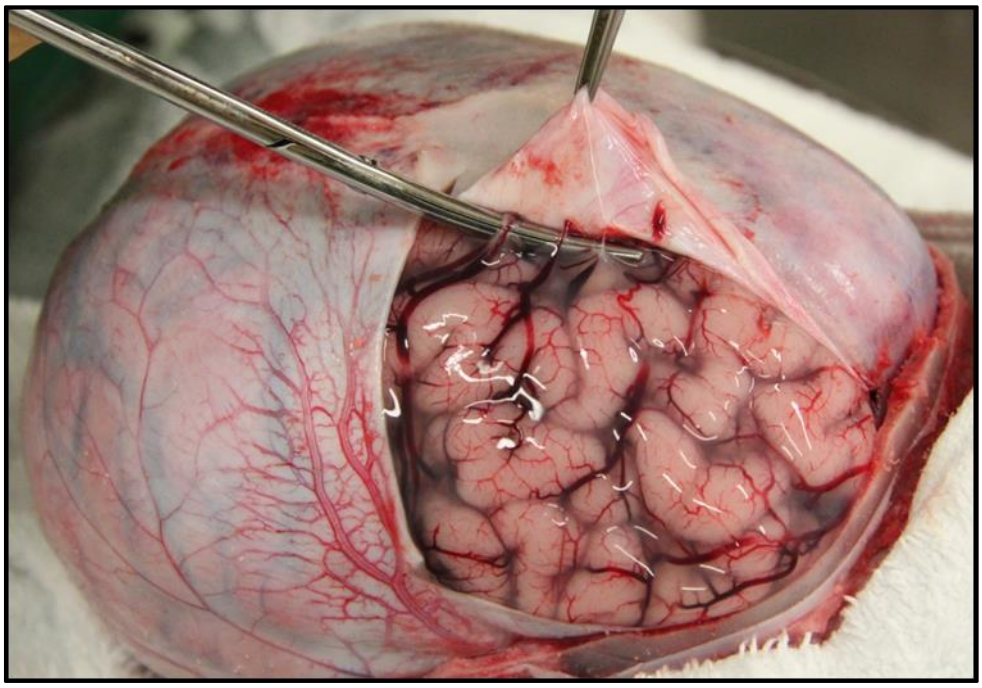


Figure 2.5 Parasagittal bridging veins close to the anterior fontanelle

One of the only demonstrations of bridging vein rupture, in what was thought to be two cases of AHT, comes from a study by Maxeiner in 2001. In his first paper in 1997, the technique for identifying damaged veins was outlined (Maxeiner, 1997). The protocol involved sawing through the skull in the fronto-occipital plane, cutting through the upper half of the brain together with the calvarium. Barium sulfate was then instilled

into the SSS by a balloon catheter and the calvarium was then x-rayed. Extravasation of the barium sulphate into the surrounding tissue indicated the presence of a ruptured vessel. In the absence of a ruptured vessel the barium sulphate would show the SSS and associated parasagittal bridging veins (Fig. 2.6a), and in the presence of a ruptured vessel the contrast agent would leak out of the vein in either one or several places either side of the SSS (Fig. 2.6b), depending on the number of damaged veins (Maxeiner, 2001). A further technique performed by this group was to fix the upper half of the brain in formalin whilst still in the calvarium and then after mounting on a piece of cork the skull would be carefully cut along its sagittal plane to demonstrate the bridging veins (Maxeiner, 1997). The authors used these techniques as an alternative to the standard autopsy methods which often damage the bridging veins, to enable observation of these delicate vessels. However, it must be said that cutting through the top of the head to view these veins is a very disruptive procedure to perform during the post-mortem examination of an infant, with potential for ethical issues, including a likelihood of extreme distress being caused to the families of these babies.

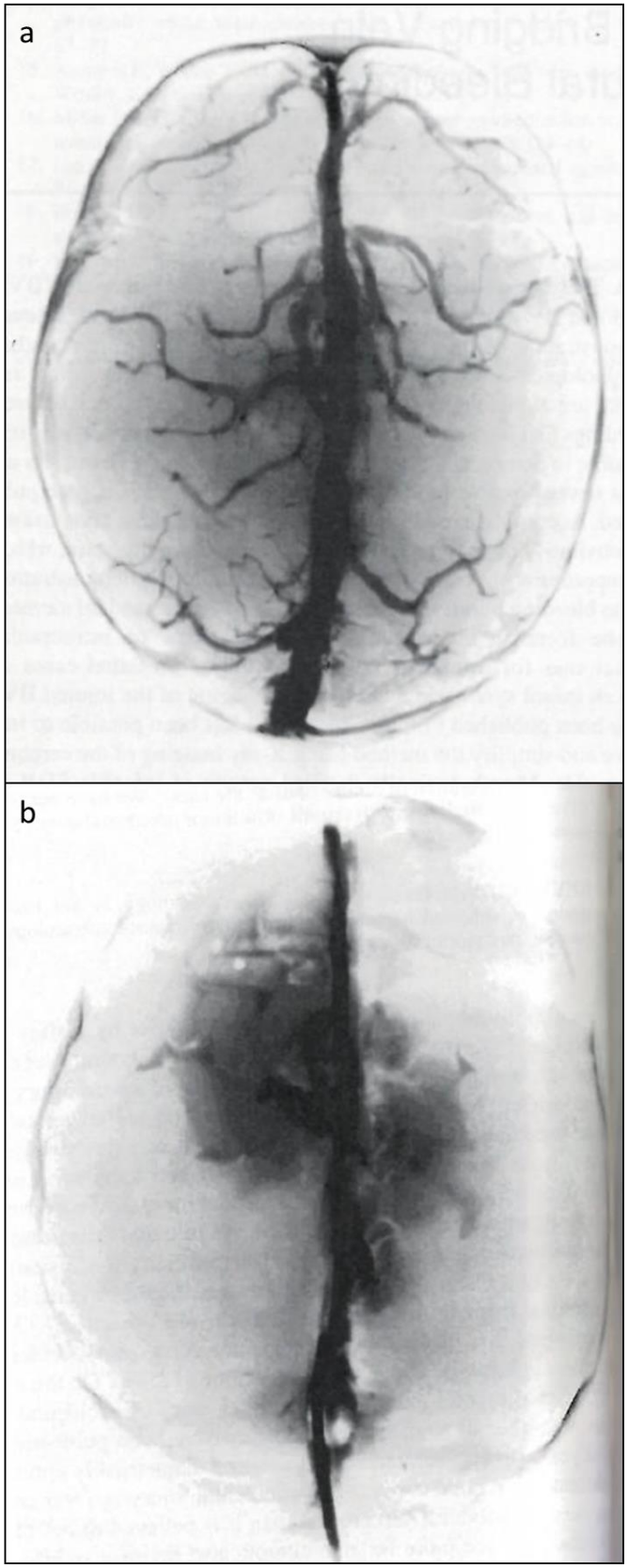


Figure 2.6 Axial post-mortem X-rays of the calvarium containing the upper half of the brain after instillation of barium sulfate in the SSS a) control case (no head injury); SSS and bridging veins clearly shown b) suggested AHT case; extravasation of contrast material and lack of filling of bridging veins suggesting several sites of rupture (Reprinted, with permission, from the Journal of Forensic Sciences, Vol. 46, Issue 1, copyright ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428)

Various text books indicate that the number of bridging veins is between six and 12 per hemisphere (Matta, 1998; Hedlund, 2015) and these texts are usually only referring to the veins draining into the SSS (the parasagittal bridging veins formed from the superior cerebral veins). Most studies on the locations and numbers of bridging veins tend to concentrate on certain areas, often the veins associated with the SSS or underneath the tentorium and close to the transverse sinus (Andrews *et al.,* 1989; Brockmann *et al.,* 2012; Han *et al.,* 2007; Gu *et al.,* 2011). Neurosurgical papers also describe the detailed locations of bridging veins for specific small areas to enable planning of suitable surgical approaches, including whether ligation of any bridging veins is necessary, and whether sacrifice of a vessel is safe (Savardekar *et al.,* 2014; Sakata *et al.,* 2000; Ueyama *et al.,* 1998). It has been suggested that sacrifice of a bridging vein could result in severe disturbance of venous return and may cause significant postoperative morbidity (Kyoshima, 2001).

There is considerable variation in the number of reported bridging veins in scientific papers (Table 2.1), with values ranging from an average of 29 veins located close to the SSS in one study (Andrews *et al.,* 1989) to only four or five in another study (Brockmann *et al.,* 2012). This might be due to differences in the approach to investigating the veins or possibly due to differences in cohort numbers and ages of the participants. When comparing imaging and anatomical dissection of cadaver studies to investigate bridging vein numbers, higher numbers are observed in cadaver research (Brockmann *et al.,* 2012; Han *et al.,* 2007; Han *et al.,* 2008; Gu *et al.,* 2011). This is most likely due to the fact that smaller bridging veins are not seen on imaging due to limitations of resolution.

Study	Location of bridging veins	Age/number of participants and investigation method (cadaver dissection/imaging	Average number of veins
Andrews et al (1989)	SSS	10 young adults, no age range given (cadavers)	29
Brockmann et al (2012)	SSS	30 adults 45-85 yrs (CTV), 9 adults 51-73 yrs (cadavers)	4 (CTV) 4.8 (cadavers)
Lee & Haut (1989)	SSS	8 adults 62-85 yrs (cadavers)	17
O'Connell (1934)	SSS	5 foetuses, 26 weeks gestation-full term (cadavers)	18.2
Han et al (2007)	SSS	30 adults (cadavers), 36 adults (DSA) 7 foetuses (cadavers)	22 (cadavers) 18 (DSA)
Han et al (2008)	Transverse sinus	30 adults 16-69 yrs (cadavers), 76 children/adults 11-90 yrs (patients) (DSA, CTV, MRI)	13.4/side (cadavers) 6.4/side (DSA) 5.6/side (CTV) 4.5/side (MRI)
Sakata et al (2000)	Tentorium	10 cadavers (no age given)	4.4
Ueyama et al (1998)	Tentorium	14 cadavers (no age given)	4.93
Gu et al (2011)	Tentorium	25 patients (MRI, CT), 20 cadavers (no age given)	3.24 (MRI/CT) 4.35 (cadavers)
Matsushima et al (1989)	Tentorium	10 cadavers (no age given)	5.67

Table 2.1 Bridging vein numbers recorded by various studies CTV- computed tomography venography, DSA- digital subtraction angiography

Several studies have been conducted with different methods to report the diameter of bridging veins, including X-ray (Ehrlich *et al.,* 2003), gross inspection light microscopy (Yamashima & Friede, 1984) and digital subtraction angiography (Han *et al.,* 2007).

There is variation between the values reported in these studies but all of them suggest that the diameter of bridging veins is between 1-4mm.

In one study, bridging veins from four cadavers where dissected out and measured to be between 1-3mm in diameter. These veins were then observed with a light microscope to measure the thickness of the vein walls. Vessel walls were shown to be relatively thin with a circumferential arrangement of collagen fibres and a lack of outer reinforcement by arachnoid trabecules. Sections of bridging veins from subdural locations had walls that varied considerably in thickness, ranging from 10 to 600µm. Sections of bridging veins with a subarachnoid location had a relatively uniform thickness in comparison, between 50 and 200µm. In both portions, the walls were composed of a layer of dense fibrous tissue encompassing the lumen and covered by thin or loose connective tissue. Next to the endothelial lining a single elastic lamina was seen, up to 7µm in thickness. (Yamashima & Friede, 1984).

In a radiological study into the anatomy of bridging veins, it was found that diameters of these veins are quite variable between different individuals, with an average of between 1.4 and 3.1mm and between 1.9 and 2.5mm in approximately two thirds of cases. Although this study included infants (age range of study was 2 months to 96 years) there was no analysis of the different age groups separately. These authors found that bridging vein diameters do not vary greatly in any one individual but that bridging vein numbers per case varied considerably. Numbers of veins ranged from nine to 31, with an average of 17 bridging veins per brain. It was also shown that an increase in the number of bridging veins of an individual usually accompanied a decrease in the diameter of the bridging veins for that person (Ehrlich *et al.,* 2003).

A final study worthy of mention is one using digital subtraction angiography (DSA) on 36 patients (11-90 years) and anatomical dissection on 30 adult and seven foetal cadavers to compare the two methods. This study investigated the diameters of bridging veins using the methods mentioned above as well as observing the entrance of the bridging veins into the superior sagittal sinus (SSS) by dividing the SSS anteriorly to posteriorly into four segments. The segments were defined by locations where bridging veins were suggested to cluster and not on specific anatomical landmarks. Therefore the length of

each segment varied between individuals, with segment 2 centered at the coronal suture and segment 4 starting approximately 3cm anterior to the lambdoid suture. Segments one and three were shown to receive the majority of bridging veins, while segments two and four were non-tributary segments, receiving few veins. Corrosion casts of the intracranial venous structures were also created from the foetal cadavers within 24 hours of death. The results from the anatomical dissection of the adult cadavers showed an average of 11 bridging veins with an average diameter of 2.5mm draining into the SSS on each side. The method using DSA showed an average of nine bridging veins on each side of the SSS, with an average diameter of 3.4mm. The smallest veins measured by DSA were 1.7mm, whilst in the dissection of cadavers, veins as small as 0.4mm were recorded. From the corrosion casts the authors noted that there was a superficial layer of small meningeal veins and venous lacunae overlapping the dural entrance of the bridging veins in segment three of the SSS. They also commented that some bridging veins drained into the meningeal vein before entering the sinus (Han *et al.,* 2007).

Of the bridging veins draining directly into the SSS, several investigators have commented on the retrograde filling of the sinus from the bridging veins. In one study, 74% of bridging veins drained into the SSS at an acute angle against the flow of blood in the sinus, 18% entered at a right angles and only 9% entered at an obtuse angle. The authors also noted that the veins always adhered to or ran parallel to the SSS before entering the sinus (Han *et al.,* 2007). A further observation has shown that the way a bridging vein enters the dural membrane can differ between vessels; entry points are described as either candelabra (veins joining together before the entry point) or separated (veins entering the same area separately).

An important observation to note is that most of the studies on bridging veins are in the adult and not the infant. There is limited data on infant bridging veins which are likely to be smaller than the vessels seen in adults, with one study of only six bridging veins suggesting outer diameters between 0.14mm and 0.72mm (Morison, 2002). There may also be variations in the number and locations of bridging veins in the younger age group. However, this cannot be known until further comparative studies are carried out in both adult and infant groups using the same methods of investigation. Infant blood

vessels underneath the dura are difficult to observe because of the attachment of the membrane to the sutures, making removal of the cranial bones without damage to the dura a challenge. There may also be difficulties in gaining ethical approval for post-mortem research in this age group and in consenting the appropriate number of cases. With traumatic damage to bridging veins being the most plausible and widely held belief of the majority of the medical community for the cause of SDH in AHT, further research on the characteristics of infant bridging veins is essential.

2.11 Differential Diagnosis and Alternative Theories for the Cause of Subdural Haematoma

The following section will describe the differential diagnosis of AHT and will discuss some of the alternative theories that have been put forward for the cause of SDH in infants with injuries suggestive of AHT. During AHT court cases, the defence may claim a number of arguments against the likelihood of AHT, which may include suggestions such as; shaking an infant is biomechanically impossible, and; that the head injuries seen in AHT would not occur without associated severe neck and spinal cord injuries. These arguments will briefly be discussed below, as well as further defence claims, some of which form part of the differential diagnosis. These include the proposed occurrence of a lucid interval, accidental injury, re-bleeding of a previous SDH, venous thrombosis, birth trauma, bleeding disorders, malignancy and metabolic or genetic syndromes.

Two of the most important theories, in terms of media coverage and the influential role that they have subsequently played in courtroom proceedings and the perception of a select few individuals working as defence expert witnesses will be discussed in more detail. The first of these theories, known as the 'Geddes' or 'Unified' hypothesis, advocates that hypoxia, alongside other factors, is the causative process responsible for the injuries seen in AHT, including SDHs and RHs (Geddes *et al.,* 2003). The second hypothesis proposes that the non-traumatic source of SDH is an 'immature dural vascular plexus'. It is suggested that blood leaks from these small networks of vessels within the dura itself which then results in SDH. As with Geddes theory, hypoxia is the process that results in 'leaky' blood vessels in this second theory (Squier & Mack, 2009; Mack *et al.,* 2009).

2.11.1 Arguments For/Against Abusive Head Trauma

During AHT court proceedings defence experts may state that there is no evidence that you can shake an infant and cause the brain injuries seen in these cases. They may also claim that an impact is required to produce the findings in AHT (Garrett, 2013). As mentioned previously, the definitive study of whether shaking an infant can cause injuries such as SDHs and RHs cannot be done for obvious ethical reasons. However, there are several studies where caregivers have confessed to shaking their babies and the typical injuries of AHT were present in these cases (Vinchon *et al.,* 2010).

The defence expert may also reference a biomechanical study from 1987 (Duhaime *et al.,* 1987) as proof that shaking an infant cannot produce the force required to produce brain injuries such as SDH without an impact (Garrett, 2013). The numerous flaws and subsequent criticism that this paper has received are discussed in an earlier section of this chapter (see 2.7.2). This study along with other biomechanical model data cannot be used to represent an infant for reasons mentioned previously, including a lack of bio-fidelity.

An argument which may be used by the defence in AHT court proceedings suggests that a shaking event would not occur without associated neck injuries (Garrett, 2013). Previously studies have demonstrated cases of children with brain injuries and no accompanying neck injuries (Brennan *et al.,* 2009). However, it is important to recognise that neck injuries may not have been described in the past because they were simply not noticed due to limited dissection of the anatomical structures of interest (Matshes *et al.,* 2011) and also because of limited spinal MRI in AHT cases (Choudhary *et al.,* 2014). Recently there has been more of a focus on spinal injuries, particularly the important role that damage to the cervicomedullary junction may play in the pathogenesis of AHT in relation to breathing difficulties. In a relatively recent study of 67 infants and young children (less than 48 months) diagnosed with AHT, spinal MRI was undertaken. Within this cohort cervical spine ligamentous injuries were present in 78%, and these injuries were statistically correlated with evidence of brain ischemia (Choudhary *et al.,* 2014).

2.11.1.1 Re-Bleeding-Defence

Re-bleeding of an existing SDH may be used during AHT court cases to argue against allegations of inflicted trauma. The birth process, a past traumatic event or hypoxia are implicated as the initial cause of the SDH which is suggested to re-bleed either spontaneously or due to a minor trauma (Garrett, 2013).

A re-bleed SDH would result from low pressure venous blood accumulation (Boos, 2006) and would not result in the rapid onset neurological symptoms and deterioration seen in AHT cases. The symptoms of a rebleeding chronic SDH are suggested to include lethargy and irritability and not the more acute, serious clinical features that result from AHT (Hymel *et al.,* 2002). The differences between the symptoms of a re-bleed and that of AHT are likely due to the focal nature of a re-bleed, as opposed to the widespread diffuse damage seen in AHT cases. There may only be a small accumulation of blood, but even in cases with more extensive bleeding the haematoma is expanding slowly, allowing the brain more time to compensate for the injury.

2.11.1.2 Lucid Interval

The 'lucid interval' is another defence previously employed in AHT cases (Garrett, 2013). A lucid interval is a period of time after a child has sustained a head injury where they may not be symptomatic, and become symptomatic at a time point-distant from the initial traumatic event, and when there will be less certainty that a defendant was alone with the infant at the time when the trauma was caused.

During a well-publicised American court case (Commonwealth v Louise Woodward (1998) 427 Mass. 659) it was stated that an infant could respond 'normally' after receiving a life-threatening abusive head injury. In response to this, more than 50 specialist child protection physicians published a letter to challenge the implication, stating that "infants simply do not suffer massive head injury, show no significant symptoms for days then suddenly collapse and die" (Alexander *et al.,* 1997).

There is no evidence of a lucid interval in infants who have sustained a non-impact head injury that results in death (Duhaime *et al.,* 1998; Gilles & Nelson, 1998). Further evidence against the occurrence of a lucid interval comes from the AHT confession

literature, where perpetrators contradict the notion of a lucid interval with accounts of immediate symptoms after abuse (Starling *et al.,* 2004).

2.11.1.3 Intracranial Venous Thrombosis

There are various terms used within the literature for the presence of a thrombus within intracranial venous structures; cerebral sinovenous thrombosis, dural sinus thrombosis, cerebral vein thrombosis, cerebral venous thrombosis, intracranial venous thrombus (Hedlund, 2013) and bridging vein thrombosis (Adamsbaud & Rambaud, 2012). These terms collectively refer to blood clotting that may occur in at least one of three compartments in the venous system; the superficial venous system (e.g. cortical dural veins), deep venous structures (internal cerebral veins) or the dural venous sinuses (Hedlund, 2013).

Spontaneous cerebral venous thrombosis is a rare event in the paediatric population, occurring in less than 1 in 100,000 over the entire paediatric age range. Neonates are most commonly affected, comprising 43% of a cohort of children (from birth to 18 years) with cerebral thrombosis in a multicentre study based in Canada, (de Veber *et al.,* 2001). The formation of any thrombosis is governed by Virchow's triad: damage to the endothelial wall, stasis and hypercoagulability. If one of these three phenomena arises, the risk of thrombosis is increased. Accordingly, venous thrombosis may be the result of trauma, infection, shock, dehydration, hypercoagulable states and tumours, to list a few. In neonates, maternal factors may predispose to thrombosis, such as maternal infection and excessive calvarial molding during vaginal delivery (Tan *et al.,* 2011).

It has been suggested that thrombus in the cortical veins/bridging veins could be a primary cause of SDH due to venous back pressure created by the clot which results in rupture of the vein (Barnes, 2011; Barnes & Krasnokkutsky, 2007). However, the theory is lacking supportive evidence. In a neuro-imaging review study of 36 patients (1 day to 19 years of age) with intracranial venous thrombosis, none had associated SDH (McLean *et al.,* 2012).

In a further study with a cohort of 45 children under 36 months that were diagnosed with AHT (91% with SDH), MRI venography demonstrated abnormality (compression, displacement, intrinsic abnormality or thrombosis) of the intracranial venous system in

31 cases (69%) (Choudhary *et al.,* 2015). Whilst these findings have been interpreted in court as primary cortical sinus or venous thrombosis with resultant SDH, the authors interpret the findings as secondary to trauma, with others agreeing that bridging vein thrombosis in AHT cases occurs as a result of tearing of the bridging vein due to traction, resulting in the inevitable in vivo stereotypical thrombus formation which ensues (Hahnemann *et al.,* 2015; Yilmaz *et al.,* 2015). Furthermore, primary venous thrombosis would be expected to demonstrate more widespread changes involving the superficial and deep venous systems, and thrombosis of vessels in areas where there is no SDH (Choudhary *et al.,* 2015).

2.11.2 Differential Diagnosis

When a child under the age of two years presents with neurotrauma and there is no obvious accidental cause, AHT, being a common cause of serious head injury in this age group, should always be considered (Sieswerda-Hoogendoorn *et al.,* 2012b).

It has been stated that no medical condition fully mimics all of the features associated with AHT (Duhaime *et al.,* 1998), but the differential diagnosis is an essential step to rule out any underlying medical conditions. Alongside radiological, clinical and laboratory investigations, the medical history and the history provided by the caregivers aid in the overall conclusions drawn by the professionals involved in suspected AHT cases. Detailed medical history, laboratory and clinical investigations can rule out the majority of the differential diagnoses.

The more common conditions that may present with findings similar to AHT include accidental and birth trauma, which will be discussed below. The differential diagnosis of two of the most common features of AHT, SDHs and RHs, either when seen together or separately, is extensive (Narang & Clarke, 2014). As well as accidental and birth trauma, the differential diagnosis includes bleeding disorders, metabolic disease, genetic syndromes, tumors, infections and congenital malformations. A list of the differential diagnoses for RHs and SDHs can be found in Appendix 1a and 1b, respectively.

2.11.2.1 Accidental Trauma

Young infants who are not independently mobile are unable to injure themselves accidentally. When infants present with serious head injury, falls are the most

commonly offered explanation (Narang & Clarke, 2014). However, the impact type trauma elucidated by a fatal or serious fall would tend to produce focal injuries, including skull fracture, epidural haemorrhage, scalp laceration or contusion (Case, 2014) which differ from the diffuse injury pattern seen in head injuries caused by inertial forces (Corey & Collins, 2011).

When falls do occur, they are normally short falls from a caregivers arms, a couch or crib (Sieswerda-Hoogendoorn *et al.,* 2012b). There are numerous studies on short falls. However, there is no standardised definition of the height of a short fall with values ranging from 10 feet to 15 feet, to less than five feet (Narang & Clarke, 2014). Well-designed studies now use a value of around four feet (the waist/chest height for most adults) or less to describe a short fall.

There may be many different elements to a fall which affect the biomechanics associated with the risk of injury. Studies using both anthropomorphic dummies and data collected from children suffering falls who enter emergency departments suggest that fall height, height of the furniture a children fell from, impact surface, and fall type (vertical free fall or the presence of additional forces) influence the severity of injuries (Ibrahim & Margulies, 2010; Ehsani *et al.,* 2010; Bertocci *et al.,* 2004; Thompson *et al.,* 2011; Joffe & Ludwig, 1998; Chiaviello *et al.,* 1994b). Softer surfaces such as carpet have been shown to absorb enough energy during impact to reduce head acceleration in the sagittal plane, whilst harder surfaces such as concrete have been shown to significantly increase head acceleration by comparison (Ibrahim & Margulies, 2010).

It has been stated that no life-threatening event or serious intracranial pathology is expected in non-complex short falls, but very rarely, falls involving stairs or infant walkers can be serious or fatal (Chiaviello *et al.,* 1994a; Chiaviello *et al.,* 1994b). In addition to the previously mentioned elements of a fall that may alter the severity of injury, incidences involving stair-ways may have factors such as the number of stairs the child fell down, or whether the child was being carried at the time of the fall. A child may be dropped by an adult and then fall independently or both may fall down the stairs at the same time. Several studies have shown that injuries to children falling down stairs

were more severe when they were being carried in the arms of a caregiver (Chiavielo *et al.*, 1994b; Joffe & Ludwig, 1998).

A further study of note was published in 2010, where 307 parents were asked if their child had fallen off a 'high surface' such as a table, bed or dresser before the age of two (Haney *et al.,* 2010). Forty percent of the parents recalled such a fall, with 59% of parents recalling more than one such fall. Among 209 reported falls, there were only two incidences of serious injury (both concussion) and no reported SDHs, RHs or death (Haney *et al.,* 2010).

Further evidence of the extreme paucity of significant injuries and fatalities from short falls comes from studies of witnessed falls in objective settings such as hospitals and day cares (Lyons & Oates, 1993).

2.11.2.2 Birth Trauma

Radiological studies have shown birth-related SDHs are relatively common (up to 46%) (Rooks *et al.,* 2008). However, smaller percentages have been reported (19%) (Looney *et al.,* 2007). Differences in the number of days after birth that imaging occurred have been suggested as the reasons for the variation in these reported figures (Rooks *et al.,* 2008). In the study by Looney et al the infants were scanned between one to five weeks after birth. It is likely that the prevalence of SDH was considerably higher than reported due to SDHs that may have been present in the first week of life but would have been undetected due to a lack of imaging during the first week of life, and also due to resolution of SDHs, especially in infants who were not scanned until the fourth/fifth week after birth (Rooks *et al.,* 2008).

In comparison to the SDHs seen in AHT, which are often found in the interhemispheric fissure and over the cerebral convexities (Minns & Lo, 2009) birth SDH is usually located posteriorly, a relatively small amount of blood, over the occipital lobe or infratentorial (Rooks *et al.,* 2008; Sieswerda-Hoogendoorn *et al.,* 2012b). Intracranial haemorrhage from birth is usually asymptomatic and resolves by one month of age (Rooks *et al.,* 2008), however, some birth SDHs are space-occupying and would result in major and immediate post-partum clinical symptoms, including seizures, hypotonia and coma (David, 2008).

2.11.3 The Unified hypothesis

One of the most influential alternative theories suggested to date, for subdural bleeding in infants, was that hypoxic injury to the brain, resulting in raised central venous pressure and brain swelling could lead to the leakage of blood from intracranial veins forming a haematoma below the dura. This hypothesis, known as the 'Geddes hypothesis' or 'Unified hypothesis' was put forward by a British neuropathologist in a series of three papers (Geddes *et al.,* 2001a; Geddes *et al.,* 2001b; Geddes *et al.,* 2003). It has been used by a select few defence expert witnesses during court proceedings for cases in which there were findings and circumstances suggestive of AHT (A Local Authority v S (2009) EWHC 2115 (Fam)).

In the first two Geddes papers in the series (Geddes *et al.,* 2001a; Geddes *et al.,* 2001b), 37 infantile cases of fatal inflicted head injury were detailed in a neuropathological study which aimed to identify axonal damage in the brain. The authors suggested that 13 of the 37 infants had widespread axonal damage due to a vascular rather than traumatic origin. Widespread axonal damage was attributed to a traumatic mechanism in only two cases, both with very clear signs of trauma. The conclusion presented by the authors was that severe traumatic axonal damage is rare in infantile AHT, unless there is a significant impact.

The main conclusion of the second paper was suggested to be that damage to the lower brainstem or upper cervical spinal cord due to stretch injury could result in apnoea, which in turn would lead to hypoxic injury and brain swelling. The authors stated that: 'nobody really knows how babies are injured, it may not be necessary to shake an infant very violently to produce stretch injury to its neuraxis'. The first part of this sentence has been recognised as an incorrect generalisation, the second section is conjecture based on unproven hypothesis.

In the final paper in the series the authors suggested the link that hypoxia, brain swelling and raised central venous pressure coupled with immaturity would lead to leakage of blood from intracranial veins. The flaws in this paper have been recognised as being numerous. The majority of the study cohort of 50 non-head injured subjects do not accurately reflect the average age of a victim of AHT, which included 17 intrauterine

deaths, three spontanoeus abortions, 21 perinatal or neonatal deaths and only nine infant deaths. These 50 subjects were then compared to three selected infants that the authors stated as presenting with classical 'shaken baby syndrome'. Even though the majority of the 50 subjects were stated as having profound hypoxia, only one had macroscopic SDH (a 25 week gestation foetus with sepsis, who died at one week of age and did not have hypoxia). The data provided by their own results did not provide evidence to support their theory that hypoxia could potentially lead to SDH. The authors also reported on the presence of microscopic intradural haemorrhage (IDH) which was seen in 36 subjects (72%). They stated that the three infants with AHT also had intradural bleeding and therefore suggested that leakage of blood may come from veins both inside the dura and below the dura, not from the traumatic rupture of bridging veins. The authors reported that microscopic IDH could be caused by hypoxia, despite there being no statistically significant relationship between hypoxia and IDH in their own study.

Hypoxia with brain swelling was also proposed as a potential cause of RHs, despite the fact that ophthalmology findings were not reported in the paper. This final comment provided a 'Unified hypothesis' for their suggested non-traumatic theory for the clinical and neuropathological findings in AHT cases.

If hypoxia were to cause SDH, one would assume that cases involving hypoxic events such as drowning or suffocation would also present with this type of bleeding. This has been investigated in several studies (Byard *et al.,* 2007; Hurley *et al.,* 2010) which directly refute the Unified hypothesis. In a retrospective study of 82 foetuses, infants and toddlers all who suffered a hypoxic episode (including but not limited to temporarily resuscitated sudden infant death syndrome, drowning, asphyxia), all had histologically confirmed hypoxic-ischaemic encephalopathy and none had macroscopic evidence of SDH (Byard *et al.,* 2007). In a further post-mortem and post-arrest brain imaging study (Hurley *et al.,* 2010), including 50 cases of children (under the age of four, 48 being less than 24 months) who presented to the emergency department or paediatric intensive care unit following an atraumatic cardiorespiratory collapse, none had significant SDH. The infants in this paper suffered severe hypoxic-ischaemic episodes and the authors found that these events were not associated with SDH.

In 2001, the suggested theory in Geddes second paper that one may not need to shake an infant with much force to produce stretch injury to the neuroaxis was described in New Scientist. This resulted in significant media coverage, with article titles such as 'Even mild shaking puts baby at risk' (Meek, 2011) and 'Gentle shaking may kill babies' (BBC News, 2001). In light of this new research that suggested convictions based on the 'triad' of injuries may be wrong and that little or no trauma may be involved, the Court of Appeal heard four appeals in London in June 2005 (Richards *et al.,* 2006). These appeals against convictions for non-accidental injury of infants (one murder, two manslaughter, and one grievous bodily harm) resulted in two appeals which were upheld, one of which was dismissed and the fourth saw a reduction in sentence from murder to manslaughter, (R v Harris, Rock, Cherry and Faulder (2005) Crim 1980).

The publication of Geddes hypothesis has been met with widespread scepticism and criticism by the majority of professionals working in the field of paediatrics, paediatric pathology, and paediatric head injury (Smith *et al.,* 2003; Punt *et al.,* 2004). In cross-examination Dr Geddes herself agreed that her research was incomplete and could no longer credibly be put forward as an alternative cause of SDH and RH (R v Harris, Rock, Cherry and Faulder (2005) Crim 1980). The hypothesis was discredited in the UK Court of appeals due to inconsistencies in the supporting research.

2.11.4 The Immature Dural Vascular Plexus Theory

A relatively recent theory put forward by Squier et al suggests that infantile SDH in non-traumatic conditions is the result of leakage from a network of vessels within the dura mater (Squier & Mack, 2009; Mack *et al.,* 2009). This network of vessels, the intradural vascular plexus, is claimed to be more extensive in the infant than adult (Mack *et al.,* 2009). The authors postulate that as in the Unified hypothesis, hypoxia is the pre-eminent factor causing immature vessels of the plexus to leak and subsequently produce SDHs (Mack *et al.,* 2009). Squier et al have only produced review papers on this subject to date and not any based on studies of patient cohorts. Consequently, their theory remains entirely untested, and currently there is no available scientific data linking an intradural vascular plexus "leakage" with SDH (Narang, 2012).

Dr Squier and others continued to reference and advocate the hypoxia theory during court proceedings (A Local Authority v S (2009) EWHC 2115 (Fam)). In a family court case in 2009 where the judge ruled that a 13 week old infant's death was the result of a mother shaking him, these expert witnesses acting for the defence concluded that AHT was possible, but unlikely in the absence of any other bony or soft-tissue injury, or other specific post-mortem evidence of trauma. Both experts agreed that the most likely primary causative event was either a choking incident or a cardiac arrhythmia combined with the effects of prolonged CPR followed by resuscitation. The judge in this case concluded that both experts had developed a scientific prejudice.

Chapter 3 : The Ethics Application Process and Obtaining Prospective Parental Consent for Paediatric Autopsy Research

3.1 Introduction

The majority of paediatric, as opposed to foetal and perinatal autopsies performed in England and Wales are undertaken on the legal authority of Her Majesty's (HM) Coroner, since hospital post-mortems with consent are now uncommon (Thayyil *et al.,* 2009). Post-mortem examination, including retention of tissue or other biological samples, for the specific purpose of investigating the cause of death, does not require consent from the next of kin. However, the Coroner's Act does not give permission for the use of tissue or data in research (Her Majesty's Stationery Office, 2005). Therefore, any post-mortem procedures or tissues/biological samples taken for use in research must be part of a study ethically approved by a recognised research ethics committee. All activities involving human tissue must also adhere to the Human Tissue Act (2004). The fundamental principle of the legislation being that consent is gained for the removal, storage and use of human organs and other tissue for scheduled purposes (Human Tissue Authority, 2010), which in the case of post-mortem research, would be provided by the next of kin.

Consenting parents in the days following the loss of their child is an extremely sensitive matter, which must be undertaken in an appropriate ethical manner. The sudden unexplained death of a previously healthy child is an extremely traumatic event and the implications for the family may be significantly different when compared to those of the death of an elderly relative (Thayyil *et al.,* 2009). Some people may also have the perception that certain tissues are special, such as the heart, brain or eyes, which may or may not be dependent on cultural or religious factors. The process of consenting is extremely important to enable valuable research, such as the study described within this thesis, which may aid the current understanding and diagnosis of AHT. An inaccurate diagnosis of AHT may lead to an unjustified loss of parental rights or imprisonment, and under-diagnosis may create a significant risk for the child and other siblings returning to

an abusive environment. This chapter will describe the ethics submission process for the paediatric post-mortem research included in this thesis, including the difficulties encountered during the application phase, as well as providing a discussion of the consenting process once approval was granted.

3.2 Method

3.2.1 Ethical Approval

Ethical approval was sought for a prospective, consented, autopsy-based research project, which would include participants requiring an autopsy, requested by the Coroner or hospital.

The online Integrated Research Approval System (IRAS) was used to apply for permissions and approvals for the research. Once some basic information about the study was entered into the IRAS, filters ensured that the data collected and collated was appropriate for the type of study, and consequently that the correct permissions and approvals were obtained.

Ethical approval was required from the National Research Ethics Service (NRES), University Hospitals of Leicester (UHL) Research and Development (R & D) offices and the University of Leicester (UoL), acting as the Sponsor's of the research project. The study would be conducted in accordance with an approved protocol (Appendix 2a), ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996 and relevant regulations and standard operating procedures (Appendix 2b).

The initial ethics application allowed for seeking consent to enable two types of Optical Coherence Tomography (OCT) to be used during post-mortem examination. OCT is a light-based imaging system that can capture micro-resolution, subsurface three-dimensional images (see Chapter 5 for further details).

Retrospective ethical approval was also obtained for the use of archived autopsy photographs of paediatric brains and bridging veins.

Observational study was undertaken on 68 infants for this thesis, including both head injured and non-head injured (Appendix 3). However, additional procedures requiring consent, outlined in the ethics application, only include non-head injured infants up to

one year of age. With such limited data in the current literature on the cerebrodural vascular system, the study of non-head injured infants would allow us to determine what the 'normal' distribution and size of these vessels are in the average infant, without damage that may have occurred from a traumatic head injury. No forensic post-mortem cases were included in this study as it would not be deemed ethical to approach parents who may be under investigation for potential neglect or abuse.

Throughout the course of the study three substantial amendments were also submitted. The first amendment was to allow removal of bridging veins for mechanical testing and histological examination and to be able to use a digital microscope for the measurement of vessel diameters. The second was to enable MRI scanning of an infant brain for the purposes of producing a three-dimensional (3D) model for mapping anatomical data points. The third amendment was to allow for the mechanical testing of infant bridging veins.

3.2.2 Consenting Process

One of the difficulties of post-mortem research, particularly for this age group, is approaching parents for informed consent in the first few days after losing their child. An important part of the ethics application was ensuring the appropriate approach would be in place for consenting cases. To minimise distress to the parents it was crucial that clear, concise and empathetic communication was given by an experienced and qualified individual. An advanced nurse practitioner, with extensive experience in consenting adult post-mortem cases for research within the East Midlands Forensic Pathology Unit (EMFPU), was responsible for speaking to parents and obtaining consent. Non-English speaking parents were not approached for inclusion as the consenter acquired informed consent via telephone conversations. During the telephone conversation, the consenter talked to the parents about the study and signed a consent form to say whether the parents did or did not wish to give consent for each procedure included in the study design. As part of the ethics application it was decided to include both a summary information sheet and a more detailed information sheet, which parents could choose whether to receive or not. The consent form and a consent script were also part of the application. The latest approved versions of these forms can be found in Appendix 2c-f.

3.2.3 Patient Confidentiality/Data Security

All data related to this project were kept in an anonymised format on an encrypted electronic device with numeric access code for confidentiality and data security purposes.

3.2.4 Participant Inclusion and Exclusion Criteria

The inclusion criteria for subjects were deceased babies (full term-one year of age), of all genders. The parents needed to be English-speaking, and willing and able to give consent for their child's participation in the study. The exclusion criteria were therefore: babies over the age of one year; non-English speaking parents; and causes of death related to a head injury or infants with suspected child neglect, abuse or trauma.

3.2.5 Subject Screening

Once a child in the appropriate age range was identified as requiring an autopsy to be carried out at the Leicester Royal Infirmary, the EMFPU would often liaise with the Coroner's Office for contact details of the parents for consenting purposes. Any parents considered inconsolable and not coping with the death of their baby were not approached for inclusion in the study. Non-English speaking parents were not approached as clear and concise communication was required to gain informed consent. Fourteen subjects were screened for inclusion in the study between September 2014 and August 2015.

3.3 Results

3.3.1 Ethical Approval

The ethics submission was successful and the project was approved by NRES, UHL and UoL. However, several difficulties were encountered which delayed the final approval. Previous post-mortem research projects within the EMFPU have been sponsored by UHL. Around the time of the ethics application for this study, it was decided between UoL and UHL that the primary employer of the chief investigator should act as the sponsor of the project. This therefore meant that the responsibility of the insurance for the project lay with the University. Ethics forms were submitted for sponsor review on 5/12/2013. There were protracted and involved delays during the sponsor review due to issues raised by the University's insurers (they had never seen a submission of this

nature before). The Insurer's letter of indemnity was received on 01/04/2014 after significant changes were made to the patient information sheets, including extensive and perhaps unnecessary detail about study procedures which the insurers believed to be appropriate. This not only resulted in a delay to the NHS ethical approval but also had an impact on the outcome of the NHS ethics review meeting, where the review board did not agree with changes to documents suggested by the University's Insurers. This lead to further document amendment. NHS ethical approval was finally granted on 04/07/2014. Subsequent substantial amendments were approved relatively quickly and no further problems were encountered.

3.3.2 Subject Screening and Consenting

Of the 14 subjects, four were not approached for consent after conversations with the Coroner's Office (Table 3.1). The Unit was informed that in one of the cases the parents were not coping with the death of their baby and were inconsolable, as were the parents in another family where the point of contact was the grandmother due to the grief of the parents. The other two cases were of babies with non-English speaking parents and therefore they did not meet the inclusion criteria.

Case Number	Approached for consent yes/no	Consent gained yes/no
1	Y	Y
2	N	N
3	Y	Y
4	N	N
5	Y	Y
6	Y	Y
7	N	N
8	Y	N
9	Y	N
10	Y	Y
11	Y	Y
12	N	N
13	Y	N
14	Y	Y

Table 3.1 Cases approached for consent

Of the 10 parents approached by the study consenter, seven gave their consent for the various research investigations, resulting in a consent rate of 70%. Two of the parents

refused to give consent as they did not want further procedures carried out on their babies, one of which was conceived via *in vitro* fertilisation. No reason for denied consent was recorded for the third infant. Some of the reasons given by the parents for agreeing to participate in the research including; seeing something positive come from the death of their babies i.e. feeling like the death was less of a waste of life, and also that they felt the research was important with potential benefits to babies in the future.

3.4 Discussion

Approval of the ethics application and subsequent amendments permitted the study of the infant cerebrodural system, by the application of additional procedures during the autopsy, which would have no overall effect on the post-mortem examination itself. These procedures included imaging techniques (OCT) and digital microscopy, used within the mortuary during the post-mortem to determine the size, location and number of bridging veins within the infant head.

Previous study of consenting the recently bereaved to post-mortem imaging of loved ones in adults has shown very high consent rates within our Unit (96.6%) (Saunders *et al.,* 2013). In this current study the consent rate was slightly lower at 70% and although conclusions cannot reliably be drawn from such a relatively small patient population, the rate is likely to reflect the sensitive nature of post-mortem research in a young age group.

We found the practice of telephone consenting to be a successful approach, as previously demonstrated in other autopsy based research (Saunders *et al.,* 2013; Millar *et al.,* 2007; Thayyil *et al.,* 2009). Consenting via telephone was deemed necessary to ensure the safety of the consenter, as face to face meetings with the parents would take place within the community in unknown environments. In addition, as the Leicester Royal Infirmary offers a regional paediatric autopsy service with a wide catchment area, the consenter would not have been able to travel long distances for the purpose of home visits considering the short time frame before the post-mortem examination.

Alongside other studies from our Unit (Saunders *et al.,* 2013) and contrary to popular belief that next of kin may be angry or upset by a telephone consenting approach, we found that the process was viewed positively by parents and no objection to being

contacted was made. Although bereavement counselling was not specifically offered, several parents expressed their appreciation about being able to talk to someone about their child's death and the autopsy procedure. Two parents who wanted to see something positive come from the death of their baby and who thought the study was of great importance wanted to make a donation towards the research.

One of the challenges to gaining consent for post-mortem research is the short time frame between finding out a post-mortem has been requested by HM Coroner and the day of the post-mortem. In some cases within this study the researcher co-ordinating the work only found out about an infant requiring a post-mortem a day or two before the autopsy. Upon receiving this information it would then often be necessary to contact the Coroner's Office for the parents contact information if the Coroner's report had not been received or had missing phone numbers. This information would then need to be relayed to the study Consenter who would then try to contact the parents. If consent was gained the researcher would then need to co-ordinate with other members of the research team for assistance and equipment kept within other departments both in the hospital and the University. Fortunately, collaborators from other departments working on the project were aware of the last minute nature of the work and limited number of participants and were available for all potential cases. Equipment was also always made available at short notice.

Chapter 4 : Post-Mortem Removal of the Calvarial Bones Using Neurosurgical Equipment

4.1 Overview

As discussed within the Introduction section of this thesis, standard post-mortem access to the brain is disruptive to the dural membrane and bridging veins. The following chapter will describe an improved method for removal of the calvarial bones in infant autopsies using specialised paediatric neurosurgical tools. This approach is minimally disruptive, allowing the dura mater to remain undamaged. This technique has become routine practice in our unit for paediatric post-mortems because it not only allows for further investigations of the infant bridging veins (detailed in Chapters 5 to 7) but also enables visualisation of the brain and its coverings before these areas are disrupted, which is particularly important in cases where a head injury is likely to have occurred.

4.2 Introduction

When an infant suffers a head injury it can be difficult to identify the precise cause as symptoms are non-specific and the history provided is often inaccurate (Piteau *et al.,* 2012). Often, there may be no externally visible injuries (Case *et al.,* 2001), although bruising and superficial abrasions occur in more than 40% of cases (Minns & Lo, 2009). There is a requirement for a meticulously documented autopsy of an infant where there is suspicion of inflicted injury since many findings may be subtle (Dunham & Perry, 2001) or may be vigorously contested in a medico-legal setting (Garrett, 2013). During autopsies on babies and young children, visualisation of the brain and its coverings, including the dura mater, is of particular importance. Avoidance of autopsy-induced artefact is essential.

Removal of the infant brain during post-mortem requires the use of a different approach and equipment than that required for the adult brain owing to differences in the structure of the skull and its attachment to the pachymeninges (dura mater).

When an adult skull cap is removed to gain access to the brain, an electric oscillating saw is usually used to cut through the relatively thick bone (Pomara *et al.,* 2010). If this is done carefully, the underlying dura mater should be left intact, enabling a clear view of this membrane for signs of injury such as epidural or subdural haematoma, or brain swelling.

In contrast, infant calvarial bones are considerably thinner than those forming the adult skull cap. The major calvarial suture lines are un-ossified and are in fibrous continuity with the underlying dura and the fontanelles (Collins, 1995). The posterior fontanelle usually closes by three months of age (Hayden *et al.,* 2008) whereas the closure of the anterior fontanelle can be extremely variable (Amiel-Tison *et al.,* 2002), usually between the 9th and 18th month (Hayden *et al.,* 2008) with closure before six months considered early and after 18 months considered late (Amiel-Tison *et al.,* 2002). By the end of the 1st year of life 38% of infant anterior fontanelles are closed and by the end of the 2nd year in 96% (Scheuer & Black, 2000). A scalpel and scissors (or special 'infant skull shears') can be used to incise within the edges of the bones (Okazaki & Campbell, 1979; Riezzo, 2010). In a modification of the Beneke method of brain removal, which creates two incisions parallel to the sagittal suture in the frontal and parietal bones and extending down the lambdoid sutures, the frontal bones are also incised along the coronal sutures (Okazaki & Campbell, 1979). It is often necessary, when employing this procedure, to break the frontal and parietal bones near the skull base in order to acquire sufficient access. This standard method of brain removal leaves a midline strip of bone approximately 1 to 2cm wide overlying the superior sagittal sinus and the falx (Fig. 4.1).

One consequence of using the standard autopsy procedure for removing the infant calvarial bones is the lack of direct vision during the incision of the dura mater, with the potential to disrupt the brain (Fig. 4.2) and bridging vessels, and artefactually inducing contamination of the surface of the brain with blood. It is obvious such artefact could complicate assessment of subdural haematoma (especially thin-film haematomas). At younger ages, it is difficult to separate the dura mater from the sutures and fontanelles and removing the calvarial bones risks damaging this underlying membrane. In this chapter, an improved approach to post-mortem removal of the skull bones of infants using commercially available paediatric neurosurgical equipment is presented. This

technique leaves the dura mater intact, allowing for the observation of intracranial injuries free from autopsy-induced artefact, as well as detailed investigation of the infant bridging veins.

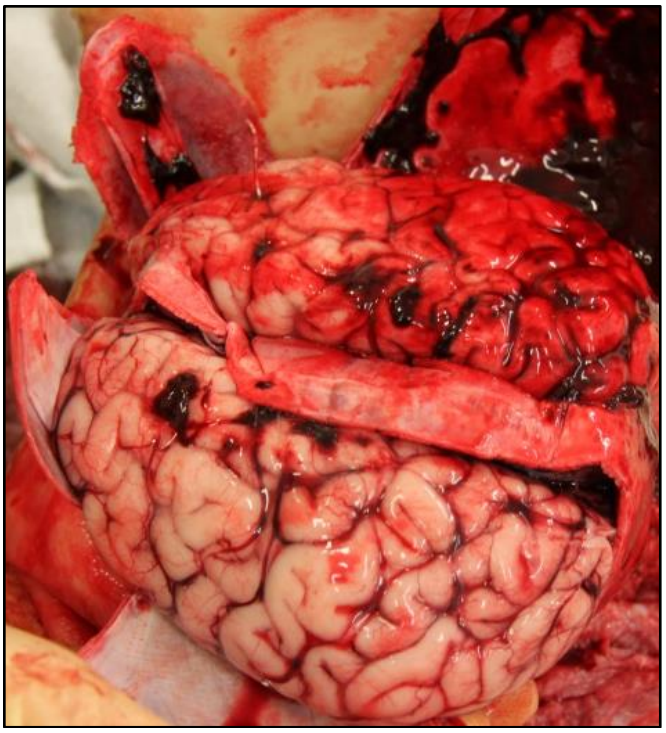


Figure 4.1 Standard method of brain removal

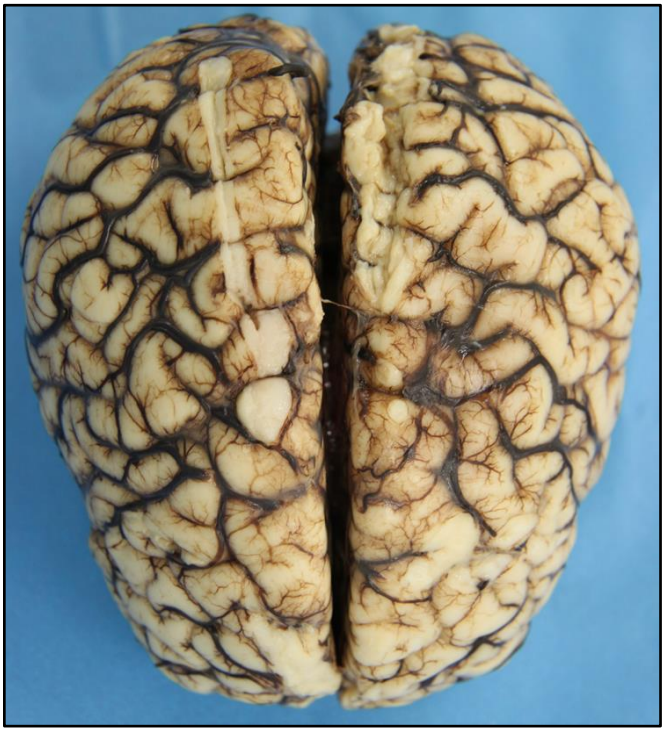


Figure 4.2 Extraction damage to the surface of the brain after using infant skull shears

4.3 Materials and Methods

The use of the neurosurgical tools in the manner described below was first identified at the Wales Institute of Forensic Medicine. Once the process had been established it was promoted for use by this Institute amongst the forensic practitioners of the United Kingdom. As both the Wales and Leicester Forensic Units are in receipt of Home Office funding for research into the causation of subdural haematomas in infants the process identified at Wales was adopted into autopsy practice at Leicester. The use of the tools to create burr holes and cut along the bone was demonstrated to the researcher by the Wales Institute of Forensic Medicine and then the researcher developed the skull removal method described below. Leicester undertakes examinations of infants and children from a wide catchment area who have either been consented by the parents for autopsy examination (so-called 'hospital autopsies') or those authorised by HM Coroner for medico-legal autopsy. In the case of medico-legal autopsies, it is up to the practitioner to decide how any evisceration procedure is undertaken, including the approach to the opening of the head.

4.3.1 Case Selection

Sixty-four consecutive neonatal, infant and early childhood autopsies (age range one day to 37 months) undertaken at Leicester between October 2012 and September 2015 as part of a regional paediatric autopsy service were included in this practice analysis (Appendix 3). Included were nine cases of head injury: six neonates (four one day olds, a six day old and a 13 day old) with perinatal head trauma and three infants (nine weeks old, 17 weeks old and 31 weeks old) with AHT.

4.3.2 Removal of the Calvarial Bones

Routine autopsy incisions were made to enable the scalp to be reflected anteriorly to the level of the superior orbital ridge and posteriorly to below the skull base. Before the cranial bones were removed the periosteum was stripped using a scalpel and dura strippers to enable direct visualisation of any skull bone fracture and to prevent subsequent clogging of the craniotome blade.

An electric high-speed surgical drill, the Midas Rex® Legend EHS Stylus® (Medtronic Xomed, Inc, Jacksonville, FL, USA) (Fig. 4.3), was employed to remove the calvarial bones

during the post-mortem examination of the head. Training in the use of the drill was undertaken at a workshop run by Medtronic in Switzerland.

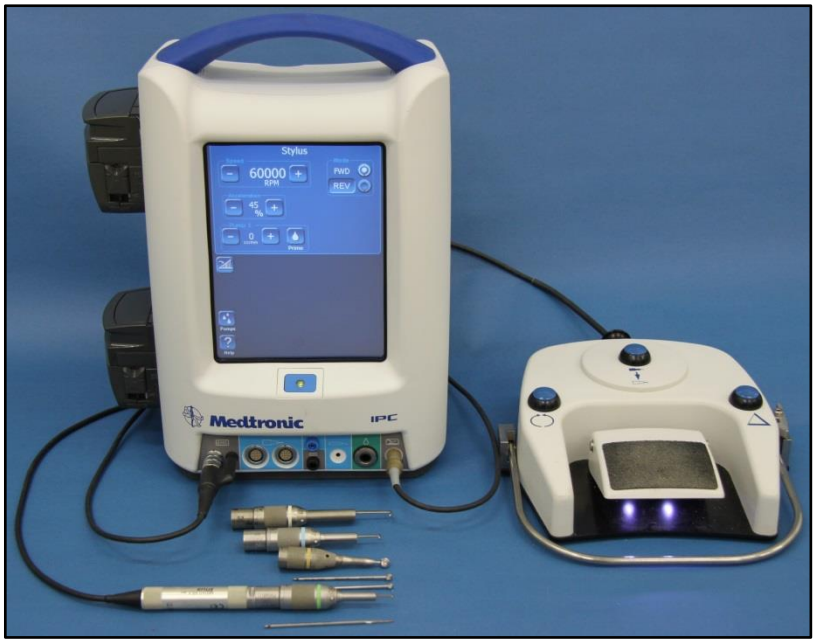


Figure 4.3 The Midas Rex® Legend EHS Stylus®, high-speed neurosurgical drill

Burr holes approximately 5-8mm in diameter, were created in each of the frontal, parietal and occipital bones (Fig. 4.4a-b). Using these burr holes (burr diameter ranging from 4mm-7mm (manufacturer's part numbers: 7BA40-7BA70)), selected according to age and therefore skull thickness of the infant, the craniotome was then used to create large windows in the calvarial bones, incising close to the edge of the bone and sutures (approximately 5-10mm) (Fig. 4.4c). After the bone windows were removed, cuts were completed in the bone perpendicular to the sutures near the skull base. This then enabled the remaining bone strips to be carefully dissected away from the dura mater using a small scalpel (Swann Morton No. 10) and leaving the dura mater intact (Fig. 4.4d). Dissection of the bone from the dura mater in one piece enabled excellent cosmetic reconstruction of the head using either glue or masking tape (Fig. 4.4e). This approach was used in all babies under the age of one year.

For the older age group (approximately 16 months and older) where the sutures had largely fused, a single burr hole was created in one of the frontal or parietal bones close to the residuum of the anterior fontanelle to allow access for the craniotome. The craniotome was then used to make a circular cut around the anterior fontanelle. After creating a few additional burr holes near the skull base, the craniotome was then used

to cut through the bone along a line parallel to the skull base to remove the skull cap (Fig. 4.4f).

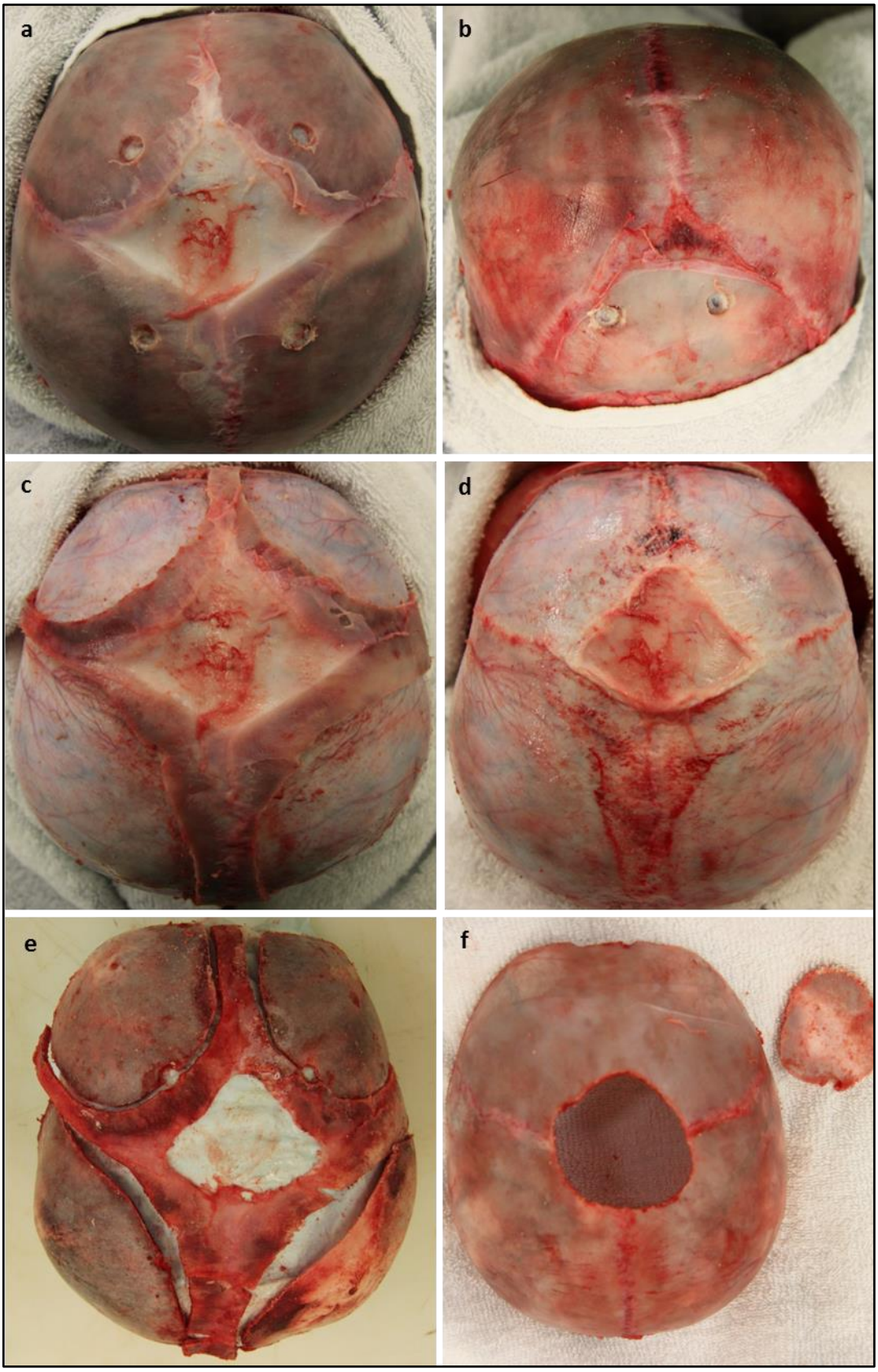


Figure 4.4 Removal & reconstruction of the calvarial bones of 5 month old male a-b) Burr holes in the frontal, parietal and occipital bones c) Removal of the bone windows d) Intact dura mater e) Reconstruction of the bones f) Skull cap of a 16 month old male

4.4 Results

Calvarial removal with the underlying dura mater remaining intact was successful in 61 of the 64 cases. Once sufficient operator experience had been gained, the entire process of calvarial bone removal, including the use of the neurosurgical tools and the careful dissection of the bone adjacent to the sutures took approximately 30 minutes. It was considered that this time scale would not present an impediment to application of this procedure in routine paediatric autopsy practice.

We found that the dura was detached sufficiently from the sutures by 16 months of age to enable removal of the calvarial bones as a skull cap. However, there was often a dural attachment to the bones adjacent to the anterior fontanelle requiring a small circle of bone including the fontanelle to be removed by careful dissection. In the case of slightly older children (two three year olds), the craniotome was used in place of the standard electric saw to remove the skull cap without the requirement for a circular incision around the anterior fontanelle. However, in one of these cases the attachment of the bone to the sagittal suture was still relatively strong and there was a small amount of damage to the parasagittal dura when removing the skull cap.

In one case in the young children group (an 18 month old male) there was damage caused to the dura. This was most likely due to the then inexperience of the operator in using the neurosurgical equipment. In one subsequent case (a 30 week old male), the dura mater was strongly adherent to the skull bone and on removal of the calvarial bones a thickening of the dura mater was observed in the areas most adherent to the bone. This strong adherence caused a slight tearing of the periosteal layer of the dura mater upon removal of one of the frontal bones and a few small perforations were occasioned in this region of the dura. Similar problems have not occurred later in the series. In all neonatal cases, complete integrity of the dura was successfully maintained during skull bone removal.

Calvarial bone removal was successful in both the perinatal and infant cases of head injury. This method in the AHT cases allowed for observation of brain swelling resulting in a flattened appearance of the gyri (Fig. 4.5), identification of exact areas occupied by

subdural haematoma and a notable lack of macroscopic intradural haemorrhage in the falcine, tentorial, parietal and basal dura mater.

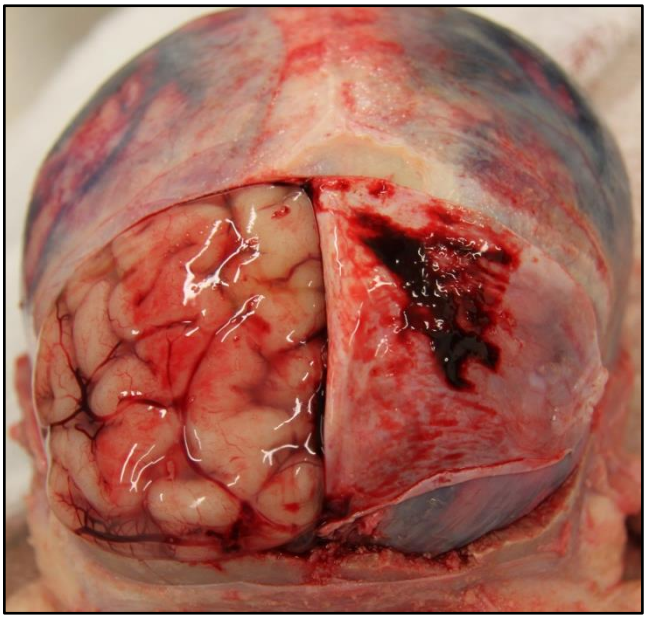


Figure 4.5 9 week old male with head injury showing brain swelling, flattened gyri and subdural haemorrhage

4.5 Discussion

A burr and craniotome combination have been used in clinical practice by neurosurgeons since the first instrumentation system was invented and developed in 1962 by Dr Forest Barber (Medtronic, 2013). Cranial and neurosurgical procedures which involve accessing the brain and operating within the skull are challenging. The neurosurgical drill is designed to provide optimum power, visibility and control for these procedures, whilst creating minimal damage to the areas around the operation site. In this chapter, the use of a clinical neurosurgical instrument has been translated into the autopsy environment.

The young infant skull bones cannot be removed as a cap as opposed to the adult calvarium which can be taken off in one piece at autopsy. Consequently, the standard procedure for infant brain removal is that documented in standard text books (Okazaki & Campbell, 1979; Riezzo, 2010; Stocker, 2011; Keeling, 2007). When cutting the infant calvarial bones, the dura mater is often incised at the same time owing to the dense attachment of the dura mater to the bone. A search of the published literature on the anatomy and development of the sutures failed to elicit reports of an exact age beyond

which there was adequate detachment of the cranial sutures from the dura mater to enable removal of the skull cap in one piece. It appears that suture biology and the exact mechanisms dictating cranial suture fusion or patency remain to be fully characterised (Slater *et al.,* 2008). The cranial suture complex has been shown to be made up from dura mater underlying the suture, the osteogenic fronts of the calvarial bone plates, the intervening cranial suture mesenchyme and the overlying pericranium (Slater *et al.,* 2008). It has been shown that the cranial sutures require tissue interactions with the dura mater to resist osseous obliteration *in vitro* (Opperman *et al.,* 1995), and that the dural fibres remain present within the sutures throughout life (Sergueef, 2007).

Minimally disruptive post-mortem investigation of the brain and surrounding meninges is a challenge in the young infant/neonate. Using the approach reported here, employing the use of motorised burrs and craniotome blades, it has been demonstrated that it is possible to visualise the entirety of the intact calvarial dura mater in all of the early childhood age groups attempted within this study. This technique is particularly beneficial in the assessment of head injuries due to the avoidance of post-mortem artefact and improved ability to directly observe and assess macroscopic pathological features of head injury (Fig. 4.6) including epidural haemorrhage, subdural haemorrhage and brain swelling prior to incision of the dura. This method also facilitates detailed dissection of the dura *in situ* and direct examination of the bridging veins (Fig. 4.7).

Figure 4.6 A 4 month old male head injury case with widespread bilateral thin film subdural haematoma and brain swelling

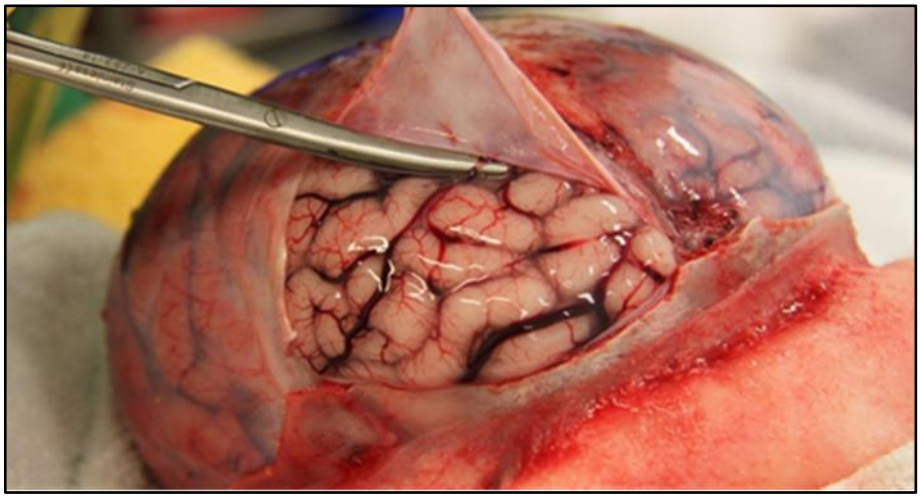


Figure 4.7 The use of the neurosurgical tool facilitates careful dissection of the dura mater to expose a bridging vein

The use of neurosurgical equipment has now become routine practice in both our unit and the Wales Institute of Forensic Medicine for the opening of the calvarium in infant and young child autopsies. In one case in this series the craniotome provided the ability to precisely excise the skull bones surrounding a fracture for decalcification and histological assessment. In several cases in this series the high speed surgical drill has also proved to be as an efficient tool for opening the spinal canal and superior orbital plates, procedures that are often necessary in the post-mortem assessment of AHT.

Chapter 5 : Optical Clearing of the Dura Mater

5.1 Overview

This chapter describes a method for increasing the transparency of the dura which not only aids optical coherence tomography (detailed in Chapter 6) but also enhances the post-mortem assessment of infant head injuries, particularly subdural haematomas.

5.2 Introduction

The dura mater is a typical fibrous connective tissue (Bashkatov *et al.,* 2000), being relatively thick and dense (Standring, 2008). It consists of an outer endosteal layer and an inner meningeal layer. In a relatively recent study, the median infant dural thickness by age was found to be 485µm at 0-90 days, 663µm at 91-180 days, 607µm at 181-270 days and 469µm at 271-365 days (Breisch *et al.,* 2010). There is a predominance of collagen fibres (over 90% of the dura's thickness (Scarr, 2008), especially in the endosteal layer, which are highly corrugated, and layered on top of each other (Van Noort *et al.,* 1981). The collagen fibres are arranged in parallel bundles with differing orientations, varying from highly aligned to apparently random and arranged in lamellae (Hamann *et al.,* 1998).

Light-based imaging systems such as optical coherence tomography (OCT), require light to penetrate through biological tissue. However, these tissues highly scatter light, decreasing the effective depth of imaging (Sudheendran *et al.,* 2010). Optical clearing agents (OCAs) are used to aid such imaging systems by decreasing the amount of scattered source light, increasing tissue transparency and increasing the depth of imaging.

To aid OCT imaging through the dura and assessment of the vasculature below and within this membrane, as part of this thesis, optical clearing using glycerol and mannitol was investigated to determine the success of these OCAs on the dura. Optical clearing of animal and human skin has previously been used in conjunction with OCT studies to enhance the imaging of subcutaneous blood vessels (Vargas 2003, Bonesi 2010, Wen 2012, Proskurin 2007). Within these studies, as well as increasing the depth of OCT

imaging, optical clearing has also been shown to increase image contrast and spatial resolution of the visualised subcutaneous skin structures (Bonesi 2010).

The composition and increasing thickness of the dura varies with age, making this membrane difficult to see through when observing the brain with the naked eye during a post-mortem examination. A further postulated advantage of the application of an OCA would be to increase the transparency of the dura mater during post-mortem examination of head injuries, aiding in the assessment of areas of bleeding associated with this membrane. As well as determining a suitable OCA to use alongside OCT, this chapter will also present the translation of a method often used for increasing imaging depth of tissues, to aid in the visualisation of the brain surface and pathologies associated with head injury through the intact infant dura in paediatric autopsies.

5.3 Method

Initial optical clearance testing was performed in the laboratory as a proof of concept experiment to ensure a change in the transparency of the dural tissue when immersed in an optical clearing agent. Once proof of concept was gained, the testing was performed in the mortuary during the post-mortem examination of the infant to enable testing of fresh tissue. Following these experiments, *in situ* testing was performed using glycerol.

5.3.1 Case Selection

Cases were identified as part of a regional paediatric/perinatal autopsy service at Leicester Royal Infirmary between July 2013 and September 2015. For all cases, tissue that was retained at autopsy for research purposes had appropriate parental consent.

5.3.2 Extracorporeal Testing of Optical Clearing of the Dura Mater in the Laboratory

Dural tissue was obtained from a hospital post-mortem case which had consent for retention of tissue for research purposes. After the post-mortem the tissue was kept in formalin at room temperature for three days prior to testing. Before testing the dura was rinsed in distilled water to remove any excess formalin. It was then cut into sections of approximately 2cm x 2.5cm. Samples of dura were immersed in vials of 100% glycerol for one hour. The tissue was removed from the OCA at timed intervals of 5, 10, 15, 30

and 60 minutes and excess glycerol was removed. The section of dura was then placed on a USAF 1951 resolution test target patterned background and digital photographs of the tissue sample were taken with a Canon EOS 600D camera with Canon EF 100mm f/2.8L Macro IS USM lens set to automatic focus and exposure, at each time point before returning the tissue to the glycerol. The photographs from each time point were then compared to determine the effect of time the dura spent immersed in glycerol on the resulting optical clearance of the tissue.

5.3.3 Extracorporeal Testing of Optical Clearing of the Dura Mater in the Mortuary

In the mortuary all digital photographs were taken using a Canon EOS 500D camera with an attached Canon EFS 17-85MM lens set to automatic focus and exposure. Photographs were taken of the dura before, during and after testing with the OCAs.

5.3.3.1 Immersion in Glycerol

Extracorporeal testing in the mortuary allowed for experiments to be performed on fresh sections of dura. In the previous testing within the laboratory it was determined that the length of time the dura was immersed in the glycerol did not make a significant difference to the optical clearance of the dura when comparing 5-10 minutes with one hour. It was therefore decided to only immerse the dura in the OCA for 10 minutes in the extracorporeal experiments within the mortuary.

Extracorporeal immersion testing using glycerol was undertaken on samples of dura from two paediatric autopsies (a nine week old male and a three day old male) and one 36 week old stillborn foetus. As part of the post-mortem examination of the brain and meninges, the dura mater was removed from the cadavers using standard autopsy technique by a specialist Consultant Paediatric Pathologist. Samples of parietal dura approximately 6cm x 6cm in size were immersed in 100% v/v glycerol or 50% v/v glycerol/distilled water for 10 minutes.

5.3.3.2 Immersion of Dura in Mannitol

In a further extracorporeal immersion experiment, similar sized samples of cadaveric parietal dura from a seven week old male were immersed in three different concentrations of mannitol (80 g/L, 160 g/L, 320 g/L) as a comparison to a sample of

dura from the same subject immersed in 100% glycerol. Samples were approximately 2cm x 3cm and were immersed for 10 minutes.

5.3.3.3 Topical Application of Glycerol

To prepare for the *in situ* topical application of glycerol to the dura, a final extracorporeal experiment was performed where the OCA was applied to the surface of sections of parietal dura from a six week old male. To model the curved surface of the brain and overlying dura when *in situ* a model doll head was used. The USAF 1951 resolution test target patterned background was secured onto the top of the model and sections of parietal dura (approximately 8cm x 11cm) were placed on top of the pattern. Two concentrations of glycerol (100% and 50%) were tested in these experiments. The OCA was applied to the dura with a soft brush every 1-2 minutes for the duration of the experiment to account for any glycerol that ran off the side of the curved surface. Digital photographs were taken of the sample of dura at 10 time points every three minutes for 27 minutes.

5.3.3.4 Quantification of the Extracorporeal Testing

For all the extracorporeal experiments, both within the laboratory and the mortuary, the USAF 1951 resolution test target patterned background was used to enable the investigator to determine the increase in transparency by observing the photographs of the dura before, during and after the application of the OCA. Within the patterned background, three horizontal and three vertical bars constitute an element block and there are six element blocks in a group. There are four groups in total (number -2 to 1). As the group and element number increases, the size of the bars and spaces between the bars decreases (Fig. 5.1). As the transparency of the dural tissue increased the clearer the patterns on the underlying target became, allowing for smaller bars on the target to be resolved.

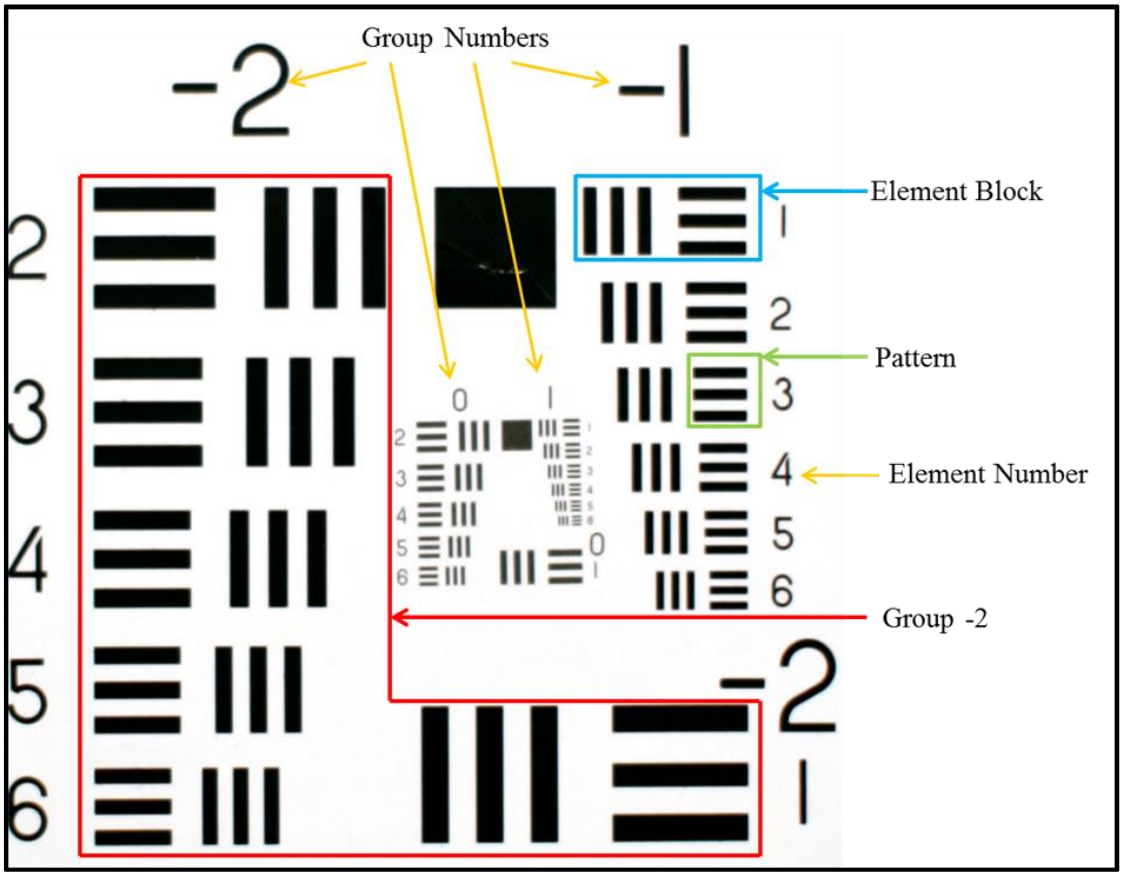


Figure 5.1 The group and element numbers on a USAF 1951 resolution test target

To improve the assessment of the effects of glycerol on the dural tissue at the two concentrations used in the extracorporeal testing, further analysis of the digital photographs was undertaken for the extracorporeal immersion experiments performed in the mortuary using the USAF 1951 resolution test target patterned background. Photographs from one of the three cases were selected for this further analysis due to the more uniform nature of the two sections of dura used to assess the two different concentrations of glycerol. Four photographs were selected in total to show a before and after shot for both the 100% and 50% glycerol testing.

Photographs were dimensionally normalised in Photoshop Elements (version 10) to ensure that the target pattern backgrounds were reproduced to the same scale. Eight independent observers with optometrically corrected vision were then asked to assess each photograph (at a size of 17.5cm x 20.5cm) to determine the smallest group and element number that they could visually resolve by separation of the vertical and horizontal bars of the element blocks viewed through the dural tissue. Photographs were viewed from a distance of 60cm on an Apple Mac Pro 2 x 3.06 GHz 6-core Intel

Xeon with an Apple LED cinema display LCD monitor with a 27″ screen and a resolution of 2560 x 1440. The element blocks were assigned numbers on a linear scale from one to 24, with one representing the smallest element block, to allow arithmetic means and standard deviations (SD) to be calculated for each photograph. Image J image analysis software (National Institutes of Health, USA) was used to determine the pixel dimensions of the spacing of the element bars in order to calculate the percentage change in size of element blocks the participants could resolve before and after immersion of the dura in glycerol.

5.3.4 *In Situ* Optical Clearing of the Dura Mater

Following successful results of the extracorporeal testing, the use of glycerol (100%) as an OCA was adopted routinely into local paediatric autopsy practice for the evaluation of subdural brain pathologies. Glycerol was applied to the dura mater *in situ* as part of the post-mortem examination of the brain in 36 neonatal, infant and early childhood autopsies. Included were 22 males and 14 females, with an age range from one day to three years with a median age of 15 weeks. There were three cases of AHT, and three cases of perinatal head injury. Initially, opportunistically selected cases were used to evaluate the effects of the glycerol on the dura. Later in the series, cases were selected where the application of glycerol was considered to enhance the viewing of specific brain pathologies.

The infant calvarial bones were removed using the method discussed in Chapter 4, which leaves the dura mater intact. The dura was then rinsed with tap water to remove surface blood and bone debris. The surface was blotted dry with a towel. Glycerol (100%) was then painted onto the surface of the dura using a soft brush. Depending on the thickness of the dura in each case, one to three applications of glycerol were applied to the dura over a period of one to two minutes.

5.3.4.1 Histology

Samples of dura from the extracorporeal experiments that were untreated and treated with 100% glycerol were processed for routine histology in a CPA accredited hospital histopathology laboratory by standard methods. Standard Hematoxylin and Eosin (H&E) sections were analysed by a Consultant Paediatric Pathologist using an Olympus BX45

clinical microscope equipped with planar objectives. Masson's trichrome, Perls' stain (for iron), glial fibrillary acidic protein (GFAP), CD68, and beta amyloid precursor protein (β-APP) staining were also undertaken. Digital micrographs were taken using a Jenoptik ProgRes CapturePro v 2.8.8 software.

5.4 Results

5.4.1 Extracorporeal Testing of Optical Clearing of the Dura Mater in the Laboratory

An obvious difference could be detected when observing the photographs of the sample of dura before and after immersion in glycerol. The smaller sized groups (0 and 1) within the test target were only slightly detectable through the dura before immersion and no element blocks could be resolved with the naked eye. After immersion the smaller groups could be seen with the naked eye. No difference was seen in the transparency of the dura throughout the timed intervals, the tissue immersed for five minutes appeared to have resolved to the same element block as the tissue immersed for 60 minutes (Fig 5.2a-c). Therefore, time did not appear to have an effect on optical clearance of the dura during extracorporeal immersion testing after the five minute time interval.

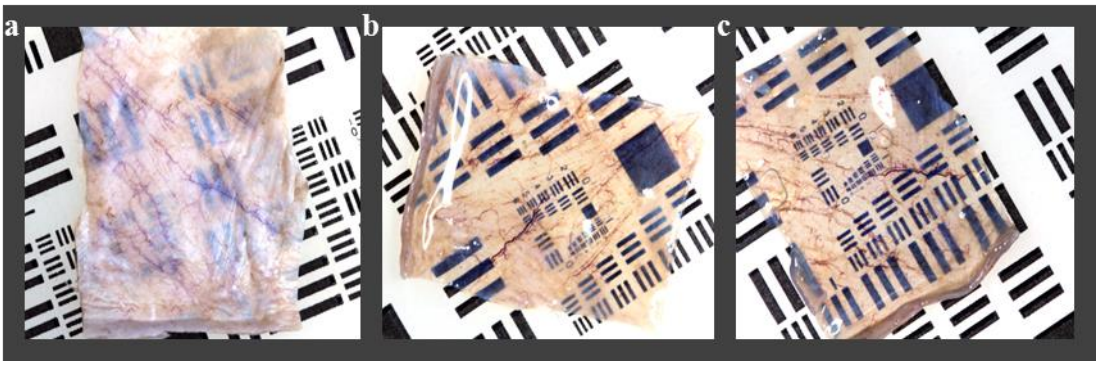


Figure 5.2 Extracorporeal laboratory optical clearing testing of dura a) Before immersion in 100% glycerol b) 5 minutes after immersion c) 60 minutes after immersion

5.4.2 Extracorporeal Testing of Optical Clearing of the Dura Mater in the Mortuary

5.4.2.1 Immersion in Mannitol

No difference could be detected with the naked eye when comparing the transparency of the dura before and after the tissue was treated with the three different concentrations of mannitol (Fig 5.3a-b). In comparison, an obvious change in a parallel

sample of dura immersed in glycerol was associated with an increase in the transparency and accompanying shrinkage and stiffening of the tissue.

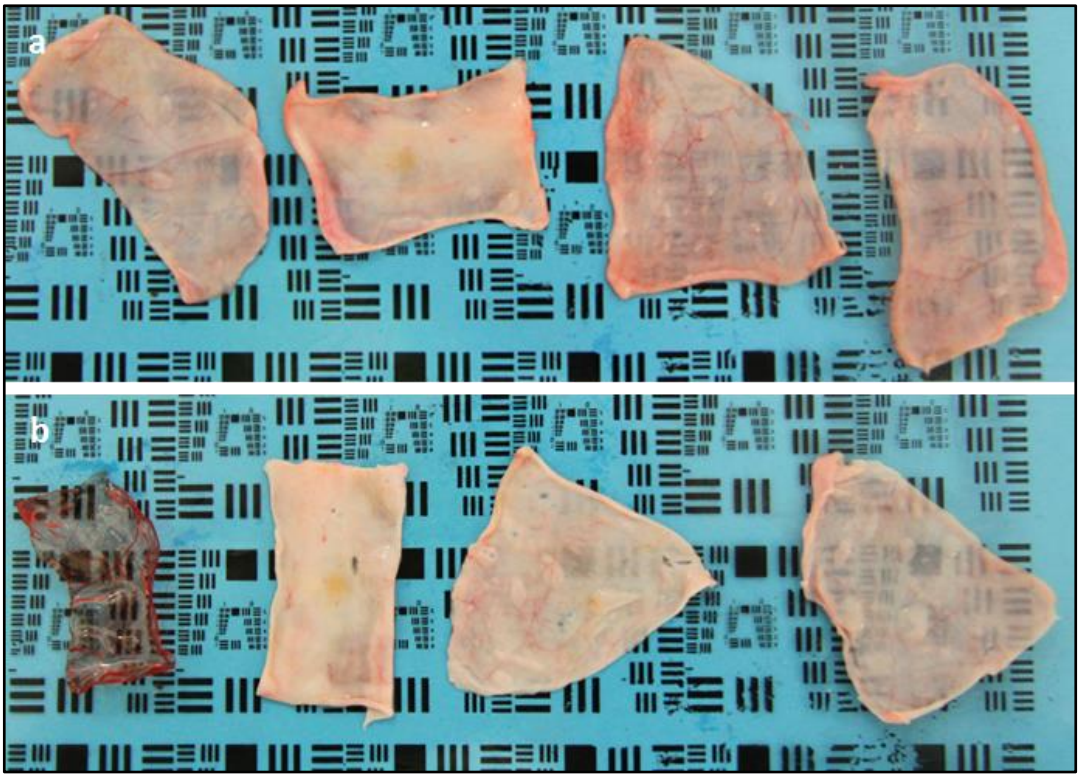


Figure 5.3 Samples of parietal dura on a USAF 1951 resolution test target background a) Before immersion in OCAs b) After immersion for 10 minutes in 100% glycerol, 80 g/L mannitol, 160 g/L mannitol and 320 g/L mannitol (from left to right)

5.4.2.2 Immersion in Glycerol

A very noticeable increase in the transparency of the dural tissue was seen during the extracorporeal experiments with both 50% and 100% glycerol (Fig. 5.4a-d). Against the USAF 1951 resolution test target patterned background, the mean number on the linear scale seen by the independent observers for the section of dura before immersion in 100% glycerol was 11.50 with a SD of 2.20, corresponding approximately to element 1 in group 0. After immersion the mean number on the linear scale was 6.75 with a SD of 0.89, corresponding approximately to element 6 in group 0. The mean number on the linear scale seen by the independent observers for the section of dura before immersion in 50% glycerol was 16.13 with a SD of 2.30, corresponding approximately to element 3 in group -1. After immersion, the mean number on the linear scale was 7.00 with a SD of 1.93, approximately corresponding to element 6 in group 0. The increase in transparency for the dural tissue before and after immersion in 100% and 50% glycerol reduced the size of the element block the observers could resolve by 5 and 9 element

blocks respectively. Using the pixel data (Table 5.1), this reduction in resolvable target size was calculated to be a percentage decrease of 60.67% and 73.59% for the 100% and 50% glycerol experiments respectively.

Sections of dural tissue immersed in 50% and 100% glycerol resolved to the same element block. However, the tissue immersed in 50% glycerol started at a larger sized element block, resulting in a larger percentage decrease for the size of bars that the participants could resolve before and after immersion in glycerol.

The 100% glycerol appeared subjectively to dehydrate the dural tissue more than the 50%, which would be expected owing to the absence of water molecules in the 100% glycerol.

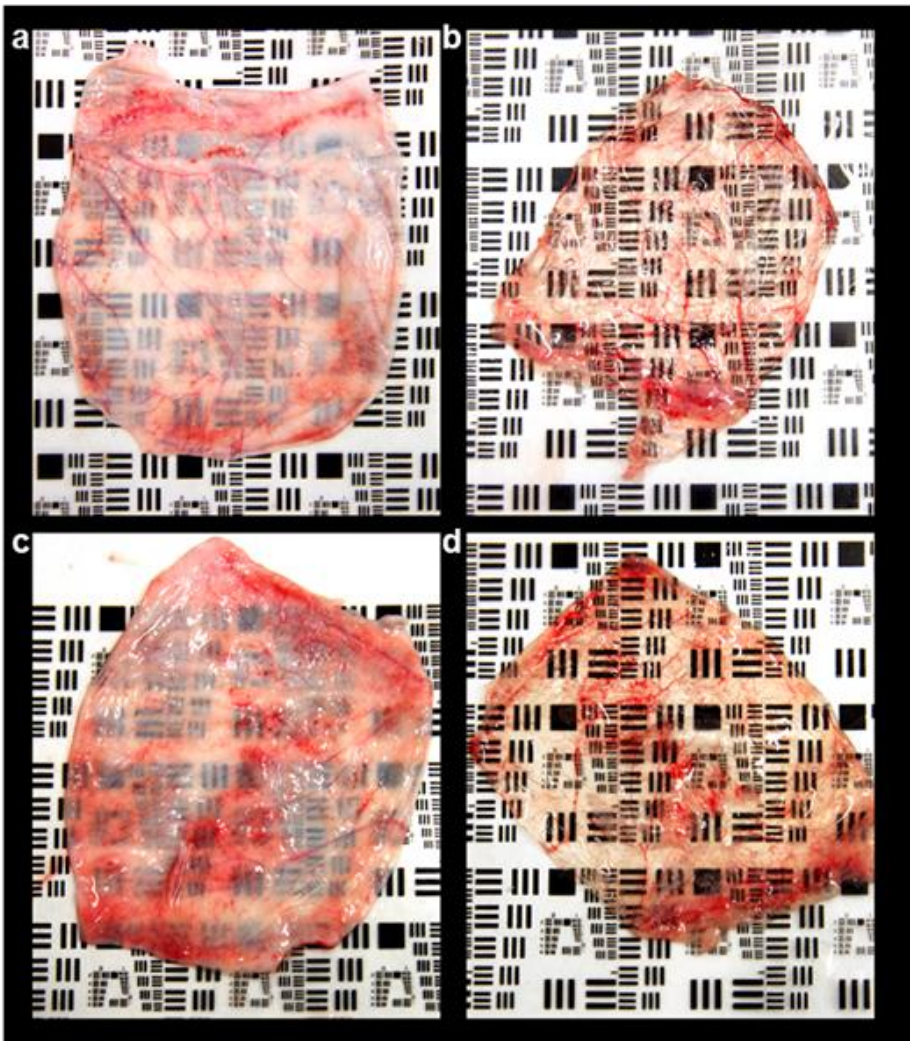


Figure 5.4 Samples of parietal dura on a USAF 1951 resolution test target background a) Before immersion in 100% v/v glycerol b) After immersion in 100% v/v glycerol c) Before immersion in 50% v/v glycerol d) After immersion in 50% v/v glycerol

Tissue sample	Mean EB (element, group)	Mean width of the spaces between the bars of the EB (pixels)
A- Before immersion in 100% G	1,0	20.95
B- After immersion in 100% G	6,0	8.24
C- Before immersion in 50% G	3,-1	31.20
D- After immersion in 50% G	6,0	8.24

Table 5.1 Mean element blocks the independent observers could resolve for dural tissue before and after immersion in 50% and 100% glycerol, and the width of the spaces between the bars of the element blocks, G-glycerol, EB-element block

5.4.2.3 Topical Application of Glycerol

When observing the digital photographs for the topical application of glycerol (Fig 5.5 & 5.6) an increase in the transparency of the dural tissue was apparent for both 50% and 100% glycerol. The sample of dura that was used in the 50% glycerol testing covered four sets of the smaller groups (groups 0 and 1) in the test target background. These groups became partially visible through the dura at time interval 4 (nine minutes), by which point the increase in transparency was relatively obvious with the naked eye. Although there does not appear to be an obvious difference in transparency between the last few photographs in the series for the 50% glycerol experiment, on closer inspection, a pattern towards the outer edge of the tissue sample has started to become apparent at time interval 9 (24 minutes) (Fig 5.5).

The sample of dura used for the 100% glycerol experiments was slightly smaller than that used for the 50% glycerol testing and therefore the dura was overlying three sets of the smaller groups (groups 0 and 1) in the test target background. These groups all became partially visible at time interval 3 (six minutes). An increase in the resolution of these groups on the target can be seen between time interval 3 and time interval 5 (12 minutes). The difference in transparency between the last five photographs appears relatively small when observing the photographs with the naked eye. However, on closer inspection, sections of the target pattern are still becoming visible towards the outer, thicker areas of the sample in the last half of the digital photographs in the series (Fig. 5.6).

The smaller groups on the pattern became visible more quickly in the 100% glycerol testing (six minutes) compared to the 50% glycerol testing (nine minutes).

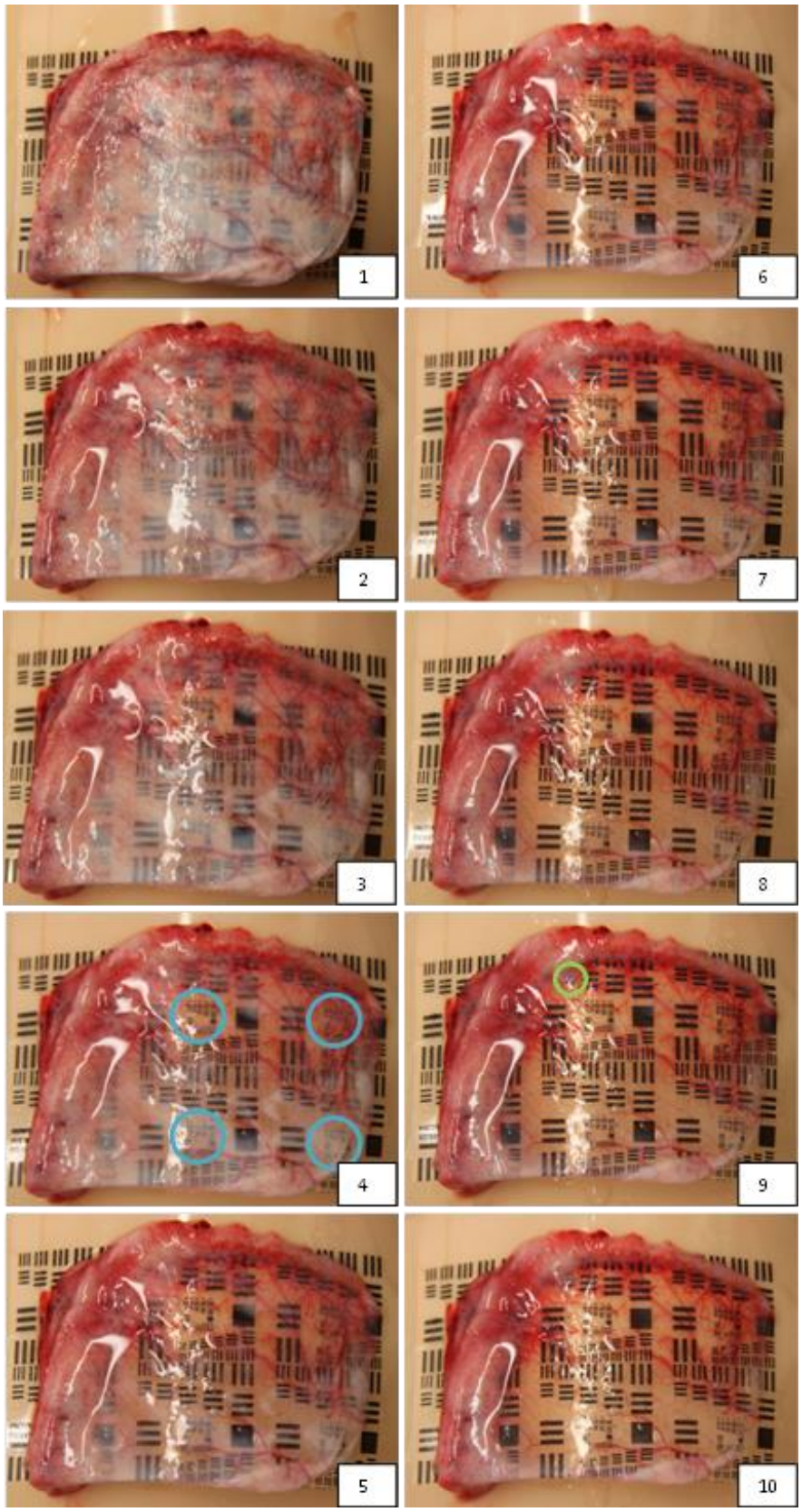


Figure 5.5 Timed interval series of digital photograph showing a sample of dura with 50% glycerol applied topically every 1-2 minutes. The first photograph showing time 0, before application of glycerol, and the last photograph showing the sample after 27 minutes. At time interval 4 all the smaller patterned groups are partially visible (blue circles). From time interval 9 onwards, a section on the pattern is becoming visible through the thicker part of the dural tissue (green circle)

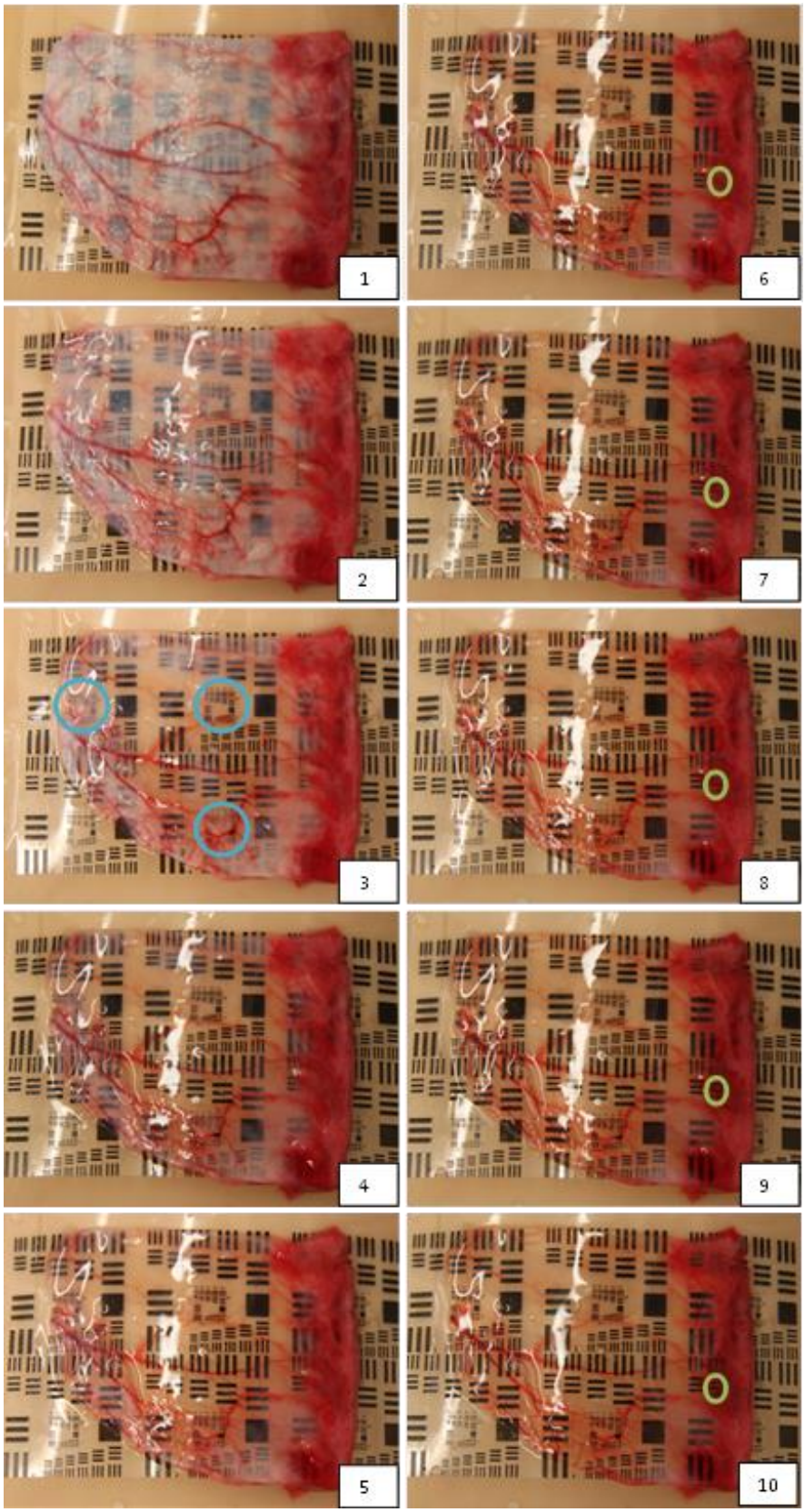


Figure 5.6 Timed interval series of digital photograph showing a sample of dura with 100% glycerol applied topically every 1-2 minutes. The first photograph showing time 0, before application of glycerol, and the last photograph showing the sample after 27 minutes. At time interval 3 all the smaller patterned groups are partially visible (blue circles). From time interval 6 onwards, a section on the pattern is becoming visible through the thicker part of the dural tissue (green circles)

5.4.3 *In Situ* Optical Clearing

As optical clearing is mediated at least, in part, by dehydration of the tissue, 100% glycerol was chosen for *in situ* autopsy practice as it was expected that the fluid in the underlying brain tissue would re-hydrate the treated dura, thereby reducing the effect of clearing. An increase in optical clearance of the dura could be seen with the naked eye within the first minute of application of glycerol during *in situ* use in all but one subject. In nearly all cases, application of glycerol resulted in the blood vessels within and below the dura mater and on the surface of the brain becoming more readily apparent (Fig. 5.7a-b). *In situ* application of 100% glycerol did not appear to dehydrate the dural tissue to the same degree when compared to that apparent in the dura mater in the extracorporeal experiments, most likely due to continued hydration of the dura from the underlying brain tissue. With prolonged application (>10 minutes or so) some adherence of the dura to the brain tissue was observed. The increase in optical clarity and the accompanying dehydration of the dural tissue, which causes a slight shrinkage and decrease in the flexibility of this membrane, can be completely and almost instantaneously reversed with the application of aqueous fluids (including tap water, 0.9% saline and 10% formalin) to the tissue (Fig. 5.7c). The clearing effect reliably lasted long enough to allow acquisition of a systematic series of macroscopic photographs of all surfaces of the dura (Fig 5.8).

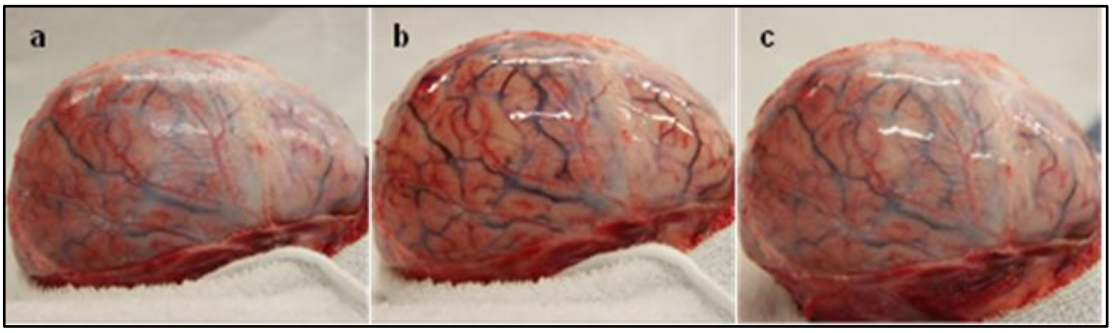
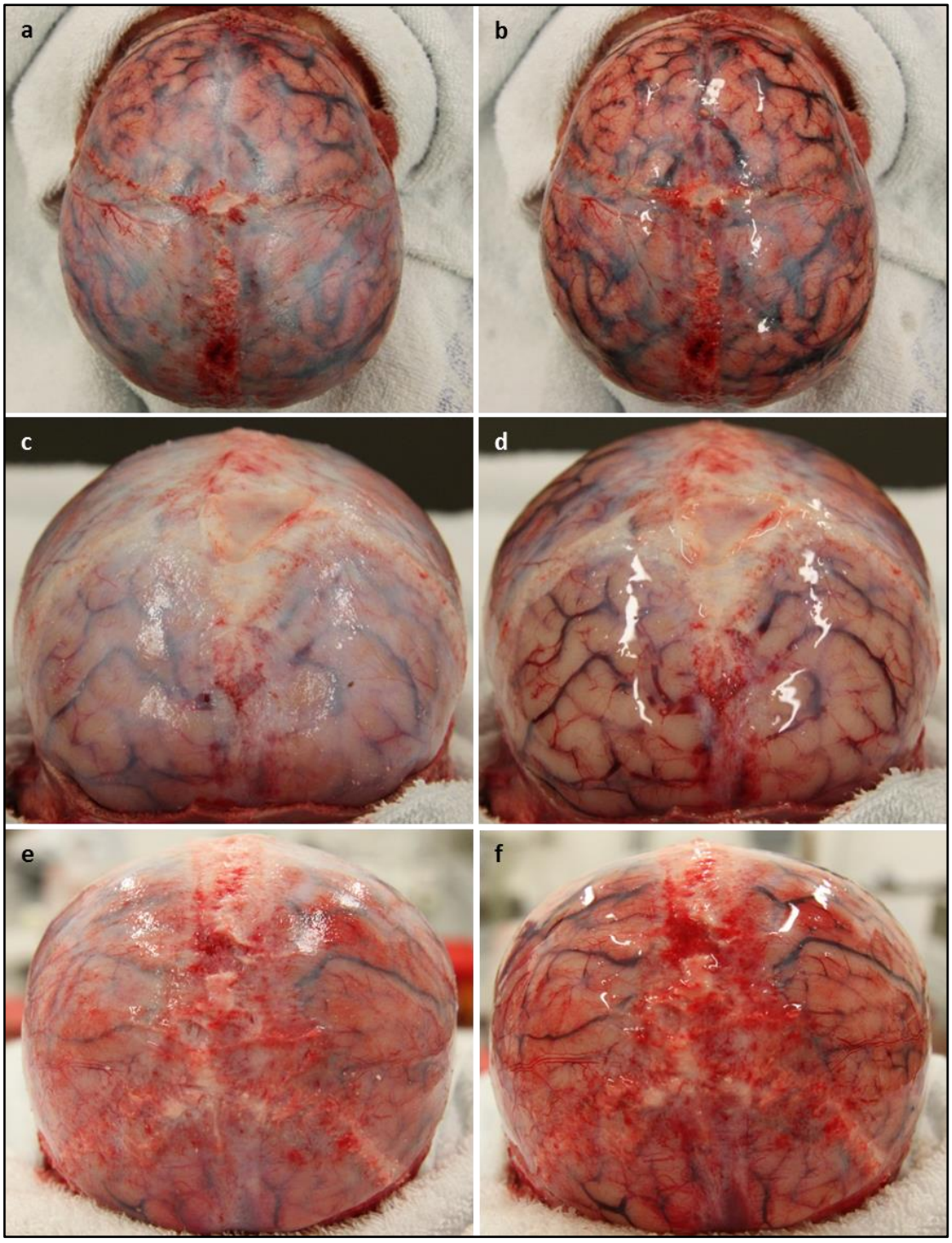


Figure 5.7 *In situ* clearing on a 9 week old male a) Before application of glycerol b) After application of glycerol c) After reversal of clearing with water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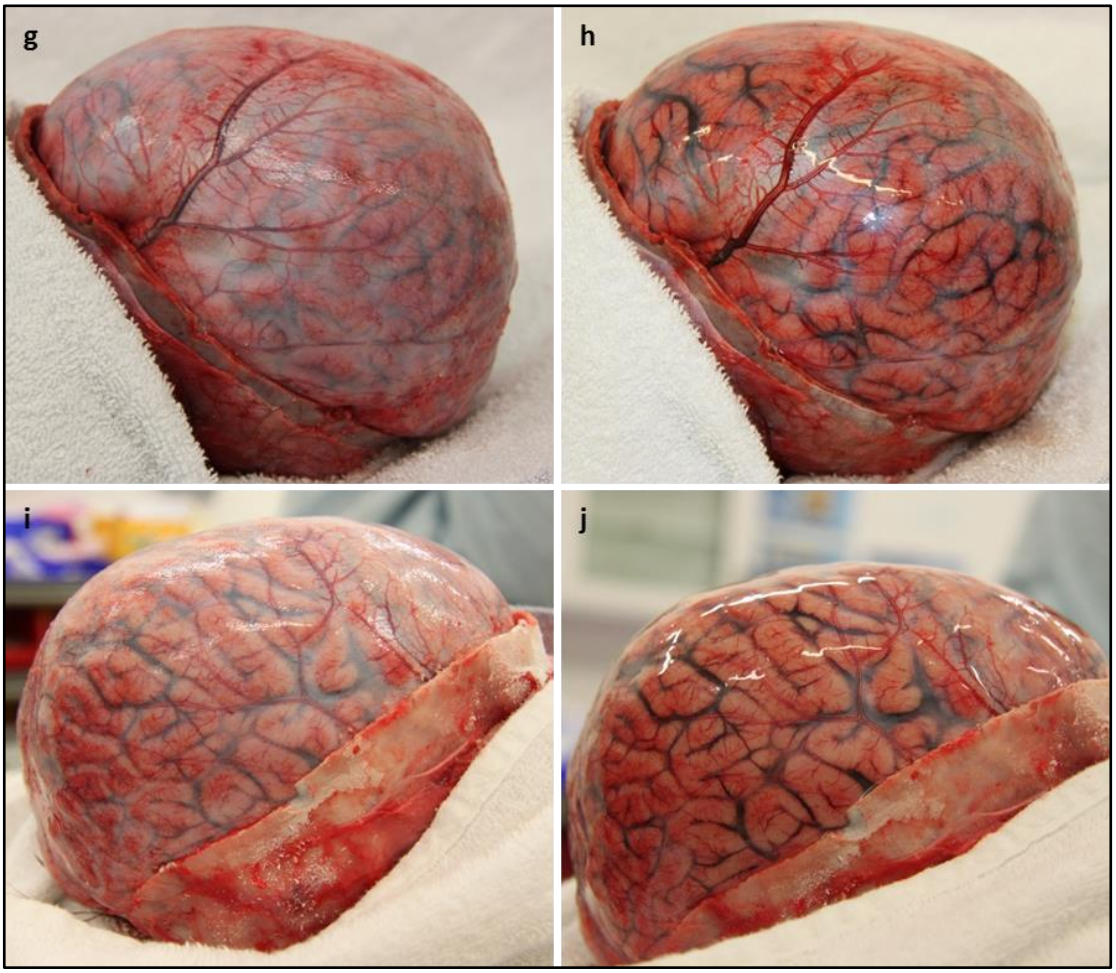


Figure 5.8 Macroscopic photographs of all surfaces of the dura a,c,e,g,I) Before treatment with 100% glycerol b,d,f,h,j) After treatment with 100% glycerol

In a single case, a partial clearing effect was produced in a two year old male severely affected by cerebral palsy where there was significant atrophy of the underlying brain tissue and prominent gelatinous leptomeninges with thickening of the dura. This partial effect is most likely due to the fluid rich-nature of the leptomeninges in this particular circumstance (Fig. 5.9).

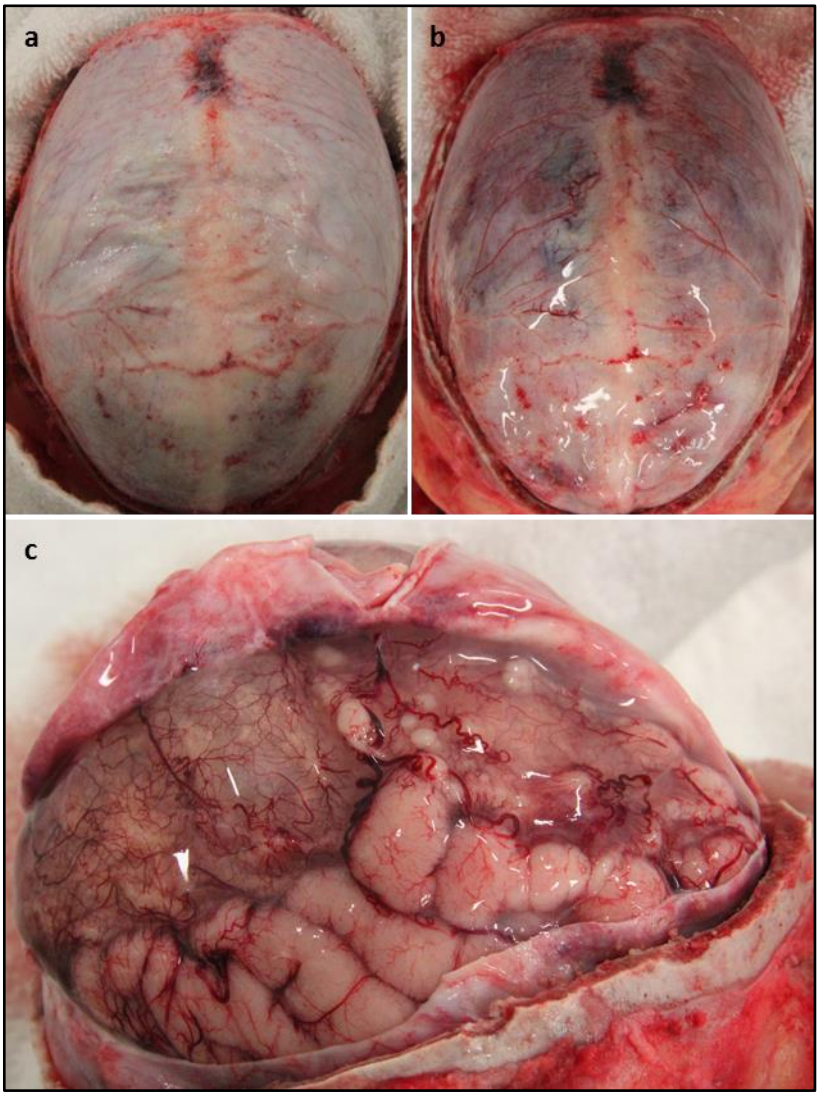


Figure 5.9 Partial clearing of the dura of a 2 year of male with cerebral palsy a) Before application of 100% glycerol b) After application of 100% glycerol c) Atrophy of the brain and gelatinous leptomeninges

In three cases of AHT (a two month old male, a four month old male and a seven month old female) with bleeding below the dural membrane, 100% glycerol was used to assess the extent of areas of haemorrhage within and underneath the dura mater (Fig. 5.10a-f). With the resulting increased transparency of the calvarial dura mater, extensive haemorrhage underneath the membrane could be seen more clearly.

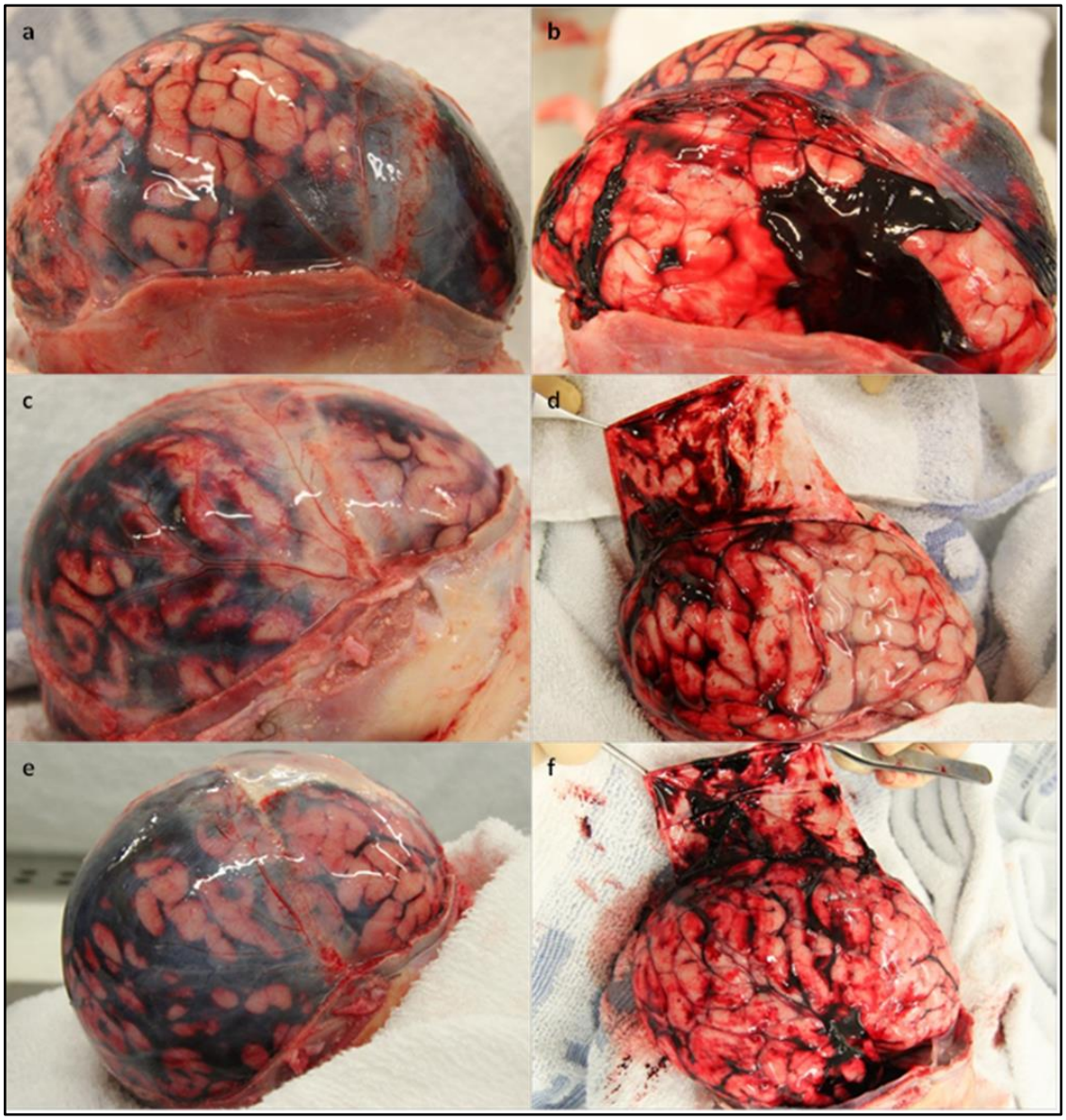


Figure 5.10 *In situ* clearing with glycerol and lifting the dura of 3 AHT cases a-b) 4 month old male c-d) 2 month old male, and e-f) 7 month old female, all showing bilateral patchy thin subdural hematoma, thickest over the sulci of the brain with extremely thin bleeding over gyral convexities

In three cases of perinatal head injury and one case of confessed overlaying, the latter having apparently led to minor crush injury, glycerol was used to determine whether subdural haemorrhage was present and to increase visualisation of obvious patterns of bleeding (Fig. 5.11a-l). In one case in the series (a four week old male), clearing of the dura enabled observation of a focus of extremely thin bleeding (best characterised as a smear of blood), over the occipital convexity (Fig. 5.11b), which was revealed to be subdural haemorrhage upon subsequent reflection of the dura (Fig. 5.11c).

No macroscopic areas of intradural haemorrhage were noted in the calvarial dura (i.e. over the convexities of the brain or near the superior sagittal sinus) before or after application of glycerol in any of the cases in this study.

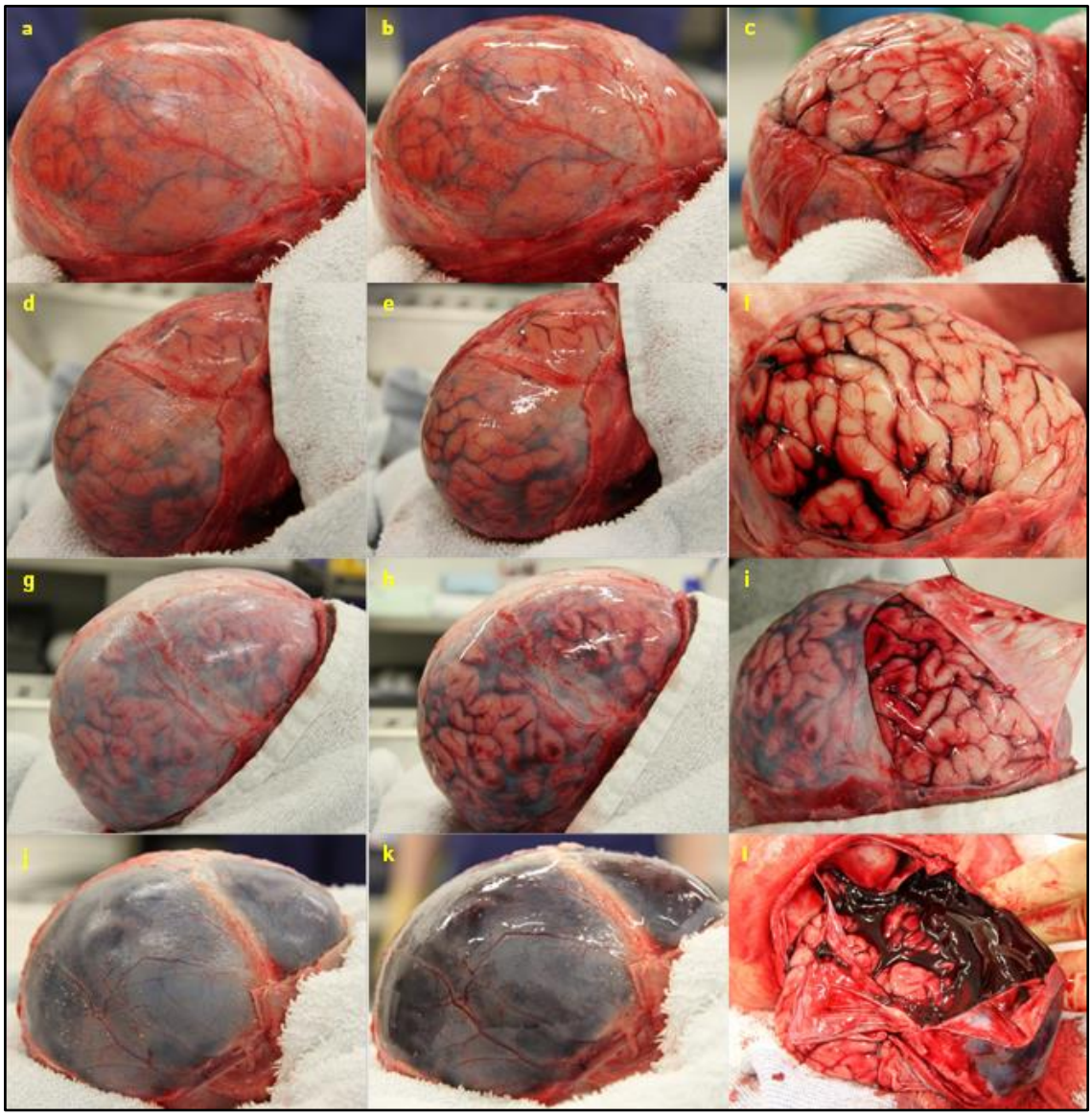


Figure 5.11 *In situ* clearing, before (a,d,g,j) and after (b,e,h,k) application of glycerol, and lifting of the dura (c,f,i,l) (a-c) 4 week old male with focal posterior subdural bleeding, revealed on lifted the dura as an extremely thin smear of blood over the occipital convexity d-f) 1 day old female with perinatal head injury born by emergency caesarean section following failed suction cup delivery showing a focal thin smear of subdural bleeding below the posterior parietal and occipital dura g-i) 3.5 month old male with minor crush injury most likely the result of confessed overlay, with very thin subdural bleeding over the right cerebral convexity j-l) 1 day old female with perinatal head injury following forceps delivery showing extensive space occupying subdural bleeding extending over the entire convexity

5.4.3.1 Histology

Histological assessment indicated that there was no significant difference between the appearances of the tissue samples by routine light microscopy when comparing untreated dura mater with samples which had previously been immersed in 100% glycerol (Fig. 5.12a-b). There appeared to be only very subtle differences in the appearance of some of the collagen bundles between dura treated and untreated with glycerol (Fig 5.12c-d). However, some variation in the appearance of dura taken from various sites is to be expected. Dural tissue treated with glycerol also showed a normal Perls' reaction for iron, and glycerol treatment of the dura did not compromise the interpretation of any of the various immunohistochemical stains undertaken on selected samples of the underlying cerebral convexities.

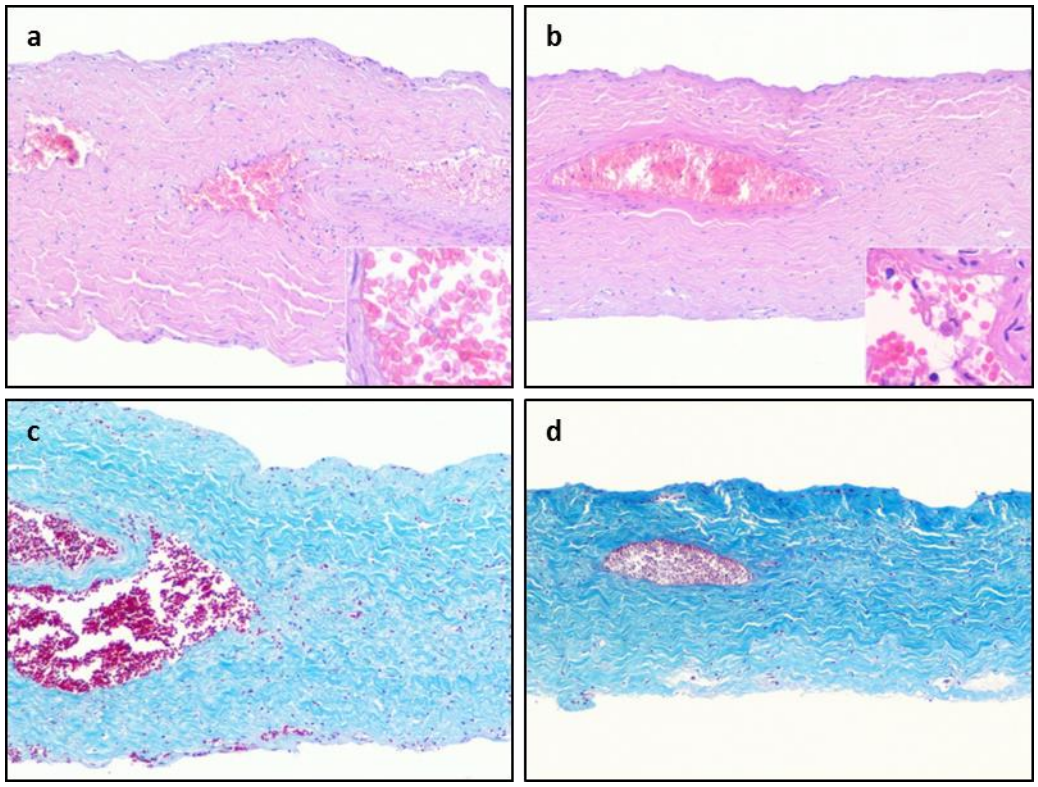


Figure 5.12 Digital photomicrographs (x10 objective and x40 insets in a-b) of parietal dura mater stained with H & E a) Not treated with glycerol b) Treated with glycerol; and Masson's Trichrome c) Not treated with glycerol d) Treated with glycerol

5.5 Discussion

Variation in refractive indices means that light travels at different speeds and angles through biological tissues (Zhu *et al.,* 2013), inducing light scattering. The components of dural tissue, such as collagen, scatter light to a large degree as they have high

refractive indices. The surrounding interstitial fluid and/or cytoplasm have lower refractive indices and therefore scatter light to a smaller degree. Optical clearing agents such as glycerol, with their high refractive indices are hyperosmotic (Yeh *et al.,* 2003) and therefore they diffuse into the tissue they are applied to, reducing variation in refractive indices, in turn reducing the amount of light scattering.

A further effect associated with the process of optical clearing is thought to be dehydration of the tissue as a result of the rate of passive diffusion of the glycerol into the tissue, which is slower than the migration of water out of the tissue because OCAs have high osmolality (Guo *et al.,* 2010). The smaller diffusion coefficient of glycerol compared to water results in the glycerol travelling into the tissue and cells at a much slower rate, causing the tissue to shrink (Vargas *et al.,* 2003). Collagen is soluble in sugar-alcohols (Yeh *et al.,* 2003) and therefore structural modification or dissociation of collagen is also thought to play a role in optical clearing (Larin *et al.,* 2012). In these experiments dissociation of collagen fibres was not observed, possibly due to a reversible effect produced by rehydration of the tissue when place in formalin. Rather, if anything, there was slight compaction of some of the collagen bundles in treated dura samples. However, these minimal changes would not be diagnostically significant.

A USAF 1951 resolution test target pattern was adapted for the extracorporeal transparency assessment within this study. Subjective observations by the naked eye against the standardised test target enabled an appropriate comparison of the dural tissue before and after immersion in the OCA. It is likely that some variation in observed resolution will occur owing to inter-observer variability, variations in the thickness of the dura, overlaying of the dura on different components of the target pattern and a variation in the vasculature of the tissue. The latter appear to have resulted in some instances where meningeal vessels have obscured the view through the tissue to the target.

After a search of the literature, the use of glycerol to optically clear the human dura mater *in situ* was not seen to have been reported previously. There are, however, publications relating to optical clearing of animal dura mater with glycerol (Yao *et al.,* 2002), in addition to experiments on human dura mater using glucose and mannitol

(Bashkatov *et al.,* 2000; Bashkatov *et al.,* 2003a; Genina *et al.,* 2000; Genina *et al.,* 2005). There are numerous studies that have used glycerol on both animal and human skin and sclera (Guo *et al.,* 2010; Vargus *et al.,* 2003; Proskurin & Meglinski, 2007; Vargus *et al.,* 1999; Genina *et al.,* 2008; Wen *et al.,* 2010; Galanzha *et al.,* 2003; Genina *et al.,* 2010). The dura mater is comparable to scleral and dermal tissue in that it has a densely fibrous structure, and therefore, it has similar optical properties to those tissues (Bashkatov *et al.,* 2003b). A study using glucose and glycerol on rabbit dura *in vitro* and *in vivo* found better results in optical clearance with glycerol compared to glucose (Yao *et al.,* 2002). For this reason and owing to the number and wide variety of other studies using glycerol on fibrous tissues, it was decided to assess and then apply the use of this particular OCA in the examination of human infant dura mater. A comparison of glycerol and mannitol was also performed on several pieces of dura from one case as mannitol is another OCA that has previously been proposed as an agent for clearing of human dura mater *in vitro* at concentrations of 160g/L [Bashkatov *et al.,* 2000; Genina *et al.,* 2005). A possible suggestion for the lack of increased optical clearance observed for the dura immersed in mannitol in our present study is a shift in pH to a more acidic level within the tissue's interstitial fluid caused by the OCA diffusion. A change in pH can result in swelling of a tissue. Swelling of collagen fibres results in an increase in their size and changes in their packing arrangement. These changes increase the scattering of light and therefore a decrease in tissue transparency is observed (Genina *et al.,* 2000).

Shrinkage of the dura due to dehydration is a consequence of the physicochemical optical clearing process. During these experiments, along with studies by others (Vargas *et al.,* 2003), it was found that the morphological effects of tissue optical clearance are completely reversible with the application of water or phosphate buffered saline and that there would be no deleterious effect on the subsequent autopsy. However, re-hydrating the dura with water is recommended before removal of this membrane and continuing with the autopsy assessment of the brain in order to re-establish the flexibility of the dura and restore the ease of its manipulation. It is also recommend that any necessary samples for toxicology be taken prior to the application of glycerol during the autopsy.

No diagnostically significant effect on the histology of the dura could be detected by an experienced paediatric histopathologist when comparing untreated dura samples with those treated with glycerol. Tissues were fixed in a 10% aqueous solution of formaldehyde which is likely to have resulted in the rehydration of the samples that had previously been immersed in glycerol. One of the suggested effects of optical clearing of tissues is stasis and dilation of micro vessels (Galanzha *et al.,* 2003). No such appearances were noted histologically in the samples of dura in this study. Indeed, glycerol-induced morphological changes in blood vessels, including vascular occlusion have been shown to be reversible upon rehydration of the tissue in *in vivo* studies on hamster skin (Vargas *et al.,* 2003). Further, there was no obvious alteration to diagnostically relevant histochemical or immunohistochemical ancillary staining properties of the dura or underlying brain tissue following the application of 100% glycerol to the dura at autopsy.

In the case of a four week old male infant and also a one day old female, the combination of a subtotal craniectomy approach to calvarial bone removal in combination with optical clearing of the intact dural membrane, allowed detection of a small focus of extremely thin subdural haematoma, best described as a 'smear' of minimal bleeding, on the convexity of the occipital lobe. It is almost certain that this finding would have been considered to represent post-mortem artefact if routine autopsy methods had been used to access the brain. Optical clearing with glycerol therefore appears to greatly increase the sensitivity of detection of bleeding below the dura mater such that all clinically significant subdural haematomas on the cerebral convexities should be assessable using this technique.

This chapter has demonstrated the application of glycerol to the intact infant calvarial dura enables the non-destructive visualisation of brain surface blood vessels and also study of the vasculature of the dura itself. One particular advantage is the enhanced ability to directly observe features of head injury including facilitation of the detailed description and photographic documentation of the distribution of subdural haematoma in infant head trauma, free from autopsy-induced artefact. Even the most trivial of convexity subdural haemorrhages can be detected.

Furthermore, the reversible nature of the quick-acting morphological effects the glycerol has on the dura make this non-hazardous sugar-alcohol solution the ideal OCA to be used in conjunction with optical coherence tomography during post-mortem research.

Chapter 6 : Optical Coherence Tomography

6.1 Overview

In order to enable optical coherence tomography (OCT) imaging of the dura and the dural venous sinuses, a combination of the skull opening and optical clearing techniques introduced in the previous chapters were used. The use of both a rotational intravascular catheter-based OCT system and a benchtop OCT system were investigated in several proof of concept experiments.

6.2 Introduction

Optical coherence tomography (OCT) is an imaging modality that provides real-time, high resolution, subsurface images of cell and tissue microstructure using near-infrared light (Boppart, 2003).

Tomographic techniques generate sequential two-dimensional (2D) slice images of 3D structures (Fercher *et al.,* 2003). This means that OCT data can be used to reproduce both 2D images as well as to generate 3D images. OCT measures the magnitude and echo time delay of backscattered light (Fujimoto & Drexler, 2008) (i.e. the amount of light reflected back from the various components within the tissue of interest). Axial scans (A-scans) measure the backreflection versus depth. The cross-sectional images are produced from a series of A-scans at different transverse positions to generate the 2D data set (B-scan). Sequential cross sectional images can then be combined to generate a 3D data set (3D-OCT) (Fujimoto & Drexler, 2008). The detection of echo time delays of light requires very high time resolution because of the speed at which light travels (light travelling from the moon to earth takes approximately 2 seconds). The time that is required is beyond the limits for direct electronic detection and therefore methods such as high-speed optical gating, optical correlation, or interferometry must be used. Interferometry techniques perform correlation or interference between light that is backreflected from the tissue and light that has travelled a known distance or time delay through a reference path. Interferometry measures the field of the light rather than its intensity (Fujimoto & Drexler, 2008).

Optical Coherence Tomography was first demonstrated in 1991 (Huang *et al.,* 1991) and one of its most common uses is in ophthalmology for diagnostic imaging of the anterior eye and retina (Hee *et al.,* 1995; Puliafito *et al.,* 1995). The development of intravascular OCT systems has also seen this imaging modality often used for imaging the coronary arteries for the characterisation of coronary atherosclerosis and to observe the responses to pharmacological and mechanical treatments (Prati *et al.,* 2010).

Depending on the imaging system, OCT can produce images with resolutions of 1-20µm (Adlam *et al.,* 2013; Fujimoto & Drexler, 2008). One of the disadvantages of OCT is that light is highly scattered by most tissues, and attenuation from scattering limits the image penetration depths to approximately 2-3mm (Fujimoto & Drexler, 2008; Boppart, 2003), unless imaging more transparent structures such as the eye, where imaging depths of 10-20mm are feasible (Boppart, 2003). Combining OCT with optical clearing techniques reduces the amount of scattering by refractive indices matching of the components of the tissue being imaged (see Chapter 5). This allows for a further depth of imaging to be achieved (Proskurin & Meglinski, 2007).

Optical Coherence Tomography has recently been translated into the post-mortem setting for the purposes of providing virtual histology of the coronary arteries as a minimally invasive autopsy technique (Adlam *et al.,* 2013). It has also been suggested that OCT could potentially provide new imaging visualisation and quantification for investigations in neuroscience and neuroimaging. Currently, research has demonstrated its use in developmental neurobiology in animal models, and image-guided surgery for the repair of peripheral nerves (Boppart, 2003).

Due to the relative thinness of the infant dural membrane (considerably less than the penetration depth of OCT), and the high resolution capabilities of this type of system, OCT was considered a suitable modality for imaging the small bridging veins below the dura, and the occurrence of any blood vessels within the membrane. Within this chapter, a benchtop OCT system and a rotational (axial) intravascular system were used to respectively image the vascular structures associated with the dural membrane and within the SSS in order to detect the bridging veins draining directly into the sinus.

6.3 Methods

6.3.1 Imaging Systems

A clinical system, the St Jude Ilumien™ Optis™ (St. Jude Medical Inc., USA) (Fig. 6.1a), was used to image within the SSS and transverse sinuses. This rotational imaging modality uses catheters with fiber-optic technology that emit near-infrared light. The Ilumien™ Optis™ imaging system provides a good imaging depth up to about 1mm but can image up to 2.5mm depending on the tissue, with a resolution of approximately 20µm.

A further clinical Fourier domain benchtop system, the Bioptigen Envisu™ C-class (Bioptigen, USA) (Fig. 6.1b) was used to image the dural membrane. This system employs a non-contact mode of use with a handheld or mounted imaging probe. The anterior chamber imaging probe of this ophthalmological OCT system was used for acquiring images. An anterior chamber imaging lens (25mm) with a field of view of 20mm was used at a working distance of 17mm, providing a lateral resolution of 21µm. The imaging depth of this system is estimated to be approximately 2.5mm.

Two types of scanning mode were employed with the Bioptigen system; a line scan (or static scan or zero pullback mode) and an area scan. For proof of concept experiments, the following areas of dura were scanned; (5 x 1) mm^2, (5 x 5) mm^2 and (10 x 10) mm^2 (width x breadth).

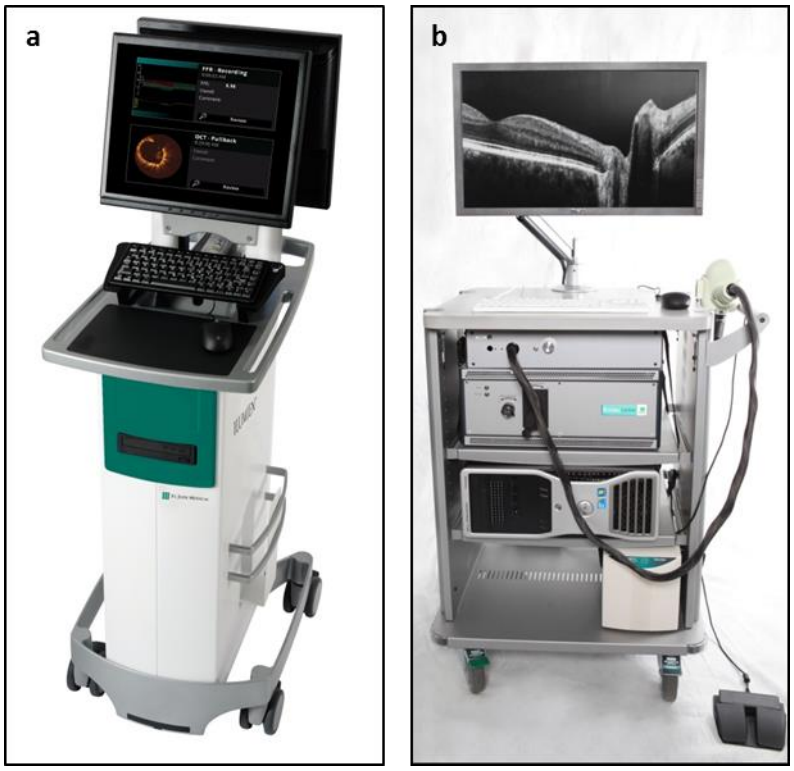


Figure 6.1 OCT imaging systems a) St Jude IlumienTM OptisTM b) Bioptigen EnvisuTM C-class

6.3.2 Case Details

Five cases were consented for the rotational OCT; two of these cases also had consented benchtop OCT performed. There were three perinatal subjects (two one day old females and a three day old male) and two infants (an eight and a nine week old male) (Appendix 3).

6.3.3 Rotational OCT

The infant calvarial bones were removed by the method describe in Chapter 4. The internal jugular veins were dissected and cannulated with Tibbs cannulae to allow perfusion of tap water into the dural venous sinuses to restore some intralumunal pressure to aid in imaging (Fig. 6.2).

Only one jugular vein was perfused to allow drainage of excess water and to mitigate swelling of the brain owing to excess retrograde perfusion of the cerebral veins. An 18-gauge needle was inserted into the SSS at the anterior fontanelle. A guide wire (0.014" diameter and with hydrophilic coating, Whisper MS by Abott Vascular) was passed through the needle and used to position the catheter (C7 Dragonfly, St Jude Medical Inc., USA) through the hole (Seldinger technique) and along the sinus to the location of

imaging. The red light visible near the tip of the catheter could be used to assess the location of the catheter tip within the sinus (Fig. 6.3).

The imaging element of the catheter performs an automatic axial traversal (a “pullback”) to image a specified length (pullback at 10mm/s (541 frames, 54mm per pullback)). Before initiating a pullback, the catheter was purged of air using saline via the 3mL syringe (Fig. 6.4).

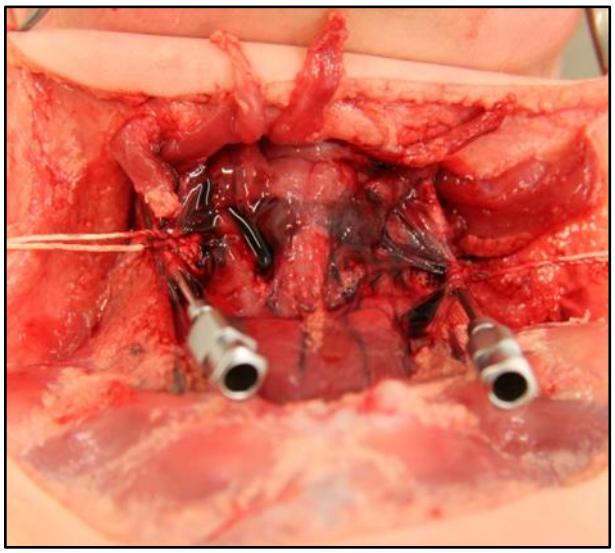


Figure 6.2 Dissection and cannulation of the internal jugular veins

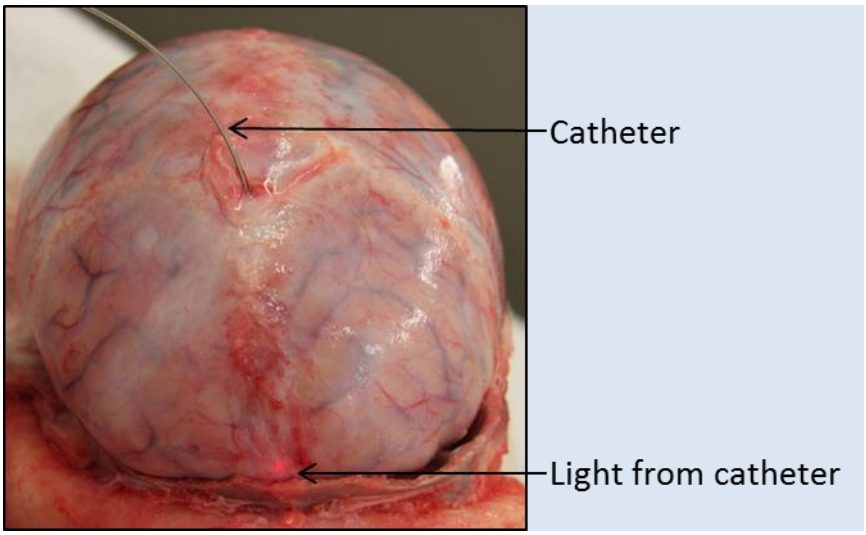


Figure 6.3 Insertion of the catheter into the SSS through the anterior fontanelle red light showing the tip of the catheter

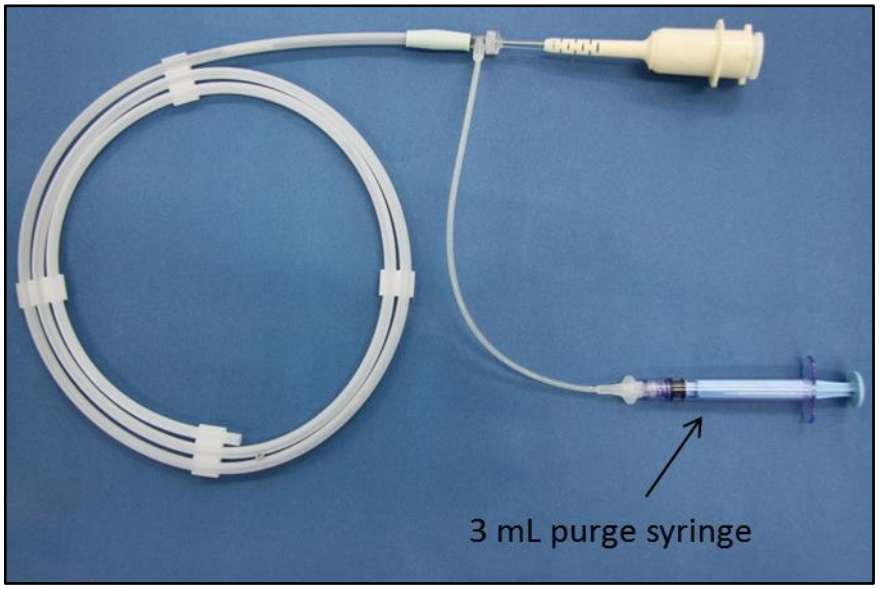


Figure 6.4 C7 Dragonfly catheter

The whole of the SSS and both the transverse sinuses were repeatedly imaged in three scans of each 54mm length of sinus with each length slightly overlapped by approximately 10mm to ensure all sections of the sinus were imaged. When the pullback was initiated, saline was infused into the sinus to open them up and improve the quality of the scans.

After rotational OCT was performed on the first few cases, it was noted that, on the video recordings of the scans, it was not possible to determine the orientation of the sinus (i.e where the external aspect of the sinus was). For this reason, a highly reflective ribbon was placed on top of the SSS to mark the orientation of the sinus in the scans (Fig. 6.5).

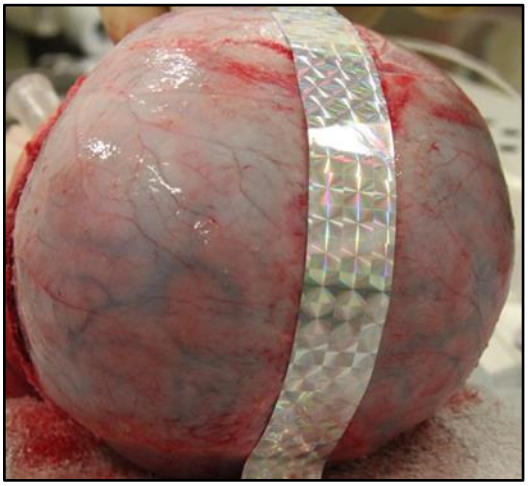


Figure 6.5 Reflective ribbon over the SSS

As each pullback video is made up of 541 frames and there were approximately 30-40 videos for each of the five cases it would not have been possible to manually analyse each frame for every single video for the five cases. Therefore, two videos of good quality (one from both an anterior and posterior section of the SSS) from the same patient (case two in Appendix 3) were selected for analysis in greater detail.

As the rotational OCT system has the capability to measure the area of the lumen in each frame, both the area and mean diameters were calculated manually for the two selected videos for every 15th frame to produce average measurements for the anterior and posterior sections of the SSS. Although the OCT system used has the facility to calculate the mean diameter and area automatically using Green's theorem, this was not used as it was obvious from viewing the still frames of the OCT data that the inbuilt function could not accurately find the walls of the SSS automatically.

6.3.4 Benchtop OCT

When the Bioptigen system is used clinically for the assessment of eyes, the imaging probe is either held by the clinician (usually when the patient is very young) or it is placed in a holder, with the lens 90° to the floor to image the eyes of a child sitting in a chair. To enable imaging of the dural membrane during post-mortems, the imaging probe needed to be held at an angle pointing towards the floor (or mortuary table). It was apparent that a stable mounting of the probe resulted in better quality images produced by the scan. A stand was therefore designed and constructed by an engineering workshop within the University of Leicester specifically to hold the imaging probe in place (Fig. 6.6).

The holder could be moved vertically on the supporting section of the stand (a relatively heavy stainless steel round section steel pillar mounted on a fabricated mobile base with four locking wheels to improve stability) as well as translated in all directions using the clamps on the arm itself to position the probe in the correct position relative to the infant head. Once the probe was placed in approximately the correct location and orientation, a micrometer stage with y-axis movement allowed fine adjustment of the probe to facilitate capture of 2D images of a small section of parietal dura close to the SSS.

Glycerol (100%) was applied to the dura (as described in Chapter 5) prior to imaging with the benchtop OCT to increase the depth of imaging.

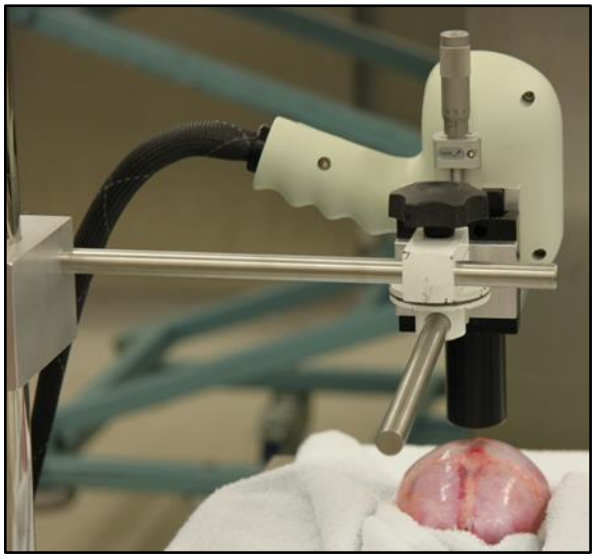


Figure 6.6 Stand for the benchtop OCT imaging probe

6.4 Results

6.4.1 Rotational OCT

6.4.1.1 General Observations

All accessible sections of the sagittal and transverse sinuses were imaged. However, on some of the frames from the videos of the transverse sinus, the entire lumen was not captured (Fig. 6.7). Although this may be due to the size of the lumen at this site, it seems more likely that it was because of the difficulty of positioning the catheter within the middle of this curved sinus.

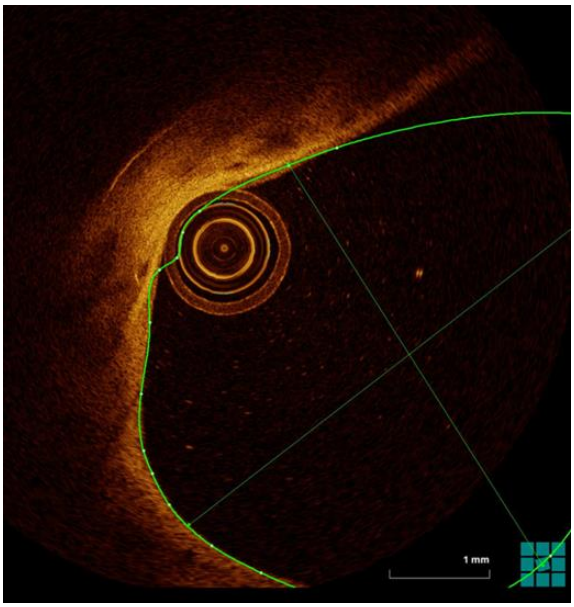


Figure 6.7 Right transverse sinus, the wall of sinus on the right side of the image is not within the frame

Numerous vessel-like structures were identified using the rotational OCT along the full length of the SSS and transverse sinus. It was not possible to confirm whether or not all the structures seen with the OCT were veins unless they were obviously seen to branch or were directly observed to be entering the SSS (Fig 6.8a-c), indicating a role in blood drainage.

The relatively larger veins draining into the SSS often joined at the apex of the triangular shaped sinus (Fig. 6.9a), whilst much smaller, vessel-like structures were seen clustering close to the walls of the sinus in both cross-section (Fig. 6.9b) and longitudinal section (Fig. 6.9c).

The reflective ribbon could be detected on the images from the OCT (Fig. 6.10). This enabled the orientation of the SSS.

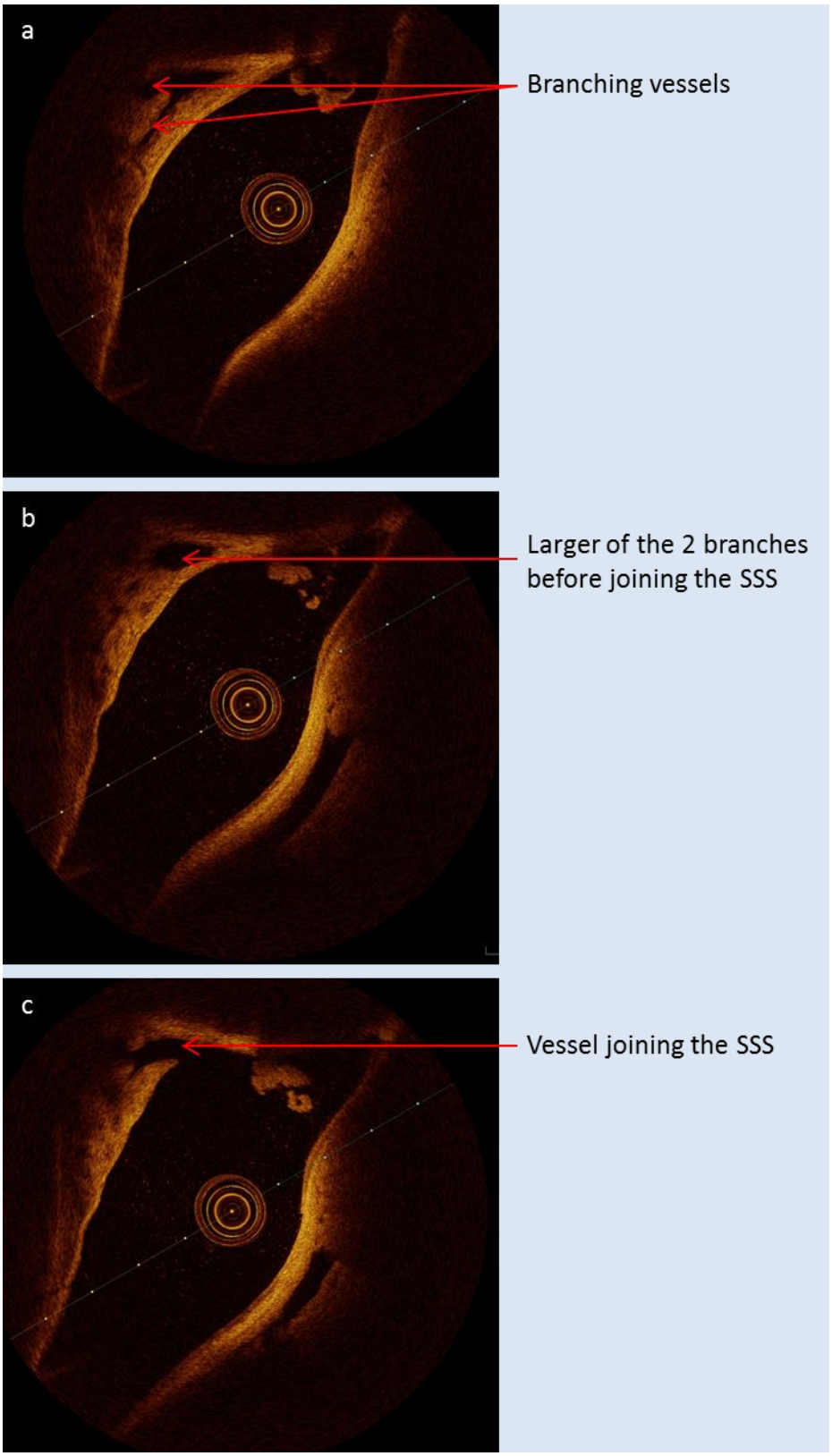


Figure 6.8 The same vein demonstrated in sequential OCT frames a) branching into 2 vessels b) just before joining the SSS c) joining the SSS

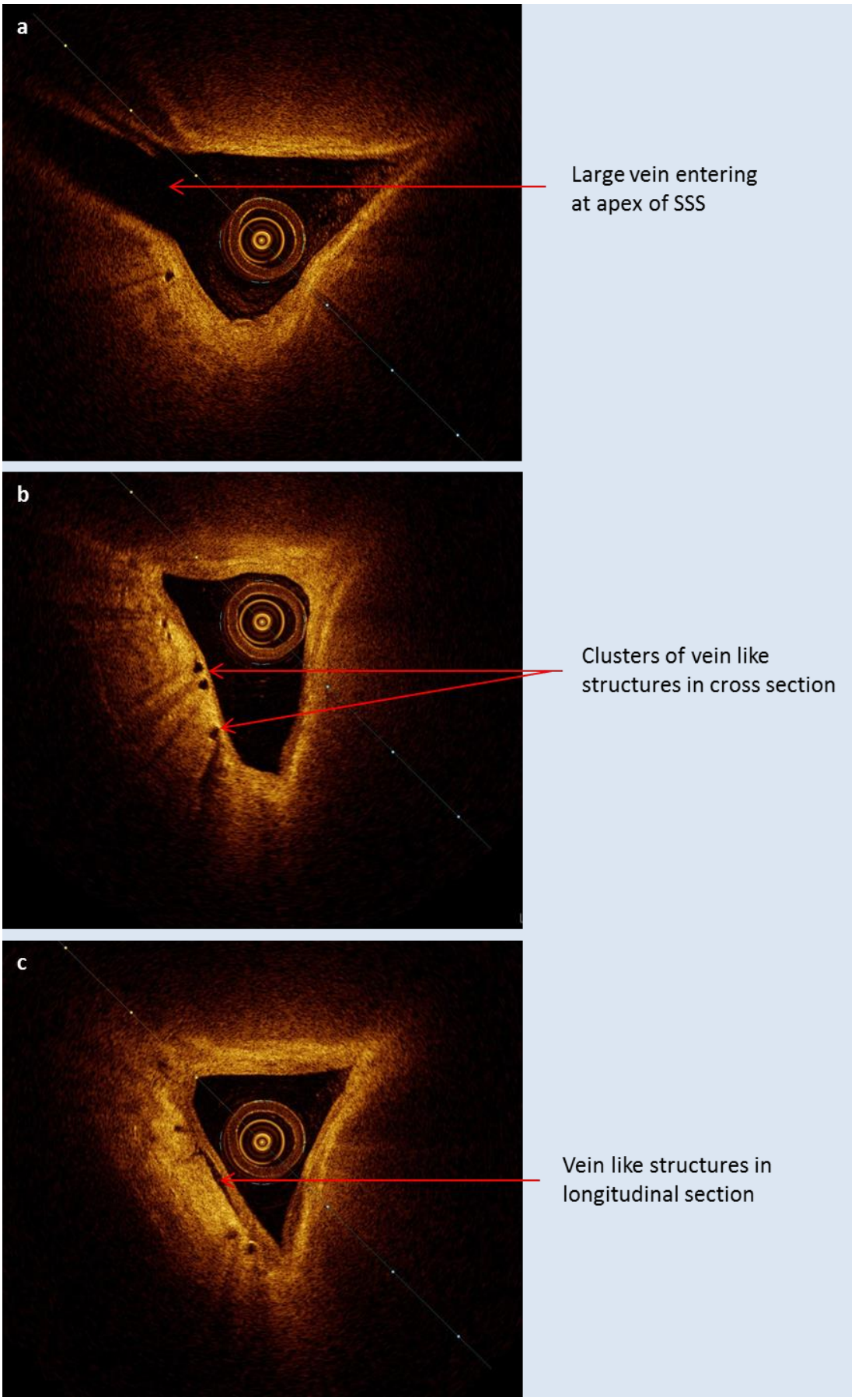


Figure 6.9 Vessel-like structures demonstrated with OCT a) large vein joining the SSS b) small vessel like structures seen in cross section and c) in longitudinal section

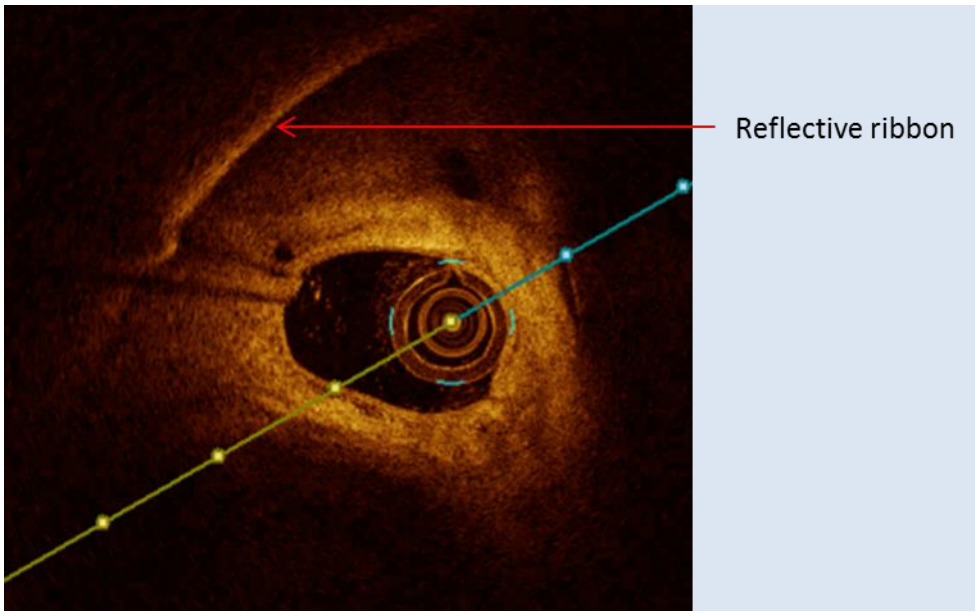


Figure 6.10 Reflective ribbon shown on OCT

6.4.1.2 Size of Vessel-like Structures

Although it is not possible to accurately measure the size of the vessel-like structures close to the SSS wall, twenty-five and twenty-three approximate diameter measurements of these structures were made for both the anterior and posterior sections respectively, when they appeared to be imaged in a cross-sectional plane to give an indication of their size (Fig. 6.11a). The mean inner diameter and standard deviation (SD) of anterior and posterior structures close to the SSS wall were 78µm (SD = 26, range 40µm to 170µm, n = 25) and 83µm (SD = 30, range 30µm to 150µm, n = 20). At the point when larger veins drain into the SSS, they do not appear in cross-section. Therefore accurate measurement of these veins was not possible. However, estimations of the diameter of the gap created in the SSS wall from the entering vein could be made (Fig. 6.11b). As it is not possible to be confident of the angle of the veins in relation to the SSS in these images, there is likely to be some variance from the true diameter in such estimates. Twenty-one and twenty-three measurements were made of gaps in the wall of the SSS where veins appeared to be entering the sinus for the anterior and posterior sections of SSS respectively. The mean diameter of these measurements were 173µm (SD = 146, range 70µm to 670µm, n = 21) and 160µm (SD = 131, range 70µm to 740µm, n = 23) for the anterior and posterior sections of SSS respectively.

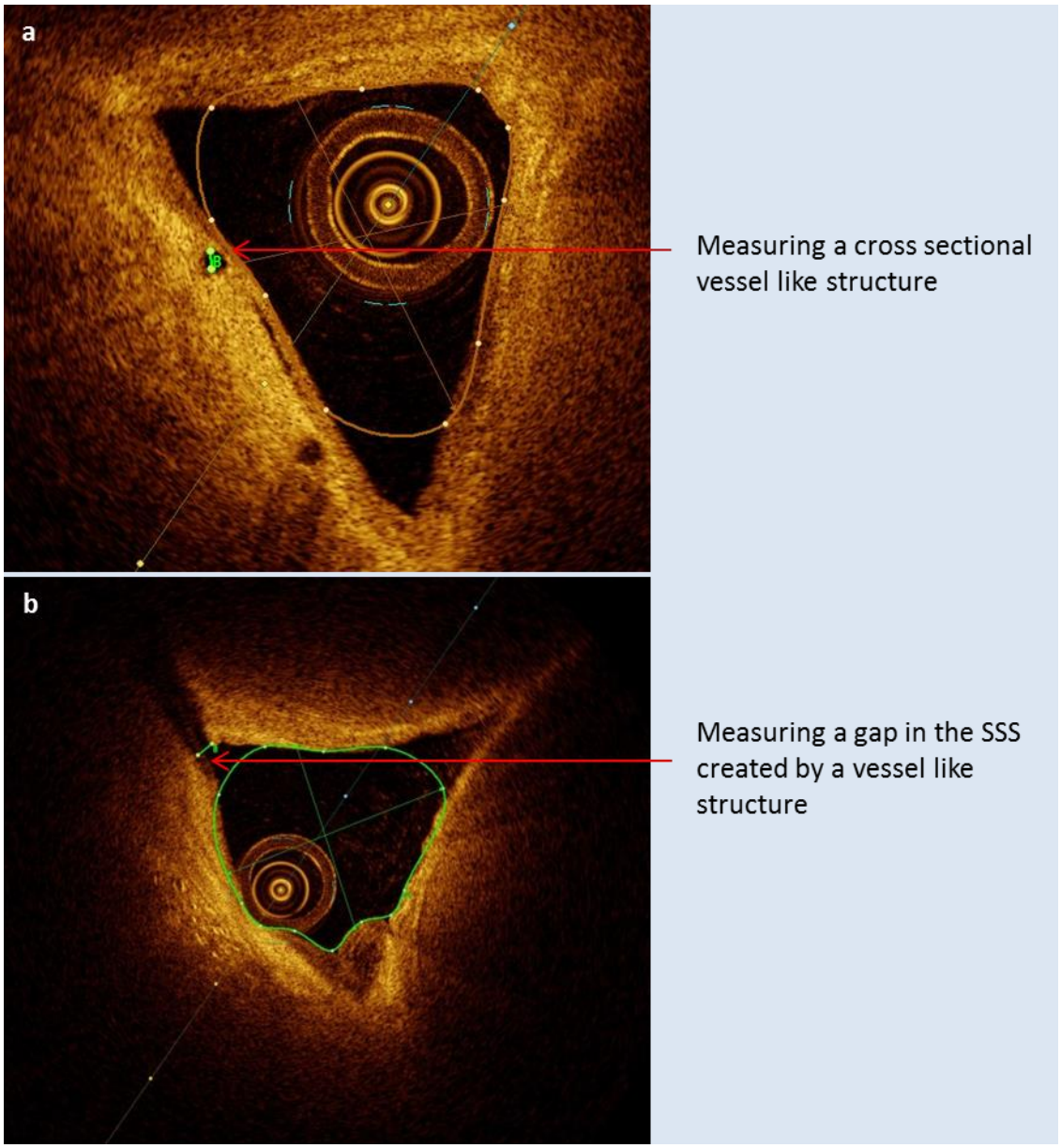


Figure 6.11 Approximate measurements of vessel-like structures using OCT a) small vessel-like structure close to SSS b) gap created in SSS wall by entering vessel

6.4.1.3 The Superior Sagittal Sinus

Drawing the outer edge of the SSS manually resulted in a mean area of 2.65mm^2 (SD = 0.61) for the anterior part of the SSS and 4.73mm^2 (SD = 1.76) for the posterior section.

The mean diameter of the anterior and posterior sections of the SSS using the manual function were 1.77mm (SD = 0.21) and 2.34mm (SD = 0.44) for the anterior and posterior sections respectively.

6.4.2 Benchtop OCT

Numerous vessel-like structures could be seen both within and below the dural membrane using the benchtop OCT (Fig. 6.12). As with the rotational OCT system, it would only be possible to confirm whether or not the structures were blood vessels if they were seen to be branching. Vessel-like structures could be seen in both cross and longitudinal sections in the OCT images. Some of these structures had relatively large diameters (larger than the dural layer) and may have been either bridging veins or cerebral veins (Fig 6.12).

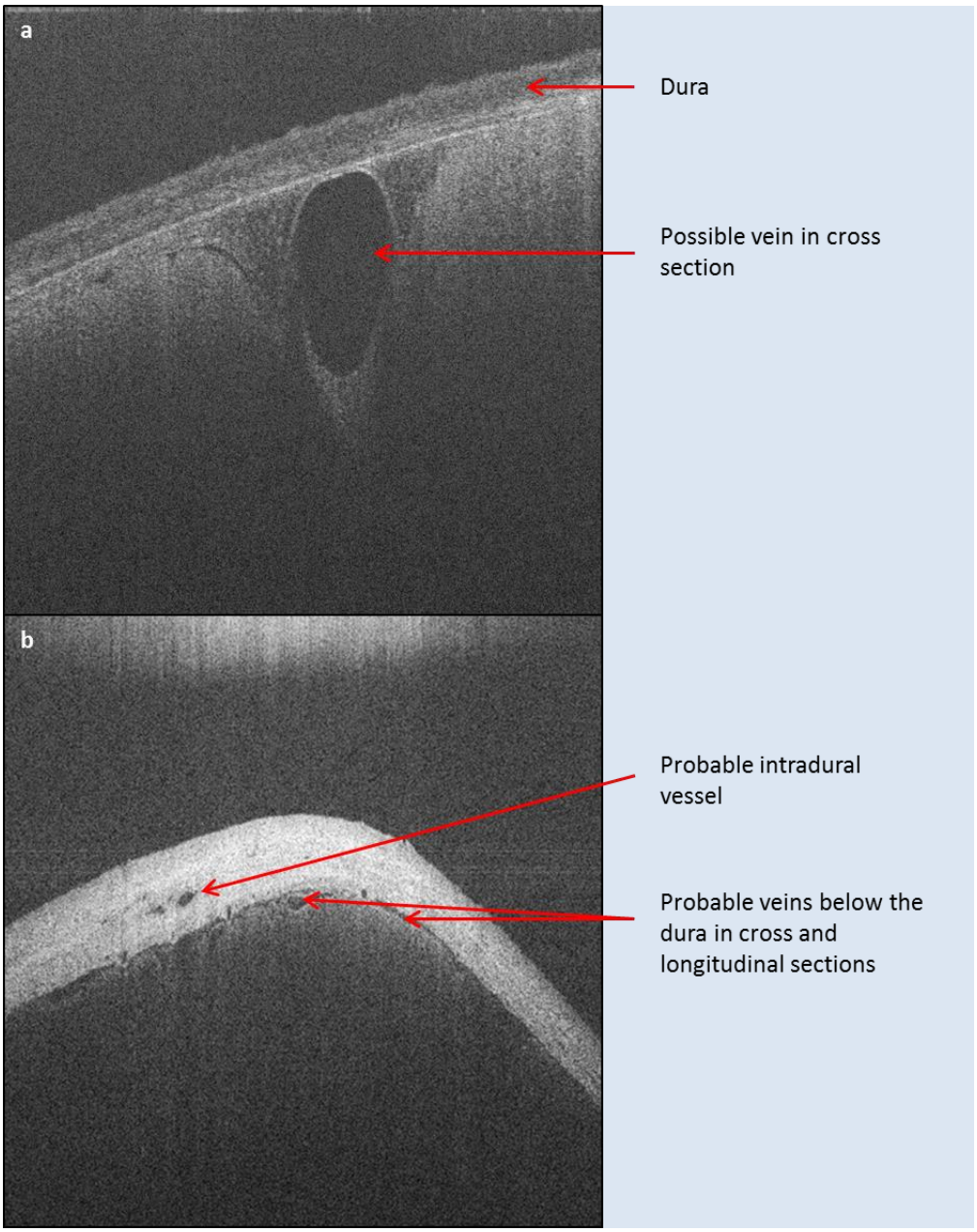


Figure 6.12 Two static images of the dural membrane using the benchtop OCT a) showing a potential large vessel below the dura and b) showing vessel-like structures within and below the dura

6.5 Discussion

Both types of OCT used within this study appeared to be capable of imaging structures that were most likely blood vessels, including bridging veins. Vessel-like structures appeared to be numerous and varied considerably in size, often appearing larger when directly entering the SSS. The estimated diameters of the veins entering the SSS (70-740µm) are similar to the measurements in another study on infant bridging veins (Morison, 2002). Although the data for infant bridging vein walls is limited, the data for adults suggests that vessel wall thickness in the subdural section of a bridging may be between 10-600µm (Yamashima & Friede, 1984).

The smaller vessels within the wall of the SSS were roughly measured at between 30 and 150µm. These structures may have included smaller bridging veins but it might be more likely that structures close to the SSS and structures seen within the dura on the benchtop OCT images were meningeal vessels as these vessels have previously been described within the dural membrane (Kerber & Newton, 1973)

Within the outer layer of the dura numerous primary anastomotic arteries (PAA) are suggested to branch from the main meningeal arteries which travel in the outer dural layer (Kerber & Newton, 1973). These arteries are stated to cross the SSS often, creating a single vascular unit for the two dural hemispheres. These PAA are approximately 100-300µm in diameter and are suggested to branch further into secondary anastomotic arteries (SAA). The SAA have been measured at approximately 20 to 40µm diameter when located upon the periosteal surface of the dura. Further SAA (arteriovenous shunts) are stated to lie in the middle of the dura and have been suggested to be plentiful and approximately 50 to 90µm, but are difficult to demonstrate. There are also penetrating vessels, which are most likely arterioles, extending deep into the dura close to the arachnoid surface which are thought to be only 5 to 15µm in diameter. The arterioles are proposed to end in an extremely rich capillary network (size of vessels approximately 8 to 12µm) which is most abundant parasagittally (Kerber & Newton, 1973). The OCT system detected vessel-like structures down to 30µm but it is likely that if smaller vessel were present, they may have been below the resolution capabilities of the rotational OCT system.

The measurements of the mean area and diameter of the anterior and posterior sections of the SSS (2.65mm^2/1.77mm and 4.73mm^2/2.34mm respectively) agree with previous studies of the adult SSS which conclude that the size of the sinus increases from the frontal region to the occipital area, however as would be expected in the adult population the SSS measurements were larger (Andrews *et al.,* 1989).

One of the limitations of the OCT experiments was the small number of recruited participants. This was due to the short time frame in which the experiments took place due to the extensive ethics application and the acquisition of the OCT machines.

6.5.1 Future Studies

It would be prudent to validate OCT for the identification of blood vessels within the dura and walls of the SSS. It is proposed that dissection of the imaged areas for histological and immunohistochemistry studies could enable confirmation of the vascular nature of the identified structures.

Once the benchtop OCT has been optimised for microvascular imaging, future studies should be aimed at the generation of 3D OCT scans of large areas of the dura by sequentially scanning small areas and combining the data through image mosaic and registration methods. These scans would require accurate automated XYZ translation of the imaging probe, using numerical control by a computer in order to scan the convex surface of the dura. The area of the parietal dura near the SSS would be of particular interest.

Chapter 7 : Post-Mortem Anatomical Mapping and Digital Microscopy of the Infant Bridging Veins

7.1 Overview

Within this chapter the dura mater, left intact through the use of the neurosurgical equipment described in Chapter 4, was carefully lifted from the surface of the brain to enable detailed photographical documentation of the location and number of bridging veins. A 3D model of an infant brain was produced from an MRI scan, which was segmented in a software application (Itk-snap). The 3D model was then transferred to a numerical computing environment (Matlab) for creation of a 3D mesh model. An interface was then produced using Matlab to enable data plotting and heat map visualisation of the location of infant bridging veins from 43 post-mortem cases. Dissection of the dura mater using the method described below, also allowed for the use of a digital microscope to record the *in situ* diameter of the veins.

7.2 Introduction

There is limited information in the medical literature on the size, number and locations of infant bridging veins. Most of the data on bridging veins comes from imaging and cadaver studies from the adult population (Andrews *et al.,* 1989; Brockmann *et al.,* 2012; Lee & Haut, 1989), and these studies often target a specific area, usually the SSS. The resolution capabilities of the imaging modalities used in these studies (such as CT and MRI) may also limit the observation of smaller veins which may be present but not clearly seen using these methods.

To aid the understanding of the source of SDH in AHT, an increased knowledge of the infant cerebrodural vasculature is of great importance. Post-mortem observations made during infant autopsies suggested that the infant bridging veins are numerous and also that they can be located in areas distant from the parasagittal region where veins are usually seen draining into the SSS. It has also been observed during post-mortems that some infant bridging veins appeared to be very small and delicate.

The purpose of this chapter is to detail novel anatomical data on infant bridging veins by photographically documenting their locations and numbers during post-mortem examination of the brain. The *in situ* use of a digital microscope also allows for the measurement of the size of some of these macroscopically visible veins.

In order to visually present the data from the photographic records made during the post-mortem and also to allow groups of cases to be analysed together, the creation of a 3D digital model was undertaken. This required the acquisition of data from a normal brain of an infant to create the 3D model. Magnetic resonance imaging was performed on an infant brain to provide this data due to the excellent soft-tissue contrast and characterisation when using this imaging modality (Lisle, 2007). Magnetic Resonance images were then segmented in a software application designed for viewing 3D medical images, before an interface was then created in a further program to allow data plotting and visualisation on the 3D model ('mapping').

As mapping occurred on a 3D brain, the location the bridging veins that were leaving the brain surface were plotted as data points, taken from the 2D photographs. Due to the fact that bridging veins are seen within the interhemispheric fissure and also in the tentorial area between the hemispheres and cerebellum, it was necessary for the 3D brain to be segmented into three separate components (the two hemispheres and the cerebellum) to allow plotting of bridging veins on all the areas of the brain surface model.

7.3 Method

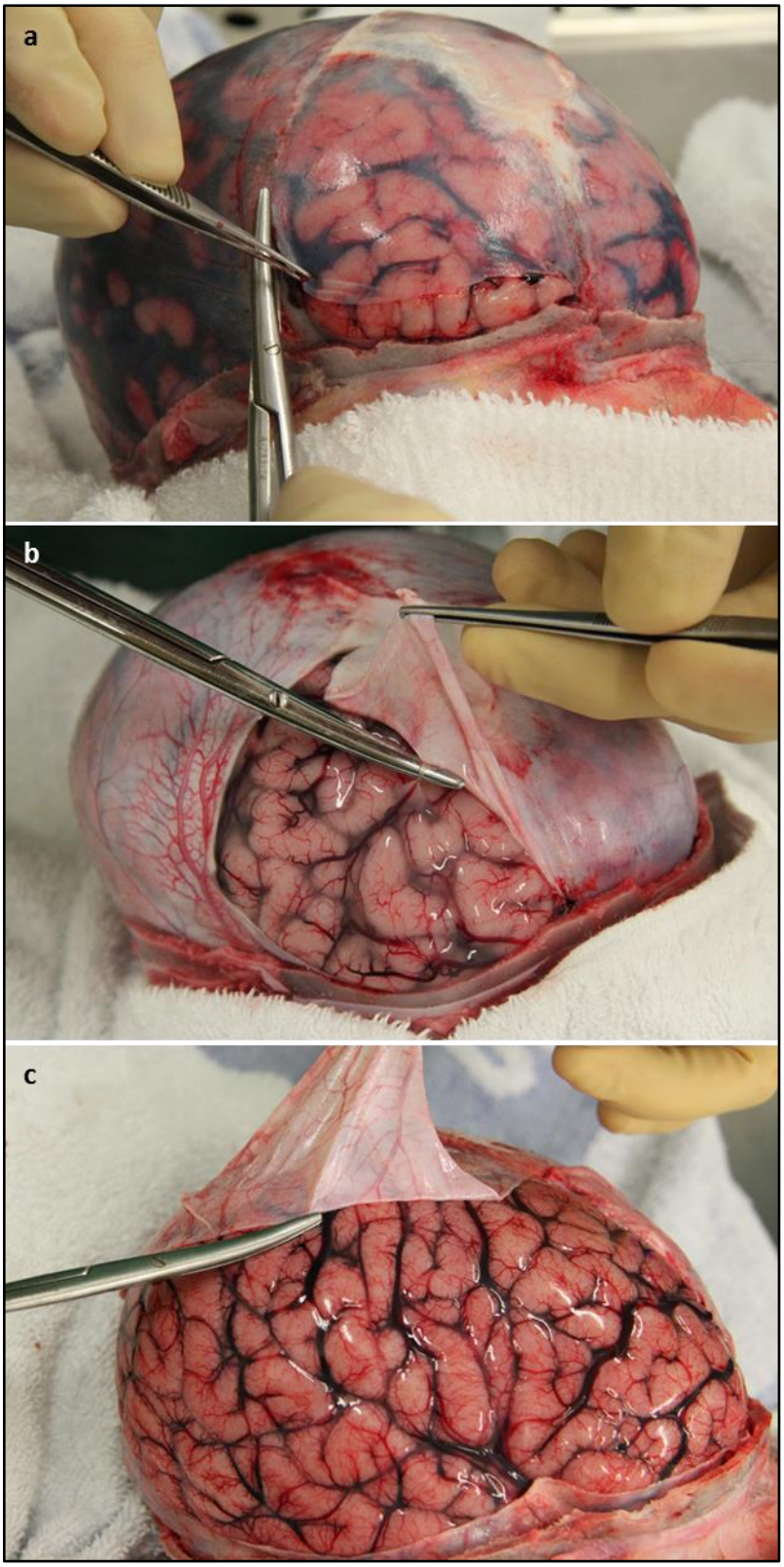
7.3.1 Post-Mortem Dissection of the Dura and Visualisation of the Bridging Veins

Once the method for infant calvarial bone removal (detailed in Chapter 4) was implemented in infant post-mortems, retrospective ethical approval was gained to enable detailed photographic mapping of bridging veins in 43 cases (Appendix 3).

The right frontal section of dura was carefully incised with a small scalpel, close to the SSS and the edge of the bone created by the cuts made with the neurosurgical equipment. An incision to the dura was made along the edge of the bone (using curved Metzenbaum scissors). The incision was then extended upwards through the dura

underlying the coronal suture towards the SSS (Fig. 7.1a). The frontal section of dura was slowly lifted to reveal the bridging veins (Fig. 7.1b) which were photographed with a Canon EOS 500D camera with an attached Canon EFS 17-85MM lens set to automatic focus and exposure. The incision in the dura was then continued along the edge of the bone at the base of the parietal dura and then upwards to follow the section of dura underlying the lambdoid suture to almost meet the SSS. This section was then slowly lifted to photograph the bridging veins over the parietal lobe (Fig.7.1c). The final, small section of dura remaining on the right convexity of the brain, over the occipital lobe, was lifted after cutting the membrane close to the bone, around to the area near the confluence of sinuses. The brain was then gently manipulated to reveal bridging veins within the interhemispheric fissure, at the frontal and temporal poles, down the sides of the temporal lobe and on the undersurface of the brain, including veins associated with the tentorium. After a thorough examination of all the areas of the right hemisphere for bridging veins entering the dura, the right hemisphere was removed by bisection of the corpus callosum and basal structures in the sagittal plane, connected with an incision into the right side of the midbrain and right optic nerve in the axial plane. Using this method of brain removal allowed for photographic documentation of the falx and tentorium (Fig. 7.1d), aiding in the ability to observe the presence of intradural bleeding and tentorial tears. A folded towel was place in the space created by the removal of the right hemisphere to stabilise the left hemisphere. The dura overlying the left convexity was carefully dissected using the same technique as that of the right hemisphere, photographically documenting bridging veins leaving all the areas of the brains surface to enter the dura. The left hemisphere was then removed by completion of the transection of the midbrain and left optic nerve, leaving behind the cerebellum (underlying the intact tentorium) and the lower brainstem. The dura was then freed from its anterior attachment of the falx to the crista galli (Fig. 7.1e). Following the anterolateral edge of the superior surface of the cerebellum, the tentorium was then incised and slowly reflected to reveal the bridging veins draining from the cerebellum (Fig. 7.1f). After the tentorium was lifted, the cerebellum was carefully manipulated to view any bridging veins draining the lateral and inferior surfaces. Vessels associated with the cranial nerves were not included in the photographic documentation of the bridging

veins. The cerebellum was then removed by transection of the cranial nerves, the vertebral arteries and the upper cervical spinal cord at the lowest point accessible.



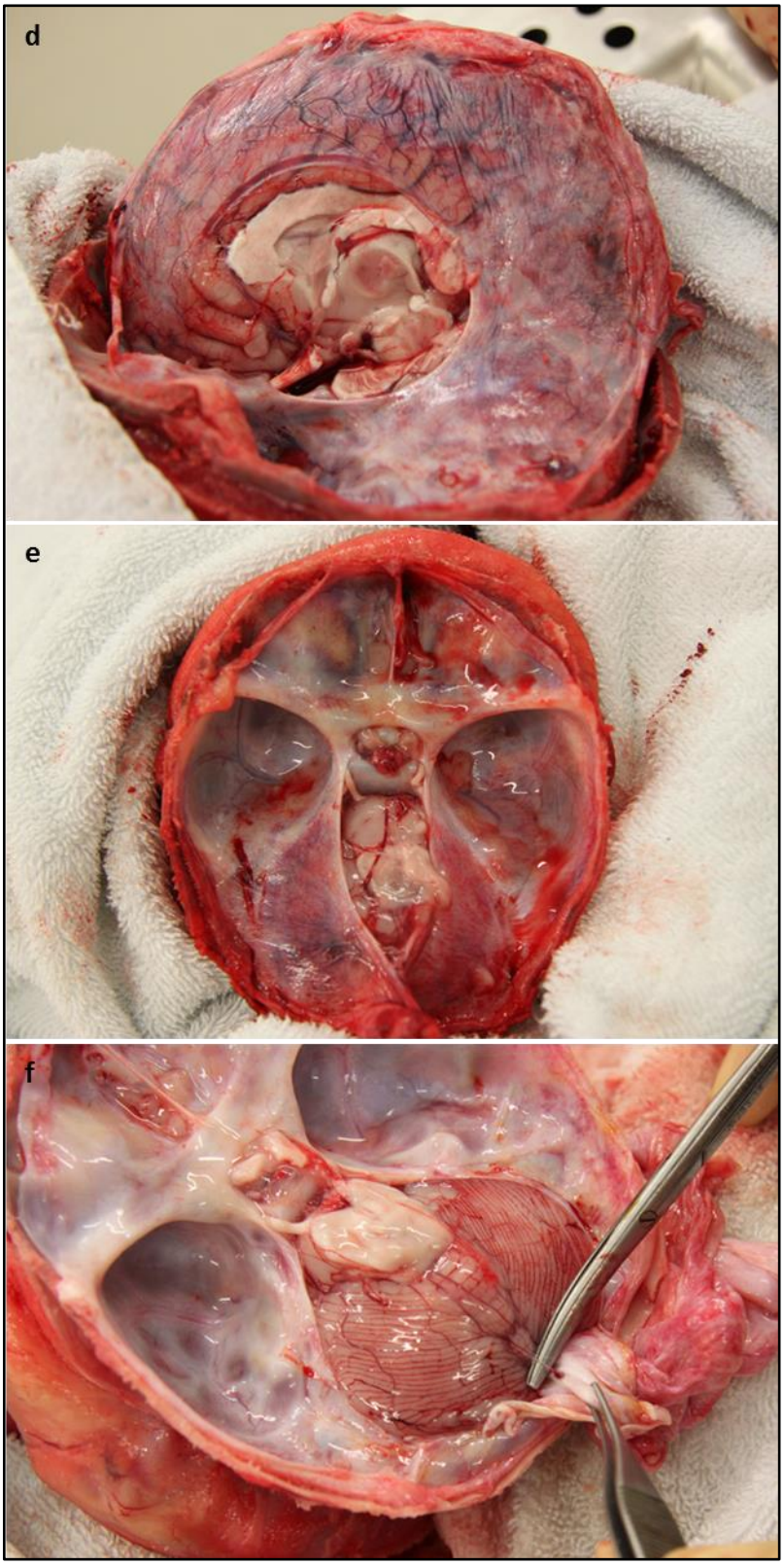


Figure 7.1 Sequential series of digital photographs showing removal of the dura mater to observe bridging veins

7.3.2 Magnetic Resonance Imaging of an Infant Brain

For the purposes of creating a 3D model of an infant brain, MRI scans were undertaken on a brain from an 11 week old infant with specific parental consent. The brain was removed as part of the standard autopsy procedure and was placed in 20% formalin for almost two weeks before scanning to allow for fixation of the tissues. The brain was suspended in a plastic container with the aid of two plastic rods to ensure the organ was surrounded by fluid, to facilitate future separation of the brain from the walls of the container on the MRI scans. Both T1-weighted and T2-weighted spin echo sequences were used to image the brain.

7.3.3 Segmentation of the Brain Using ITK-SNAP

Using the T2 weighted MRI images, (as the edges of the brain appeared to be more distinct in this sequence), the two hemispheres and the cerebellum were digitally isolated from each other and the plastic container/rods by a combination of semi-automatic and manual segmentation using an open-source multi-platform software application called "ITK-SNAP" (version 3.2.0, Yushkevich *et al.,* 2006), which is designed to segment structures in 3D medical images. The ITK-SNAP interface displays three orthogonal views (Fig.7.2) that intersect at a given point by the "3D cursor", a tool that can be used to determine the exact location of an area of interest within a volume. Each view can be zoomed, aiding in the manual segmentation process. Each part of the brain (hemispheres and cerebellum) were semi-automatically segmented initially, by outlining a 3D point of interest and by applying an algorithm to this area based on pixel intensity and image edges. Further manual segmentation was then required for two reasons; firstly, the areas of the brain requiring segmentation had similar pixel intensities resulting in some overlap of segmented areas and secondly, the algorithm was unable to detect two small sections on the lateral aspects of the hemispheres due to reduced quality of the MRI images in those areas. Each segmented area of the brain was given a numerical label. The 3D brain was exported from ITK-SNAP as a mesh stl file. The numerical labels would enable the three separate components of the brain to be recognised by Matlab, a numerical computing environment which would be used for the next stage in this chapter; data plotting and visualisation on a 3D brain model.

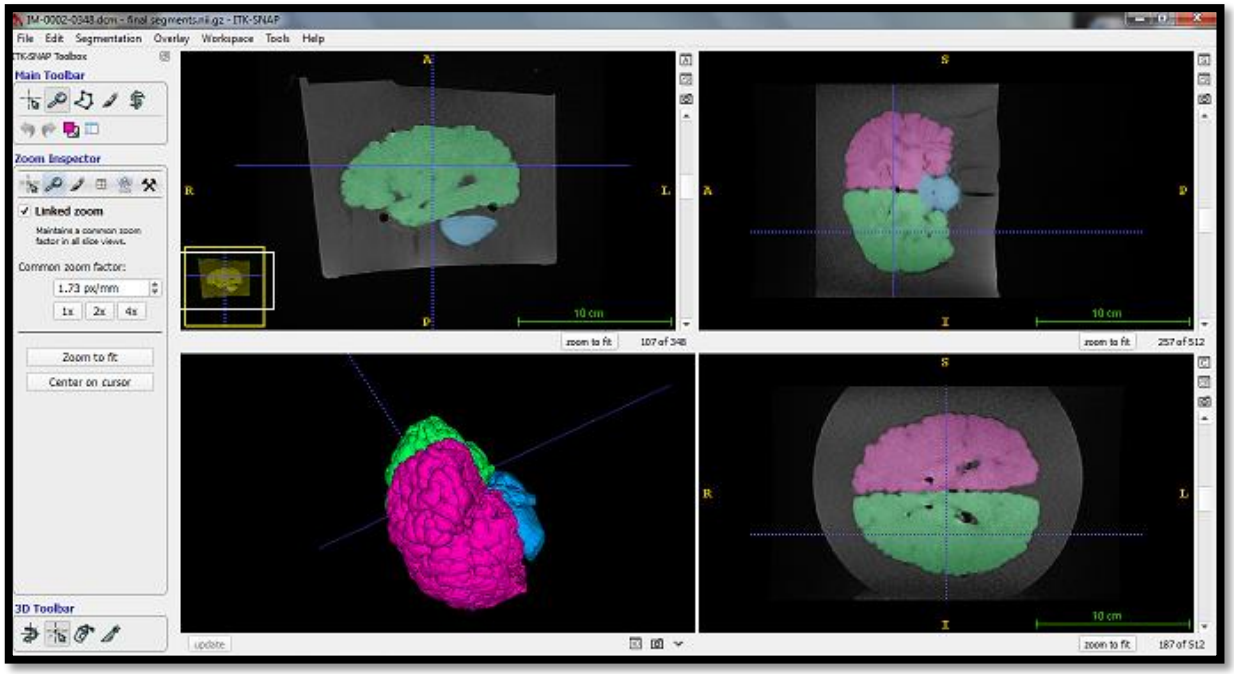


Figure 7.2 The ITK-SNAP interface

7.3.4 Creation of a Matlab Interface for Plotting Bridging Vein Locations on a 3D Brain

Assistance was sought from the Mathematics department of the University of Leicester for development of a user interface that would enable the post-mortem photographic data to be mapped onto the 3D brain produced in ITK-SNAP. A researcher from within the University of Leicester was employed to undertake the development of the interface using Matlab, a high-level technical numerical computing environment.

The 3D brain data from ITK-SNAP was imported into Matlab as binary stl files which created a 3D model made of small triangular faces.

One of the requirements of the interface was that the brain could be viewed as its three separate components, to enable plotting of data points on surface areas that would be hidden if the brain was seen as a whole organ (i.e. the interhemispheric fissure and the tentorium). Further requirements necessary for data plotting included; rotation of both the whole 3D brain and the separate hemispheres/cerebellum and the ability to save and load separate cases and groups of cases. To enable enhanced visualisation of the cluster pattern of the data points the application of heat maps to the brain was also required.

7.3.5 Counting Bridging Veins During the Post-Mortem Examination

After photographically documenting the bridging veins in several infant post-mortems, the difficulties of capturing these small vessels were realised. With the manipulation of the brain to allow viewing of all the surface areas, it is likely that some of the smaller vessels had been disrupted before a photograph could be taken. Veins located in small crevices without much light were also difficult to photograph. In order to aid validation of the number of vessels counted in the photographs, a tally counter was also used to provide values for the number of veins in 28 cases. If there was uncertainty as to the number of bridging veins in an area due to clustering or reduced light, the smaller estimate of the total number in that area was recorded both when using the tally counter, and when mapping from the digital photographs onto the 3D model.

7.3.6 Statistical Analysis of the Two Counting Methods

When measuring a biological variable in each of a number of individuals, a certain amount of variability is to be expected. Variation is likely to occur between individuals as well as within the same individual if the measurements are repeated. When assessing a measuring technique (such as counting) it is important to explore the variability of the data to gauge the reliability of the methods (Petrie & Sabin, 2009). It is most unlikely that different methods will agree exactly, by giving an identical result for all individuals (Bland & Altman, 1986). It is important to consider by how much a method may vary from another. The reliability (also known as agreement) of the two methods of measurement used to count bridging veins (tally counter and digital photographs) were accessed using a paired t-test, a Bland Altman plot and the intraclass correlation coefficient (ICC).

A paired t-test was undertaken to see if the results from the two counting techniques agreed on average over the whole data set. If the average difference is zero then there is no systematic difference between the pairs of results (i.e. on average the duplicate results agree).

The ICC is an index of reliability often used to measure repeatability and reproducibility and takes a value from 0 (no agreement) to 1 (perfect agreement). The ICC is the between variance expressed as a proportion of the total variance of the observations

(Petrie & Sabin, 2009). A two-way mixed ICC was performed in SPSS (a statistics software package) as this model is used for random participants where the types of measurement are fixed.

The Bland Altman plot is an alternative to the ICC or can be used to provide additional support for the level of agreement. Initially, the raw data from the two different counting methods were plotted against each other on a scatterplot. A line of equality was then drawn. This line is where the data points would lie if the two measures gave exactly the same reading every time, and gives a rough idea of the degree of agreement between the measurements. The Bland Altman plot is a more informative plot and required the plotting of the differences between the methods against their mean for each of the individuals. On this scatterplot, there should be no pattern to the data points. The next step in the Bland Altman plot is to produce limits of agreement. The British Standards Institution repeatability coefficient (2SD) is calculated, and this value is the maximum difference which is likely to occur between two measurements. Assuming a normal distribution of the differences, it is expected that approximately 95% of the differences in the population lie between đ ± 2SD where đ is the mean of the observed differences. The upper and lower limits of this interval are called the limits of agreement. These limits were calculated and plotted on the Bland Altman diagram, which allows the agreement between the sets of data to be assessed subjectively. This plot also enabled detection of any outliers in the data (Bland & Altman, 1986; Petrie & Sabin, 2009).

7.3.7 Digital Microscopy of Bridging Veins

In two post-mortems (a one day old female and a 16 day old male) (Appendix 3) *in situ* measurements of the diameter of 11 bridging veins were made. The dura was reflected and the brain was positioned under a Keyence VHX-900F digital microscope to capture a still image of the vein. Measurements were then taken using the software (VH s30k) provided with the digital microscope, of the outer diameter of the bridging veins at the location the vein entered the dura/sinuses, the section of the vein where it was seen to leave the brain surface (the arachnoid), and in the middle of the vessel. Digital photographs were also taken to ascertain the location of the bridging veins when

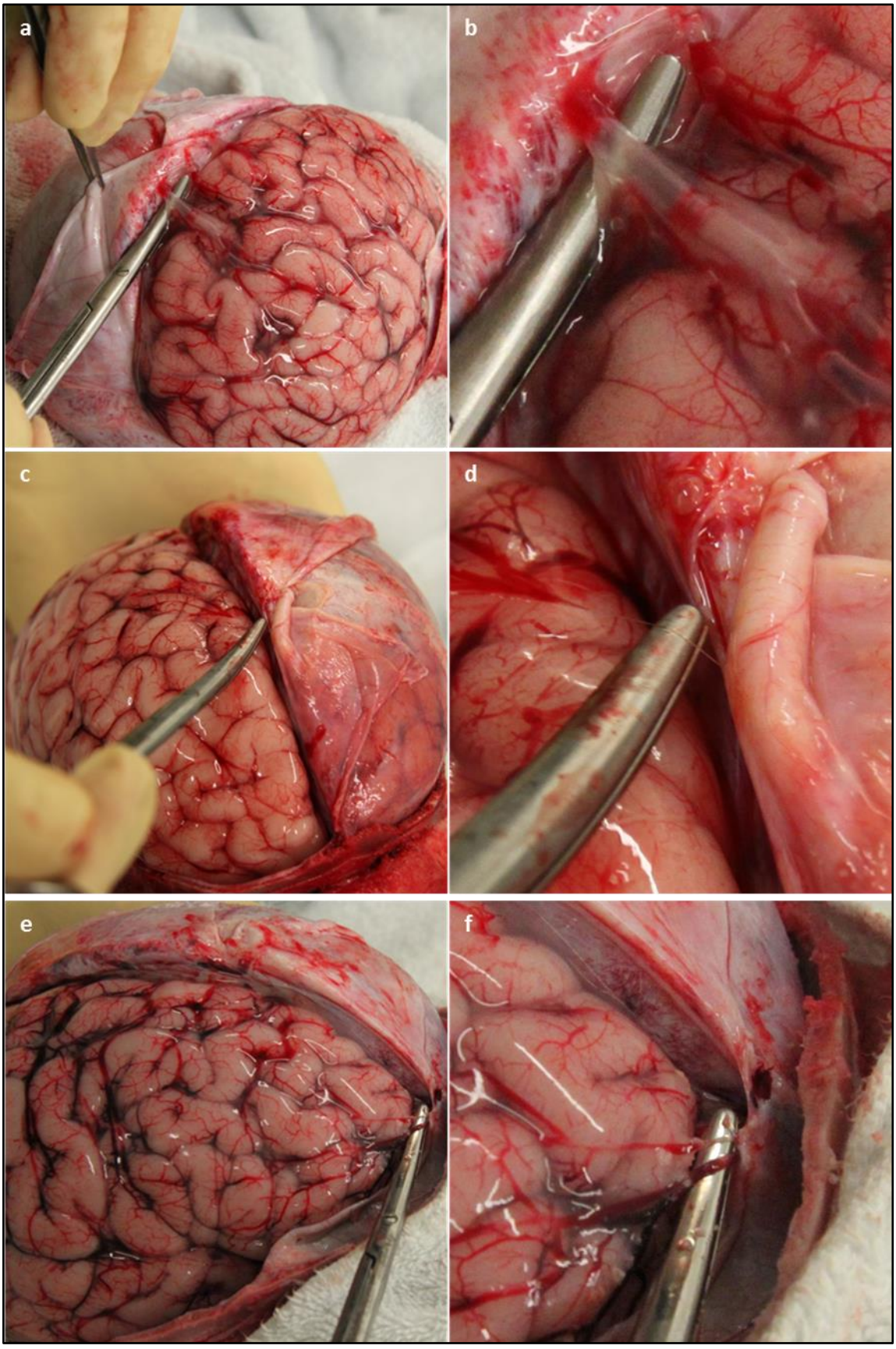
measuring the diameters by matching the image number for both the digital microscope and camera.

7.4 Results

7.4.1 Post-Mortem Observations of the Infant Bridging Veins

Bridging veins were seen close to the SSS, including vessels that were directly entering the sinus. Bridging veins appeared to vary greatly in size, with the larger veins often seen close to the SSS (Fig. 7.3a-b). However, smaller veins were seen in various locations that were almost as small as a hair's breadth and could be easily missed with the naked eye (Fig. 7.3c-d). Veins were often seen at the frontal and temporal poles (Fig. 7.3e-h), and were also often seen in clusters on the inferior aspect of the temporal lobe (Fig. 7.3i-j). Although veins were often seen close to the venous sinuses, vessels were also seen on the cerebral convexities, away from any sinuses (Fig. 7.3k-l). Bridging veins either entered a sinus directly or appeared to come into contract with the inner aspect of the dura without obviously entering the membrane and travelled parallel to the membrane to the nearest sinus (Fig. 7.3m-n). Bridging veins entered the sinus/attached to the dural membrane singularly, or anastomosed beforehand, either within the 'subdural space' (Fig. 7.3o-p) or as cerebral veins before they pierced through the arachnoid membrane. On reflection of the tentorium, bridging veins were frequently seen leaving the surface of the cerebellum. Most commonly, veins appeared to bridge away from the cerebellum surface at the folium section of the superior vermis (Fig. 7.3q-r) and the anterosuperior border of the cerebellar hemispheres (Fig. 7.3s-t).

In the three cases of AHT in the series, it was observed that the bridging veins generally appeared to be more obviously engorged with blood than is usual and on application of slight pressure, such engorged veins did not blanch (Fig. 7.3u-v) as was usually seen in the bridging veins from non-head injured cases. Around the parasagittal region the blood in the engorged vessels often extended back into the cerebral section of the veins (Fig. 7.3w-x). Engorged vessels appeared to be adjacent to areas of SDH.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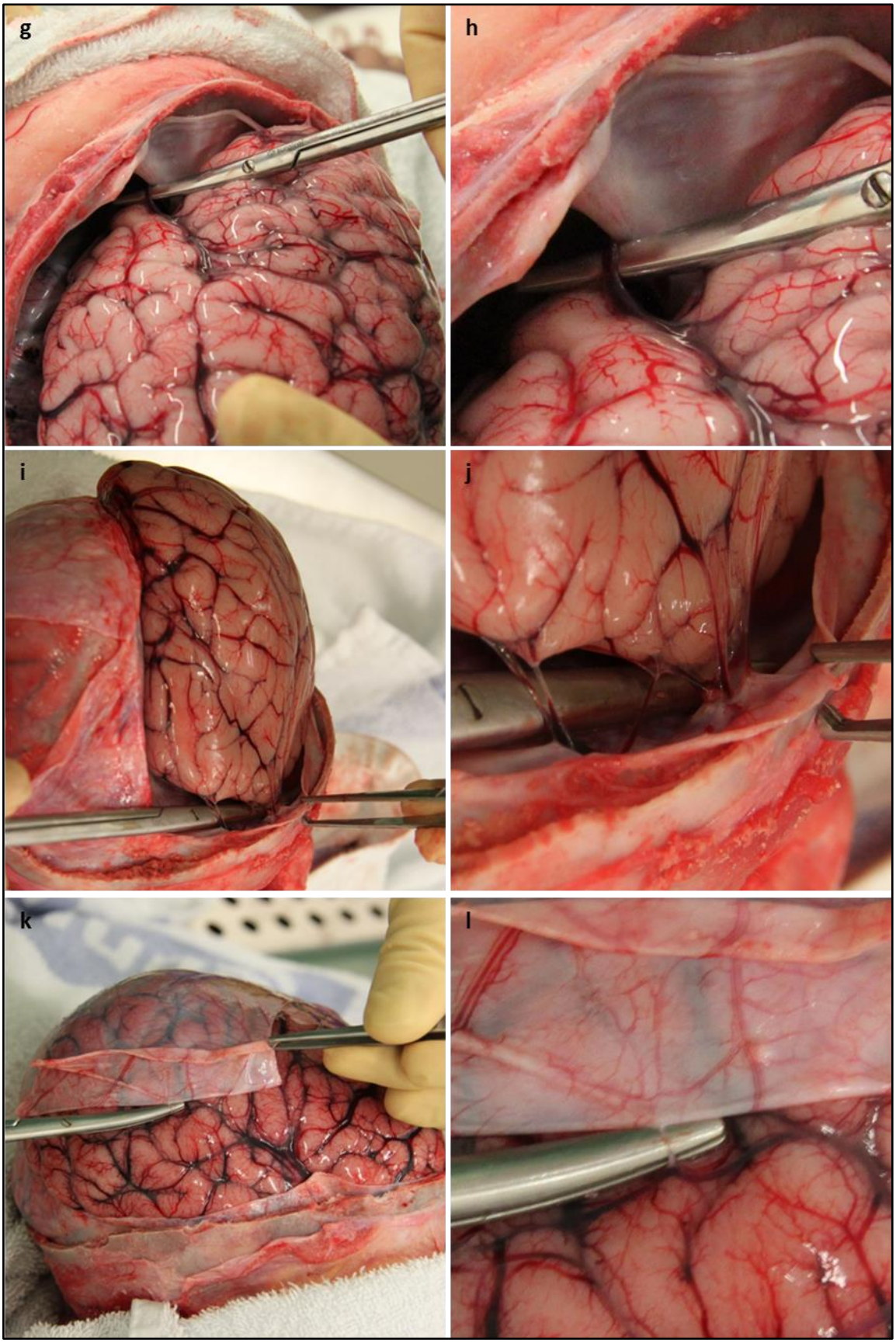
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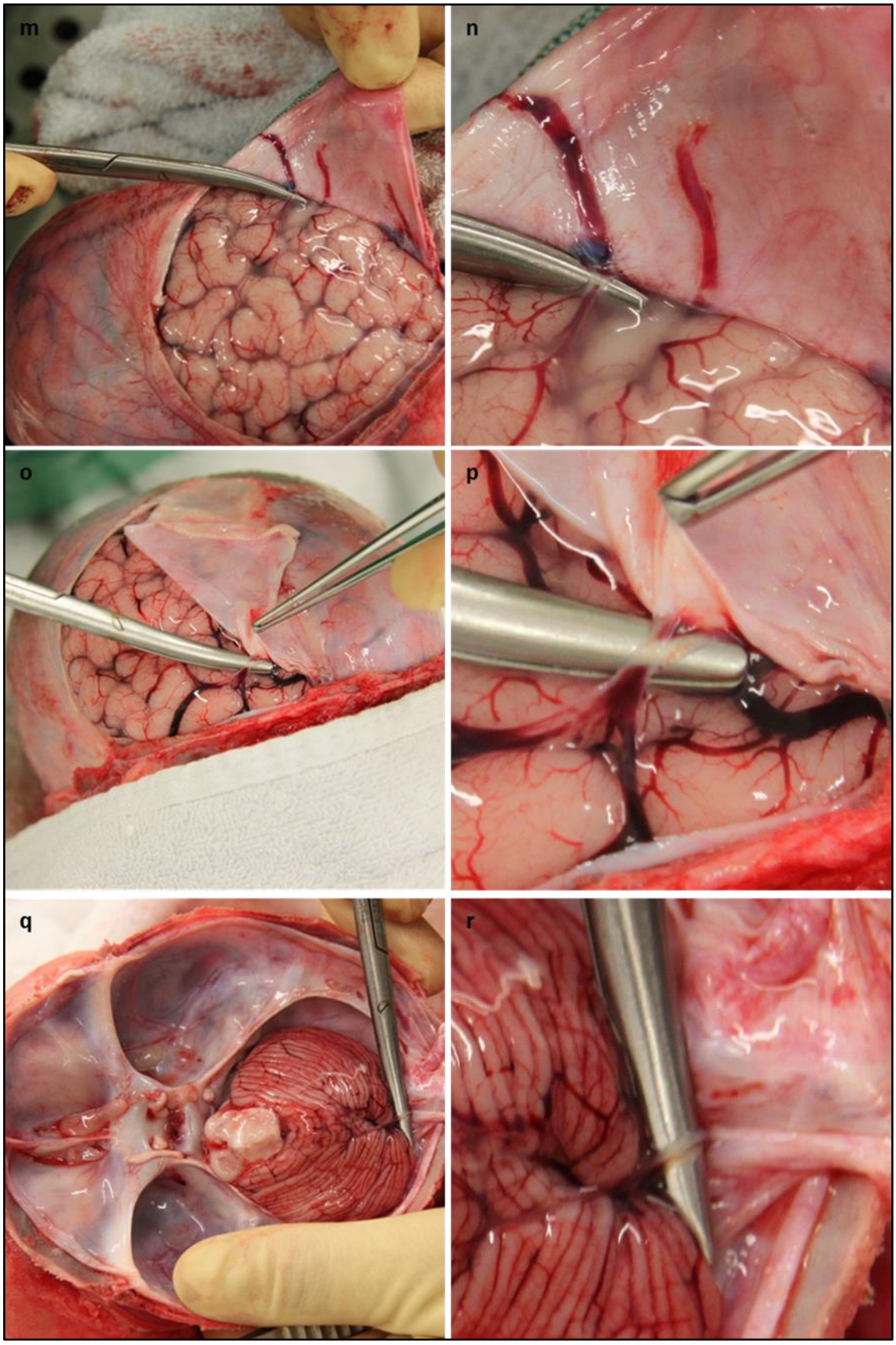
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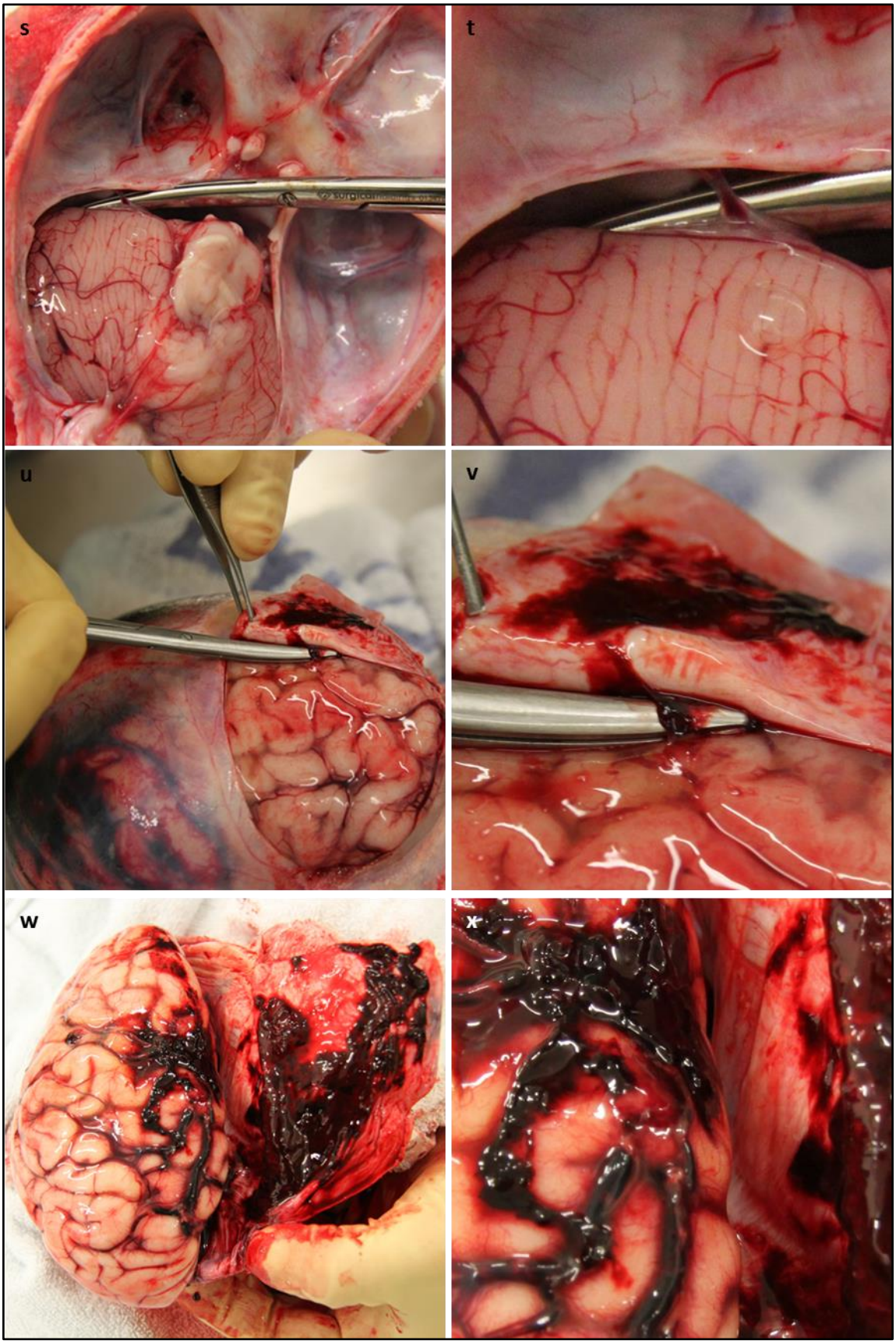


Figure 7.3 Infant bridging veins, original images on left hand side, with x2 and x4 magnifications on the right hand side

7.4.2 The Matlab Interface

An interface was created that met the specified requirements. The interface could be viewed in two separate windows, one that showed the whole brain (Fig. 7.4) and one that split the object into its three separate components (Fig. 7.5). The interface also showed the basic statistics (number of data points on each hemisphere, on the cerebellum and in total) for whichever case was loaded at that time. To enable 3D visualisation of the whole brain, the additional feature of taking AVI videos of the brain rotating both horizontally and vertically was also included within the interface (Appendix 6), along with the ability to alter the transparency of the brain model (if viewing the whole brain) in order to allow all the data points to be seen. A heat map tuning bar and transparency bar enabled tuning of the heat map and alterations to the opacity of the brain model. The heat map was plotted by gradually colouring around the data points using "knnsearch" and "rangesearch" algorithms. Knnsearch finds the nearest neighbouring data point, whilst rangesearch finds all neighbouring points in a specified distance. The greater the number of data points clustered in a certain area the more intense the heat map colour (red indicating the most highly populated area). The heat-mapping effect could be altered by 'tuning' which changes the Euclidean distance for the area around every point. The Euclidean distance is the "ordinary" i.e. straight line distance between two points in Euclidean space. Euclidean space is a two or three-dimensional space in which points are designated by coordinates (one for each dimension) and the distance between two points are given by a distance formula (Encyclopaedia Britannica, 2015).

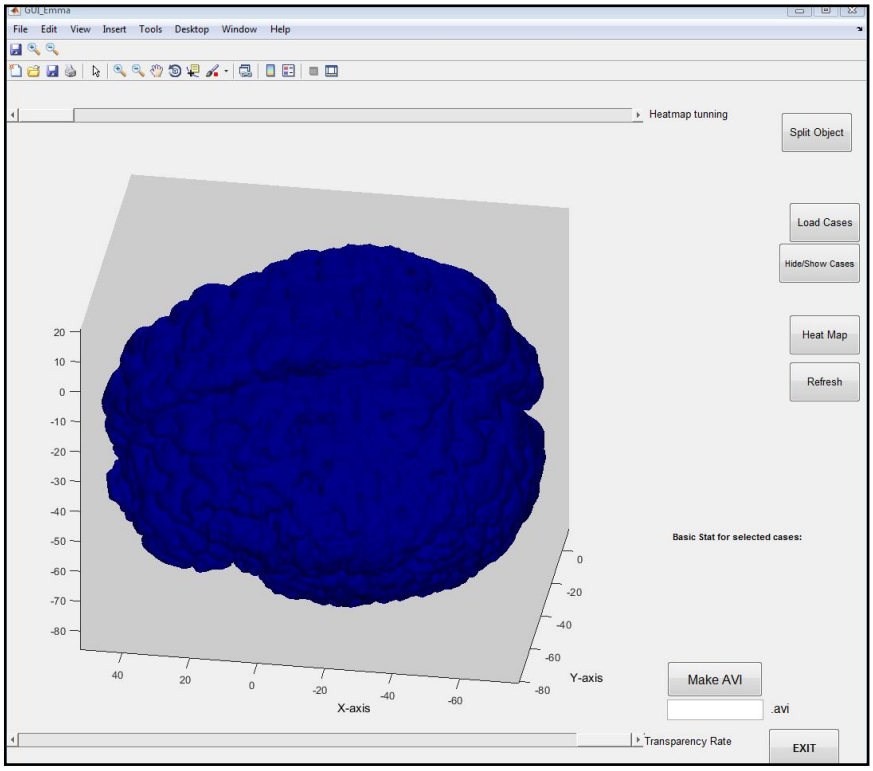


Figure 7.4 The Matlab interface showing the whole brain

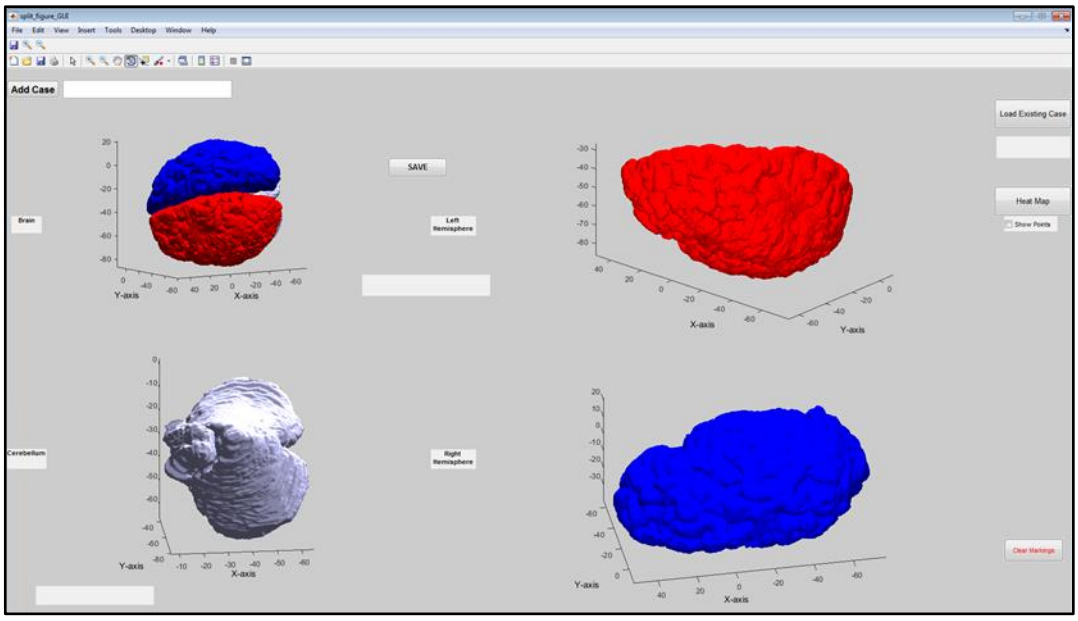


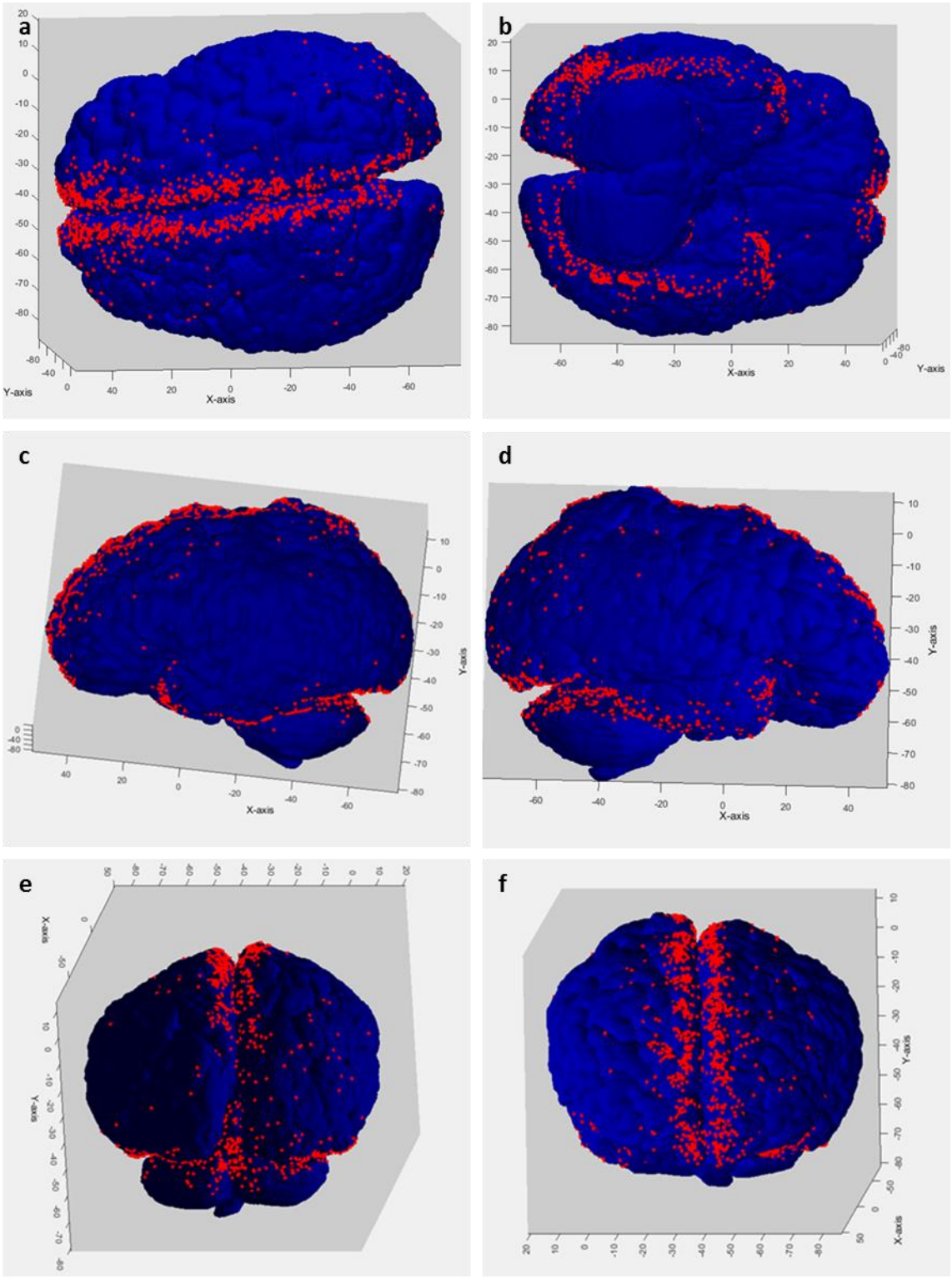
Figure 7.5 The split object view of the Matlab interface

7.4.3 Distribution of the Data Points and Heat Map Visualisation

The data points for all 43 cases were plotted together on the 3D brain to provide a general distribution pattern for all the age groups (neonates, infants and young children) including both head injured and non-head injured cases (Fig. 7.6). A heat map was then produced illustrating the distribution (Fig. 7.7) (Appendix 6).

The dot scatter plots (Fig. 7.6) showed that the majority of infant bridging veins are located near the SSS and transverse sinuses. However, bridging veins are also seen remotely from the sinuses, extending out over the cerebral hemispheres. The heat map allowed for a more detailed view of where the bridging veins cluster. Areas of high heat included: close to the superior sagittal sinus, particularly along the parietal and frontal lobes; the frontal and temporal poles; alongside the transverse sinus on the temporal and occipital lobes; and around the folium section of the superior vermis and the anterosuperior border of the cerebellar hemispheres.

As there were only three cases of AHT in the study series, a heat map could not be created for this small group (data not shown). However, bridging veins were still observed in the expected locations on the dot scatter plots, including: the parasagittal region, the frontal and temporal poles, the underside of the temporal lobe and the top of the cerebellum (Fig. 7.8). A few veins were also seen in the interhemispheric fissure and on the cerebral convexities away from the SSS. Dividing the cases into age groups also resulted in groups being too small to analyse collectively (data not shown).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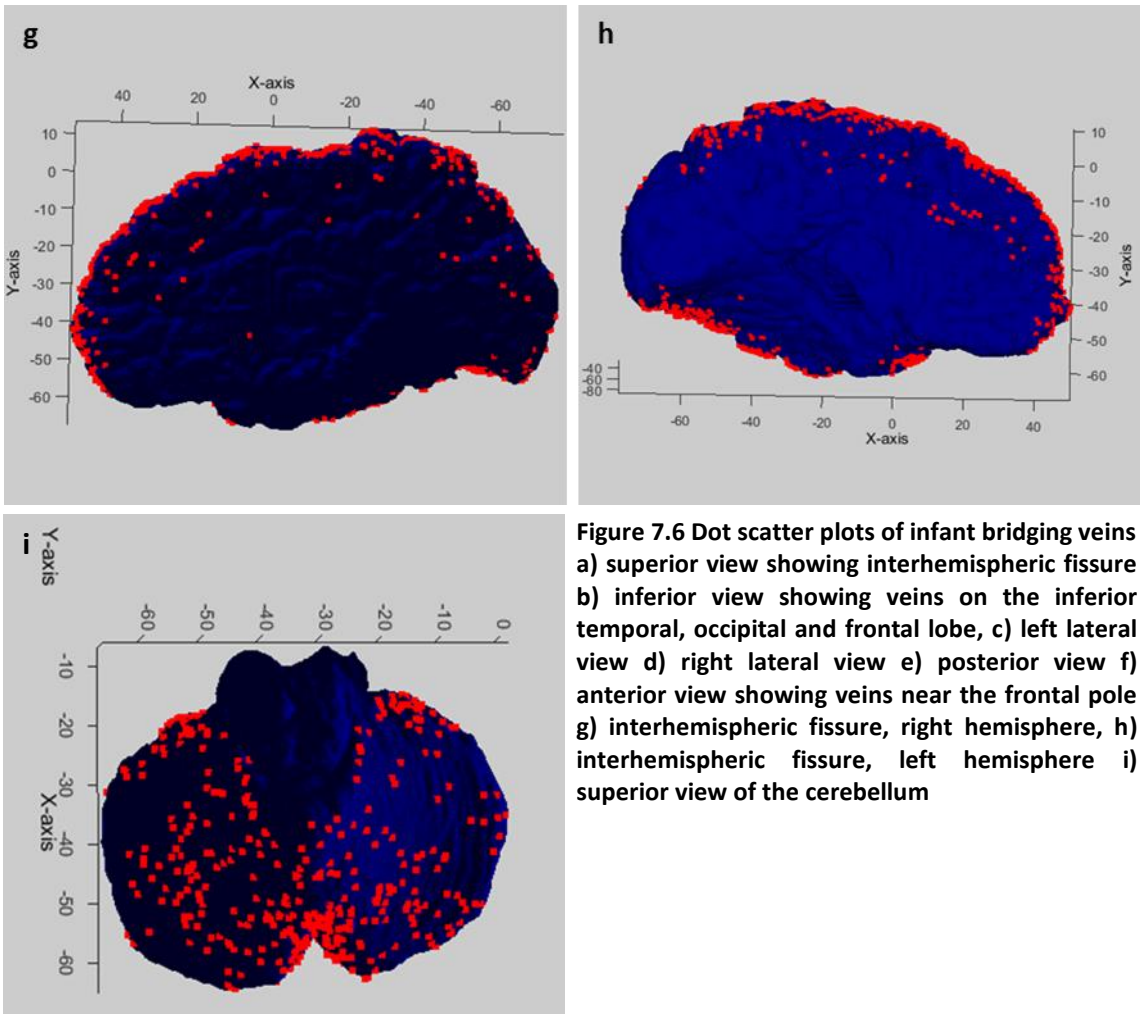
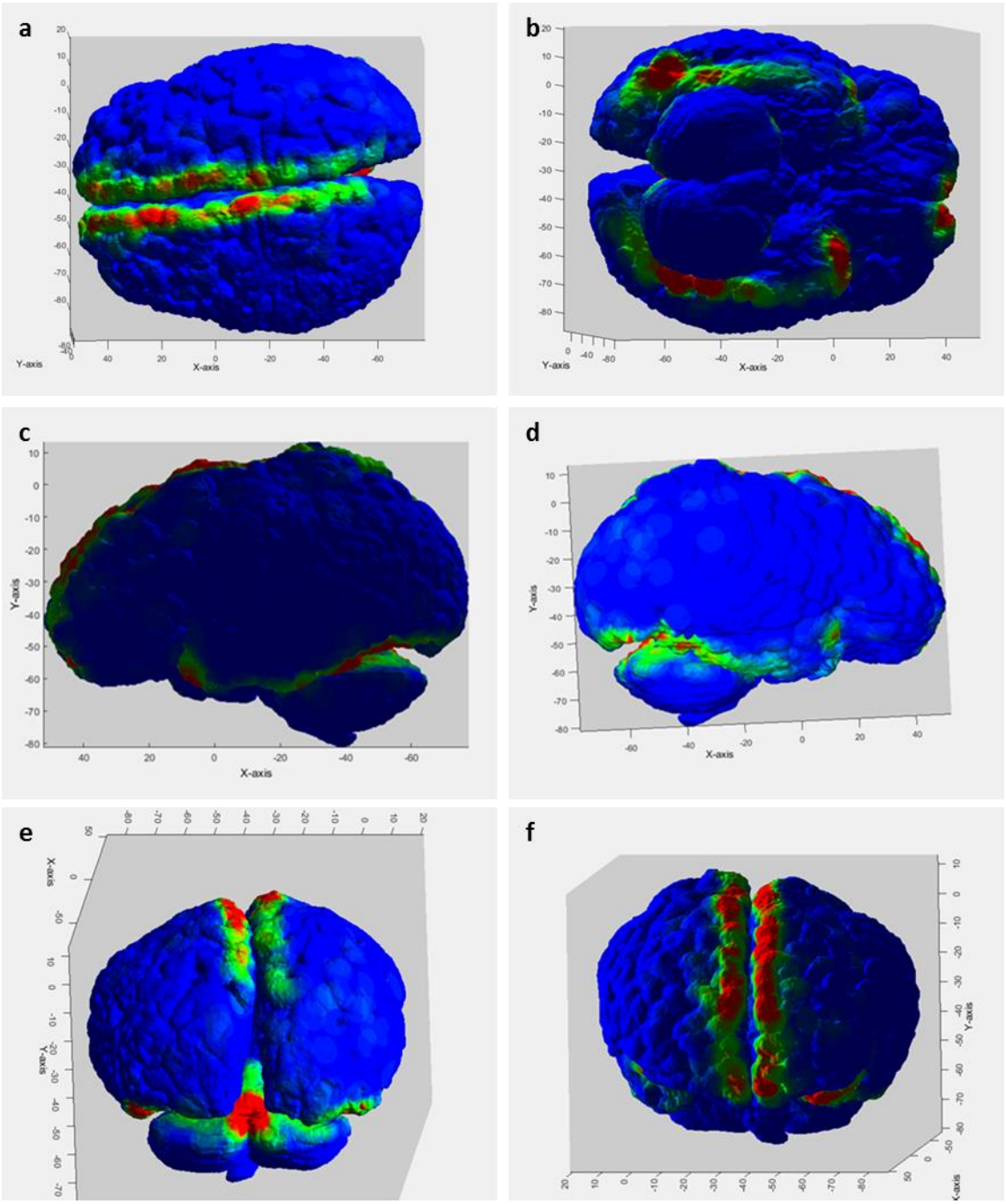


Figure 7.6 Dot scatter plots of infant bridging veins a) superior view showing interhemispheric fissure b) inferior view showing veins on the inferior temporal, occipital and frontal lobe, c) left lateral view d) right lateral view e) posterior view f) anterior view showing veins near the frontal pole g) interhemispheric fissure, right hemisphere, h) interhemispheric fissure, left hemisphere i) superior view of the cerebellum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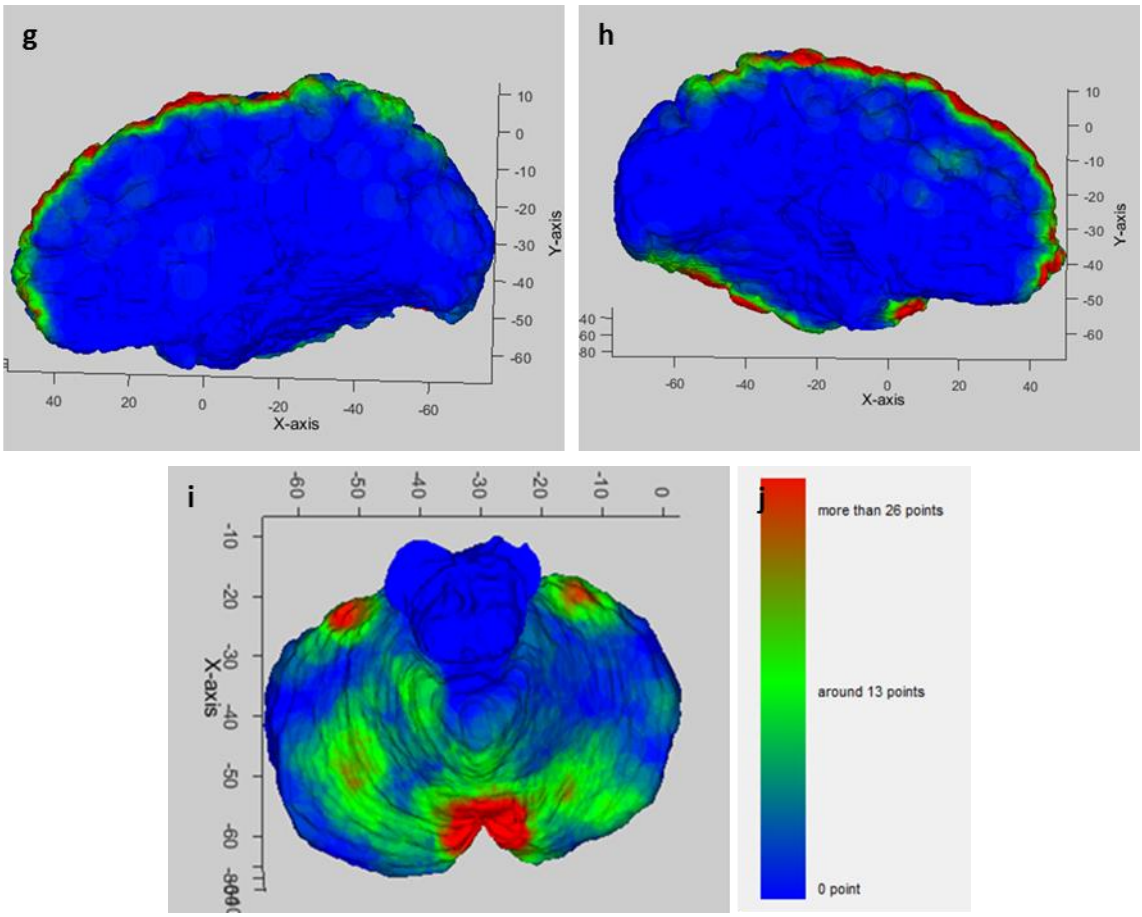
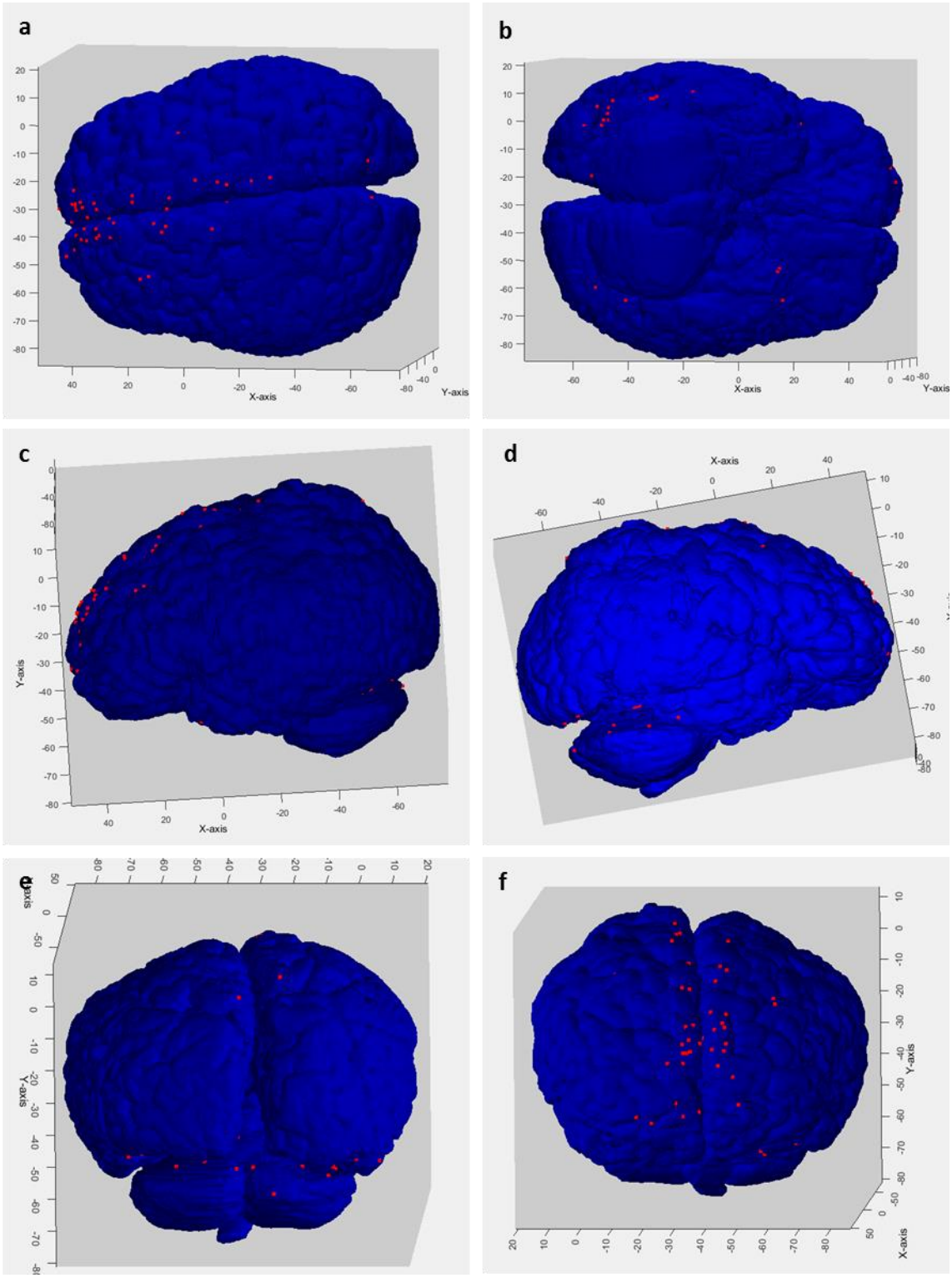


Figure 7.7 Heat map distribution of infant bridging veins a) superior view showing interhemispheric fissure b) inferior view showing vein distributions on the inferior temporal, occipital and frontal lobe, c) left lateral view d) right lateral view e) posterior view f) anterior view showing veins near the frontal pole g) interhemispheric fissure, right hemisphere, h) interhemispheric fissure, left hemisphere i) superior view of the cerebellum j) Heat map intensity bar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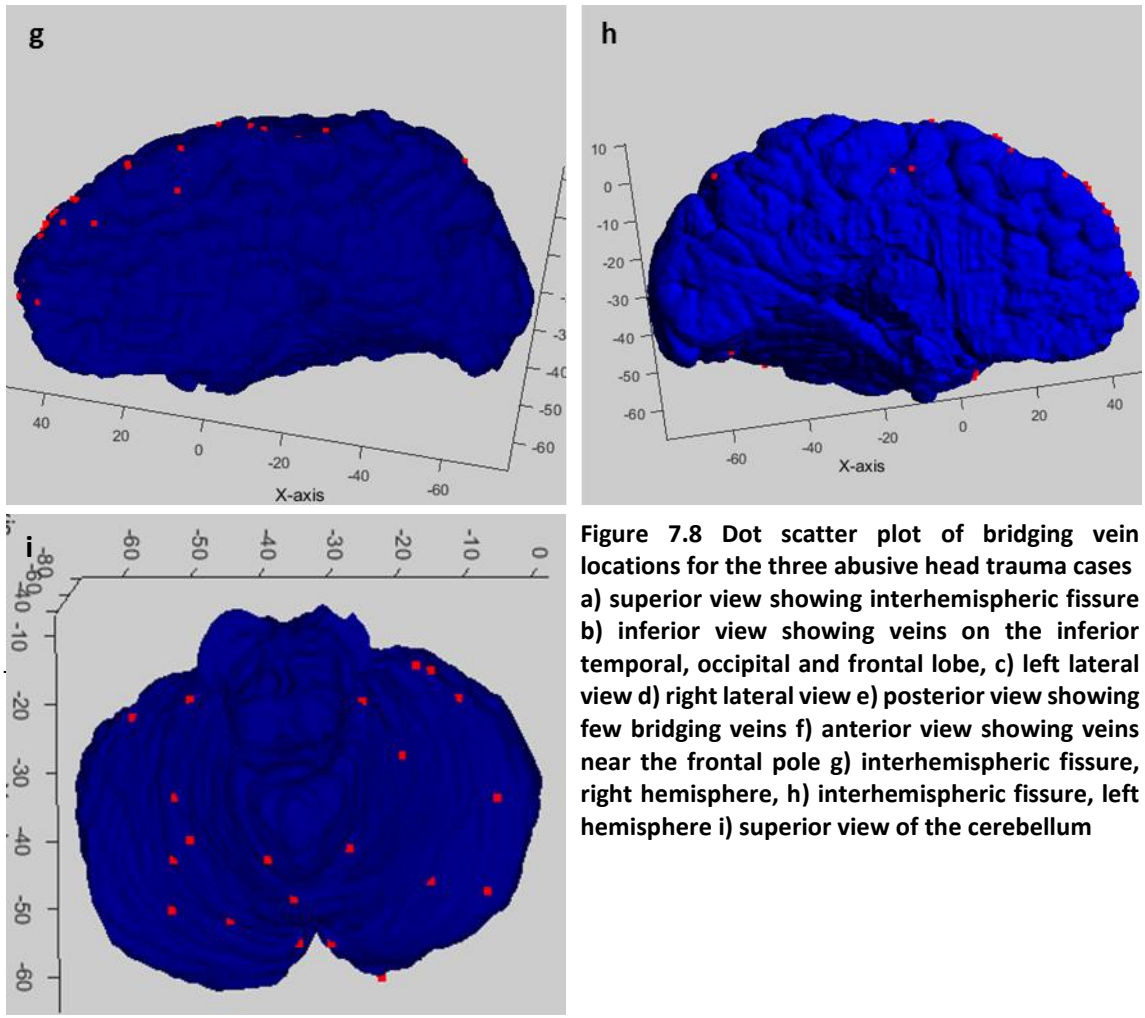

Figure 7.8 Dot scatter plot of bridging vein locations for the three abusive head trauma cases a) superior view showing interhemispheric fissure b) inferior view showing veins on the inferior temporal, occipital and frontal lobe, c) left lateral view d) right lateral view e) posterior view showing few bridging veins f) anterior view showing veins near the frontal pole g) interhemispheric fissure, right hemisphere, h) interhemispheric fissure, left hemisphere i) superior view of the cerebellum

7.4.4 Vein Counts From the Interface

The raw data for the vein counts can be found in Appendix 4.

The total number of bridging veins for the whole brain surface for all the cases was 2326. The mean number of bridging veins was 54.1 ± 2.2 (range 27 to 94, n = 43). A histogram of the frequency distribution of the number of cases and veins shows a normal distribution (Fig. 7.9) with 32.6% of cases having between 48-57 veins.

The five lowest counts for total bridging veins were seen in the three cases of AHT (27, 30, 34) and two cases of perinatal head trauma (30, 33). All five of these cases had SDH covering a significant amount of the cerebral surface and within the posterior fossa. Veins in these cases were also engorged with blood in both the subdural section and backwards along the cerebral length of the vein. The sample size of the AHT cases would have been too small to provide a statistically valid comparison of the number of veins observed in these cases to that seen in the non-head injured cases.

The total number of bridging veins for the left and right hemispheres for all the cases were 976 and 957 respectively. The mean number of bridging veins on the left hemisphere for all the cases was 22.7 ± 1.2 (range: 7 to 42, n = 43) and the mean for the right was 22.3 ± 1.0 (range: 11 to 40, n = 43). Histograms of the frequency of veins show normal distributions for both the left and right hemispheres (Fig. 7.10 & Fig. 7.11), with 55.8% of cases having between 18 and 27 veins on both the left and right hemispheres.

The total number of bridging veins for the cerebellar surface for all the cases was 393. The mean number of bridging veins was 9.1 ± 0.5 (range: 3 to 17, n = 43). A histogram of the frequency distribution of the number of cases and veins shows a normal distribution (Fig. 7.12) with 65.1% of cases having between 7-12 veins.

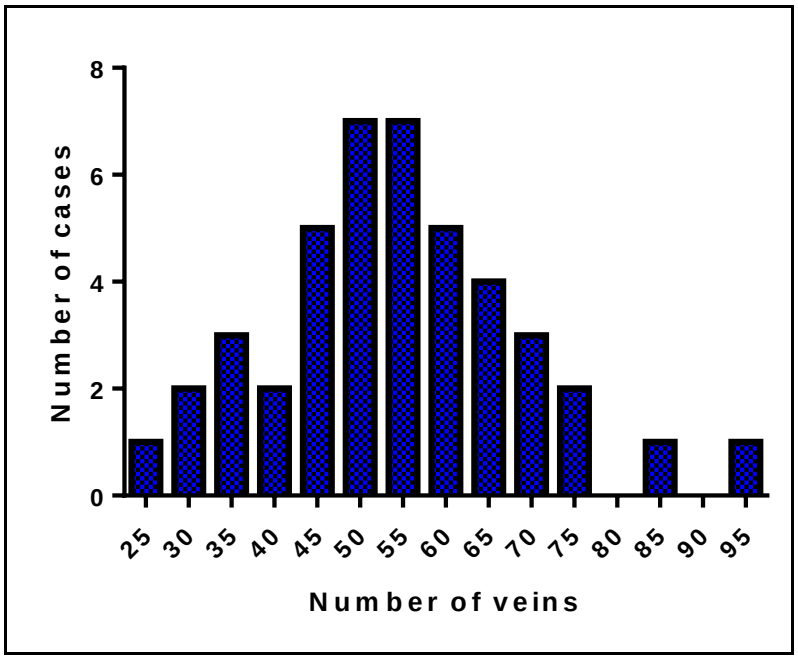

Figure 7.9 Histogram of frequency distribution of veins

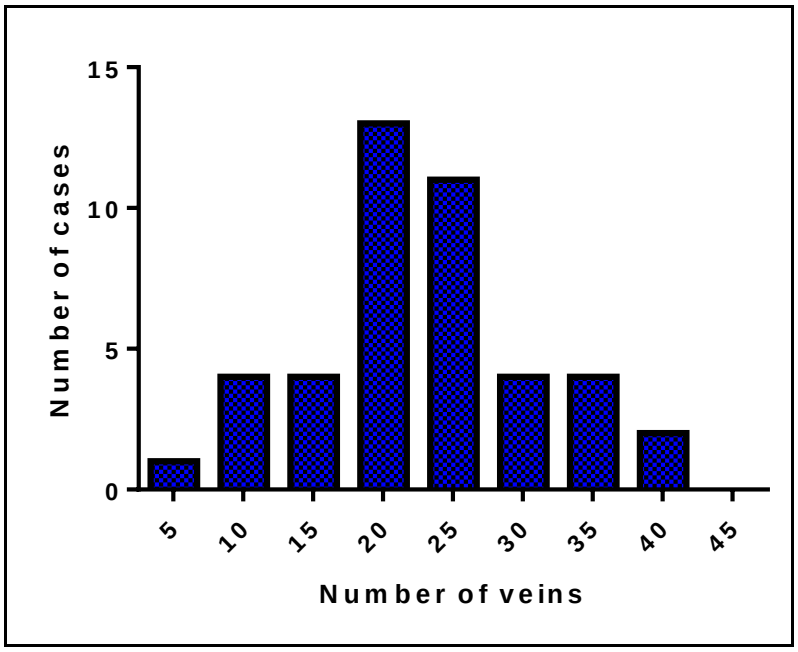

Figure 7.10 Histogram of frequency distribution of veins on the left hemisphere

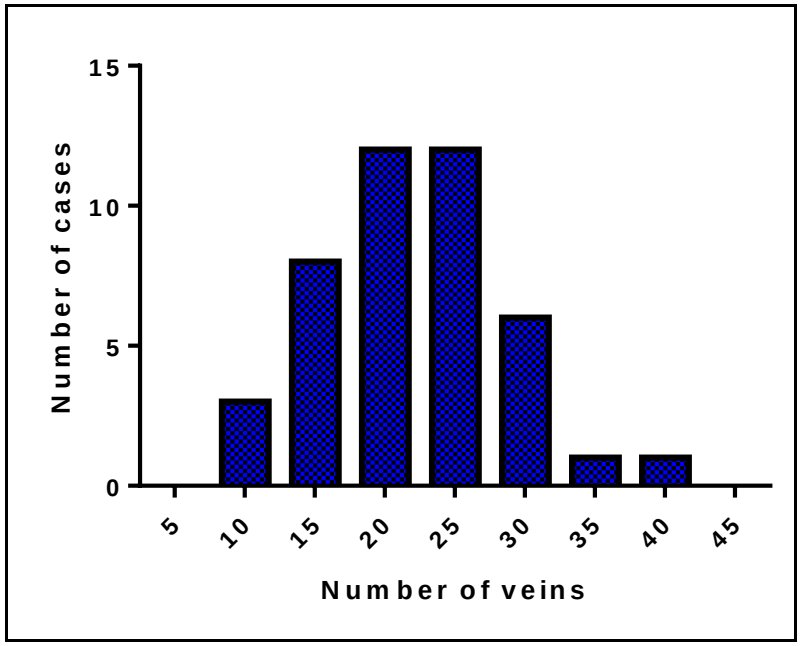

Figure 7.11 Histogram of frequency distribution of veins on the right hemisphere

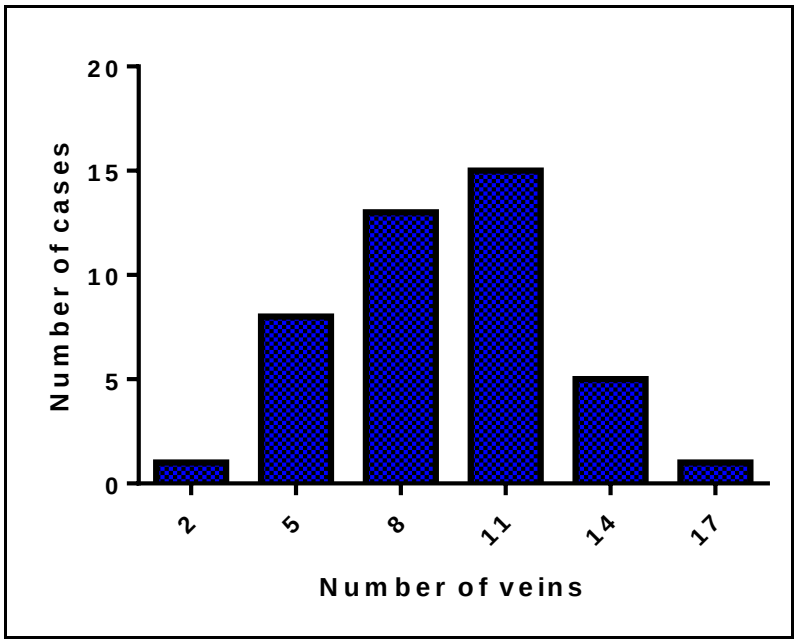

Figure 7.12 Histogram of frequency distribution of veins on the cerebellum

7.4.5 Statistical Comparison of the Two Counting Methods

The number of bridging veins, and the means and differences, for the two counting methods for the 28 cases is shown in Table 7.1. The mean number of bridging veins was 55.7 ± 2.8 and 57.8 ± 3.1 for the digital photographs and tally counter respectively.

The paired t-test showed that there was a significant difference ($p < 0.05$) in the average count of bridging veins for the digital photographs (M = 55.7, S.D = 15.0) and the tally counter (M = 57.8, S.D = 16.5), conditions; t (27) = 2.30, p = 0.03.

The average ICC value shows how consistent the counting techniques were from person to person. There was excellent agreement between the two methods, with an ICC of 0.97.

The scatterplot (Fig. 7.13) gives an indication of the level of agreement between the two counting methods. The data points are scattered close to the line of equality but only one data point falls directly on the line. If there was perfect agreement between two methods, all the data points would fall on the line of equality. A more informative plot (Bland Altman plot) is required to assess the inter-method variability.

The mean difference (đ) and standard deviation (SD) of the number of veins counted with the digital photographs and the tally counter was -2.04 and 4.68 respectively.

Using the equation below, the upper and lower limits of agreement were calculated as 7.32 and -11.4. These limits were drawn on to the Bland Altman plot (Fig. 7.14).

Mean of the observed differences (đ) ± The British Standards Institution repeatability coefficient (2SD).

-2.04 + (2 x 4.68) = 7.32 (upper limit)

-2.04 – (2 x 4.68) = -11.4 (lower limit)

The Bland Altman plot shows extremely good agreement between the two counting methods, with all the data points lying between the upper and lower limits of agreement. There appears to be no obvious difference in the distribution of the larger means compared to the smaller ones. However, there are twice as many data points below zero on the x-axis as there are above zero.

Case Number (from Appendix 4)	Measurement type		Means	Differences
	Digital photographs	Tally counter		
2	67	60	63.5	7
4	30	37	33.5	-7
6	71	81	76	-10
7	45	49	47	-4
8	51	53	52	-2
9	51	49	50	2
11	53	60	56.5	-7
13	64	70	67	-6
15	56	55	55.5	1
18	54	51	52.5	3
19	43	44	43.5	-1
20	58	63	60.5	-5
21	66	74	70	-8
22	62	66	64	-4
23	57	58	57.5	-1
32	51	48	49.5	3
34	27	22	24.5	5
36	58	54	56	4
37	50	51	50.5	-1
39	71	74	72.5	-3
41	56	54	55	2
43	39	40	39.5	-1
51	85	90	87.5	-5
52	44	46	45	-2
54	54	62	58	-8
56	34	34	34	0
58	69	80	74.5	-11
59	94	92	93	2
	Mean = 55.7	Mean = 57.8		đ = -2.04 S_d = 4.68

Table 7.1 The number of bridging veins, and the means and differences, for the two counting methods

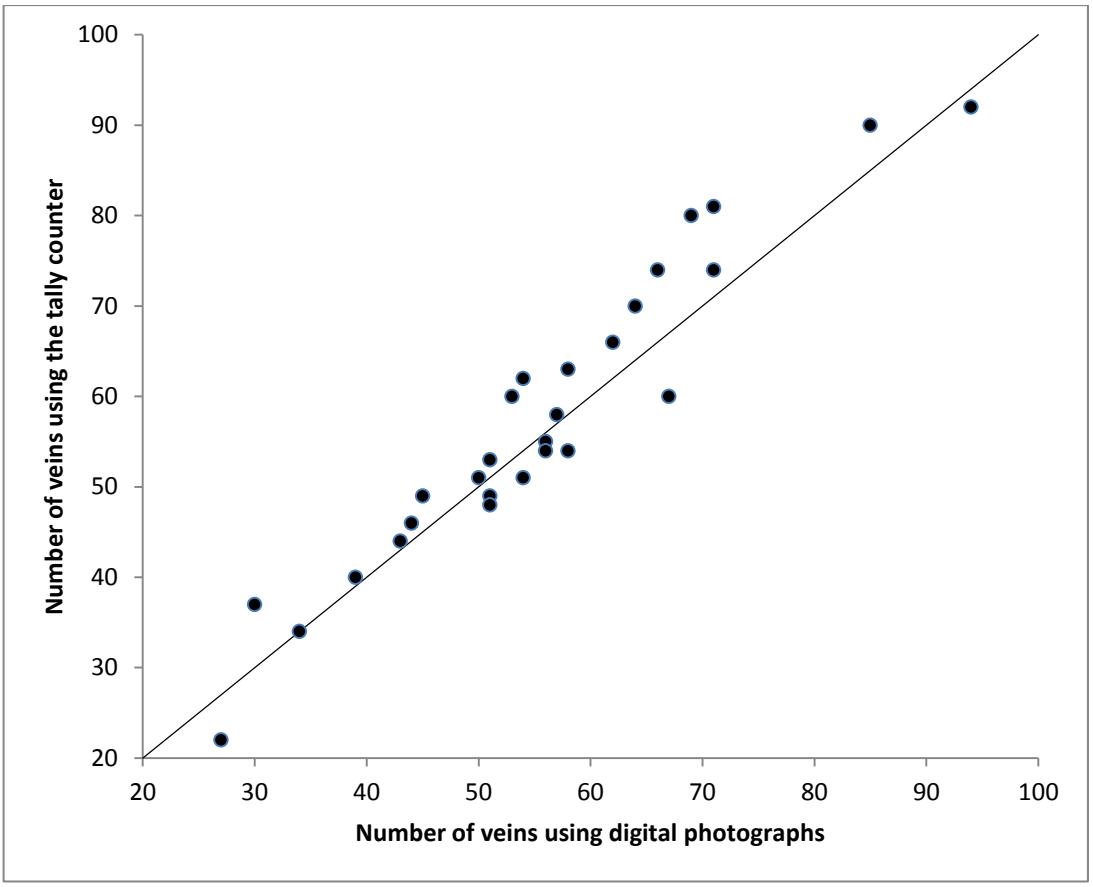

Figure 7.13 Scatterplot with line of equality of the number of veins counted using a tally counter and from digital photographs

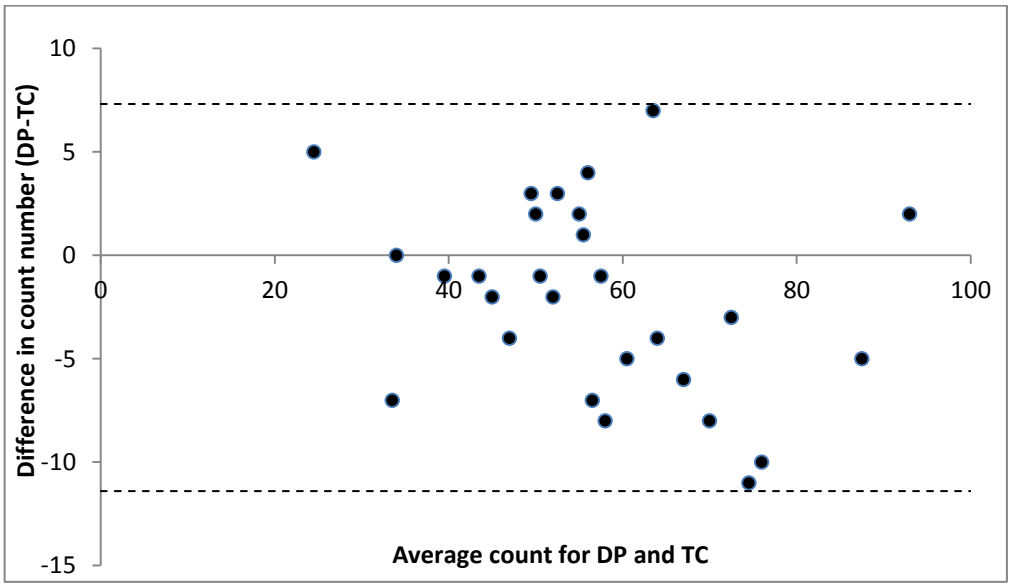

Figure 7.14 Bland Altman plot of the difference between the counting methods and the average

7.4.6 The Size of Infant Bridging Veins Using Digital Microscopy

Digital photographs were successfully taken of 11 bridging veins. Measurements were made from the section entering the brain, the dura, and the middle of the vein (Fig. 7.15 and Table 7.2). The mean ± SEM for the brain, dura and middle of the vein were 1.01 ±

0.17mm, 1.01 ± 0.18mm and 0.65 ± 0.10mm respectively. The overall mean for all the measurements was 0.89 ± 0.09mm (range: 0.29mm to 2.38mm).

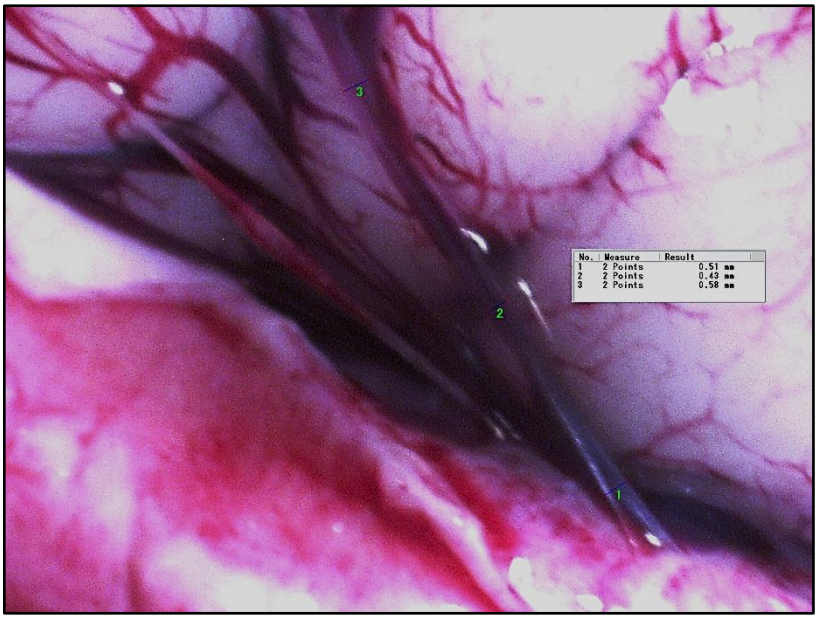

Figure 7.15 A left parietal parasagittal bridging vein

Vein Number	Vein location	Vein diameters (mm)			
		Dura	Middle	Brain	Mean
1	right parasagittal, near anterior fontanelle	1.35	0.63	1.26	1.08
2	right parasagittal, parietal	0.40	0.34	0.87	0.54
3	right frontal pole	0.75	0.61	0.59	0.65
4	right inferior temporal lobe, entering tentorium	0.37	0.37	0.75	0.50
5	left parasagittal, parietal	0.51	0.43	0.58	0.51
6	left parasagittal, parietal	1.31	0.29	0.30	0.63
7	left temporal pole	1.22	0.72	1.07	1.00
8	parasagittal	1.08	0.47	0.42	0.66
9	parasagittal	0.78	0.92	1.34	1.01
10	parasagittal	1.07	1.06	2.38	1.50
11	inferior temporal lobe	2.30	1.32	1.52	1.71
	Mean	**1.01**	**0.65**	**1.01**	**0.89**

Table 7.2 *In situ* diameter measurements of bridging veins at the dural and brain sections of the vessel and midway between these two points

7.5 Discussion

Several studies have attempted to investigate bridging vein numbers, often comparing cadaver dissection with imaging methods such as computed tomography venograhy (CTV), digital subtraction angiography (DSA) and magnetic resonance imaging (MRI) (Brockmann *et al.,* 2012; Han *et al.,* 2008). Greater numbers of vessels have been demonstrated in cadaver dissections than in studies employing neuro-imaging methods, with the average diameter of these vessels being significantly smaller than those seen in imaging studies. It has been suggested that this difference in average size is due to the fact that smaller veins are not identifiable on neuro-images (Han *et al.,* 2008) due to limitations in resolution.

No studies could be found in a comprehensive literature review on the subject of the number of bridging veins in the infant. One cadaveric study was found relating to five foetuses (six months gestation to full term) that had an average number of 18.2 bridging veins located near the SSS (O'Connell, 1934). The average number of bridging veins found in the studies detailed in this chapter were 55.7 and 57.8 when using the digital photographs and tally counter respectively. The average bridging vein numbers are considerably higher than those reported in adult studies (Andrews *et al.,* 1989; Brockman *et al.,* 2012; Han *et al.,* 2007), most likely because previous studies have not reported the number of veins located over the whole brain surface and have only focused on specific areas, often confined to the region near the SSS.

The number of bridging veins in the three AHT cases were considerably lower than the average (27, 30, 34). This is potentially a very important observation in respect of the assessment of the source of subdural bleeding as ruptured veins are the most widely believed cause of SDH in AHT. Once ruptured, smaller bridging veins are extremely difficult, if not impossible to locate macroscopically at autopsy. Intact bridging veins were engorged with blood in these cases, and a further important question presents itself as to whether or not a complete break of these vessels is necessary for blood to leak from these possibly injured, but not disrupted vessels. Although engorged blood vessels were mostly seen adjacent to SDH it would not be possible to describe a relationship between the two based on observations from only 3 cases. SDH makes assessment of bridging vein rupture and numbers difficult, particularly when large clots

are present. It is therefore not possible to confirm at this time whether the reduction in the number of the veins in these cases was due to rupture as a consequence of ante-mortem trauma or that the observation of the veins was complicated by the presence of blood clots, rendering artefactual disruption of bridging veins during assessment a possible source of artefact. That only three cases of AHT became available for study during the active phase of this project is also considered to represent too small a group to effectively eliminate the possibility that the reduction of numbers of bridging veins in AHT occurred by chance alone. A consequence of the small available study group was that no robust statistical analysis could be done to compare the number of veins in the head-injured cases to the non-head injured cases.

It is possible that the statistically significant difference of the average number of veins counted by the tally counter and the digital photographs, calculated by the paired t-test, could have resulted from the fact that bridging veins were more easily seen by the naked eye than in the photographs taken at autopsy. This would also explain the higher number of data points below zero than above on the Bland Altman plot. The paired t test was only used to assess whether the two sets of measurements agreed on average. The t test takes the mean score of all the subjects and has the potential to provide misleading estimates (Rankin & Stokes, 1998). To assess the agreement between the two counting methods, the difference between within-subject numbers was of interest and for this reason the ICC was calculated and the Bland Altman plot was used. Both methods produced very high levels of agreement for the two methods and therefore it is considered that either method could be used in place of the other when reliably counting the number of bridging veins present in any individual case.

Detailed study of the infant bridging veins is extremely challenging. This may account for the limited data in the literature on the size, number and locations of these vessels. It is likely that the number of bridging veins reported in this chapter were under-estimated due to the technical difficulties of examining these small veins. The soft infant brain was very difficult to stabilise once the dura was reflected and although attempts were made to examine the veins as thoroughly as possible, the process was carried out relatively quickly to prevent excessive distortion of the tissue. Bridging veins located in cranial recesses with poor lighting with the naked eye were difficult to capture

photographically and these may have been missed from the total count. As the dura had to be incised to gain access to the bridging veins it is possible that a small number of vessels may have been cut along with the dura. To avoid this as much as possible, cuts were made in areas where the bridging veins appeared not to cluster. Despite these limitations, relatively numerous bridging veins were typically observed during the post-mortem examinations. It is likely, however, that a proportion of the smaller bridging veins were missed due to their size and possible rupture when handling the brain due to extreme fragility.

Previous studies on the locations of bridging veins have targeted certain areas including: the SSS, the cerebellum, and temporal anterior frontal cortical areas (Brockmann *et al.,* 2012; Ueyama *et al.* 1998, Sakata *et al.,* 2000; Sampei *et al.,* 1996). No adult study could be found that documented bridging veins on all the surfaces of the brain, let alone a similar study with an infant cohort.

Bridging veins near the SSS were easier to document as they were often larger calibre vessels. When veins were seen on the cerebral convexities, away from the SSS, they appeared significantly smaller than vessels near the SSS. These vessels were very delicate and may have been prone to rupture when reflecting the dura. This could have resulted in an under-estimation of veins in these locations. The majority of the bridging veins were observed close to the sinuses, as is expected owing to their role in drainage of blood coming from the brain into these venous channels.

Most studies in adults report the diameter of bridging veins to be between 1 and 4mm (Ehrlich *et al.,* 2003; Yamashima & Friede, 1984; Han *et al.,* 2007), with one study observing veins as small as 0.4mm (Han *et al.,* 2007). Only one study could be found where the diameter of infant veins were reported. That study of six infant bridging veins (from a 34 week old foetus and 10 and 12 week old infants) measured outer diameters between 0.14mm and 0.72mm (Morison, 2002), considerably smaller than most the values reported for adult vessels.

In this chapter, 11 bridging veins were successfully measured with a mean outer diameter of 0.89 ± 0.09mm (range: 0.29mm to 2.38mm). However, due to technical

difficulties described below, only the larger bridging veins could be measured with the digital microscope.

During assessments, the brain needed to be supported under the microscope to prevent excessive movement with the help of an experienced Consultant Paediatric Pathologist. Too much movement resulted in stretching and possible breakage of the veins, especially of the smaller vessels. The microscope needed to be focused manually and then the image needed manipulating to capture the best picture. The technique required a second person to set up the microscope, take the images and save the data and to also take pictures of the vein *in situ* with the digital camera to enable future referencing to the location of the measured veins. The capture of sufficiently high-quality images from which it was possible that measurements could be made was also challenging owing to the convex surface of the brain and the varying optical planes of the structures visualised within each image frame.

In some of the digital microscope images, it was difficult to determine whether veins were singular or branching at one end due to differences in the amount of blood in the lumina of the veins, especially in images where there was extravasated blood behind the vein on either the brain or dural tissue. In cases where there was not much difference in the contrast between the vein and the background a somewhat subjective estimate had to be made of where the outer edge of the vessel was. It was also difficult to ensure that the subject vein was not placed under excessive tension at the time that the image was taken, or beforehand.

In conclusion, this chapter provides novel anatomical data on the locations, numbers and diameters of infant bridging veins. Bridging veins are not only more numerous than previously reported but can also be found in areas not previously mentioned in the literature. There is variation in the calibre of bridging veins, even within individuals. Some bridging veins are very small and delicate and not “robust” as they have sometimes been referred to as in the medical literature. These findings may have important implications for biomechanical engineers when testing the tensile strength of these smaller veins, and when designing finite element models of AHT.

Chapter 8 : The Feasibility of Removal of Infant Bridging Veins for Mechanical Testing

8.1 Introduction

Currently, there is no human data on the magnitude of the minimal inertial forces required to injure biological tissues to the extent that SDHs are induced. As the definitive experiment of shaking an infant to determine injury thresholds for tissues such as bridging veins cannot be undertaken for obvious ethical reasons, biomechanical engineers turn to various models to simulate real life situations.

As discussed within Chapter 2 of this thesis, finite element models (FEM) are frequently used to simulate traumatic brain injury. To increase the accuracy of FEMs, accurate knowledge of the material characteristics of intracranial tissue, including bridging veins is essential (Delye *et al.,* 2006).

The only published studies performed on bridging veins to assess their biomechanical properties have either been performed on adults or older children (Lowenhielm, 1974; Monea *et al.,* 2014; Delye *et al.,* 2006). As access to undisrupted infant bridging veins has now become possible as a result of this current work, the potential to dissect these vessels for the purposes of mechanical tensile testing was investigated with the aim of providing empirical data to enable the improvement of current FEMs of AHT.

8.2 Method

With the objective of assessing potential procedures to provide infant bridging vein samples for future tensile testing, one case was consented for the removal of bridging veins during this project (case 17 in Appendix 3).

In order to facilitate removal of the bridging veins; to prevent them from being damaged in transit and to enable attachment of the vessel to the testing machine; several sample surgical approximator clamps were trialled. Both stainless steel clamps (frame lengths 8, 11 or 16mm) (Acland, Germany) (Fig. 8.1) and plastic single-use clamps (Mini straight, pressure 10-15 grams) (VASCU-STRATT®, USA) (Fig. 8.2) were tested.

The clamped vessels were taken to a tensile testing machine at the Materials Centre, University of Leicester, to determine the most suitable method for transferring the veins to the clamps on the machine (Fig 8.2a & 8.2b).

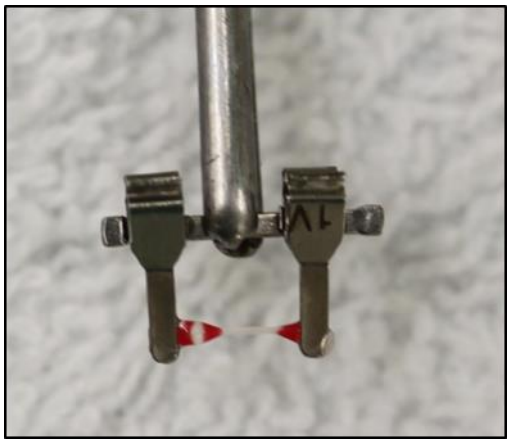

Figure 8.1 Acland approximator clamp (frame length 8)

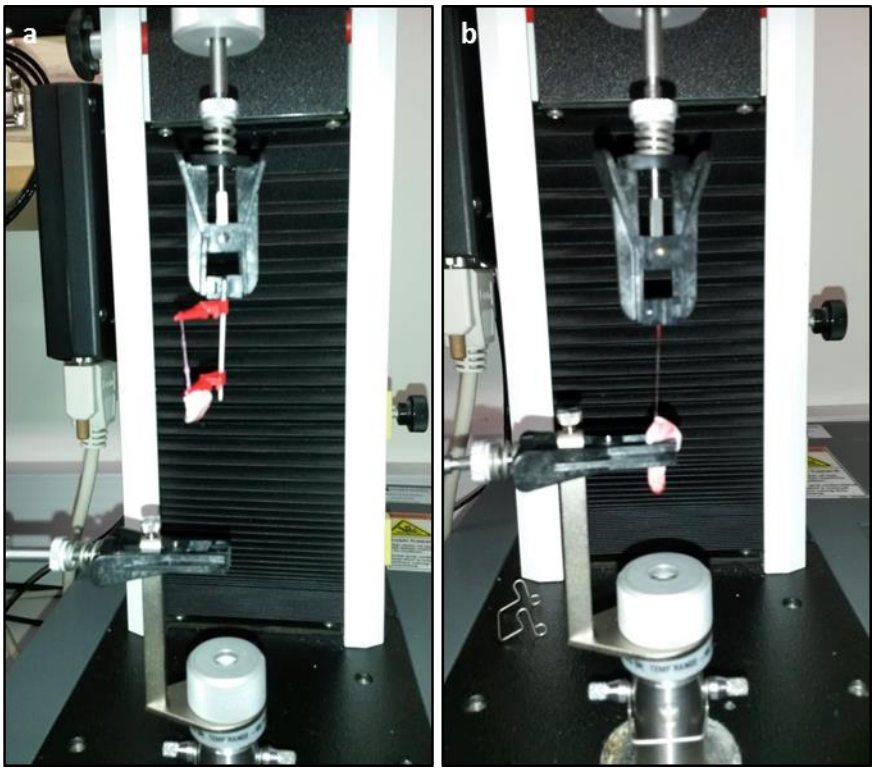

Figure 8.2 Tensile testing machine a) Bridging vein attached with VASCU-STRATT clamp b) vein attached directly to machine without approximator clamp

8.3 Results

Seven bridging veins were successfully dissected, two with the plastic clamps and five with the metal clamps. One of the bridging veins become detached at one end of a metal clamp during transit to the material centre. Of the remaining six veins, four were

successfully attached to the tensile testing machine. However, it was found that it was very challenging to both remove the vessels during the post-mortem and to attach the veins to the tensile machine without stretching them.

The plastic clamps could be rotated on the separating bar (not a feature of the metal clamps) and therefore the vein could be positioned and attached to the testing machine without removal from the approximator (Fig. 8.2a). However, veins had to be removed from the metal clamps for attachment to the tensile tester (Fig 8.2b), which was extremely difficult.

It was also found that the plastic clamps were easier to manipulate when clamping the veins *in situ* compared to the metal clamps. They also had an improved ability to grip the veins compared to the metal clamps, probably due to the serrated edges on the clamping surfaces.

8.4 Discussion

This case assisted in the identification of practical difficulties associated with dissection and handling of extremely small vessels both within the mortuary and when attaching the vein to a testing machine.

Dissection of infant bridging veins without damaging or over-stretching them prior to tensile testing has proved to be extremely challenging. The veins are easily broken, are attached to the brain which is very soft, and once the dura is incised to gain access to the veins the brain may move, stretching the vessels.

Although no data analysis was carried out for the tensile testing (because it was suspected that the veins had been significantly stretched during both the post-mortem dissection and attachment to the tensile testing machine), this brief exercise has shown that future mechanical testing of infant bridging veins appeared to be possible and has indicated the various technical improvements that will be necessary to carry out future experimentation.

Such suggested improvements include: additional assistance in the mortuary to stabilise the head during sample acquisition and targeting of specific bridging veins (most likely starting with the parasagittal veins) for dissection to enable small windows of both

cranial bone and dura mater to be removed. Limited dissection of these layers will help to secure the brain and prevent excessive movement of the organ which would result in stretching of the bridging veins.

For future experiments only plastic clamps will be used for dissection of the veins due to their ease of manipulation and ability to securely hold the vessels and directly attach to the testing machine, preventing pre-stretching of the vein before testing. Also, to prevent dehydration of the vein during tensile testing (which may alter its biomechanical properties) and to replicate some of the conditions of the internal human body, a temperature controlled water/saline bath may be used.

Finally, a method for marking certain locations on the veins (subdural section, arachnoid entry point) should be developed alongside the use of high-speed video recording of the tensile testing to demonstrate which section of the vein is breaking, possibly indicating areas of weakness in the vessel structure.

Chapter 9 : Discussion, Future Work and Conclusions

9.1 Discussion

A significant proportion of traumatic brain injuries in infants and children under 2 years of age are from inflicted injuries (Chiesa & Duhaime, 2009), with a clinical series suggesting that up to 80% of deaths from head trauma are from inflicted injuries in this age group (Bruce & Zimmerman, 1989).The diagnosis of AHT is a very challenging, complex and emotive process, often deliberated over at length in the criminal and family justice systems. Controversy has been created around the diagnosis of AHT, with questions arising about the events that may lead to a SDH and the potential sources of the bleeding. Currently, there is a lack of quality research on the infant vasculature system within the head to assist in answering these questions. In the past, unproven hypotheses have been used during court proceedings which have not been supported by reliable and reproducible scientific evidence.

Autopsy research on the infant cerebrodural vasculature system is crucial in aiding the understanding of AHT but it is an extremely challenging and sensitive area of work. It may well be that this is the main reason that there is such limited information on the characteristics of infant bridging veins.

The purpose of this thesis was to attempt to overcome some of the difficulties of research in the area of AHT and to establish novel methods for current and future investigations of the infant cerebrodural system and pathologies associated with head injuries in the young.

One of the problems in conducting research in this area is the requirement for ethical approval to carry out this work which is of a very sensitive and potentially controversial nature. A successful ethics application was made to NRES and the University of Leicester in order to carry out the work described in this thesis. However, the application procedure was a relatively intense and drawn out process, due in part to the reluctance of the insurers to approve the sponsorship agreement as they had never been involved in underwriting a study of this type before. Once approval was eventually obtained from

the insurers, the study had no further difficulties in obtaining approvals from NRES. The process of applying for ethics and the review process took approximately one year for this study.

To enable post-mortem research in the young, collaboration with pathologists employed by a relatively large paediatric autopsy service provider was very important to enable access to cases. Initially, it was suggested that approximately 24 cases would be consented for the OCT procedures described in Chapter 6. However, due to the extensive ethical application process, the acquisition of the appropriate equipment, and the establishment of the neurosurgical and clearing techniques to enable the OCT, only 10 parents could be approached for inclusion of their babies into the study within the available time. Of these 10 infants and neonates, seven were consented for the various procedures outlined in the ethics application. It is likely that future research within our Unit would see the recruitment of larger case numbers now that the techniques are more established and the appropriate equipment has been sourced.

It was observed early on in this study, during infant post-mortem examinations, that bridging veins in the young appear to be numerous and often very small and delicate, contrary to the previously held belief of some authors that infant veins are 'large' (Squier & Mack, 2009). As these observations have important implications in relation to AHT and the forces that may be required to damage bridging veins and cause SDH, a number of methods were developed to investigate these vessels. The novel approach to calvarial bone removal using neurosurgical equipment, first demonstrated by the Wales Institute of Forensic Medicine, and developed in Chapter 4 was one of the most important methods developed within this project. This minimally disruptive technique allowed for the subsequent studies to be conducted as outlined in Chapters 5 to 8, and has also become routine practice in our Unit for access to, and observation of, the brain and cranial meninges during post-mortem examinations of neonates, infants and young children.

Within both the OCT and anatomical mapping chapters, the infant bridging veins appeared to be more numerous and in locations previously unreported in the literature within studies on adult vessels. Infant bridging veins also appeared to be smaller and

more delicate than is often alluded to in scientific journals (Squier & Mack, 2009) and anatomy text books. These findings have extremely important implications for medical and legal professionals involved in AHT cases. It may be that with these new observations on the nature of infant bridging veins and the implementation of the techniques outlined in this thesis that future research may be able to answer the questions of where the subdural bleeding originates from in infantile head injuries and whether or not the cause of the haemorrhage is traumatic in any particular case.

As investigation of AHT cases requires a multidisciplinary team approach in terms of diagnosis, care and courtroom proceedings, so does the approach to research into AHT. This thesis required collaborations to be managed between various organisations and departments both within and outside the University of Leicester. Without a cohesive group of individuals from different disciplines and departments working together with the same goals in mind, post-mortem research into AHT would be impossible.

Quality scientific investigations to prove or disprove a traumatic cause of infantile SDH are of great importance to the criminal justice system to aid in the accurate diagnosis and conviction of AHT beyond any reasonable doubt.

9.1.1 Limitations

Only three AHT cases were encountered during the course of this project. This was somewhat lower than anticipated but is likely to have resulted largely from the delay in commencement of the project. This resulted in case numbers too small to make statistical comparisons of this group to the non-head injured group. As AHT occurs relatively infrequently compared to other non-head injury causes of death, it would most likely take several additional years to build up a database large enough to statistically analyse AHT as a separate group (short of the development of multicentre collaborations).

Several parts of the studies outlined in Chapters 5-7 involved subjective assessments. Within the optical clearing Chapter participants were recruited to assess the increase in transparency of dural tissue after application of glycerol to improve the objectivity of the analysis of the results. To further improve the scientific accuracy of the clearing effects guidelines for the testing were put in place. These included: participants having

optometrically corrected vision, being blinded to the treatment of each piece of dural tissue being assessed, and sitting at a set distance from the computer screen.

As opposed to the clearing experiments, the OCT image analysis and the mapping of vessel locations onto the 3D brain from the digital photographs were performed by one person. Although this resulted in all cases being subjected to the same assessment, it did not allow for independent evaluation by different individuals. Due to the lengthy process of analysing the OCT data and the 3D mapping, it was not feasible to recruit further individuals to perform these tasks for the purposes of this thesis. Additional observers would also need to have previous experience in viewing OCT data or have knowledge of brain anatomy for each of the two separate tasks. Such training may, of course, also introduce bias.

9.2 Future Work

This thesis has developed minimally disruptive techniques for the post-mortem visualisation of the infant brain and cranial meninges which has allowed for proof of concept investigations, including OCT for the investigation of the infant cerebrodural vasculature at autopsy. Now that these methods are in place, there is scope for future work into AHT.

9.2.1 Inclusion of Further AHT Cases and Additional Uses for the 3D Brain Interface

Future work based on this project would ideally see the recruitment of additional AHT cases to enable statistical comparisons between head-injured and non-head injured infants for the number and locations of bridging veins. One potential outcome of this work may be that identifiable bridging veins might be lesser in number in AHT than in non-head injured children. If damaged, bridging veins may recoil, making these vessels difficult to identify once ruptured in a shaking event, especially the veins that are small in length and diameter. However, the difficulty of examinations of the brains in the presence of subdural blood clots may provide further technical challenges in this regard.

For the purposes of post-mortem documentation of injuries, various patterns of subdural bleeding in the cases within this thesis were digitally photographed. Subdural haemorrhages in the AHT cases were observed to present in a patchy distribution

covering most of the cerebral convexity, whereas bleeding from birth-related trauma was often (but not always) seen in the posterior region. Adaptation of the 3D interface in Chapter 7 should allow for 3D mapping of subdural bleeding regions which would not only allow for comparison between cases of potential distribution patterns of bleeding in AHT, but would also enable mapping of bleeding regions with reference to the locations of bridging veins. This may aid in the identification of the source of SDH in AHT, particularly if intact veins are not demonstrated in areas of SDH. However, this may be difficult to prove, as it is likely that some subdural blood would be transferred away from its origin, over the convexities owing to gravity, although it is possible that rapid brain swelling in AHT cases may limit the movement of subdural blood if the brain is under pressure and forced up against the dural membrane. This pressure could potentially account for the patchy distribution of subdural bleeding often seen in AHT cases.

9.2.2 Optical Coherence Tomography

As outlined in Chapter 6, OCT was tested in several proof of concept experiments to determine whether this type of imaging modality has the capability to image the vessels of the cerebrodural system. Both types of OCT tested in this thesis appeared to identify vessel-like structures. Although complete verification of the nature of the structures could not be provided within the scope of this project, it is very likely that the formations draining into the SSS (seen with the rotational OCT) were most likely bridging veins due to their branching behaviour and the fact that they were anastomosing with a known venous drainage channel.

The next step in evaluating the potential use of OCT would be the addition of targeted histological sections and immunohistochemical studies of imaged areas to confirm that the structures observed on imaging are indeed blood vessels when viewed under a microscope.

As discussed in Chapter 6, the next assessment of the utility of the benchtop OCT system would be the optimisation of the scanning method for imaging of microvascular structures. Once optimised, future studies could be aimed at the generation of 3D OCT scans of large areas of the dura, by sequentially scanning small areas and combining the data sets. These scans would require the engineering of a numerically controlled

automatic computer system for the XYZ translation of the imaging probe over the convex dural surface and adaptation of software systems to facilitate manipulation and analysis of the image data.

9.2.3 Mechanical Testing of Bridging Veins

As demonstrated in Chapter 8, it may now be possible to mechanically test excised infant bridging veins. This work is still in the very early stages of development within our Unit and to date has only been carried out on one case to determine the practical difficulties of handling very small veins. For the development of this area of AHT research, further collaborations will need to be established with mechanical engineers to ensure the advancement of robust and reliable testing methods and data analysis.

9.3 Conclusions

- Infant and young children post-mortem research into AHT is practically and organisationally very difficult and requires a multidisciplinary team approach.

- This project has seen the development of a method that enables reliable and minimally disruptive access to the bridging veins as well as providing the enhanced ability to observe pathological features of head injury, free from post-mortem artefact.

- Neonatal, infant and young children bridging veins are more numerous than those previously described in studies in the adult population, with average numbers of veins per case typically recorded as ranging 56-58 per case in non-head injured children.

- Neonatal, infant and young children bridging veins are present in locations that have previously not been reported, including over the cerebral convexities and away from the dural venous sinuses.

- A proportion of bridging veins are relatively small and delicate. Of the veins large enough to manipulate in order to position under the digital microscope, the smallest recorded diameter was 0.29mm.

- With these new techniques of post-mortem calvarial bone removal and optical clearing of the dural membrane, the scope is extensive for future autopsy-based research into AHT. As a result of this, light may finally be shed on some of the most important questions surrounding AHT, such as the origin of SDH and whether or not the cause of the bleeding can be proved to be traumatic.

Appendices

Appendix 1A

Differential diagnosis for retinal haemorrhage (Narang, 2012).

Trauma	**Anemia**
Non-accidental	**Carbon monoxide poisoning**
Accidental	**Vasculitis**
Birth-related	**Hypoxia/hypo or hypertension**
Metabolic Diseases	**Papilledema/Increased intracranial pressure**
Glutaric aciduria type I	**Tumours**
Hemophagocytic lymphohistiocytosis	Lymphoblastic leukemia
Nutritional deficiencies	Cerebral aneurysm
Genetic Syndrome	Hemangioma
Osteogenesis imperfect	**Infections**
Ehlers-Danlos Syndrome type II	HSV meningoencephalitis
Coagulopathies	Bacterial meningitis
Hemophilia	
Hemorrhagic disease of the newborn	

Appendix 1B

Differential diagnosis for subdural haemorrhage (Adapted from David, 2008; Hymel *et al.,* 2002; Narang, 2012).

Trauma	**Blood coagulation disorders**
Non-accidental	Anticoagulant therapies
Accidental	Haemophilia A and B
Birth-related	Von Willebrand disease
Prenatal, perinatal and pregnancy-related conditions	Factor V deficiency
	Factor XII deficiency
Intrauterine trauma e.g. domestic violence to mother	Idiopathic or drug-induced thrombocytopaenic purpura
Idiopathic intrauterine subdural haematoma	Haemorrhagic disease of the newborn (vitamin K deficiency)
Intrauterine isoimmune thrombocytopaenic purpura	Disseminated intravascular coagulation
Maternal pre-eclampsia	Acquired inhibitors of plasma clotting factors
Postnatal cerebral infarction	Coagulopathy related to cirrhosis of the liver
Metabolic diseases	Ginko biloba ingestion
Glutaric aciduria type I	Hermansky-Pudlak syndrome
Hemophagocytic Lymphohistiocytosis	Alpha 1-antitrypsin deficiency
Nutritional deficiencies	**Infectious diseases**
Congenital malformations	Haemophilus influenza meningitis
Intracranial arteriovenous malformations	Streptococcus pneumonia meningitis
Cerebral aneurism	Kawasaki disease
Osler-Weber-Rendu syndrome	Endocarditis, leading to septic emboli of a cranial artery casuing aneurimal rupture of the vessel
Arachnoid cyst	
Encephalocoele or meningocoele	
Spontaneous rupture of a cerebral artery	Chronic otitis media
Schizencephaly or porencephaly	Malaria
Genetic diseases	Herpes simplex encephalitis
Osteogenesis imperfect	Congenital toxoplasmosis
Ehlers-Danlos syndrome type II	HSV Meningoencephalitis
Hereditary hemorrhagic telangiectasia	**Autoimmune disorders**
Sickle cell anaemia	Lupus erythematosus
Alagille syndrome	**Poisons/toxins/drug effects**
Menkes kinky hair syndrome	Lead poisoning
Autosomal dominant polycystic kidney disease	Cocaine
	Anticoagulant therapy
Malignancy	Other
Meningeal carcinomatosis	Haemorrhagic shock and encephalopathy
Leukaemia	Spontaneous intracranial hypotension
Solid tumours of the central nervous system	Wegener's granulomatosis
Primary mucosa-associated lymphoma of the dura	Hyperostosis frontalis interna
	Bone marrow transplant
Mass lesions in the subdural space	Moyamoya disease

Appendix 2: Ethic's documents

2A: Study Protocol

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Study Title: A Study of the Dural Venous Vasculature in Infants Under One Year of Age

Ethics Ref: 14/EM/0169

Date and Version No: 21 May 2015 Version 4

Chief Investigator:	Professor Guy Rutty Email: gnr3@le.ac.uk Phone: 0116 2523221
Investigators:	Emma Cheshire, Dr Tim Jaspan, Dr Roger Malcomson, Dr David Adlam, Dr Ryk James, Dr Shiju Joseph
Sponsor:	University of Leicester
Funder (if applicable):	Home Office
Signatures:	 

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, host NHS Trust (s), regulatory authorities, and members of the Research Ethics Committee.

Table of Contents

1. Amendment History

Amendment submission number	Individual Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	1	2	18/08/2014	Prof Guy Rutty	Additional procedure for measuring vessel diameters using digital microscopy
1	2	2	18/08/2014	Prof Guy Rutty	Removal of bridging veins for stretching experiments
2	3	3	02/02/2015	Prof Guy Rutty	The use of anonymised photographs for location mapping of bridging veins
2	4	3	02/02/2015	Prof Guy Rutty	Radiographical scanning for creation of a 3D brain model
2	5	3	02/02/2015	Prof Guy Rutty	Extension of study end date
3	6	4	21/05/2015	Prof Guy Rutty	Sending bridging veins to Dept. Mechanical Enginnering, KU Leuven,Belgium for stretching experiments

2. Synopsis

Study Title	A Study of the Dural Venous Vasculature in Infants Under One Year of Age
Internal ref. no.	UNOLE 0434
Planned Sample Size	24
Primary Objective	To determine the size and number of blood vessels in the dura mater.

3. Abbreviations

AHT	Abusive Head Trauma
CT	Computed Tomography
GCP	Good Clinical Practice
NHS	National Health Service
OCT	Optical Coherence Tomography
REC	Research Ethics Committee
SDH	Subdural Haematoma

4. Background and Rationale

At present, there are multiple theories regarding the source of subdural bleeding in infants who have suffered accidental or non-accidental traumatic head injury (a.k.a infant abusive head trauma and previously known as the 'Shaken baby syndrome'). One controversial subject concerns the mechanism of how violently shaking an infant causes subdural bleeding. Cases of suspected abusive head trauma (AHT) often present with a triad of injuries, including subdural haematoma (SDH), retinal haemorrhages and encephalopathy.

Historically, the most widely assumed source of subdural bleeding in these cases is the rupture of bridging veins which extend through the subarachnoid space to penetrate the dural margin of the major venous sinuses. This theory has been questioned due to biomechanical models which have suggested that the amount of force required to shear bridging veins is estimated to be greater than that of inertial forces (such as those produced by vigorous shaking). Direct demonstration of disrupted bridging veins by imaging of surviving patients, during surgery or at autopsy has also been limited.

Establishing a diagnosis of AHT is a very challenging, complex, controversial and emotive process, often deliberated over at length in the criminal and family justice systems. Without accompanying evidence of inflicted trauma, such as unexplained bruises or fractures, convictions based on the triad alone have been called into question, resulting in cases being overturned on appeal. This study will consider alternative hypotheses for the source of bleeding related to infant SDHs. Our research will focus on the anatomy of the dura mater, a membrane that has a very rich and intricate vasculature, a fact not often alluded to in the SDH literature. The forces associated with damage to bridging veins will also be investigated by stretching experiments to measure the mechanical failure characteristics of these vessels.

The aim of this area of work is ultimately to aid health, law enforcement and legal professionals in the correct recognition of cases of abusive infant head injury, based on detailed scientific study and not on unproven theory. This prospective, consented, autopsy-based study would last for 1-2 years and would be part of a studentship funded by the Home office. The research would take place in the Leicester Royal Infirmary mortuary and would include a cohort of newborn to 1 year old human infants (the most common age range for

AHT victims). A minimally destructive technique for removal of the skull vault bones will be used at autopsy. The vessels within the intact dura mater will be visualised using various imaging techniques, including optical coherence tomography (similar to a CT scan only using near-infrared light instead of x-rays). This will allow measurement of the calibre of vessels within and around the dura mater as well as anatomical mapping of the vessels in relation to the brain surface. In addition, the diameters of the vessels will be measured using a digital microscope. Veins will also be removed during the post-mortem and clamped to a mechanical testing machine to enable stretching experiments to find the mechanical failure properties of these vessels.

As understanding of the importance of the bridging veins in infant head injury has increased in recent years it has become standard autopsy practice within our unit to photographically document these vessels during infant post-mortems as an aide memoire for the purposes of generating autopsy reports. The photographs are maintained in an anonymised archive on a secure NHS server. Observational study of vessels from non-head injured infants would allow us to understand the usual size, locations, numbers and anatomical features of bridging veins in 'normal' babies. Anonymised data from these observations would aid understanding of what is anatomically 'normal' for the purpose of medico-legal expert work, as there is currently no published dataset to assist medico-legal professionals or the courts in this critical evidential area. It has now come to our attention that this unique series of archived, anonymised photographic data could be used alongside our imaging techniques to anatomically map the locations of these vessels and to inform the wider medical community as to their location and frequency.

We now seek permission to use these archived anonymised photographs to enable data plotting on one standardised 3D brain model to help determine patterns of vessel location. As this data is anonymised and cannot be traced back to the original participant by the primary researcher, we do not feel it is appropriate to retrospectively consent the use of these images for the purposes of pooling these data points onto one model as it will likely cause un-necessary inconvenience and potential distress to the parents.

To create a 3D brain replica, initially, computer aided design (CAD) brain models were reviewed. However, only adult CAD brains could be sourced. Due to the anatomical variance

between infant and adult brains, it is important that the 3D replica brain be that of an infant within the age group of this study.

In order to assimilate the data from the anonymised photographs, an infant brain would undergo a radiographical scan (e.g. MRI, CT), within the Leicester Royal Infirmary Radiology Department, or the Leicester University Engineering Department, to produce a 3D digital model of a brain. After a telephone conversation between Prof Guy Rutty and the NRES Committee, East Midlands, verbal approval was given to radiographically scan one consented infant brain for the purpose of obtaining a 3D brain model, on the understanding that a substantial amendment be submitted at the earliest convenience. During the standard infant post-mortem the brain is retained and sectioned after approximately 7 days before being returned to the body, during these 7 days the brain would be scanned, ensuring no delays would be incurred to the release of the body from the mortuary. The 3D scan data would then be put into a mathematical/statistical program to create a '3D mesh brain' to allow the photographical data locations of vessels to be plotted and statistically analysed.

The MRI/CT scans will occur outside of patient hours, and the appropriate approval has been granted from the Radiology Department. Guidance on the statistically analysis of this data will be sourced from a University of Leicester employed statistician. Any additionally incurred costs from the scans will be covered by the research project grant.

Research into the anatomy of the dura is very important to aid in the diagnosis of infant abusive head trauma. It is also crucial that the model used for anatomical study is an infant in the age range when infant abusive head trauma occurs. This is due to the fact that there is no alternative model that accurately reflects the anatomy of an infant's brain, including all animal models or using an adult human. It is essential that research carried out during the post mortem will not delay the process of the subject being released from the mortuary and that the procedures will have no side effects or complications to the participant. The skull will be opened using a neurosurgical technique that has become our practice which results in minimal disruption of dural blood vessels and bridging veins, and allows for the assessment of sources of subdural bleeding. This procedure will facilitate imaging studies and careful visual identification of bridging veins. Two types of imaging system (benchtop OCT and rotational OCT) will be used on participants. Benchtop OCT is a non-invasive imaging

system, rotational OCT will require a small incision to be made into the superior sagittal sinus. No additional external incisions will be made to the scalp and any incision into the superior sagittal sinus will not be visible externally once standard reconstruction of the head has occurred at the end of the autopsy examination. After the OCT imaging the digital microscope will be used to capture an image of each blood vessel *in situ* so that after the post-mortem these images can be analysed by software to acquire the diameters of each vessel. Once all the imaging is complete, several bridging veins will be excised for stretching experiments which will occur shortly after the post mortem. Microscopy will occur prior to, and after mechanical testing.

Objectives

5.1 Primary Objective

To determine the size, locationnumber of blood vessels in the dura mater.

5.2 Secondary Objective

To find the mechanical failure properties of veins associated with the dura mater

6. Research Design

6.1 Summary of Research Design

This research will be a prospective, consented, autopsy-based study that will last for 1-2 years. The study will only require participants to be involved during their autopsy, requested by the coroner/hospital and will only take an additional 1-2 hours.

When an individual that is identified as being eligible for inclusion in the study is brought to the mortuary the consultant paediatric pathologist will be informed. The parents will then be approached by telephone by the East Midlands Forensic Pathology Unit's consenter, an advanced nurse practitioner, for the infant to be included in the study. If consent is obtained the participant will be put forward for the study, the procedures for which will occur at the post-mortem. During the post-mortem the skull vault bones will be removed using a

minimally destructive technique. A sugar based clearing solution (glycerol) will then be applied to the dura every few minutes until the tissue has cleared and become relatively more transparent. The clearing has no discernable effect on any histology of the dura and is readily and rapidly reversible upon re-contact with water/moisture. The dural vessels will be assessed using two types of imaging system; one that will require a small probe to be inserted via a small incision into the superior sagittal sinus and the other that will be placed over the surface of the dura. After OCT imaging the vessel diameters will be measured *in situ* by placing the lens of a digital microscope above each vessel. Vessels will also be photographically documented as part of our standard infant post-mortem practice. Following this, several veins will be excised from the dura mater and brain and retained. Once operational the mechanical testing will be done in the University of Leicester, Engineering Department. After mechanical testing and microscopy the tissue will be brought to the histology lab, Level 3, Robert Kilpatrick building for storage till the end of the study. All tissue will be tracked by the EMFPU tracking system from receipt to disposal. At the end of the study all tissue will be sensitively disposed of using the University human tissue disposal system. In the meantime, until mechanical testing is fully operational at Leicester, as there are currently no institutes in the UK that are mechanically testing bridging veins, additional mechanical testing may also be done with the Department of Mechanical Engineering, KU Leuven, Belgium, as this group already have experience and optimised testing protocols in place for this work. Veins that are to be tested in KU Leuven will be brought to the histology lab, Level 3, Robert Kilpatrick building immediately after the post-mortem to be snap frozen in isopentane. They will then be stored in the -80 °C freezer whilst delivery of the samples is arranged. Samples will then be sent by DHL logistics company to KU Leuven. After mechanical testing, bridging veins will be sent back to University of Leicester for disposal. To obtain a 3D brain model to map the anatomical locations of vessels in the photographs, an infant brain that has been removed and placed in formalin as part of the post-mortem examination, with be taken to the Radiology Department in the Leicester Royal Infirmary or the Engineering Department within Leicester University for radiological scanning (MRI/CT). The brain will be scanned outside of patient hours and returned to the mortuary before the brain is due to be sectioned and returned to the deceased. The additional procedures should not extend the post mortem by more than 2 hours. There are no side effects or complications to the participant with the additional OCT imaging, application of the glycerol or digital microscopy. There will also be no delay to the

release of the participant from the mortuary (which ordinarily occurs at least seven days following an infant autopsy owing to the requirement for fixation and detailed examination of the removed whole brain). Disruption of the skull vault bones is ordinarily required at post mortem for access to the brain and dura; therefore the only additional procedures required for this study would be the application of the glycerol and the application of the imaging modalities. The design of this study has been chosen owing to the minimally destructive techniques used to enable visualisation of the dural vessels. This new and innovative access method that could potentially also be used as part of routine paediatric medico-legal autopsy practice to more precisely identify potential indicators of abusive cranial trauma, such as subdural haemorrhage and torn blood vessels, whilst eliminating some autopsy technique-induced artefacts.

6.2 Outcome Measures

The size and number of blood vessels will be measured using optical coherence tomography. This system will create 3D images of the blood vessels, creating a map of where they interact with the dura mater. From these images vessel diameter can be measured and numbers of vessels can be calculated. The digital microscope will also offer an alternative method for measuring vessel diameters. Stretching experiments using bridging veins will measure the mechanical failure properties of these vessels. Number and location of vessels will also be analysed from anonymised post-mortem photographs using a 3D brain model, created from a radiographical scan.

6.3 Research Participants

6.3.1 Overall Description of Research Participants

Research participants will be new-born to 1 year olds, requiring a post mortem that has consent from parents for inclusion in the study.

6.3.2 Inclusion Criteria

- Participant's parents speak English and are willing and able to give informed consent for participation in the study
- Deceased male or female babies (full term)-one year of age

- All causes of death that do not involve head injuries, and suspected child neglect, abuse or trauma

6.3.3 Exclusion Criteria

The participant may not enter the study if ANY of the following apply:

- No participants with non-English speaking parents as consenter needs to be able to have phone conversations in English with parents
- No participants that are less than full term or over the age of one
- No participants with head injuries
- No participants with suspected child neglect, abuse or trauma

6.4 Study Procedures

Participants will undergo OCT imaging and digital microscopy during post-mortem. Radiographical scanning of the brain may also occur in a select few participants, once the brain has been removed for post-mortem fixation in formalin. Several vessels will also be excised for stretching experiments and microscopy, to take place shortly after the post-mortem (within 1-2 days). Depending on consent, participants will either have benchtop OCT, rotational OCT, digital microscopy or a combination of these procedures (further details in Post Mortem Optical Coherence Tomography Protocol,Digital Microscopy Protocol, Histology Protocol and Mechanical Testing of Bridging Veins Protocol). Depending on consent, participants may also have several bridging veins excised. Participation in the study will only be required for a few hours during the post mortem.

6.5 Study End Date

August31st 2017

7. Informed Consent

An advanced nurse practitioner or medical consultant from the East Midlands Forensic Pathology will seek telephone verbal consent and sign and date a consent form during the conversation with parents. The reason the parents will be approached by phone and not in

person is the time period between the unit being notified that an autopsy was requested by the HM Coroner/hospital and the undertaking of the examination is very short. The unit is not allowed to do anything that will slow down the process of HM Coroner. As there will be several requests to different families on the same day, it is not possible for the unit to visit each and every person personally, although we would wish to, especially if parents live outside the immediate City of Leicester boundaries. During the phone conversation the person taking verbal consent will sign to say that they have spoken to the parents and that the parents gave informed verbal consent for their baby's participation in the study. They will confirm whether the parents do/do not wish for their baby to have benchtop OCT, rotational OCT, digital microscopy and radiological scanning. They will confirm whether the parents do/do not wish for retention of bridging veins for mechanical testing and microscopy. They will also confirm whether the parents do/do not give informed verbal consent for the study images to be used for teaching and training purposes. They will also sign to confirm whether the parents wish to receive the information sheet. The latest approved version of the verbal informed consent form must be signed and dated by the consenter before any study specific procedures are performed.

The parent's information sheet will provide information on the exact nature of the study, the implications and constraints of the protocol, and the known side effects and any risks involved in taking part (of which there are none).

It will be clearly stated that the parents are free to withdraw their baby from the study at any time for any reason and it will not affect the autopsy that is to be undertaken, and with no obligation to give the reason for withdrawal. Due to the time scale set by HM Coroner for the autopsy it is possible that the parents may have decided not to allow their baby to take part in the study after the procedure has occurred. In this situation all the baby's data will be removed from the study.

The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief/Principal Investigator. A copy of the signed Informed Consent will be given to the parents. The original signed form will be retained at the study site.

8. Screening and Eligibility Assessment

All English speaking parents of babies under the age of one that have had a HM's Coroner autopsy requested will be approached to consider whether or not they will allow their baby into the research programme. No parents will be approached of their baby has suffered a head injury or is suspected of suffering from child neglect, abuse or trauma.

9. Direct Access to Source Data/Documents

Direct access will be granted to authorised representatives from the sponsor, host institution and the regulatory authorities to permit study-related monitoring, audits and inspections.

10. Quality Control and Quality Assurance Procedures

The study will be conducted in accordance with the current approved protocol, ICH-GCP and relevant regulations and standard operating procedures.

Regular monitoring will be performed according to ICH GCP. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following written standard operating procedures, the monitors will verify that the study is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

The University, as Sponsor, operates a risk based monitoring system to which this study will be subject.

11. Ethics

The main ethical issue will be any distress to the parents when obtaining consent. It is important that clear, concise and empathetic communication is given to the parents by the consenter, an advanced nurse practitioner or medical consultant

11.1 Declaration of Helsinki

The Investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki (last amended October 2000, with additional footnotes added 2002 and 2004).

11.2 ICH Guidelines for Good Clinical Practise

The Investigator will ensure that this study is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996.

11.3 Approvals

The protocol, informed consent form and parent information sheet will be submitted to an appropriate Research Ethics Committee (REC), and host institution(s) for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

11.4 Patient Confidentiality

The only individuals that will be involved in this study are deceased infants. The data will be anonymised and keep in a secure facility.

12. Data Handling and Record Keeping

All data related to the project to be kept on an encrypted external hard drive with coded anonymity. External hard drives containing all study data/images to be kept in a secure, locked storage cabinet located in Professor Rutty's room, in the East Midlands Forensic Pathology Unit. Access to the room is via a proximity key card and additional unique door lock with limited user access. The room is alarmed and has a camera monitoring the external aspect of the door 24 hours a day.

13. Financing and Insurance

Financing of the study will be through a grant from the Home Office.

Arrangements for insurance will be made through the University of Leicester and NHS indemnity scheme.

14. Publication Policy

Please refer to University of Leicester's publication policy.

2B: Standard Operating Procedures

University of Leicester

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Chief Forensic Pathologist
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Post-mortem Optical Coherence Tomography Protocol

Author: Professor G N Rutty
Version: 1
Date: 09/01/2014

Research:

A Study of the Dural Venous Vasculature in Infants Under One Year of Age
NRES Committee East Midlands-Leicester
Study Number: 14/EM/0169

The following protocol will apply to the undertaking of Optical Coherence Tomography (OCT) of baby cadavers undergoing autopsy at the Leicester Royal Infirmary.

All research team members undertaking any procedure related to OCT must have been trained by a medical member of the research team and considered competent by their trainer before undertaking the procedure on their own.

1. Under the legal authority of H.M.Coroner or through the hospital permission autopsy route, the deceased baby who requires an autopsy examination is identified.
2. Consent is acquired by a medical member of the East Midlands Forensic Pathology Unit (EMFPU) or the project consenter for the cadaver to undergo OCT investigation during the autopsy.
3. Consent is acquired for one or both procedures.

Benchtop OCT

1. The skull vault bones are removed from the deceased as part of the normal invasive autopsy examination. This procedure will be done using a minimally disruptive technique with a specialist drill to ensure the dura mater underneath the skull bones remains intact.
2. An optical clearing agent is then applied to the dura mater to make the tissue transparent.
3. The internal jugular veins are then identified with an incision in the neck and cannulated. Water is then flushed into the veins via 2 syringes, up into the vessels in the head.
4. Whilst creating a flow of liquid within the vessels using the water the benchtop OCT is placed over the dura mater to begin mapping the location of vessels.

Rotational OCT

1. The skull vault bones are removed in the same procedure for benchtop OCT above.
2. A needle is then inserted into the anterior fontanelle to remove the blood within the sagittal sinus. A small incision is then made into the sinus and a catheter is inserted to facilitate the introduction of the OCT device for the length of the sinus.

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Digital Microscopy Protocol

Author: Professor G N Rutty
Version: 1
Date: 18/08/2014

Research:

A Study of the Dural Venous Vasculature in Infants Under One Year of Age
NRES Committee East Midlands-Leicester
Study Number: 14/EM/0169

The following protocol will apply to the undertaking of digital microscopy of bridging veins of infant cadavers undergoing autopsy at the Leicester Royal Infirmary (LRI).

All research team members undertaking any procedure related to digital microscopy must have been trained by a member of the research team and considered competent by their trainer before undertaking the procedure on their own.

This protocol applies to autopsies undertaken on the authority of H.M.Coroner. There are few (<1 a year) hospital requested autopsies in the age range of this study. All hospital consented post-mortems have a section relating to use of tissue for research. Only those consented hospital cases which specifically have consent for research on tissues will be used for the study. We do not anticipate that there will be a sufficient number of cases to study without using the Coroner requested autopsies.

1. Under the legal authority of H.M.Coroner or through the hospital permission autopsy route, the deceased baby who requires an autopsy examination is identified.
2. Consent is acquired by a medical member of the East Midlands Forensic Pathology Unit (EMFPU) or the project consenter for the cadaver to undergo bridging vein measurement, using the digital microscope, during and after the autopsy. This will take place in the Leicester Royal Infirmary mortuary which is a Human Tissue Authority licensed facility.

Digital Microscopy in the mortuary

1. The skull vault bones are removed from the deceased as part of the normal invasive autopsy examination. This procedure will be done using a minimally disruptive technique with a specialist drill to ensure the dura mater underneath the skull bones remains intact.
2. The dura mater is slowly lifted from the brain surface to visualise the bridging veins.
3. When a bridging vein is observed the digital microscope lens is placed above the vessel so that an image can be captured *in situ* for measurement of diameter. The digital microscope is attached to a laptop with software for viewing and measuring the bridging veins.

Digital Microscopy and mechanical testing

1. See step 1 above.
2. The dura mater is slowly lifted from the brain surface to visualise the bridging veins. Bridging veins consented for removal for mechanical testing (see mechanical testing of bridging veins protocol) will be carefully excised by cutting away a very small section of tissue where the vein enters the dura and the brain.
3. The excised veins will be orientated and temporarily preserved in a standard saline solution
4. Prior to mechanical testing the veins will be measured using the digital microscope, either in the mortuary, Robert Kilpatrick building or the engineering department.
5. The vein will be examined again by digital microscope after mechanical testing. Veins will then be fixed in histological preservative and brought to the histology lab, Level 3, Robert Kilpatrick building for storage till the end of the study.
6. All tissue will be tracked by the EMFPU tracking system from receipt to disposal.
7. At the end of the study all tissue will be sensitively disposed of using the University human tissue disposal system.

University of Leicester

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Histology Protocol

Author: Professor G N Rutty
Version: 1
Date: 18/08/2014

Research:

A Study of the Dural Venous Vasculature in Infants Under One Year of Age
NRES Committee East Midlands-Leicester
Study Number: 14/EM/0169

The following protocol will apply to the undertaking of microscopy of bridging veins of infant cadavers who have undergone autopsy at the Leicester Royal Infirmary (LRI).

All research team members undertaking any procedure related to microscopy must have been trained by a member of the research team and considered competent by their trainer before undertaking the procedure on their own.

This protocol applies to autopsies undertaken on the authority of H.M.Coroner. There are few (<1 a year) hospital requested autopsies in the age range of this study. All hospital consented post-mortems have a section relating to use of tissue for research. Only those consented hospital cases which specifically have consent for research on tissues will be used for the study. We do not anticipate that there will be a sufficient number of cases to study without using the Coroner requested autopsies.

1. Under the legal authority of H.M.Coroner or through the hospital permission autopsy route, the deceased baby who requires an autopsy examination is identified.
2. Consent is acquired by a medical member of the East Midlands Forensic Pathology Unit (EMFPU) or the project consenter for the removal of bridging veins to undergo microscopic evaluation after the autopsy. The removal of veins will take place in the Leicester Royal Infirmary mortuary which is a Human Tissue Authority licensed facility.

Histology

1. The skull vault bones are removed from the deceased as part of the normal invasive autopsy examination. This procedure will be done using a minimally disruptive technique with a specialist drill to ensure the dura mater underneath the skull bones remains intact.
2. The dura mater is slowly lifted away from the surface of the brain to visualise the bridging veins.
3. When a bridging vein is observed it is carefully excised by cutting away a very small section of tissue where the vein enters the dura and the brain.
4. The tissue will be orientated and placed in formalin for laboratory preparation and production of histological slides.
5. The preserved tissue will be taken directly by the researcher to the Robert Kilpatrick Building, histology laboratory, 3rd floor, where all processes will be undertaken. This is a Human Tissue Authority licenced facility.
6. Slides will be produced for histological examination of the veins. The assessment will be undertaken by the paediatric pathologist member of the research team.
7. All tissue, blocks and slides will be tracked by the EMFPU tracking system from receipt to disposal.
8. At the end of the study all tissue, blocks and slides will be sensitively disposed of using the University human tissue disposal system.

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Magnetic Resonance Imaging Protocol

Author: Professor G N Rutty
Version: 1
Date: 02/02/2015

Research:

A Study of the Dural Venous Vasculature in Infants Under One Year of Age
NRES Committee East Midlands-Leicester
Study Number: 14/EM/0169

The following protocol will apply to the undertaking of Magnetic Resonance Imaging (MRI) of brains from infants who have undergone autopsy at the Leicester Royal Infirmary (LRI).

All research team members undertaking any procedure related to MRI must have been trained by a member of the research team and considered competent by their trainer before undertaking the procedure on their own.

1. Under the legal authority of H.M.Coroner or through the hospital permission autopsy route, the deceased baby who requires an autopsy examination is identified.
2. Consent is acquired by a medical member of the East Midlands Forensic Pathology Unit (EMFPU) or the project consenter for the brain to undergo MRI after the post-mortem whilst the brain is being fixed in formalin, and before the brain is examined by a paediatric pathologist as part of the normal autopsy examination.

MRI protocol

1. The brain is removed from the deceased and placed in formalin for fixation as part of the normal invasive autopsy examination. The brain will be stored within the LRI mortuary as per normal autopsy protocol.
2. The brain will remain in formalin until examined by a paediatric pathologist (approximately 7 days). During these 7 days the brain will be taken to the Radiology Department within the Leicester Royal Infirmary for MRI. The examination will be undertaken outside normal clinical hours by a radiographer trained in MRI at a time convenient to the MRI department.
3. Prior to scanning the brain will be signed out of the mortuary by a member of the research team to allow for tracking of the organ and signed in when it is returned from scanning.
4. The brain will be taken direct to MRI by a member of the research team who will remain with the brain during scanning.
5. After scanning, the brain will be returned to the mortuary by a member of the research team. It will be stored by the mortuary prior to examination by the paediatric pathologist.
6. The images generated from the MR scanning will be retrieved from the UHL PACS by a medical member of the research team. They will be made anonymous. They will be analysed by appropriate imaging software which will be used to generate 3D CAD data for the anonymous building of a 3D CAD brain map.

Magnetic Resonance Imaging Protocol
Ref: 14/EM/0169

02/02/2015 Version 1

University of Leicester

School of Medicine

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Mechanical Testing of Bridging Veins Protocol

Author: Professor G N Rutty
Version: 2
Date: 21/05/2015

Research:

A Study of the Dural Venous Vasculature in Infants Under One Year of Age
NRES Committee East Midlands-Leicester
Study Number: 14/EM/0169

The following protocol will apply to mechanical testing of bridging vessels removed from infant cadavers undergoing autopsy at the Leicester Royal Infirmary (LRI).

All research team members undertaking any procedure involving the removal of bridging veins and mechanical testing must have been trained by a medical member of the research team and considered competent by their trainer before undertaking the procedure on their own.

This protocol applies to autopsies undertaken on the authority of H.M.Coroner. There are few (<1 a year) hospital requested autopsies in the age range of this study. All hospital consented post-mortems have a section relating to use of tissue for research. Only those consented hospital cases which specifically have consent for research on tissues will be used for the study. We do not anticipate that there will be a sufficient number of cases to study without using the Coroner requested autopsies.

1. Under the legal authority of H.M.Coroner or through the hospital permission autopsy route, the deceased baby who requires an autopsy examination is identified.
2. Consent is acquired by a medical member of the East Midlands Forensic Pathology Unit (EMFPU) or the project consenter for retention of bridging veins during the autopsy. The removal of the tissue will take place in the Leicester Royal Infirmary mortuary which is a Human Tissue Authority licensed facility.

Removal of bridging veins and mechanical testing

1. The skull vault bones are removed from the deceased as part of the normal invasive autopsy examination. This procedure will be done using a minimally disruptive technique with a specialist drill to ensure the dura mater underneath the skull bones remains intact.
2. The dura mater is slowly lifted back from the surface of the brain. When a bridging vein is observed it is carefully excised by cutting away a very small section of tissue where the vein enters the dura and the brain.
3. The excised bridging veins will be orientated and temporarily preserved in a standard saline solution.
4. The tissue will be transported to the University of Leicester, Department of Engineering where they will undergo mechanical testing. Prior to mechanical testing the vein will be examined using microscopy (see microscopy and digital microscopy protocols). Some veins may be sent to the Department of Mechanical Engineering, KU Leuven, Belgium for stretch testing. These veins will be taken to the histology lab, Level 3, Robert Kilpatrick Building immediately after the post-mortem. They will be snap frozen in isopentane and stored in the -80 °C freezer whilst delivery arrangements are made to send the veins to KU Leuven. Samples will then be transported on dry ice.
5. Each end of the vein will be clamped to a mechanical testing machine and the vein will then be stretched until mechanical failure of the vessel occurs. Mechanical characteristics will be measured by the machine during the procedure.
6. The remains of the tissue will then be examined again by microscopy (see protocols). It will then be brought to the histology laboratory, Level 3, Robert Kilpatrick Building for storage till the end of the study.
7. All tissue will be tracked by the EMFPU tracking system from receipt to disposal.
8. At the end of the study all tissue will be sensitively disposed of using the University human tissue disposal system.

2C: Parent Summary Information Sheet

University of Leicester

School of Medicine

Department of Cancer Studies & Molecular Medicine

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Chief Forensic Pathologist
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Parent Information Sheet
OCT telephone verbal consent information sheet
Version 3 February 2015

SUMMARY SHEET

A Study of the Dural Venous Vasculature in Infants Under One Year of Age

Thank you for agreeing to allow your baby to take part in this research study. We are extremely grateful to you for this.

The following document is a short summary of the study which either a doctor or the study consenter from the East Midlands Forensic Pathology Unit has already talked to you about. There is a more detailed information sheet available to you should you wish to read it.

Questions
It is important to us that you understand the purposes of this study. However we appreciate that this is a very sensitive and emotional subject to try and explain to you at this time. We have tried to do so in a way that provides you with sufficient information to understand the study but at the same time do not add further distress to you at your time of loss. We have tried to write this information sheet in non-medical terms but there may be terms used within it that you may still feel unsure about.

We advise that this summary sheet should be explained to you by your General Medical practitioner who will be able to explain any terms that you do not understand and provide support to you whilst reading this document.

Section 1. General information

The following section of the document provides general information as to why you have been approached by the research team. Information about the research study is found within the second section of this document.

Why has my baby been chosen for this study?
As an autopsy is to be undertaken on your baby you have been approached to consider whether or not you will allow us to undertake an additional research procedure during the autopsy examination.

Why did you not come and see me in person?
We have approached you by phone and not in person because the time between us being notified that an autopsy was requested, and the examination is very short. We are not allowed to do anything that slows down this process.

Do I have to allow my baby to take part?
As you have already decided to allow them to take part we have sent you this information sheet, as requested by you, to keep and consider. You are free to withdraw your baby from the study at any time, for any reason and with no obligation to give a reason for the withdrawal. If you decide this all your baby's data will be removed from the study.

What do I have to do?
The only thing for you to do is to consider this document to ensure that your questions have been fully answered.

What are the alternatives for diagnosis or treatment?
None. We are unable to undertake the study without the assistance of the relatives of the deceased. There is no alternative model or age group we can consider to answer these important questions.

What are the side effects of any procedure to your baby when taking part?
There are no adverse effects to your baby taking part in this study.

What are the possible disadvantages and risks of taking part?
None

What are the possible benefits of taking part?
There is no direct benefit to your baby in taking part.

What happens when the research study stops?
If you do not permit the information gathered during the research to be retained for teaching and training purposes they will be destroyed at the end of the study.

What if something goes wrong?
If your baby is harmed by taking part in this research project, there are no special compensation arrangements. If your baby is harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or your relative treated during the course of this study, the normal National Health Service complaints mechanisms would be available to you.

Will my taking part in this study be kept confidential?
The answer to this question is "yes".

What will happen to the results of the research study?
The results from this study will be presented and published to advance medical knowledge. The results from this study will **NOT** be used to assist in the diagnosis of the cause of your baby's death. It will not be released to HM Coroner or yourself.

Who is organising and funding the research?
The work is at present being funded by the Home Office.

Who has reviewed the study?
The local NHS Research Ethics Committee and the relevant University of Leicester sponsor committee.

Section 2. Research request information

The following section of the document provides information related to the research which has been requested to be undertaken on your baby.

What is the purpose of the study?
By using modern day clinical imaging devices and microscopy we wish to study the anatomy of the tiny veins that are found under the thick membrane inside the head that covers the brain (the "dura") and which drain into a large vessel that runs along the top of the inside of the head known as the "sagittal sinus". We also wish to removal several veins for mechanical investigations. Tests will be performed to assess the "stretch" of vessels and microscopy evaluation of the veins will be done before and after these tests. In doing this we hope to try and identify veins which, for a variety of reasons, and ***not*** in your baby's case, can cause bleeding to occur inside the head of babies.

What is the research procedure?
During the autopsy examination we wish to examine the anatomy of the dural veins using a medical device which has a short hand name of "OCT". This will be used to image the tiny veins related to the dura. We also wish to take several of these veins for further microscopic and stretch investigations in the days after the post mortem examination. We may also wish to perform a scan of the brain itself using a patient imaging system known as 'MR'.

What will happen to my baby if I allow them to take part?
By consenting for your baby to take part in this study we will attempt to image the veins of the dura with one or both types of OCT clinical device. We may image the brain using MRI. All images and data will be made anonymous.

Contact for Further Information
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East Midlands Forensic Pathology Unit
University of Leicester

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2D: Parent Detailed Information Sheet

University of Leicester

School of Medicine

Department of Cancer Studies & Molecular Medicine

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Chief Forensic Pathologist
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Parent Information Sheet
OCT telephone verbal consent information sheet
Version 5 May 2015

A Study of the Dural Venous Vasculature in Infants Under One Year of Age

Thank you for agreeing to allow your baby to take part in this research study. We are extremely grateful to you for this.

Although a doctor or the study consenter has talked to you by telephone already it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask us if there is anything that is not clear or if you would like more information. Our contact information is found at the top of this sheet and at the end of this document. Please take time to decide whether or not you wish to continue to take part this study.

Questions
It is important to us that you understand the purposes of this study. However we appreciate that this is a very sensitive and emotional subject to try and explain to you at this time. We have tried to do so in a way that provides you with sufficient information to understand the study but at the same time do not add further distress to you at your time of loss. We have tried to write this information sheet in non-medical terms but there may be terms used within it that you may still feel unsure about.

We advise that this information sheet should be explained to you by your General Medical practitioner who will be able to explain any terms that you do not understand and provide support to you whilst reading this document.

Section 1. General information

The following section of the document provides general information as to why you have been approached by the research team. Information about the research study is found within the second section of this document.

Why has my baby been chosen for this study?
Her Majesty's Senior Coroner has requested that an autopsy is to be undertaken on your baby. As a result of this you have been approached to consider whether or not you will allow us to undertake an additional research procedure during the autopsy examination.

We understand that this is a very sensitive request and one that will be difficult to consider. However the answer to the study question outlined in section 2 can only be sought by the examination of human babies at autopsy and as we have no other means of undertaking such research other than making this request we believe, despite the sensitivity of our request, that this request is appropriate to make to you.

Why did you not come and see me in person?
The reason that we approached you by phone and not in person was because the time period between us being notified that an autopsy was requested by HM Coroner, and the undertaking of the examination is very short. We are not allowed to do anything that slows down the process of HM Coroner. There will be other research requests to different families on the same day from our Unit, so although we would wish to meet with you, it is not possible for us to visit each and every person personally, especially if relatives live outside the immediate City of Leicester boundaries. Thus we have had to take the option of using the telephone to speak to you.

Do I have to allow my baby to take part?
It is up to you to decide whether or not to allow your baby to take part.

As you have already decided to allow them to take part we have sent you this information sheet, as requested by you, to keep and consider. If you decide not to allow them to continue to take part in this study, it will not affect the autopsy that is to be undertaken on your baby.

You are free to withdraw your baby from the study at any time, for any reason and with no obligation to give a reason for the withdrawal. Due to the time scale set by HM Coroner for the autopsy it is possible that you may decide not to allow your baby to take part in this study after the procedure has occurred. In this situation all your baby's data will be removed from the study.

What do I have to do?
The only thing for you to do is to consider this document to ensure that your questions have been fully answered.

What are the alternatives for diagnosis or treatment?
None. We are unable to undertake the study without the assistance of the relatives of the deceased. There is no alternative model or age group we can consider to answer these important questions.

What are the side effects of any procedure to your baby when taking part?

- There are no side effects or complication to your baby of the research technique
- There will be no effect on the choice of cremation or burial of your baby.
- There will be no delay incurred prior to the autopsy examination that could delay the release of your baby from the mortuary.
- Whether or not your baby takes part in the study they will still have the autopsy requested by the Coroner's Office.

What are the possible disadvantages and risks of taking part?

None

What are the possible benefits of taking part?

There is no direct benefit to your baby in taking part. The benefit will be to the medical profession in the consideration to the answer to the research question.

What happens when the research study stops?

If you do not permit the information gathered during the research to be retained for teaching and training purposes they will be destroyed at the end of the study.

What if something goes wrong?

If your baby is harmed by taking part in this research project, there are no special compensation arrangements. If your baby is harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or your relative treated during the course of this study, the normal National Health Service complaints mechanisms would be available to you.

Will my taking part in this study be kept confidential?

All information which is collected concerning your baby during the course of the research and teaching/training will be kept strictly confidential. Any data gathered during this research in relation to your baby which leaves the hospital will have their name and address removed so that they cannot be recognised from it.

What will happen to the results of the research study?

The following will happen:

- The anonymised results of the study will be made available to the Home Office as they are funding the research.
- The anonymised results will be used for higher degrees (Doctorates, PhD's) which will be held within the University of Leicester Library
- The anonymised results will be presented at local, national or international scientific meetings
- The anonymised results will be published within medical peer reviewed journals

The results from this study will **NOT** be used to assist in the diagnosis of the cause of your baby's death. It will not be released to HM Coroner or yourself.

Who is organising and funding the research?
The work is at present being funded by the Home Office.

Who has reviewed the study?
All research that involves NHS patients or staff, information from NHS medical records or uses NHS premises or facilities must be approved by an NHS Research Ethics Committee before it goes ahead. It has also been reviewed by the relevant University of Leicester sponsor committee. Approval does not guarantee that you will not come to any harm if you take part. However, approval means that the committee is satisfied that your rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits and that you have been given sufficient information on which to make an informed decision.

Section 2. Research request information

The following section of the document provides information related to the research which has been requested to be undertaken on your baby.

What is the purpose of the study?
By using modern day clinical imaging devices we wish to study the anatomy of the tiny veins that are found under the thick membrane inside the head that covers the brain (the "dura") and which drain into a large vessel that runs along the top of the inside of the head known as the "sagittal sinus". In doing this we hope to try and identify veins which, for a variety of reasons, and ***not*** in your baby's case, can cause bleeding to occur inside the head of babies. When this happens it can make a baby very sick and on occasion may be life threatening. The commonest type of bleeding that is encountered has a medical name of a "sub-dural haematoma" which can be shortened to "SDH". This type of bleeding accumulates below the dura. Although it is accepted that the bleeding occurs from a vein, not an artery, the source of this bleeding is disputed within the medical literature. When these veins bleed, it is thought to be due to "stretching" of the vessel. We wish to study this stretching in more detail by mechanically testing these veins to the point of failure (i.e. when the vessel breaks). We may also wish to scan the brain itself by using a medical imaging machine known as MRI, to enable us to create a 3D model of a brain to show the locations of the veins between the surface of the brain and the dura.

What is the research procedure?
During the autopsy examination we wish to examine the anatomy of the dural veins using a medical device which has a short hand name of "OCT".

OCT is a clinical imaging system that uses near-infrared light to produce very detailed three-dimensional images of translucent or opaque materials. This type of imaging is currently used for example for the clinical examination of children's eyes as well as the blood vessels that supply blood to the heart in those suspected of having a heart attack. OCT has the potential to inform us of the anatomy of the dural veins of babies

both from the outside and the inside of the vessels in more detail than we can see with our own eyes.

As part of the process of using OCT a sugar based solution will need to be applied to the dura shortly before OCT imaging. This will cause the dura to become translucent. There will be no lasting effect of this process as the dura will return to its natural state with the application of water after OCT imaging.

To mechanically test the "stretch" and failure limit of the vessels, several veins will be removed during the autopsy. Tests will then be performed to assess the stretch of these vessels. The veins will undergo microscopic evaluation before and after stretching.

During the autopsy the diameter of veins will be measured using a non-contact digital microscope. Veins removed for stretching will also be measured by the digital microscope.

When the brain is temporarily retained following the autopsy examination we may perform a magnetic resonance imaging (MRI) scan. This type of scan produces very detailed two and three-dimensional images of the body. It is used in day-to-day clinical practice to examine patients for the presence of disease in the organs and bones of the body and it produces more information than traditional x-ray examinations.

What will happen to my baby if I allow them to take part?

By consenting on the telephone for your baby to take part in this research programme they will first be allocated a unique code by a coding officer to ensure that your baby's details are kept anonymised within the study.

The dura will be examined by the naked eye during the normal autopsy procedure. It is only because we want to examine this area in more detail that we need to use imaging systems and as this is not part of the standard autopsy we need to ask for your permission.

What occurred during the study depends upon what was discussed with you on the phone and what you consented to. Several different scenarios are presented here which may or may not have occurred. **Please be assured that only those procedures you have consented to happened.**

- If you consented for a rotational OCT to be used then the OCT probe will have been passed down the sagittal sinus to visualise the vessel.
- If you consented for a benchtop OCT to be used then the sugar solution would have been applied to the dura. A small amount of water was injected into two large veins and the veins were imaged using the benchtop OCT system.
- If you consented to digital microscopy during the autopsy a microscope lens was placed over each bridging vein to measure the diameter.
- If you consented to removal of bridging veins, several veins were dissected and stored in saline for microscopy evaluation and stretching tests.

- If you consented to MR scanning, the brain was placed in a MR machine and scanned prior to examination by a paediatric pathologist as part of the autopsy examination.

After the examinations have concluded;

- If you have consented for the images to be used for future teaching and training they will be stored at the EMFPU. This is a secure storage system. No identifying details will be available on the images so these images cannot be identified as coming from your relative.

Contact for Further Information

Professor Guy N Rutty,
Chief Forensic Pathologist
East Midlands Forensic Pathology Unit
University of Leicester

Tel: 0116 252 3221
Fax: 0116 252 3274
Email: gnr3@le.ac.uk

Thank you for reading this.
You will be given a copy of the information sheet and a signed consent form to keep

2E: Consent Form

University of Leicester

School of Medicine

Department of Cancer Studies & Molecular Medicine

East Midlands Forensic Pathology Unit
Level 3, Robert Kilpatrick Building
Leicester Royal Infirmary
PO Box 65
Leicester LE2 7LX, UK
Tel: +44 (0)116 252 3221
Fax: +44 (0)116 252 3274

Chief Forensic Pathologist
Professor Guy Rutty
MBE MBBS MD FRCPath DipRCPath(Forensic) FFSSoc FFFLM

Study Number: 14/EM/0169
Patient Identification Number for this trial:

TELEPHONE VERBAL CONSENT FORM
Coroner's cases
Version 4 February 2015

Title of Project:

A Study of the Dural Venous Vasculature in Infants Under One Year of Age

Name of Researcher:

Professor Guy N Rutty,
Chief Forensic Pathologist
East Midlands Forensic Pathology Unit
University of Leicester
Tel: 0116 252 3221
Fax: 0116 252 3274
Email: gnr3@le.ac.uk

To be completed by the Study Consenter or medical member of the EMFPU during the time of the telephone consenting process. Please initial each box to confirm verbal consent procedure and consent was given.

I confirm that I spoke by phone to (insert parents name)…… on (insert date)……	
I confirm that the parent of the deceased gave informed verbal consent for their baby's participation in this study.	
I confirm that the parent of the deceased *did* or *did not* (delete as appropriate) give informed verbal consent for benchtop OCT for the purpose of this study.	
I confirm that the parent of the deceased *did* or *did not* (delete as appropriate) give informed verbal consent for rotational OCT for the purpose of this study.	
I confirm that the parent of the deceased *did* or *did not* (delete as appropriate) give informed verbal consent for the removal of bridging veins for the purpose of this study.	
I confirm that the parent of the deceased *did* or *did not* (delete as appropriate) give informed verbal consent for digital microscopy for the purpose of this study.	
I confirm that the parent of the deceased *did* or *did not* (delete as appropriate) give informed verbal consent for the mechanical stretching tests of bridging veins for the purpose of this study.	
I confirm that the parent of the deceased *did* or did *not* (delete as appropriate) give informed verbal consent for the transportation of bridging veins to KU Leuven for mechanical testing for the purpose of this study.	
I confirm that the parent of the deceased *did* or *did not* (delete as appropriate) give informed verbal consent for the microscopic examination of bridging veins undergoing mechanical stretching tests.	
I confirm that the parent of the deceased *did* or *did not* (delete as appropriate) give informed verbal consent for MR imaging.	
I confirm that the parent of the deceased *did* or *did not* (delete as appropriate) give informed verbal consent for the study images to be used for teaching and training purposes.	
I confirm (delete as appropriate) *yes or no* that the parent of the deceased wished to receive the summary information sheet (Version 3, dated 02/02/2015).	
I confirm that the parent of the deceased gave informed verbal consent for the research records/medical notes to be viewed by the Sponsor or their representative and/or the UHL for the purposes of monitoring/audit.	

Name of Person taking verbal consent	Date	Signature

Insert address for information sheet to be sent to if different from that on coroner's officer's death report:

2F: Consent Script

University of Leicester

School of Medicine

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Chief Forensic Pathologist
Professor Guy Rutty
MBE MBBS MD FRCPath DipRCPath(Forensic) FFSSoc FFFLM

Script for OCT telephone verbal consent
Coroner's cases
Version 1 January 2014

A Study of the Dural Venous Vasculature in Infants Under One Year of Age

The following script provides a guide line for the consenter of the study entitled above when approaching parents via the telephone. While all aspects of the script will be thoroughly covered by the consenter the script is only provided as a guild line, each parent approached by the consenter will be treated as an individual ensuring every parent has a full understanding of what they are being asked during a difficult time of grief.

1) General Introductions. The name and contact telephone number for the parent will have been provided by HM Coroner. The Coroner's office will already have been in contact with the parent to inform them of a possible phone call from the Unit. The consenter will begin by explaining who they are and that they work as part of the Unit.
2) Confirm identify of the parent and ask if convenient to speak to them. The consenter will ensure the person receiving the phone call answers to the correct name and will also check whether the Coroner's office have been in contact. This will aid the consenter in verifying who they are speaking to.
3) Express condolences, sympathetic listening if parent wishes to talk about matters surrounding the death.
4) Explain post mortem examination is scheduled to take place and clarify that the parent understands why this is being undertaken (i.e. Coronial law).
5) Explain current research. The consenter will take into account the educational level and language ability of the parent they are speaking to and the use of complex medical and scientific terminology will be avoided unless deemed appropriate.

6) Explain nature and stages of the trial, advise that no delay to the post mortem or release of the body will occur. Ensure that the parent is aware that they can withdraw from the study, however the procedure may already have occurred due to the time restriction of the autopsy set by HM Coroner. Explain that if they withdraw their baby from the study all data will be removed.
7) Obtain oral consent for their baby to enter the study, and for each stage (if applicable):
    a. Benchtop OCT
    b. Rotational OCT
    c. Use of anonymised images for teaching and research
8) Record any opinions or questions given by the parent
9) If permission is not granted, express thanks and re-assure their wishes will be honoured.
10) If consented to all, or parts of the study, ask if parent would like an information sheet sent to them in the post the next working day.
11) Ask if they have any further questions regarding the study or the post mortem.
12) If questions raised regarding general bereavement issues or questions outside the scope of study then individual advised to contact the coroner's office.
13) Consent form signed by the consenter documenting whom consent was obtained from, the date of consent and what was consented to e.g. 1 or both types of OCT.

Appendix 3

Case details for all 68 patients, including; sex, age, cause of death and associated features, and types of procedure undertaken.

Case Number	Sex	Body weight (g)	Age	Cause of death/associated features	Neurosurgical skull removal	*In situ* clearing	Extracorporeal clearing	Dura Histology	Bridging vein histology	Photographical mapping of bridging veins	Digital Microscopy	Mechanical Testing	MRI	Rotational OCT	Surface OCT
1	M	3948	1 day, 36 wks GA	Antepartum asphyxiation (stillbirth).	×	×	✓g	✓	×	×	×	×	×	×	×
2	F	4505	1 day	Birth trauma, instrumental delivery (suction cup) subgaleal haemorrhage	✓	✓	×	×	×	✓	×	×	×	✓	✓
3	M	2795	1 day	Report to be completed, SDH, most likely birth-related injury	✓	✓	×	×	×	×	×	×	×	×	×
4	F	4941	1 day	Perinatal asphyxiation and head injury	✓	✓	×	×	×	✓	✓	×	×	✓	×
5	M	3617	1 day	Perinatal head injury	✓	×	×	×	×	✓	×	×	×	×	×
6	M	3592	3 days	HIE, perinatal asphyxiation, uteroplacental insufficiency	✓	×	×	×	×	✓	×	×	×	×	×
7	M	3412	3 days	HIE, uteroplacental insufficiency and ruptured vasa previa	✓	✓	×	×	×	✓	×	×	×	✓	✓
8	F	3137	3 days	Pulmonary haemorrhage; subtle congenital anomalies	✓	×	×	×	×	✓	×	×	×	×	×
9	M	3195	3 days	Persistent pulmonary hypertension of the newborn, patent ductus arteriosus	✓	×	×	×	×	✓	×	×	×	×	×

10	M	2828	3 days	HIE, perinatal asphyxiation, uteroplacental insufficiency	×	×	✓g	✓	×	×	×	×	×	×	×
11	M	2561	3 days	HIE	✓	×	×	×	×	✓	×	×	×	×	×
12		2615	6 days	Bowel perforation, perinatal head trauma	✓	×	×	×	×	✓	×	×	×	×	×
13	F	5615	8 days	Lung dysplasia	✓	×	×	×	×	✓	×	×	×	×	×
14	M	3369	10 days	Unascertained, co-sleeping	✓	✓	×	×	×	×	×	×	×	×	×
15	F	2263	12 days	Positional asphyxia, co-sleeping	✓	✓	×	×	×	✓	×	×	×	×	×
16	F	3352	13 days	Herpes simplex virus encephalitis, perinatal head trauma	✓	×	×	×	×	×	×	×	×	×	×
17	M	4820	16 days	Report to be completed, no obvious head injury	✓	×	×	×	×	×	✓	✓	×	×	×
18	M	4405	17 days	HSV infection	✓	×	×	×	×	✓	×	×	×	×	×
19	M	4553	24 days	Pulmonary haemorrhage	✓	✓	×	×	×	✓	×	×	×	×	×
20	F	4028	26 days	Unascertained, SUDI, co-sleeping	✓	✓	×	×	×	✓	×	×	×	×	×
21	M	3470	4 weeks	Pulmonary haemorrhage	✓	✓	×	×	×	✓	×	×	×	×	×
22	M	2983	4 weeks	Unascertained, SUDI	✓	✓	×	×	×	✓	×	×	×	×	×
23	M	4544	4 weeks	Report to be completed, subarachnoid haemorrhage	✓	×	×	×	×	✓	×	×	×	×	×
24	F	5708	5 weeks	Report to be completed, no obvious head injury	✓	×	×	×	×	×	×	×	×	×	×
25	F	3070	6 weeks	Unascertained, co-sleeping, possible positional asphyxiation	✓	✓	×	×	×	✓	×	×	×	×	×
26	M	5126	6 weeks	Unascertained, pulmonary haemorrhage	×	×	✓g	×	×	×	×	×	×	×	×
27	M	5283	6 weeks	Mitochondrial defect	✓	×	×	×	×	×	×	×	×	×	×
28	M	6158	7 weeks	Unascertained, SUDI, co-sleeping	✓	×	✓m	×	×	×	×	×	×	×	×
29	M	4410	7 weeks	Unascertained, SUDI, co-sleeping	✓	✓	×	×	×	×	×	×	×	×	×
30	F	2477	8 weeks	Unascertained, SUDI, prematurity, twin	✓	✓	×	×	×	×	×	×	×	×	×
31	M	5136	8 weeks	SIDS	✓	✓	×	×	×	✓	×	×	×	×	×

32	M	5004	8 weeks	Unascertained, SUDI, co-sleeping	✓	×	×	×	×	✓	×	×	×	✓	×
33	F	4826	9 weeks	SIDS	✓	×	×	×	×	✓	×	×	×	×	×
34	M	5461	9 weeks	Head injury, suspected AHT	✓	✓	×	×	×	✓	×	×	×	×	×
35	M	2763	9 weeks	Unascertained, dysmorphic	×	×	✓g	×	×	×	×	×	×	×	×
36	M	4648	9 weeks	External airway obstruction, co-sleeping	✓	✓	×	×	×	✓	×	×	×	✓	×
37	M	4789	9 weeks	Unascertained, SUDI, co-sleeping	✓	×	×	×	×	✓	×	×	×	×	×
38	F	4581	11 weeks	Positional asphyxia, co-sleeping	✓	×	×	×	✓	×	×	×	✓	×	×
39	M	3525	14 weeks	Overlaying, co-sleeping	✓	✓	×	×	×	✓	×	×	×	×	×
40	F	4799	15 weeks	Haemopericardium secondary to Kawasaki disease	✓	✓	×	×	×	×	×	×	×	×	×
41	M	5841	15 weeks	SIDS	✓	✓	×	×	×	✓	×	×	×	×	×
42	M	6059	15 weeks	SIDS	✓	✓	×	×	×	✓	×	×	×	×	×
43	F	4965	16 weeks	Positional asphyxia, restrictive seating device	✓	×	×	×	×	✓	×	×	×	×	×
44	M	7264	17 weeks	AHT	✓	✓	×	×	×	✓	×	×	×	×	×
45	M	5124	20 weeks	Positional asphyxia, restrictive seating device	✓	×	×	×	×	×	×	×	×	×	×
46	M	5355	21 weeks	Unascertained, failure to thrive	✓	✓	×	×	×	×	×	×	×	×	×
47	M	7614	21 weeks	Unascertained	✓	✓	×	×	×	×	×	×	×	×	×
48	M	9095	21 weeks	SIDS	✓	×	×	×	×	✓	×	×	×	×	×
49	M	8324	23 weeks	Unascertained, SUDI, viral URTI, hyperthermia	✓	×	×	×	×	×	×	×	×	×	×
50	F	6894	23 weeks	Unascertained, SUDI, co-sleeping	✓	✓	×	×	×	✓	×	×	×	×	×
51	M	9916	25 weeks	Unascertained, SUDI, co-sleeping	✓	×	×	×	×	✓	×	×	×	×	×
52	F	7240	27 weeks	Dog attack, head injury	✓	✓	×	×	×	✓	×	×	×	×	×
53	M	8878	28 weeks	Unascertained, SUDI, co-sleeping	✓	✓	×	×	×	×	×	×	×	×	×
54	F	6339	29 weeks	Smoke inhalation	✓	✓	×	×	×	✓	×	×	×	×	×

55	M	4642	30 weeks	Unascertained, SUDI, failure to thrive	✓	✓	×	×	×	×	×	×	×	×	×
56	F	8041	31 weeks	Head injury (possible AHT)	✓	✓	×	×	✓	✓	×	×	×	×	×
57	F	9160	43 weeks	Unascertained, SUDI, co-sleeping	✓	×	×	×	×	✓	×	×	×	×	×
58	M	7131	45 weeks	RSV bronchiolitis	✓	×	×	×	×	✓	×	×	×	×	×
59	F	13000	58 weeks	Unascertained, possible external airway obstruction	✓	✓	×	×	×	✓	×	×	×	×	×
60	M	14000	14 months	HIE, cause unascertained	✓	✓	×	×	×	✓	×	×	×	×	×
61	M	9600	16 months	Unascertained, SUDIC	✓	✓	×	×	×	×	×	×	×	×	×
62	M	12045	18 months	Unascertained, SUDIC	✓	×	×	×	×	×	×	×	×	×	×
63	F	13778	18 months	Unascertained, SUDI, prone sleeping, recurrent febrile convulsions	✓	×	×	×	×	✓	×	×	×	×	×
64	M	12500	18 months	Unascertained, SUDIC	✓	✓	×	×	×	✓	×	×	×	×	×
65	F	11200	20 months	Sharp force extracranial trauma	✓	✓	×	×	×	✓	×	×	×	×	×
66	M	8190	29 months	Cystic encephalomalacia and epilepsy	✓	✓	×	×	×	✓	×	×	×	×	×
67	F	12296	37 months	SUDEP	✓	✓	×	×	×	×	×	×	×	×	×
68	M	15000	37 months	Sepsis and pneumothorax, congential anomalies	✓	×	×	×	×	×	×	×	×	×	×
				Total Case Numbers	64	36	5	2	2	43	2	1	1	5	2

g- glycerol, m- mannitol, GA- gestational age, HIE- hypoxic-ischaemic encephalopathy, HSV- herpes simplex virus, SUDI- sudden unexpected death in infancy, SUDIC- sudden unexpected death in childhood, SIDS- sudden infant death syndrome SUDEP- sudden unexpected death in epilepsy, URTI- upper respiratory tract infection RSV- respiratory syncytial virus

Appendix 4

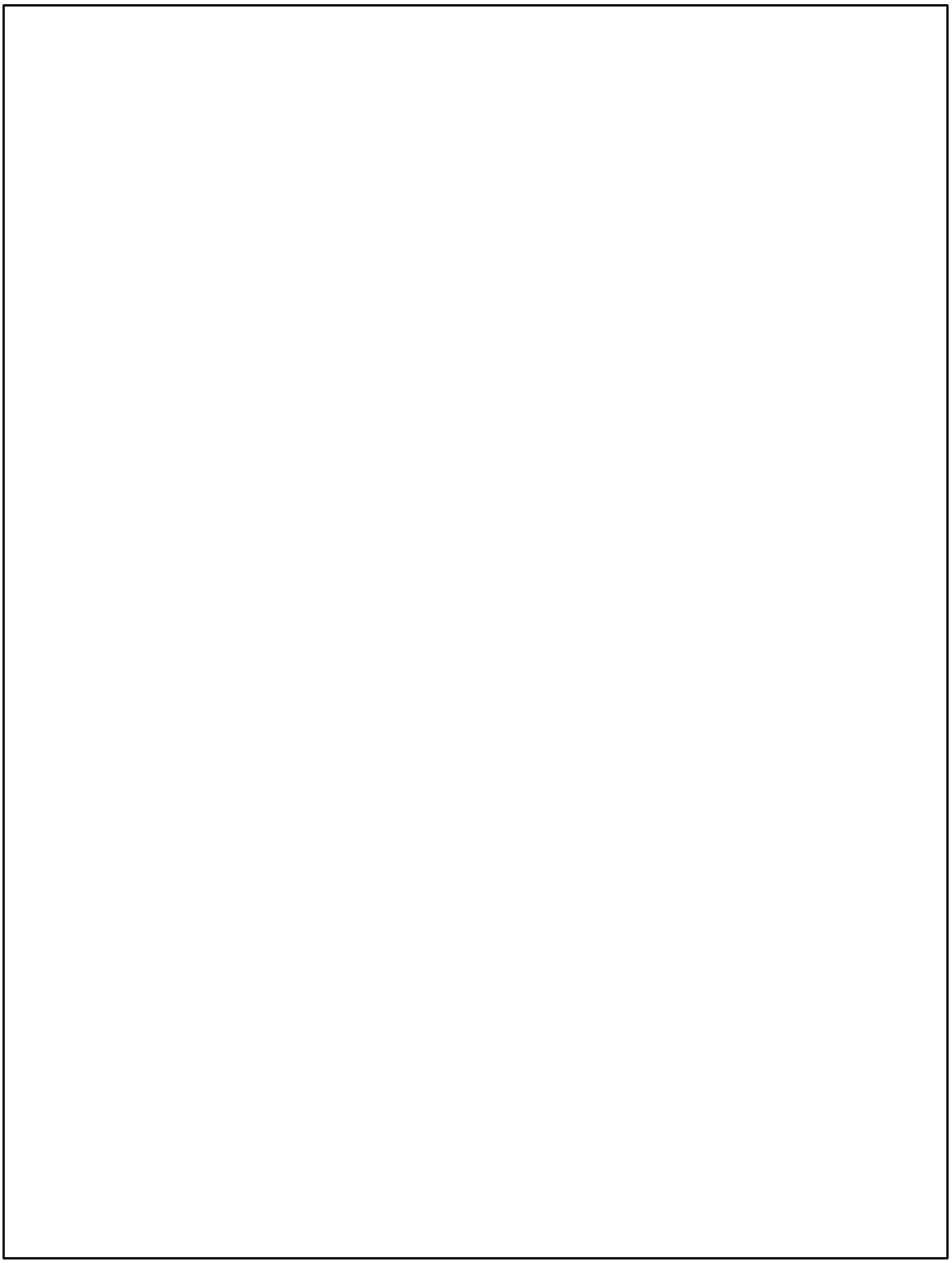
Raw data for vein counts using digital photographs and tally counter.

Case number from appendix 3	Vein counts from the digital photographs				Total with tally counter
	left hemisphere	right hemisphere	cerebellum	total	
2	28	31	8	67	60
4	13	12	5	30	37
5	19	22	4	45	n/a
6	24	30	17	71	81
7	18	22	5	45	49
8	21	19	11	51	53
9	21	20	10	51	49
11	23	19	11	53	60
12	15	11	7	33	n/a
13	28	25	11	64	70
15	23	27	6	56	55
18	23	23	8	54	51
19	15	23	5	43	44
20	25	25	8	58	63
21	37	18	11	66	74
22	18	30	14	62	66
23	25	27	5	57	58
25	21	17	10	48	n/a
31	23	23	11	57	n/a

32	19	17	15	51	48
33	21	31	8	60	n/a
34	7	13	7	27	22
36	24	26	8	58	54
37	23	21	6	50	51
39	35	24	12	71	74
41	28	20	8	56	54
42	29	21	13	63	n/a
43	15	16	8	39	40
44	12	15	3	30	n/a
48	22	20	7	49	n/a
50	19	14	7	40	n/a
51	37	35	13	85	90
52	21	19	4	44	46
54	26	19	9	54	62
56	11	12	11	34	34
57	21	27	10	58	n/a
58	27	31	11	69	80
59	42	40	12	94	92
60	38	26	11	75	n/a
63	11	17	8	36	n/a
64	11	29	12	52	n/a
65	21	14	10	45	n/a
66	36	26	13	75	n/a
Totals	**976**	**957**	**393**	**2326**	**1390**

n/a- cases early on in the series, before the post-mortem use of the tally counter

Appendix 5: Publications



The following published articles have been removed from pages 211-225 of the electronic version of this thesis due to copyright restrictions:

Visualisation of the intact dura mater and brain surface in infant autopsies: a minimally destructive technique for the post-mortem assessment of head injury

Cheshire, E et al. Int J Legal Med (2015) 129: 307. doi:10.1007/s00414-014-1110-1

Optical clearing of the dura mater using glycerol: a reversible process to aid the post-mortem investigation of infant head injury

Cheshire, E et al. Forensic Sci Med Pathol (2015) 11: 395. doi:10.1007/s12024-015-9691-7

The unabridged version can be consulted at the University of Leicester Library.

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