Post-mortem magnetic resonance imaging in fetuses, newborns and children

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DECLARATION OF ORIGINALITY

The contents of this thesis are original material, except where indicated in the text for illustrations and background material recreated from the referenced sources.

This thesis accounts solely for my own work, except where indicated in the case of work done in collaboration with others, in particular:

- Magnetic resonance imaging was reported by a team of paediatric radiologists (Cardiac MR by Prof Andrew Taylor, Chest and Abdomen MR by Dr Cathy Owens and Dr Oystein Olsen, Brain MR imaging by Dr Kling Chong and Dr Rox Gunny, Musculoskeletal MR imaging by Dr Amaka Offiah)

- Autopsies were performed and reported by Prof Neil Sebire, Dr Tom Jacques (Paediatric neuropathology), Dr Michael Ashworth (Paediatric cardiac pathology), Dr Marione Malone and Dr Rosemary Scott.

- Rapid prototyping models were built by Dr Silvia Schievano

- Matlab program for T_1 relaxometry was originally developed by Dr Enrico deVita

- Some of the conventional MR images were performed by Rod Jones and Wendy Norman (Research radiographers, GOSH)

- Dr Mark Lythgoe, Dr Antony Price and Jon Cleary assisted in acquiring high field MR images at Centre for Advanced Biomedical Imaging (CABI) and Dr Harry Parkes helped in acquiring high field MR images at Institute of Neurology (Ion).

- Parental consent was obtained by Angie Scales

- Statistical advice was provided by Dr Angie Wade

ABSTRACT

My thesis explores the feasibility and utility of whole body post-mortem magnetic resonance (MR) imaging as an alternative for conventional autopsy in fetuses, newborns and children. The thesis starts with a systematic review of the existing literature on post-mortem MR imaging to identify the knowledge gaps. This is followed by the development of an effective recruitment model and a comparative study on the accuracy of less invasive autopsy by post-mortem MR imaging with conventional autopsy in 200 fetuses, newborns and children. The cause of death was accurately identified in more than 90% of cases by less invasive autopsy, following a hospital death, unexplained stillbirth or an unexpected death under HM Coronial investigation. Post-mortem MR imaging of the brain had a very high negative predictive value for excluding major neuropathological lesions; opening of the head can be avoided if post-mortem MR imaging of the brain is normal. High-resolution, 3D post-mortem cardiac MR imaging accurately detected structural heart diseases in larger fetuses, newborns and children. However, the accuracy of post-mortem lung MR imaging was poor; renal lesions required histological examination for definitive diagnosis. Furthermore, post-mortem MR imaging cannot differentiate between the normal death process and ante-mortem hypoxic brain injury due to the changes in T_1 and T_2 relaxometry values occurring after death. The diagnostic utility and image quality at 1.5 Tesla MR imaging was poor in smaller fetuses, however high field MR imaging at 9.4 Tesla provided satisfactory MR images in this sub group. In addition, visceral organ weights were accurately estimated from post-mortem MR data sets and anatomical models of these organs reconstructed by rapid prototyping. My thesis concludes by demonstrating the proof of principle of MR guided percutaneous biopsy in a piglet model.

In summary, less invasive autopsy by post-mortem MR imaging may be a satisfactory alternative to conventional autopsy; however accurate methods of percutaneous tissue sampling need to be developed and validated for adequate histological examination of visceral organs, particularly lungs, kidneys and heart.

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Dedicated

to the memory of

my father, Dr TV Sudhan

For

Monica

and

Ashwini

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CHAPTER 1: OUTLINE OF THESIS

1.1 BASIS OF THE THESIS WORK

Autopsy has an undisputed role in confirming or refuting ante-mortem diagnosis, quality assurance and advancing scientific knowledge. Despite this there has been a global reduction in autopsy rates in the past few decades. This has lead to the development of less invasive autopsy techniques that may be more acceptable to parents. Most promising of such techniques is the use of whole body post-mortem magnetic resonance (MR) imaging. Even though, the feasibility of post-mortem MR imaging as an alternative for conventional autopsy has been reported more than a decade ago, it is not yet used in clinical practice.

In 2001, the UK Chief Medical Officer recommended post-mortem MR imaging should be explored systematically as an alternative for conventional autopsy. The UK Department of Health subsequently funded a large prospective study to examine the utility of post-mortem MR imaging in fetuses, newborns and children, which forms the basis of my thesis.

I hypothesised that whole body magnetic resonance imaging can provide an accurate, detailed, three-dimensional post-mortem record of structural abnormalities and the disease processes of the whole body in the fetus, neonate and child, with comparable diagnostic information to a conventional autopsy

1.2 CHAPTER CONTENTS

The thesis is organised in the following way.

Chapter 2 and 3 are introductory chapters. Chapter 2 describes evolution and utility and components of autopsy and the reasons for declining autopsy rates. Chapter 3 describes various less invasive techniques that have been described as an alterative for conventional autopsy. Chapter 4 is a systematic review and meta-analysis of the published literature to examine the accuracy of post-mortem MR imaging. Chapter 5 describes the consenting and recruitment model that I used for this study and the parental and professional attitudes towards participation in post-mortem research. The study protocol including transfer of the infants for scanning, database and the MR sequences used is described in chapter 6. Chapter 7 is a prospective study comparing post-mortem MR imaging with conventional autopsy on 200 fetuses, newborns and children and describes the large database that was developed for this purpose. Chapter

8 describes a retrospective study in a subgroup of infants with hypoxic ischemic encephalopathy. This study examines whether ischemic brain injury in MR imaging of brain can be differentiated from normal death process can be differentiated from hypoxic ischemic changes. Chapter 9 examines some of the issues raised in chapter 8 and examines the normal changes in post-mortem T_1 and T_2 relaxometry. Chapter 10 examines the utility of high resolution post-mortem cardiac MR imaging. Chapter 11 describes estimation of organ weights using post-mortem MR imaging in a non-invasive way. Chapter 12 examines the utility of high field post-mortem MR imaging in smaller fetuses. Chapter 13 reports the feasibility of rapid prototyping for accurate reconstruction of visceral organs and demonstration of pathological lesions in a 3D model. Chapter 14 examines the feasibility of post-mortem MR guided biopsy in an animal model. Chapter 15 summarises the study and suggests future directions for research. Chapter 16 summarises the original research findings. Referances are listed in chapter 17. Parent information leaflets, consent form and ethics approaval is given in the appendix.

1.3 RESEARCH OUTPUT DIRECTLY ARISING FROM THIS WORK

1.3.1 Protocol Review

1. Thayyil S, Robertson NJ, Chitty LS, Sebire NJ, Taylor AM. Protocol 08PRT/5409: Post-mortem magnetic resonance imaging in the fetus, infant, and child: a comparative study with conventional autopsy (UKCRN: 6794). Lancet 2009:http://www.thelancet.com/protocol-reviews/08PRT-5409.

1.3.2 Original Papers

1. Thayyil S, Schievano S, Robertson NJ, Jones R, Chitty LS, Sebire NJ, Taylor AM. A semi-automated method for non-invasive internal organ weight estimation by post-mortem magnetic resonance imaging in fetuses, newborns and children. Eur J Radiol 2009;72(2):321-6.
2. Thayyil S, Robertson NJ, Scales A, Sebire NJ, Taylor AM. Parental consent for research and sudden infant death. Lancet 2008;372(9640):715.
3. Thayyil S, Robertson NJ, Scales A, Weber MA, Jacques TS, Sebire NJ, Taylor AM. Prospective parental consent for autopsy research following sudden unexpected childhood deaths: a successful model. Arch Dis Child 2009;94(5):354-8.

4. Thayyil S, Cleary JO, Sebire NJ, Scott RJ, Chong K, Gunny R, Owens CM, Olsen OE, Offiah AC, Parks HG, Chitty LS, Price AN, Yousry TA, Robertson NJ, Lythgoe MF, Taylor AM. Post-mortem examination of human fetuses: a comparison of whole-body high-field MRI at 9.4 T with conventional MR imaging and invasive autopsy. Lancet 2009;374(9688):467-75.
5. Thayyil S, Chandrasekaran M, Chitty LS, Wade A, Skordis-Worrall J, Bennett-Britton I, Cohen M, Withby E, Sebire NJ, Robertson NJ, Taylor AM. Diagnostic accuracy of post-mortem magnetic resonance imaging in fetuses, children and adults: A systematic review. Eur J Radiol 2010;75(1):e142-8
6. Schievano S, Robertson NJ, Sebire NJ, Taylor A, Thayyil S. Reconstruction of fetal and infant anatomy using rapid prototyping of post-mortem MR images. Insights Imaging 2010:(in press).

1.3.3 Review Articles and Book Chapters

1. Thayyil S. Less invasive autopsy: an evidenced based approach. Arch Dis Child 2010:(Epub ahead of print).
2. Thayyil S, Chitty LS, Robertson NJ, Taylor AM, Sebire NJ. Minimally invasive fetal post-mortem examination using magnetic resonance imaging and computerised tomography: current evidence and practical issues. Prenatal Diagnosis (in press)
3. Thayyil S, Robertson NJ, Sebire NJ, Taylor AM. Post-mortem MR and CT imaging in fetus, newborns and children. Current Diagnostic Histopathology (in press)
4. Weber MA, Thayyil S, Sebire NJ. Perinatal autopsy: In Text Book Of Neonatology (JM Rennie) (in press)

1.3.4 Successful Grants Funded From This Work

(Total Grant income = £295,067)

1. Post-mortem MR guided biopsy (Chief Investigator): Foundation for Sudden Infant Deaths £3600 (2009-2010)
2. Molecular autopsy for cardiac ion channelopathies in unexplained stillbirth. (Chief Investigator): Well being of Women £95,865 (2010-2012)

3. High field (9.4T) magnetic resonance microscopy in fetal and neonatal brain and heart (Principal Investigator): CBRC £195,602 (2009-2010) (Chief Investigator–Dr NJ Robertson)

1.3.5 Presentations/ Posters

1. Thayyil S, Schievano S, Sebire NJ, Robertson NJ, M Lythgoe, A Taylor. Non invasive organ weight estimation by post-mortem MRI, Neonatal Society, Mar 08
2. Thayyil S, Scales A, Sebire NJ, Robertson NJ, M Lythgoe, A Taylor. Parental consent and SIDS: A new model: SIDS International Conference, Portsmouth 08
3. Thayyil S, Cleary J, Sebire NJ, Robertson NJ, Lythgoe MF, Taylor AM. Magnetic Resonance Microscopy in fetuses, ISMRM, Toronto 08
4. Thayyil S, Cleary J, Sebire NJ, Robertson NJ, Lythgoe MF, Taylor AM. Less Invasive autopsy by high field MR Microscopy, International Women's Health Conference, London 08
5. Yousry T, Thayyil S, Robertson NJ, Lythgoe MF, Taylor AM. High-field 3d whole body post-mortem magnetic resonance microscopy at 9.4 Tesla: initial experience in human fetuses. ESNR, Athens 2009
6. Parkes HG, Thayyil S, Cleary J, Price AN, Yousry T, Robertson NJ, Lythgoe MF, Taylor AM. High-field Whole Body Post-mortem Magnetic Resonance Microscopy of Human Fetuses at 9.4 T. ESMRMB, Antalya, 2009
7. Thayyil S, Robertson NJ, Gunny R, Chong WK, Chitty LS, Sebire NJ, Taylor AM. Less Invasive autopsy by Post-mortem MR imaging of brain and spinal cord in fetuses, newborns and children. Paediatric Academic Societies Meeting, Baltimore 2009
8. Thayyil S, Chandrasekhar M, Ashworth MA, Chitty LS, Sebire NJ, Robertson NJ, Taylor AM. High resolution 3D post-mortem cardiac imaging is a satisfactory alternate to conventional autopsy in fetuses, newborns and children Paediatric Academic Societies Meeting, Baltimore 2009
9. Thayyil S, Chandrasekhar M, Scales A, Skordis-Worrall J, Cohen M, Whitby EW, Sebire NJ, Robertson NJ, Taylor AM. Diagnostic Accuracy, Acceptability And Economic Evaluation Of Less Invasive Autopsy By Post-mortem Magnetic Resonance Imaging: A Systematic Review And Meta-Analysis. ESMRMB, Antalya, 2009

10. Thayyil S, Chandrasekhar M, Ashworth MA, Chitty LS, Sebire NJ, Robertson NJ, Taylor AM. Post-mortem 3D Cardiac Imaging. ESMRMB, Antalya, 2009

1.3.6 Invited Lectures

1. Post-mortem MR imaging of newborn infants. Neurological Investigations Course, Nov 2008, London
2. Post-mortem MR guided biopsy, Foundation for the Study of Infant Deaths (FSID) and the Scottish Cot Death conference, Sept 2008, Greenwich
3. Post-mortem neuroimaging. European Paediatric Neuroradiology Conference, Mar 2008, London
4. Less Invasive Autopsy. Medical Grand rounds, UCL, London, Jan 2009
5. Post-mortem MR Imaging. Royal College of Radiologists, London, Sept 2010.
6. Post-mortem Neuroimaging. European Academic of Paediatric Societies (EAPS), Copenhagen, Oct 2010

1.3.7 Press Releases

1. Specialist Nurses boost consent to post-mortem research (http://www.ucl.ac.uk/news/news-articles/0903/09031201)
2. High field MRI comparable with invasive autopsy (http://www.ucl.ac.uk/news/news-articles/0908/09080701)
3. Lancet Podcast of high field post-mortem MR imaging (http://podcast.thelancet.com/audio/lancet/2009/9688_08august.mp3)

CHAPTER 2: EVOLUTION & UTILITY OF AUTOPSY

2.1 SUMMARY

Autopsy has been used for more than 2000 years and has played a crucial role in the progress of medicine. Despite advances in medical diagnosis and technology, autopsy continues to have an important role in contemporary medical practice in the 21st century; namely in around 30% of cases, clinically significant additional information that was not available during life, is obtained at autopsy. However, autopsy rates have steadily declined over the past few decades and current neonatal and paediatric autopsy rates are less than 20% of those who die in most developed countries. While there are several factors influencing the decline in the autopsy rates, the primary factor appears to be parental refusal. Autopsy is a complex procedure, nevertheless the components of autopsy can be arbitrarily categorised as invasive (i.e. open dissection and histological examination of internal organs) and non-invasive or minimally invasive (imaging, cytogenetic testing, placental examination, percutaneous biopsy). Parental refusal and clinicians reluctance for requesting autopsy consent appears to stem primarily from the invasive components of autopsy. Whilst, every effort needs to be made to improve conventional autopsy consent rates, it has become clear that such measures alone, may not significantly improve the autopsy rates in the UK. Therefore, accurate and less invasive approaches to autopsy, that may be more acceptable to parents, need to be developed and validated.

2.2 ORIGINS AND EVOLUTION OF AUTOPSY

One of the earliest reports on dissection of the human body is a classic treatise on human anatomy by Herophilus, a famous teacher in Alexandria (Greece) in 300 BC (Eldelstein 1967). There have been several reports of autopsy examinations since. Perhaps one of the greatest medical compilations of all time is a series on 3000 autopsies with almost 450 authors including Galen, published in 1679 –'Sepulchretum of Theophilus Bonetus'. These autopsies were primarily intended for teaching anatomy rather than understanding the disease process (Eldelstein 1967; King et al. 1973).

Xavier Bichet (1771-1802)–best known as the 'father of histology', drew the attention of the clinicians from macroscopic organs to examination of tissues during autopsy. Bichet identified 21 different types of tissues in the human body by various physiological and biochemical experiments; interestingly, he did not use a microscope to describe these tissues. Soon autopsy started having a more comprehensive integration with clinical and other pre clinical medical subjects like anatomy and physiology. During the 18th and early 19th century, hospital medicine progressed rapidly; clinicians meticulously observed their patients while alive and carefully autopsied them when dead. Corroboration of the autopsy data with clinical data resulted in rapid advances in medical science. Improvement of the microscope opened up new avenues for investigating form and function at a cellular level. Karl Rokitansky's and Rudolf Virchow's work using the microscope and the publication of Die Cellularpathologie in 1858 resulted in a unprecedented progress in pathology (Breathnach 2002); soon histological examination became a routine part of autopsy (Eldelstein 1967; King et al. 1973).

Autopsy continued to have an important role in the 20th century, when outstanding clinicians spent considerable amount of time in the autopsy room. Good hospitals were recognised by their high autopsy rates and poor hospitals by low rates. It was generally considered that by not performing an autopsy doctors were attempting to bury their mistakes (King et al. 1973). By 1930, pathology began to develop as a clinical discipline in its own right. Though it should be noted that no consent from next of kin was required to perform autopsies at this time and autopsy rates continued to be very high! Soon with further development of post-mortem examination techniques, pathologists started branching out and sub specialising. Medicine at this

time was dominated by pathology and clinico-pathological conferences often had the last word in medicine.

Autopsy started to become a focus of attack towards later half of 20th century. In an editorial in the Journal of American Medical Association (JAMA), Sir Isaac Starr questioned the values of routine autopsy (Starr 1956) and pointed out large volumes of information gathered in the morgue contributed little to research or clinical practice. At this time, in America, consent from next of kin was required to perform autopsy, which was often refused. Therefore, American medical students often flew to Germany to attend autopsies, where no such consent was required to perform the autopsy. Defenders of autopsy however, claimed that autopsy information was still useful and it was important to perform autopsy as a quality assurance of clinical management (Rosahn 1956). The Royal College of Pathologists in the UK was established in 1962 to co-ordinate further development in pathology and to maintain high standards of British Pathology.

I conclude the discussion about the history of autopsy with a quotation from another editorial in JAMA in 1965; this is of great relevance to how we approach post-mortem examinations in 21st century – *"It is a pernicious misconception that the mere performance of post-mortem dissection leads to progress in medical science. Progress depends not on the autopsy but on the person who is examining the material. Those who believe that the more autopsies we perform, the more medical science will progress, are pleading not for more autopsies but for more persons who can profitably utilize the data of autopsies, persons who have imagination, originality, persistence, mental acuity, sound education and background, the indispensable "prepared mind" without which observations are quite sterile. It is a grave disservice to confuse the performance of autopsies with the spark of insight, which the autopsy may trigger. We want the insight; and autopsies alone, no matter how numerous, are not the equivalent"*(King 1965).

2.3 AUTOPSIES IN THE 21st CENTURY

Although the basic principle of autopsy has not changed, it still has a vital role in 21st century medicine as a means of studying new diseases, evaluating therapies and providing information to the family of the diseased. Autopsy is now regarded as a professional activity that requires extensive knowledge and technical ability, in order

to identify important findings within a wide range of clinical contexts. Medicolegal autopsies have a crucial role in death investigations and necessitate highest possible standard of practice. Similarly, clinical autopsy represents one of the few occasions where clinicians elect to submit their management of patients to detailed assessment by other doctors. In this context confirmation of ante-mortem diagnosis and findings are no less important than demonstration of a discrepancy (RCOG 2001; RCPCH 2002).

Current guidelines in the UK (RCOG 2001; RCPCH 2002) recommend that all perinatal and paediatric deaths be offered the option of an autopsy and these examinations should be performed by specialist paediatric or perinatal pathologists. The Royal college of Pathologists specifies a minimum data set that need to be collected in various clinical scenarios including fetal autopsy, neonatal autopsy, sudden unexpected death of an infant (SUDI) and in cases where a cardiac cause of death is suspected. Another important role of autopsy is in forensic practice, as a part of criminal death investigations.

2.4 EVIDENCE BASE FOR UTILITY OF AUTOPSY

There are differences in opinion as to whether advances in technology and availability of sophisticated diagnostic methods have resulted in a reduction of medical errors, that might have resulting in patient harm and death (Goldman et al. 1983; Landefeld et al. 1988). A careful meta-analysis of studies with high autopsy rates have reassuringly shown a reduction in class I errors (i.e. major findings that, if known before death, would have altered clinical management and might have resulted in cure or prolonged survival) detected at the autopsy in the past 4 decades (relative risk reduction of 33.4% per decade (95% CI–8.4%-51.6%) (Shojania et al. 2003). Despite this reduction, class I errors continue to be reported in 8.5% to 12% and class II errors (major findings that, if known before death, would not have changed clinical management because the treatment was not available or was being already given) in 13.6% to 29% of neonatal and paediatric autopsies (Stambouly et al. 1993; Elder et al. 2005b; Cardoso et al. 2006). Unexpected iatrogenic injuries are recorded at autopsy in 41% of extremely premature babies; in 14% this was the direct cause of death (Elder et al. 2005a).

Despite the rigorous antenatal investigations in life, similar data is available for fetal autopsies. In a review of 27 such studies, the perinatal post-mortem examination resulted in a "change in diagnosis" or "additional findings" which might have influenced management or counselling in 22-76% of cases (Gordijn et al. 2002), the rate varying from 28-75% for stillbirths and 22-49% for terminations of pregnancy. Importantly, up to 35% of antenatal ultrasound diagnoses were modified by additional findings at autopsy. Furthermore, a study of 309 terminations of pregnancy for abnormalities reported that, of the 132 cases which were chromosomally normal but found to have a structural defect on antenatal ultrasound examination, and in which the parents consented to post-mortem examination, the autopsy revealed additional findings which modified the recurrence risk in 27% (Boyd et al. 2004). The utility of autopsy from few selected recent case series is shown in Table 1.

In assessing the utility of autopsy it is important to examine the autopsy rates in the cohort. A low autopsy rate may potentially introduce a selection bias; the cases selected may be when the clinician is unsure of the ante-mortem diagnosis. Indeed the information from autopsy may be more important in such cases; therefore, a falsely high utility of autopsy may be seen. Nevertheless, it is unclear if clinicians are able to select the cases, which would give a high yield form autopsy (Shojania et al. 2008).

2.5 ROLE OF AUTOPSY

There are at least six main areas where a non-forensic autopsy makes a valuable contribution as detailed below.

2.5.1 Determination of Cause of Death

In many cases a post-mortem examination will establish the cause of death. However, it is noteworthy that up to 75% of clinically unexpected stillbirths will remain unexplained after post-mortem examination, highlighting the need for further research into the causes of intrauterine fetal demise in the third trimester (Thali et al. 2003b). Similarly, up to half of all sudden unexpected early neonatal deaths will remain unexplained following detailed post-mortem examination, a situation analogous to sudden infant death syndrome (SIDS) in older infants (Weber et al. 2008c). Whilst the currently available evidence suggests that additional information will be revealed in a significant proportion of perinatal and neonatal autopsies (Table 1), it is also important to recognise that even negative findings may be of benefit to parents. The

mere confirmation of the underlying cause of death and clinical issues will be reassuring to clinicians and parents, and it is likely that this knowledge may assist some parents in their grieving process.

Post-operative deaths and deaths occurring following a period of intensive care treatment are challenging and often require specific questions to be answered with regards to why the child failed to respond to the clinical management. A close liaison with clinicians and detailed review of ante-mortem investigations and history is mandatory for effective autopsy in these cases.

2.5.2 Future Pregnancies and Siblings

As outlined in Table 1, in approximately 20% of cases, additional information will become available following autopsy, which has a direct effect either on the recurrence risk or counselling for siblings or future pregnancies. For example, the detection of additional malformations may lead to a syndromic diagnosis or the identification of a hereditary disorder, and as such will modify genetic counselling and/or management of the next pregnancy. The detection of such underlying genetic or metabolic disorders may also have implications for other family members, who may require additional screening investigations. Likewise, placental examination may reveal potentially recurrent disorders, including massive perivillous fibrin deposition and chronic histiocytic intervillositis, both of which are associated with an adverse pregnancy outcome and carry a high risk of recurrence (Bonetti et al. 2010).

2.5.3 Research and Clinical Practice Development

Post-mortem studies have led to a better understanding of a variety of neonatal diseases, including pulmonary hypoplasia (Endo et al. 1995), bronchopulmonary dysplasia and diffuse axonal injury in preterm babies (Haynes et al. 2008). With the proportion of stillbirths having not significantly decreased over recent years, the need for further research into possible causes thereof is clearly highlighted (Thali et al. 2003b). Such autopsy based research is also important to elucidate the effects of new treatment modalities and therapeutic interventions, including possible complications and side effects, the assessment of new diagnostic procedures, and the pathological spectrum of rare or emerging new infectious diseases, such as viral epidemics, thus providing important epidemiological information.

Table 1: Utility of fetal, neonatal and childhood autopsies from recent selected case series.

Author, Year	Population	N (%)	Utility of autopsy
Termination of pregnancy for chromosomal abnormalities			
(Szigeti et al. 2007)	Fetuses terminated for downs syndrome	184 (89%)	83% had structural abnormalities at autopsy. Major new findings at autopsy in 34.2%. Sono graphic findings refuted at autopsy in 9%. Agreement between US and autopsy was 65% for brain malformations, 67% for cardiac, 46% for abdominal, 50% for renal and <5% for other malformations.
(Papp et al. 2007)	Fetuses terminated for trisomy 21, 18 or 13	305	Total 611 major structural abnormalities identified at autopsy. Complete correlation of US and autopsy findings only in 36%. Additional findings at autopsy in 64%.
Termination of pregnancy for antenatally diagnosed fetal malformation			
(Piercecchi-Marti et al. 2004)	All fetuses terminated for malformations	352	Malformation of brain –22%, heart –15%, urogenital –9%. In 51% of cases autopsy finding were decisive for genetic counseling. Brain autolysed in 28/78 of cases terminated for a brain malformation
(Boyd et al. 2004)	All fetuses terminated for malformations	132 (43%)	Autopsy confirmed antenatal US finding in 72%. In 27% major additional finding that changed recurrence risk noted.
(Johns et al. 2004)	All fetuses terminated for malformations	47 (31%)	Complete agreement of antenatal US and autopsy in 47%, In 28% of cases additional major findings at noted at autopsy.
(Parkar et al. 2009)	All fetuses terminated for malformations	198	99% correlation between antenatal US and PM finding
(Dickinson et al. 2007b)	Termination of pregnancies	809 (80%)	Autopsy confirmed the prenatal diagnosis in 63.5%. In 1.1% autopsy added major diagnostic information, and in 15.1% significant information was provided.

Table 1 (Continued)

Author, Year	Population	N (%)	Utility of autopsy
Still births			
(Killeen et al. 2004)	Birth weight >500g	130	Confirmed ante-mortem diagnosis in 51% of early and 33% of late still births, New diagnostic findings in 38% of early and 29% of late still births.
(Kock et al. 2003)	Gestation >28 weeks	177	Confirmed ante-mortem diagnosis in 90%, new diagnostic findings in 10%, cause of death changed from explained to unexplained in 6.8%. 39% remained unexplained even after autopsy.
(Rasmussen et al. 2003)	Gestation >28 weeks and Birth weight>1000g	325	Cause of death changed from unexplained to explained in 6% of cases. 66% remained unexplained even after autopsy.
Preterm and term in neonatal intensive care unit			
(Elder et al. 2005a)	Gestation <28 weeks and age <28 days	29 (54%)	Class I-8.3%, Class II- 28% of cases. Iatrogenic injury was cause of death in 14%.
(Elder et al. 2005b)	16 cases of neonatal encephalopathy	16(80%)	Class I-0%, Class II 62.5%
Infants and children in paediatric and cardiac intensive care units			
(Stambouly et al. 1993)	Children 1 to 17 yrs	50(55%)	Class I diagnosis 10%, Class II diagnosis–18%.
(Gatzoulis et al. 1996)	Children with congenital heart disease	59(55.6 %)	Class I finding in 8.5% cases. Class II–13.6% cases.
(Cardoso et al. 2006)	Median age 21 months.	114(55 %)	Class I in 12%, Class II in 29%
Sudden and unexpected death of an infant (SUDI)			
(Weber et al. 2008a)	Sudden infant death referred by HM Coroner	546	37% explained by the autopsy findings, of which 58% were infective. Cause of death determined by histological examination in 46%, macroscopic examination in 30%, microbiological tests 19%, clinical history 5%. 63% remained unexplained.

2.5.4 Audit, Quality Control and Teaching

The post-mortem examination plays an important role in auditing. Comparisons between post-mortem findings and imaging during life, including antenatal ultrasonography, are imperative in order to improve diagnostic acumen. Regular discussion and feedback at multidisciplinary team meetings on discrepancies between clinical diagnoses and post-mortem findings should be encouraged to continually improve patient care and service provision. The post-mortem examination may also play an important role in teaching medical staff, including surgeons, trainee pathologists and undergraduate medical students (Burton et al. 2007), to achieve the highest possible standards of care.

2.5.5 Medicolegal Issues / Malpractice Litigation

Increasingly, the pathologist is requested to perform a coronial autopsy if there is risk of litigation relating to an intrapartum event or a post-operative death. In such instances, questions usually relate to the timing of events, such as the timing of hypoxic-ischaemic brain injury, meconium staining, or presence of iatrogenic injury, such as related to the placement of long lines or other therapeutic interventions.

2.5.6 Disease Epidemiology

Autopsy examination has a very important role in reporting of accurate mortality statistics and disease burdens. This is particularly important as newer diseases and epidemics emerge. Verbal autopsy has been attempted as a way of epidemiological data collection, in situations where formal autopsy is not possible. However, the accuracy and utility of such a procedure at an individual level is questionable (Byass et al. 2009).

2.6 FACTORS INFLUENCING THE YIELD FROM AUTOPSY

There are many factors that may influence the usefulness of the autopsy: for example, the antenatal diagnostic approach, sophistication of ante-mortem imaging and level of clinical expertise may decrease the likelihood of identifying additional findings at post-mortem; on the other hand, tertiary care centres are likely to be referred more complex cases, which tend to yield more additional information (Gordijn et al. 2002; Gordijn et al. 2007). The expertise of the pathologist is likely to be important, with

autopsies performed by specialist perinatal / paediatric pathologists in tertiary centres being more likely to reveal significantly useful information (Gordijn et al. 2002; Gordijn et al. 2007).

Recent alterations to the post-mortem examination procedure and consent process may often reduce the amount of information available, especially for central nervous system abnormalities in fetuses. Until recently, the usual practice was to remove and fix the brain before dissection, a process that could take up to 3 weeks. Parents now frequently request that all organs must be replaced before burial; adequate fixation is difficult within this time. In many cases, the brain has to be examined following a suboptimal period of fixation, a state that makes interpretation difficult especially in the immature, developing brain. Adequate brain examination is even more difficult for the pathologist if there is a delay between intrauterine death and delivery leading to maceration of the fetus. The Royal College of Obstetricians and Gynaecologists (RCOG) guidelines suggest that any pregnancy terminated after 22 weeks gestation should be accompanied by fetocide to ensure that the fetus is not born alive. This procedure is usually accompanied by the administration of mifepristone (a cervical ripening drug), which has its optimum efficacy in shortening the time between induction and delivery after 48 hours. This effectively means that most fetuses undergoing termination of pregnancy after 22 weeks gestation will have been dead for at least 48 hours, rendering post-mortem examination of the brain difficult. The brain may be completely autolysed in up to 36% of such cases.

2.7 COMPONENTS OF THE POST-MORTEM EXAMINATION

Autopsy is a complex process and involves careful integration and analysis of the information drawn from a variety of sources, including ante-mortem investigation, detailed external and internal examination of the body and a wide range of histological, microbiological, immunohistochemical and cytogenetic investigations. For the purpose of my thesis it is useful to categorise the components of autopsy into separate compartments; (a) Non-invasive or minimally invasive components; (b) Invasive components that require extensive dissection of the body.

2.7.1 A. Non Invasive and Minimally Invasive Components

1. External and Macroscopic Examination

This includes a careful review of the clinical history and ante-mortem investigations. The fetus or child is weighed and basic measurements are taken, including head circumference, crown-rump length, crown-heel length and foot length, which are compared to standard reference tables. Assessment of fetal biometry allows identification of intrauterine growth restriction/small-for-gestational age infants and macrosomic/large-for-gestational age infants. The degree of maceration or post-mortem change is assessed, which includes documenting the extent of skin discolouration, blistering and skin slippage, allowing the pathologist to make an approximate estimate of the duration of time since death, although such estimates may not be precise. Particular emphasis is placed on identifying possible dysmorphic features, although the external appearances should be interpreted in the context of the gestational age and subtle syndromic abnormalities may not be readily discernable in mid-trimester fetuses. Routine photographs are usually taken and stored as part of the infant's medical record, with more detailed photographs to document specific abnormalities if noted. This is often useful when for subsequent reviews, for example, review by a geneticist (Keeling 2007).

2. Post-mortem Imaging

Most perinatal autopsies are usually preceded by whole-body X-ray examination, usually by using a faxitron radiographic unit. If there are suspected skeletal abnormalities, particularly for suspected skeletal dysplasias, detailed whole-body radiography is mandatory. In suspected non accidental injuries and sudden infant deaths, detailed skeletal survey is undertaken and reported by a specialist radiologist prior to autopsy. Information from X-rays and post-mortem examination is complementary in such cases, as it is likely some fractures may be missed if X-rays are not undertaken. Alternatively, post-mortem examination may reveal some fractures (for example fresh rib fractures) there were not seen in the X-ray examination. Increasingly CT scans are being used to examine the skeletal system (Keeling 2007).

3. Placental Examination

Placental examination forms an integral part of the perinatal post-mortem examination, and it is important that the placenta be transferred with the body to the pathologist, if possible. In a significant proportion of cases, the cause of intrauterine

fetal demise is only revealed by examination of the placenta. Placental examination may also reveal potentially recurrent disorders, such as massive perivillous fibrin deposition, chronic intervillositis or villitis of unknown aetiology, and may confirm the presence of chorioamnionitis and funisitis, viral pathogens, or fetal thrombotic vasculopathy, some of which may have implications for future siblings or pregnancies. Moreover, targeted specialist investigations can be performed, such as vascular injection studies in complicated monochorionic twin placentas (Bonetti et al. 2010)

4. Other Ancillary Investigations

Other important ancillary investigations include microbiological and virological analyses, as well as metabolic studies (blood and bile spots for acylcarnitine profiling by tandem mass spectrometry, or enzyme assays using cultured fibroblasts harvested from a post-mortem skin biopsy), and cytogenetic and DNA analysis. Samples for this can be collected from placenta, blood and skin of the fetus or infant is often sufficient to perform these investigations and therefore are considered as non-invasive or minimally invasive.

2.7.2 B. Invasive Components Of Autopsy

1. Dissection and Direct Internal Examination of Visceral Organs

Dissection of the body and detailed macroscopic investigation of the visceral organs is an integral part of the autopsy. Access to the thoracic and abdominal viscera is traditionally gained via a midline incision through the anterior thorax and abdomen. A careful inspection of the internal organs is then carried out, which are then removed, weighed and dissected. All abnormalities are described and documented photographically, after which small samples are routinely taken for histological examination. The organs are then returned to the body, which is reconstructed prior to release (Keeling 2007).

All organs are routinely weighed, and the weights are compared to reference tables against the expected gestational age. More helpful are weight ratios, such as the brain: liver weight ratio (which is increased in intrauterine growth restriction), and the combined lung: body weight ratio (which is reduced in pulmonary hypoplasia) (Keeling 2007).

If the brain is to be examined, the scalp is incised posteriorly, either by means of a coronal incision or vertical midline incision from the vertex to the occiput. The skull is usually opened by following the non-fused cranial suture lines, although in older infants cranial bones may have to be cut. The brain is usually examined following a period of formalin fixation, which may be several weeks for complex brain anomalies. Reagents for rapid fixation of brain thereby shortening the duration of brain retention to days instead of weeks are currently available. Examination for skeletal abnormalities and injuries require extensive dissection of the region (Keeling 2007).

2. Microscopic Examination

The standard post-mortem examination involves taking small tissue samples of organs for microscopic examination to confirm or exclude the presence of disease. In upto a quarter of paediatric autopsies final diagnosis is made on the basis of histological examination. Histological examination of heart for myocarditis and lungs for pneumonia are particularly important (Weber et al. 2008a; Weber et al. 2008b). Macroscopic examination of lungs is notoriously inaccurate for making a diagnosis of pneumonia and can be easily confused with pathologies like hyaline membrane disease in premature infants. Conversely, if the cause of death is not detected on macroscopic examination of the brain, it is unlikely that histological examination of brain would detect a cause of death. Nevertheless, neuropathological examination is of crucial importance in improving the current understanding about a wide range of malformations, developmental and degenerative diseases. Most disorders that affect humans from cradle to grave are diseases of the central nervous system.

However, this is less of an issue in cases of fetuses terminated for malformations, particularly of the brain, which are almost always structural and in which routine histological examination of many internal organs provides relatively little additional diagnostic information. Obvious exceptions would be specific lung, liver and renal pathologies associated with syndromic defects (Corabian et al. 2007)

Apart from routine staining with haematoxylin and eosin, the tissue sample may also be examined with a wide range of histochemical and immunohistochemical stains in selected cases to allow for detailed characterisation of the underlying disease process. In some cases in-situ hybridisation techniques and occasionally electron microscopy may be performed.

2.8 THE POST-MORTEM REPORT

Current guidelines recommend that a preliminary report is submitted to the clinician within 24-48 hours of the post-mortem examination, followed by a final report which incorporates the histological findings and results of further investigations (RCOG, 2001), usually within four to six weeks. The post-mortem report must document macroscopic and microscopic findings, as well as the results of all ancillary investigations performed. Importantly, the salient (positive and negative) findings should be summarised, followed by a detailed, directed and appropriate clinicopathological correlation. If possible, photographs of macroscopic or histological abnormalities, as well as copies of X-ray radiographs, should also be incorporated into the post-mortem report. Parents are entitled to a copy of the report, but it is recommended that the contents of the report be discussed with them by their clinician prior to receipt, preferably in person, as some parents may find the technical jargon (and photographs) used in such reports insensitive and distressing (Keeling 2007).

2.9 HUMAN TISSUE ACT AND AUTOPSY CONSENT

The Human Tissue Act (2004) received royal assent in November 2004 and came into force on 1 September 2006; its implementation was overseen by the Human Tissue Authority (HTA), an independent statutory regulator that provides advice and guidance relating to the Act, and which is responsible for defining a minimum set of standards, published as Codes of Practice. In addition, the HTA also oversees the licensing of organisations and establishments that deal with human tissue (see http://www.hta.gov.uk).

The Act requires consent for the removal, storage and use of human tissue for anatomical examination; determining the cause of death; establishing, after a person's death, the efficacy of any drug or other treatment administered to them; obtaining scientific or medical information, which may be relevant to any other person now or in the future; public display; research in connection with disorders, or the functioning, of the human body; transplantation; clinical audit; education or training relating to human health; performance assessment; public health monitoring; and quality assurance. This applies to all tissue removed at post-mortem, including small samples

such as blocks and slides and samples that might be kept as part of the infant's medical record.

The Act (HTA Code of Practice 1) defines the giving of consent as a positive action; the absence of refusal is not evidence of adequate consent. In order for consent to be valid, it must be given voluntarily by an appropriately informed person who has the capacity to agree to the activity in question. The Act defines who may give consent, which in the setting of perinatal post-mortem examinations will usually be one or both parents. For stillbirths and neonatal deaths, it is recommended that, if possible, consent is obtained from the mother, and that, where appropriate, both parents are involved. Under the Act, consent from one parent is sufficient; however, if there is disagreement between the parents, it is recommended that this be sensitively discussed with both parents before proceeding with the post-mortem examination. It is important that the consent process is not viewed as a single act, for example, the signing of a consent form, but rather, it should be seen as an ongoing process in which parents can discuss the issue fully, ask questions and make an informed choice, which includes the option of withdrawing consent at a later stage.

It is usually the treating clinician's responsibility to seek consent, who should be sufficiently senior and well informed, with a thorough knowledge of the post-mortem procedure. It is recommended that they be trained in the management of bereavement and should have witnessed a post-mortem examination. As valid consent can only be given if appropriate communication has taken place, information leaflets and consent forms should be available in the main local community languages for patients whose first language is not English, and interpreters should be used. Written consent is not required by law, although usually required by the local hospital policy.

2.10 DECLINE IN AUTOPSY RATES

Despite the potential benefits of the autopsy outlined above, post-mortem rates have decreased the past few decades. According to data released by the Confidential Enquiry into Maternal and Child Health (CEMACH, 2005), the perinatal autopsy rate (including late fetal losses, stillbirths, and neonatal and postneonatal deaths) decreased from 48% of potential cases in 2000, to 39% in 2003 (Table 2)

Interestingly, there is marked regional variation of the perinatal autopsy rate, with a reported uptake of perinatal post-mortem examinations of almost 50% in the North

East of England, compared to only 27% in the North West (CEMACH, 2007). Latest data reveal a plateau for the proportion of neonatal deaths referred for consented post-mortem examination, with 28% in 2000, 22% in 2003, 21% in 2006, and 21% in 2007 (CEMACH, 2009). Unfortunately, the decline in autopsy rates has continued despite an increase in the number of cases where autopsy is offered by clinicians.

Table 2 Post-mortem examination of neonatal deaths

Year	Still birth			Newborn		
	Autopsy proportion	Declined by parents	Not offered	Autopsy proportion	Declined by parents	Not offered
2000	54.7%	33.1%	11.2%	28.2%	40.8%	22.8%
2001	*48.8%*	*35.2%*	*15.1%*	*21.8%*	*41.7%*	*27.5%*
2002	45%	33.6%	20.0%	21.4%	39.8%	29%
2003	*45.3%*	*36.6%*	*16.9%*	*21.7%*	*41.6%*	*28%*
2004	44.5%	36%	18.2%	20.6%	41%	29.3%
2005	*44.3%*	*36.3%*	*18.5%*	*19.8%*	*41.1%*	*29.6%*
2006	42.7%	48.4%	7.5%	21.3%	52.9%	17.9%
2007	*45%*	*47.1%*	*6.8%*	*21.1%*	*51.6%*	*18.3%*

There has been considerable debate among professionals regarding the reasons for declining autopsy rates. It is important to recognize and respect difference in the opinions among clinicians, pathologists and general public as to why the autopsy rates have declined. Jan Zijlstra in Lancet (2007) gives an interesting anology for about these discussions – *"Believers give reasons to stay in the church, but non-believers slip out through the back door without listening or giving arguments. The believers stay behind, guessing about the reasons for their empty church*"(Zijlstra 2007).

One question that is rarely asked is whether autopsy rates were ever high in situations that required explicit consent from next of kin. Quite possibly, very high autopsy rates were only achieved early in the 20th century when no consent was required for autopsy following a hospital death. In certain countries like Austria, Hungary, and Trieste (Italy), the law allows autopsies to be performed without the consent from next of kin for medical, scientific and educational reasons. Therefore, in these countries the autopsy rates remain high. In countries where a detailed and explicit

consent is required for autopsy from next of kin, the autopsy rates have significantly dropped (e.g. US, UK and Australia). Clearly, such laws should take account of both the larger interests of the community and the interests and rights of the family. This could be compared to the 'opting out' option that is used for organ donation in many European countries, in an effort to increase the number of organs available for transplant, which in turn looks at the larger interest of the community. But the balance between larger interest of the community and that of individual is a grey zone. One way of viewing the organ retention controversies in the UK is that the clinicians and pathologists were too focused on the larger interests of the community, than the interests of individual families. Enquiries into alleged organ retention without formal parental consent in the Bristol Royal Infirmary Inquiry (Kennedy 2001) and the Royal Liverpool Children's Inquiry (Redfern 2000) have indeed resulted in further decline of autopsy rates. However, the decline in autopsy rates appear to started much earlier (Table 3).

Table 3: Global decline in autopsy rates (Burton and Underwood 2007a)

	Initial autopsy rate (period)	Subsquent autopsy rate (period)
Australia	21% (1992-93)	12% (2002-03)
France	15.4% (1988)	3.7% (1997)
Hungary	100% (1938-51)	68.9% (1990-02)
Ireland	30.4% (1990)	18.4% (1999)
Jamaica	65.3% (1968)	39.3% (1997)
Sweden	81% (1984)	34.0% (1993)
UK	42.7% (1979)	15.3% (2001)
USA	26.7% (1967)	12.4% (1993)

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2.10.1 Attitude of Bereaved Parents

Undoubtedly the attitude of bereaved parents and clinicians play a crucial role in determining whether autopsy consent is granted or not. The two main reasons often quoted as reasons for parents declining the autopsy are (1) the "dread of the child being mutilated", often coupled with the notion that, in cases where the child has been in the intensive care unit, the infant has been through enough already; and (2), the

feeling by parents that there were "no unanswered questions," an attitude that may more likely reflect the opinion of the consenting clinician (McHaffie et al. 2001).

Parents' decision not to consent to a post-mortem examination may also be influenced by cultural and religious considerations, although most major religions do not explicitly prohibit autopsy, especially if there is a perceived benefit to public health in practice (Burton et al. 2007). Recent data from the UK show that the proportion of parents declining an autopsy following a neonatal death is similar among different ethnic groups, ranging from 52% of white parents, to 55% of Asian parents, and 56% of Chinese and black parents (CEMACH 2007). It is noteworthy that in a small survey of women's reactions to perinatal autopsy, 30% of women who had refused an autopsy subsequently regretted not having agreed to one, possibly since questions remained unanswered. In contrast, only 6% of those who agreed for autopsy regretted it subsequently (Rahman et al. 1995).

Discussion of autopsy is undoubtedly a stressful experience for parents and very sensitive handling by an experienced person is required. For the consent to be fully informed, the benefits of autopsy need to be explained clearly. Some parents are driven by an altruistic motive and may be denied this opportunity if autopsy and participation in post-mortem research is not offered in a sympathetic way. Qualitative studies have shown that the caution exercised by most clinicians for the fear of causing additional distress to newly bereaved parents may not be entirely appropriate (Snowdon et al. 2004b; Snowdon et al. 2004c).

2.10.2 Clinicians' Attitudes

Whilst there are recent improvements in the proportion of clinicians offering parents the option of an autopsy (see Table 2 above), there is little doubt that neonatologists may find discussing the option of a post-mortem examination with bereaved parents difficult,– "distasteful and distressing"(Snowdon et al. 2004a). Clinicians' attitudes may be further exacerbated by the lengthy consent forms now required in the UK, which are very detailed, time-consuming and possibly confusing. It has been reported that younger clinicians regard autopsy as less useful compared to their senior colleagues, suggesting that junior staff are no longer directly familiar with the post-mortem examination and its potential benefits, in part a likely reflection of the markedly reduced emphasis on (autopsy) pathology teaching in the undergraduate

curriculum (Burton et al. 2007). Finally, clinicians' perceptions are also likely influenced by pathologists' attitudes, with delays in issuing a final post-mortem report, lack of clinicopathological correlation, and paucity of appropriate multidisciplinary team meetings undoubtedly contributing to the notion that the autopsy is of limited value in the immediate and subsequent management of the patient and/or parents (Snowdon et al. 2004a)

Current guidelines regarding the consenting procedures and detailed explanation to be given regarding the invasive nature of the autopsy process, undoubtedly make discussions around post-mortem examination stressful for both newly bereaved parent and for the clinicians.

In conclusion, the autopsy process has evolved over the years and the needs of the society at large have had an important role in this evolution. Autopsy continues to have an important role in the 21st century. The components of autopsy can be divided into invasive examinations and non-invasive examinations. Parental refusal and clinicians' reluctance for requesting autopsy consent appear to stem primarily from the invasive components of autopsy. Whilst every effort needs to be made to improve conventional autopsy consent rates, it has become clear that such measures alone, may not significantly improve the autopsy rates in the UK. One potential solution may be the development and use of less invasive approaches to autopsy. In the next chapter, I will discuss various options for a less invasive autopsy.

CHAPTER 3: TECHNIQUES FOR LESS INVASIVE AUTOPSY

3.1 SUMMARY

Several less invasive alternatives to conventional autopsy have been proposed in the past few decades. This includes needle autopsies (with or without image guidance), X-ray, ultrasound, computerised tomography (CT), magnetic mesonance (MR) imaging and endoscopic autopsies. The pros and cons of each of these methods are discussed in this chapter, with particular reference to perinatal and paediatric autopsy.

3.2 DEVELOPMENT OF LESS INVASIVE AUTOPSY

The only non-invasive methods of autopsy are radiological autopsy (including X-rays, CT and MR imaging) and the 'view and grant system' proposed by Rutty and colleagues (Rutty et al. 2001). Both these approaches are legally acceptable methods of autopsy under the HM Coronial system in the UK (Rutty et al. 2001).

However, many important ancillary post-mortem investigations can be performed on easily accessible tissues like blood, skin or placenta without dissection of the body and such examinations are unlikely to be objected to by the parents (Wright et al. 2004). Therefore, the terms 'less invasive autopsy' (Parker 2004) or 'minimally invasive autopsy' (Weustink et al. 2009) have been used to describe the combined approaches using radiological autopsy and these ancillary post-mortem investigations. I have used both these terms synonymously in my thesis, even though the latter term 'minimally invasive autopsy (MIA)' may be a more meaningful description.

Parental refusal towards the conventional autopsy is primarily related to the invasive nature of autopsy, particularly towards opening of the head. Needless to say, both macroscopic and microscopic examination of internal organs are integral parts of autopsy, even though relative utility of these examinations may vary in each setting. The purpose of macroscopic examination of internal organs is to visualise gross pathology, malformations or any color changes in the visceral organs or skeletal system. On the other hand, histological abnormalities may not be always macroscopically visible and therefore microscopic examination of the tissues may be important to provide a complete picture.

In view of declining autopsy rates, several methods for minimally invasive autopsy procedures (Wright et al. 2004) have been described in the literature. Many of these techniques are complementary to each other and full information can be obtained only by a combination of various methods. For example imaging methods like X-rays and computerised tomography (CT) scan are primarily suited for examination of skeletal abnormalities and injuries, whilst MR imaging may be more suited for soft tissues and visceral organs. In principle, visceral organs biopsies can be obtained either by a percutaneous needle or endoscopically for histological diagnosis. Ultrasound may not provide a useful post-mortem imaging modality on its own, but it may be useful in guiding the needle accurately for biopsy. Endoscopic biopsies have an added

advantage of visual inspection of internal organs. Following is a brief summary of each of these potential less invasive autopsy techniques, with a particular focus on perinatal and paediatric autopsies.

3.3 X-RAY EXAMINATION

Post-mortem X-ray examination is used as an integral part of perinatal and paediatric autopsies, to examine pathologies in the skeletal system. It is widely available and inexpensive. Post-mortem radiology is particularly useful in cases of skeletal dysplasias and skeletal injuries. The final diagnosis in some cases of skeletal dysplasias can be made on the basis of X-ray abnormality alone; in others an abnormality in the X-ray may direct the pathologist for sampling of the particular area e.g. costochondral junctions (Olsen 2006); however specific diagnosis of skeletal dysplasias are not easy and radiological interpretation requires substantial expertise.

Certain findings that may be noted on X-ray like oedema, contractures, cranial malformations like anencephaly or soft tissue masses may be of little value as a definite diagnosis cannot be based only on the radiography due to poor tissue resolution. Likewise, lung opacities on post-mortem X-rays have little correlation with histologically diagnosed pneumonia (Olsen 2006). By itself radiography has little potential to be an alternative to conventional autopsy.

Post-mortem X-ray examination has a definite role in non accidental injuries (NAI); skeletal surveys are therefore routinely performed as a part of unexpected sudden death investigations. Rib fractures at post-mortem due to non accidental injuries need to be differentiated from resuscitation related fractures; resuscitation related rib fractures often occur antero-laterally, as opposed to fractures from NAI, which may occur antero-laterally or posteriorly (Weber et al. 2009). Whilst X-ray is able to detect 93% of healing rib fractures as opposed to 22% of fresh fractures; stripping of pleura and detailed examination of each rib at autopsy is required to identify fresh fractures accurately (Weber et al. 2009).

3.4 NEEDLE AUTOPSY

Needle autopsies were introduced in the 1950's as a more acceptable and less invasive alternative to conventional autopsy. Several pathologists have reported the use of needle biopsy without image guidance ('blinded needle biopsy'), mostly without

appropriate autopsy comparisons (Underwood et al. 1983). One of the earliest report on needle biopsies as a method of less invasive autopsy was published by Richard Terry in 1955 (Terry 1955). The author successfully performed needle biopsies and detected several liver pathologies, in a highly selected case series involving a variety of liver diseases in adults. Subsequently several series of blinded needle biopsy in adults have been reported; only few had autopsy comparisons and almost all the studies reached a predictable conclusion–needle autopsies are less accurate than conventional autopsy, however they may be useful when conventional autopsy is refused (Underwood et al. 1983; Benbow et al. 2003).

To date, two good quality prospective studies have been reported comparing the accuracy of needle biopsy in a blinded way to an independent gold standard i.e. conventional autopsy (Breeze et al. 2008b). The accuracy was reported in two ways (a) number of cases in which adequate sample volume for histological diagnosis was obtained (b) detection of pathological lesions when compared with open dissection and direct block histology.

The first study included 25 newborn infants who died in a tertiary care neonatal unit (Garg et al. 2009). The authors were able to obtain adequate volumes of lung and liver biopsy in 84% and 92% of cases respectively (Table 4). Adequate brain biopsy was obtained in 68% of cases. Success rate from other organs varied between 0% to 54%. In cases where adequate biopsy was obtained, lung biopsy detected most cases of diffuse lung diseases like meconium aspiration syndrome (MAS), hyaline membrane disease (HMD) and extensive pulmonary haemorrhage, even though focal lesions were missed. However, all congenital heart diseases (CHD), renal and gastrointestinal pathologies were missed.

The second study examined the utility of needle biopsy in 30 fetuses referred for autopsy to a perinatal centre (Breeze et al. 2008b). The cases included unexplained intra uterine deaths and terminations of pregnancies. In three of the cases, the authors used ultrasound guidance (Table 5).

Table 4. Accuracy of blinded needle biopsy in newborns

Organ	Adequate	Pathology	Biopsy
Brain	17 (68%)	Meningitis-1	Meningitis-1
Lungs	21 (84%)	Pulmonary haemorrhage in 11, MAS in 7, HMD -1	Pulmonary haemorrhage -8, MAS-5, HMD-1
Liver	23 (92%)	2 abnormal lesions	Both missed
Spleen	5 (20%)	5 normal	5 normal
Left kidney	14 (56%)	1 cystic dysplasia	Missed
Right kidney	6 (24%)		
Heart	Not given	CHD-6	All cases missed

CHD=Congenital heart disease, MAS=Meconium aspiration syndrome, HMD=Hyaline membrane disease

Table 5. Accuracy of blinded needle biopsy in fetuses

Organ	Adequate	Pathology results	Biopsy results
Brain	Did not try		
Lungs	86%	5 abnormal cases (chronic inflammation, focal necrosis-2, interstitial infiltrate-1, poorly formed air space 1)	3 of this biopsy in adequate, 2 reported as normal
Liver	76%	Congested liver-1	Reported as normal
Spleen	17%	Haemorrhagic spleen-1	Reported as normal
Kidney	34%	Multicystic dysplastic kidneys-1	Biopsy inadequate
Thymus	17%	Depletion-2, Cyst-1	Biopsy inadequate 2, reported as normal-1
Heart	52%	Focal necrosis-1	Biopsy not available
Adrenal	41%	Haemohrrage-1, Congested-1	Biopsy not available-1, Reported as normal -1

Even though the authors could obtain adequate tissue from lungs and liver in 86% and 76% of cases respectively, none of the pathological lesions were detected from these organs. Adequate biopsy volumes were obtained only from 17% to 52% from other organs, and again all pathological lesions were missed (Table 5).

The lower yield of needle biopsy in perinatal cases may be related to the small size of the fetus and newborns (compared to adults and children) and difficulty in hitting the target accurately. In addition maceration may be often present in fetal cases, which makes sampling difficult. Nevertheless, it is appears that blinded needle biopsies have little role in this population.

3.4.1 Needle Biopsy Under Ultrasound Guidance

A Spanish group have reported very high accuracy of ultrasound guided post-mortem needle biopsy, termed as 'Echopsy'(Farina et al. 2002). In a prospective study of 100 cases (19 children, 81 adults), Juliana Fariña et al claimed that in 100% of the cases adequate sampling was obtained from liver, spleen, lungs, kidney and brain. Adequate samples from gastrointestinal tract were obtained in 21% and from thyroid in 76%. With regards to the main diagnosis, the authors report a concordance of 83% between needle biopsy and conventional autopsy. No specific details of the cases are provided in the paper with regards to the nature of pathologies detected; therefore it is difficult to interpret the results of this study. There has not been any subsequent work confirming this study from the same group or any other groups. Considering the poor resolution of ultrasound images in post-mortem fetal and neonatal setting, it is unlikely that this technique would be of significant utility in perinatal autopsy.

3.4.2 Needle Biopsy Under Computerised Tomography Guidance

Aghayev et al have reported feasibility of computerised tomography (CT) guided biopsy in adults (Aghayev et al. 2008a). The authors obtained adequate samples from cerebrum, cerebellum, heart, liver, spleen and kidney. However, it is difficult to interpret the utility of these observations due to a number of major limitations in the study design and reporting. For example the authors have not provided the exact number of cases included in the study, neither did they perform conventional autopsy in these cases. No pathological lesions were seen in the biopsied samples, nevertheless it is difficult to know if these represent true negatives or false negatives,

in the absence of conventional autopsy. There is limited published data on CT guided biopsy in fetuses and children.

3.5 ENDOSCOPIC AUTOPSY

Post-mortem endoscopies can be performed by insufflation of abdomen by carbon dioxide and insertion of the endoscope using a trochar. Contents can either be viewed using the endoscope or on a video screen. A group from Tel Aviv published 3 separate papers in 1995 involving endoscopic autopsy in 25 adults (Avrahami et al. 1995a; Avrahami et al. 1995b; Avrahami et al. 1995c). Most these were trauma related deaths. The authors reported good correlation of laparoscopic and thoracoscopic finding with conventional autopsy, accuracy was less for retroperitoneal pathology. Another endoscopic autopsy study in adults reported that pulmonary embolisms, myocardial infarction, aspiration pneumonia, small renal tumors, liver hemangiomas, and colonic polyps may be missed by an endoscopic approach (Cacchione et al. 2001). Biopsy by endoscopic approach appears to be easier and more accurate than needle biopsies (Cacchione et al. 2001). If the abdominal wall integrity is lost, as in some trauma cases, endoscopy becomes difficult to perform due to difficulty in insufflation. More recent series have reported higher accuracies of laparoscopy autopsy following a traumatic death (Peyvandi et al. 2009) and in unexplained adult deaths referred by the coroner, using a combined thoracoscopic, laparoscopic, endoluminal or endovascular approach (Fan et al. 2010). The endoscopic approach is quite promising, however there is very little data on fetuses, newborns and children.

3.6 POST-MORTEM COMPUTERISED TOMOGRAPHY

The first published post-mortem CT scan appears to be a case report following a gun shot injury to the head (Wu¨llenweber et al. 1977). Several forensic groups have subsequently explored use of post-mortem CT scan in traumatic and unnatural deaths alongside autopsy; increasingly it is becoming an integral part of forensic autopsy (O'Donnell et al. 2008).

The published work supporting the use of post-mortem CT imaging is primarily focussed on trauma related death in adults; the largest published blinded comparative study of post-mortem CT and conventional autopsy included 57 cases (56 adults and 1

infant) (Yen et al. 2007). Post-mortem CT detected only 75% of skull fractures; however it detected 7 maxilla and 3 mandibular fractures which were missed at autopsy. Thin subdural bleeds were missed on CT imaging and false positive sub arachnoid bleeds were reported in 11 cases. Only 53% of cerebral grey matter contusions were detected by post-mortem CT imaging. Cause of death (when related to a brain injury) was established in up to 79% of cases by post-mortem CT imaging (Yen et al. 2007). Several case reports and series related to use of post-mortem CT imaging in traumatic injury have been published by the same group and others, particularly following strangulation deaths, drowning, ruptured internal vessels and penetrative injuries; however the data on non-forensic and paediatric use is very limited.

Post-mortem CT imaging appears to be particularly useful in the following scenarios: (a) poly trauma cases with base of skull fracture or facial injuries; autopsy requires extensive dissection in such cases. Interestingly, in animal models it is seen that CT scan has a higher sensitivity in detecting skull fractures than conventional radiology and autopsy, when compared with the gold standard of skeletonisation (by extreme maceration). On the other hand, CT scan may be less sensitive than X-rays and conventional autopsy for detecting rib fractures (Cattaneo et al. 2006); (b) 3D reconstructions for demonstration of skeletal injuries in the court; (c) Intracranial injuries; and (d) gas and air fluid collections (O'Donnell et al. 2008).

A recent systematic review of post-mortem CT imaging in trauma cases concluded that there is insufficient evidence at present to use CT as an alternative for conventional autopsy; nevertheless it has an important role as an adjunct post-mortem investigation in forensic cases. Not surprisingly, many forensic centres around the world have installed CT scanners in the mortuary and are routinely performing CT imaging prior to autopsy (O'Donnell et al. 2008).

3.7 POST-MORTEM MR IMAGING

MR imaging of the excised brain, spine (De Groot et al. 2001; Pfefferbaum et al. 2004) and heart (Meyer-Wittkopf et al. 1996) has been successfully performed. The use of whole-body post-mortem MR imaging is still controversial, even though the first report of post-mortem whole body MR imaging was published more than two decades ago (Ros et al. 1990). Six cadavers (3 stillbirths, 1 infant, 2 adults) were

scanned on a 0.5 Tesla MR scanner before autopsy in this study. Good tissue contrast and resolution was obtained in most cases and the authors were able to identify large macroscopic abnormalities and air fluid collections. A few years later, Brookes and colleagues from University College London reported a small case series comparing whole body MR imaging with conventional perinatal autopsy. This study was intended to be a feasibility study; nevertheless it generated a great deal of controversy among some pathologists for two reasons (a) the authors seemed to imply normality on MR imaging equated to normality on conventional autopsy, ignoring the histological contribution or contribution from ancillary post-mortem investigations (b) Some of the post-mortem MR imaging findings like small intra ventricular bleeds had no correlates at autopsy, and therefore the authors suggested that MR imaging may be even superior to conventional autopsy. However, other pathologists took a more balanced view and suggested scepticism towards such a new technology may be a reflection of paranoia towards change (Rutty et al. 2004).

Several studies have been published on post-mortem MR imaging since then, particularly in fetuses. In most studies imaging of the central nervous system (CNS) proved the most accurate, whilst body imaging, in particular imaging of the heart proved more problematic (Breeze et al. 2006). Central nervous system anomalies account for 20% of fatal congenital abnormalities in fetuses, and a recent study, specifically looking at the fetal and stillborn CNS, suggested an MR sensitivity of 100% and specificity 92% for detecting CNS abnormalities when compared with conventional post-mortem examination (Griffiths et al. 2005). Other studies have confirmed the accuracy of CNS fetal post-mortem MR(Whitby et al. 2006). Imaging of the other body systems has been less well documented. For the heart, the potential for using 3D cardiac post-mortem MR has been demonstrated, though its relatively poor accuracy in all the studies to date may reflect limited experience of MR imaging in congenital heart disease and the use of sub-optimal MR sequences for cardiac imaging (Breeze et al. 2006).

Furthermore, MR imaging has grown in clinical importance in the living fetus and newborn infant (Glenn 2009; Glenn et al. 2009)(29), especially for brain anomalies. There is now extensive literature describing the normal MR imaging appearance of the in utero fetal brain from around 17 weeks gestation and the ex-utero preterm infant brain from around 25 weeks gestation. In the fetus, MR imaging is used as an

adjunct to detailed ultrasound examination to define pathology in many organs, although as this use of the technology is relatively new there is still debate about its use in this role (Glenn 2009; Glenn et al. 2009).

Following the recommendation of Chief Medical Officer, Alistair Parker on behalf of the Department of Health examined possible options for less invasive autopsy and suggested that MR imaging offered the best prospect. In response to this, the Department of Health funded a large prospective study to examine the accuracy of post-mortem MR imaging as an alternative for conventional autopsy. This study forms the basis of my thesis work. My thesis begins with a systematic review of the published data on post-mortem MR imaging, which is described in the next chapter.

CHAPTER 4: POST-MORTEM MR IMAGING: A SYSTEMATIC REVIEW AND META-ANALYSIS

4.1 SUMMARY

*This study describes a systematic review and meta-analysis of published studies to examine the diagnostic accuracy of less invasive autopsy by post-mortem MR imaging, in fetuses, children and adults. Medline, Embase, the Cochrane library and reference lists were searched to identify all studies comparing post-mortem MR imaging with conventional autopsy, published between Jan 1990 and March 2009. **A** random effects model was used for meta-analysis and chi square test to examine heterogeneity. I identified a total of 539 abstracts; 16 papers met the inclusion criteria; data from 9 studies were extracted (Total: 146 fetuses, 11 children and 24 adults). In accurately identifying the final cause of death or most clinically significant abnormality, post-mortem MR imaging had a sensitivity and specificity of 69% (95% CI–56%, 80%) and 95% (95% CI–88%, 98%) in fetuses, and 28% (95% CI–13%, 47%) and 64% (95% CI–23%, 94%) in children and adults, respectively. Sensitivity for detecting traumatic brain lesions and visceral organ pathology, particularly cardiac defects, was poor. There is insufficient data to recommend use of post-mortem MR imaging as an alternative for conventional autopsy. Post-mortem MR imaging may miss an unnatural cause of death in children and adults, if used as the only post-mortem investigation.*

4.2 INTRODUCTION

There has been increasing pressure from certain religious groups to use post-mortem MR imaging as an alternative for conventional autopsy in the UK. The Department of Justice (UK) has recently announced a commitment for wider implementation of post-mortem MR imaging in the NHS, following reports of 99% accuracy from a group in Manchester (Bereaved Jews and Muslims now can opt for scans instead of post mortems in Manchester). Therefore, I wanted to examine the published literature of post-mortem MR imaging in a systematic way.

Systematic reviews are considered as scientific investigations in themselves, as these have a with pre-planned methods and an assembly of original studies as the "subjects." Multiple primary investigations are stratified using strategies that limit bias and random error (Cook et al. 2007). These strategies include a comprehensive search of all potentially relevant articles and the use of explicit, reproducible criteria in the selection of articles for review. Primary research designs and study characteristics are appraised, data are synthesized, and results are interpreted. Such reviews provided a more unbiased opinion than conventional narrative reviews (Cook et al. 2007).

In this review and throughout my thesis, I have used the term forensic autopsy to include suspicious and traumatic deaths only and not sudden unexpected non-suspicious deaths. In England, the HM Coroners investigate the latter and paediatric or general pathologists perform autopsies in such cases. However, the practice elsewhere in the world may vary and often forensic pathologists undertake autopsies on all sudden unexpected deaths including suspicious and traumatic deaths.

4.3 AIMS

The aim of this analysis was to systematically and quantitatively compare the diagnostic accuracy of post-mortem MR imaging with conventional autopsy in fetuses, children and adults by reviewing the published literature prior to 2009.

4.4 METHODS

I followed the guidelines from the Cochrane Diagnostic Test Accuracy Working Group for undertaking and reporting this systematic review and meta-analysis. I

searched Medline and Embase databases (January 1990 and March 2009) using the medical index subject heading (MeSH) search terms "Autopsy" AND "magnetic resonance imaging". In addition, I performed key word searches using the terms "necropsy", "post-mortem imaging" and "post-mortem radiology". No language restrictions were used. Articles were initially identified based on their title. Abstracts of all the identified studies were examined and full papers obtained on potentially eligible studies. References and bibliographies from retrieved articles were also manually examined. Studies that were reported only as conference abstracts were not included. Case reports and case series, and studies examining forensic cases were excluded.

4.4.1 Data Extraction

Two reviewers (myself and M Chandrasekhar) independently extracted the data on study design, population, index test and reference test from all eligible studies into an Access database (Version 2003, Microsoft Inc, USA) as given in Figure 1. Disagreements were resolved by consensus. Individual patient data in the original papers was examined carefully and the primary overall diagnosis/cause of death and the primary malformation/pathology identified from each organ system from MR imaging and conventional autopsy was used to construct separate 2x2 tables, according to a predefined protocol. We contacted the authors for any missing information.

4.4.2 Quantitative Data Synthesis

I performed the meta-analysis in two levels. For the first level, I estimated the accuracy of post-mortem MR imaging with regards to the final diagnosis i.e. identification of the most significant pathology in fetuses or cause of death in children and adults. For the second level, agreement for each organ pathology was examined separately in fetuses, children and adults. Autopsy was considered as the gold standard, in both these comparisons. Small amounts of fluid collections in serous cavities (e.g pleural effusion) were considered as post-mortem changes and of little clinical significance (Spitz 2006); therefore these were excluded from analysis of abnormalities.

Multilevel models were used to estimate sensitivities and specificities with centre as a random effect. Both adjusted and unadjusted pooled diagnostic indices were

calculated. Unadjusted refers to the estimates without taking into account correlations between individuals from the same centre/paper. Exact confidence intervals based on F distribution were used to calculate confidence intervals for sensitivity and specificity (Leemis et al. 1996). If any study had a zero value in any cell of its 2x2 table, a value of 0.5 was added to all cells of the table. This correction does not apply to calculations of sensitivity and specificity. The heterogeneity between the studies was investigated using the chi-squared test. The I^2 index was used to describe the percentage of total variation across studies that was due to heterogeneity rather than chance. SPSS Version 16.0 for Macintosh (SPSS Inc, Chicago, Illinois) and MetaDisc Version 1.4 (Ctr.de.Colmenar Viejo, Madrid) were used for statistical analysis.

The quality of each study was assessed by QUADAS (Quality Assessment of Diagnostic Accuracy Studies) criteria as recommended by the Cochrane Diagnostic Review Group and by STARD (Standards for the Reporting of Diagnostic accuracy studies) checklist.

4.5 RESULTS

I reviewed 539 abstracts identified by electronic searching. Full text articles of 101 relevant studies were examined of which 16 met the eligibility criteria for examination of diagnostic accuracy. Of these 9 studies were included (5 on fetuses, 1 on children, 3 on adults) (Table 6). This comprised of individual patient data from a total of 181 cases (146 fetuses, 11 children, 24 adults).

Seven studies were excluded because individual patient data of 2x2 table data could not be extracted (Brookes et al. 1996; Woodward et al. 1997; Breeze et al. 2006; Cohen et al. 2007; Weustink et al. 2009), cases reported were included in another paper (Cohen et al. 2008) or not all cases underwent autopsy (Ezawa et al. 2003) and data on the ones who had autopsy could not be separately extracted (Table 7).

4.5.1 Population Characteristics of Included Studies

Five studies examined post-mortem MR imaging in fetuses and newborns, however two of these excluded fetuses less than 16 weeks. One study examined fetuses terminated for congenital malformations only and one study used a case control design of 11 fetuses with spinal abnormalities and 30 without abnormalities. Only one study, which limited MR imaging to the brain, evaluated use of post-mortem MR

imaging in children. This study included 11 infants (age 1 month - 1 year) referred by HM Coroner for autopsy, following a sudden and unexpected death.

Three studies evaluated use of post-mortem MR in adults. Two of these were in a setting of sudden unexpected death, referred by HM Coroner for autopsy. One was following hospital deaths, where next of kin had consented for a hospital autopsy.

4.5.2 Diagnostic Utility of Post-mortem MR Imaging

4.5.2.1 Accuracy in identification of the most important abnormality or cause of death

Pooled data from all 5 studies on fetuses showed an overall sensitivity of 69% (95% CI– 56%, 80%) and specificity of 95% (95% CI–88%, 98%) for detection of the most clinically significant abnormality. However, significant heterogeneity was seen on sensitivity of PM MR imaging (chi-square $p<0.001$), thus limiting the utility of pooled results (Figure 2). Pooled data from 4 studies on children and adults showed sensitivity of 28% (95% CI–13%, 47%) and specificity of 64% (95% CI–23%, 94%) for identification of the cause of death. No heterogeneity was seen in the pooled results (Figure 3).

4.5.2.2 Accuracy for identification of pathological lesions in brain and spinal cord

Meta-analysis was then performed at the second level, after grouping individual cases in the studies according to specific organ pathology in fetuses, children and adults separately. For detection of pathology in fetal brain and spinal cord (data from 5 studies), post-mortem MR imaging had a sensitivity of 88% (95% CI–74%, 96%) and specificity of 94% (95% CI–86%, 98%). No significant heterogeneity was identified in this meta-analysis. However, pathological lesions in the included cases were limited to congenital structural malformations and no data was available on other fetal brain pathology (e.g. ischaemic, traumatic, haemorrhagic lesions).

Figure 1. Flow chart for identification of the studies for the review.

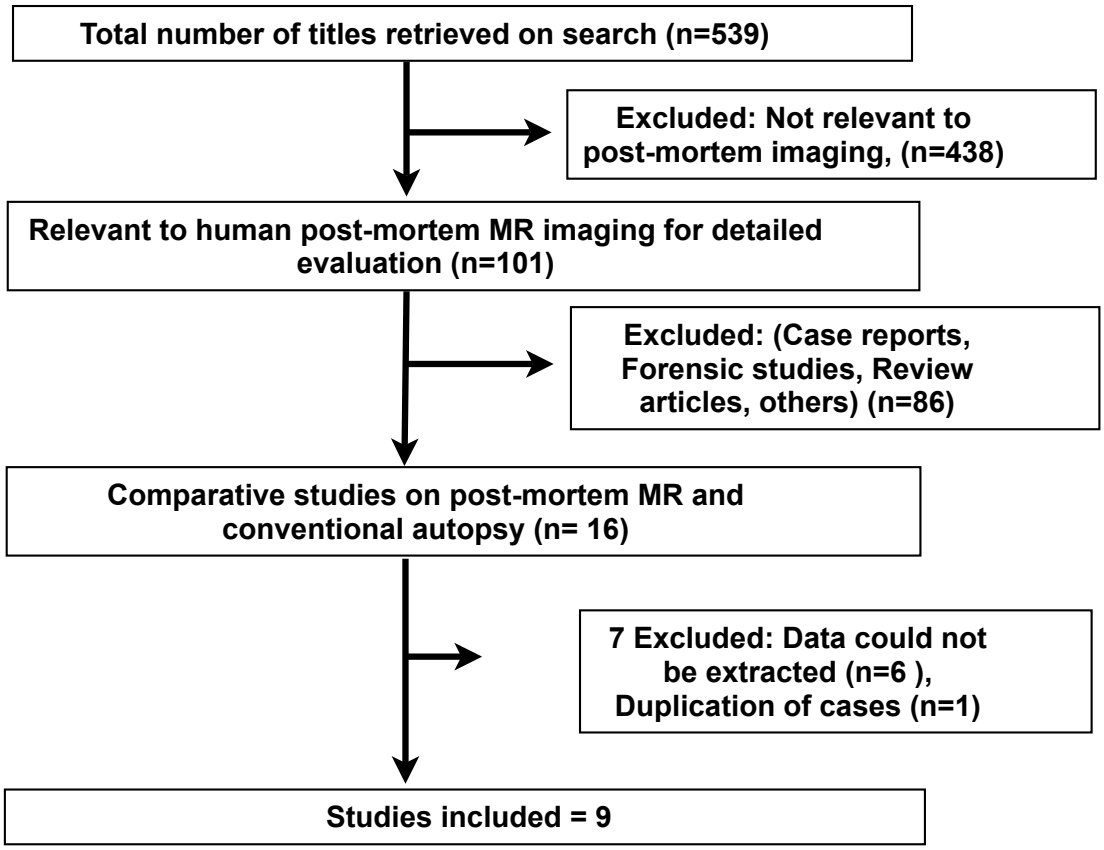

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Table 6. Details of included studies for examination of diagnostic accuracy of post-mortem MR imaging

Study	Design	Population	MR sequence	N^1	N^2	N^3	N^4	Blinding			Organs examined	Type of lesions reported
								Rad-Clin	Rad-Path	Path-Rad		
(Huisman et al. 2002)	Prospective	ToP only (14-32)	1.5T, 2D T_1 and T_2W	NA	10	10	10	Y	Y	UC	Whole body	Congenital malformations
	Prospective	ToP, miscarriages (16-40)	1T MR, 2D T_1W in 16, 2D T_2 W in 10	58	34	27	26	Y	Y	Y	Whole body	Congenital malformations
(Griffiths et al. 2003)	Prospective	ToP and miscarriages (14-42)	1.5T, 2D T_2W	NA	40	40	32	N	Y	Y	Brain and spine	Congenital malformations
(Hagmann et al. 2007)	Unclear	ToP and miscarriages and newborns	1.5T, 2D T_2W	NA	37	37	37	N	Y	UC	Kidneys	Congenital malformations
(Widjaja et al. 2006)	Unclear	Fetus-11 cases (ToP), 30 controls	1.5T, 2D T_2W	NA	41	41	41	N	Y	UC	Spine	Congenital malformations

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Table 6. Details of included studies for examination of diagnostic accuracy of post-mortem MR imaging (continued)

Study	Design	Population	MR sequence	N^1	N^2	N^3	N^4	Blinding			Organs examined	Type of lesions reported
								Rad-Clin	Rad-Path	Path-Rad		
(Hart et al. 1996)	Prospective	Children with suspected NAI	1.5T, 2D T_2W	33	11	11	11	N	Y	N	Brain	Subdural, SAH, Contusions
(Bisset et al. 2002)	Retrospective	Sudden unexpected death in adults (54-96 years)	NA	53	53	6	6	N	UC	UC	Whole body	Ischemic heart disease, pneumonia
(Roberts et al. 2003)	Prospective	Sudden unexpected death in adults	1.5T, 2D T_2W	NA	10	10	10	N	Y	UC	Whole body	Pneumonia, LVF, Bronchial Ca, perforated viscera
(Patriquin et al. 2001)	Prospective	Adults (mean age 64 yrs), hospital deaths	1.5T, 2D T_2W	NA	8	7	7	N	Y	Y	Whole body	IHD, ARDS, Pneumonia, Lungs cysts, Cirrhosis

NA=Not available, UC=Unclear, N^1=Eligible population, N^2=Recruited cases, N^3=Cases that had full autopsy, N^4=Cases included in meta-analysis.

Rad-Clin=Radiologist blinded to clinical details, Rad-Path=Radiologist blinded to autopsy details, Path-Rad=Pathologist blinded to radiology details.

ToP – Termination of pregnancy, SAH – Sub arachnoid haemorrhage, LVF – Left ventricular failure, IHD – Ischemic heart disease, ARDS – Adult respiratory distress syndrome, Bronchial Ca – Bronchial carcinoma

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Table 7. Details of excluded diagnostic accuracy studies.

Study	Population	N	Organs examined	Results	Reason for exclusion
(Brookes et al. 1996)	Fetus	20	Whole body	90% of cases MR provided equivalent information to that of conventional autopsy	2x2 data not extractable
(Woodward et al. 1997)	Fetus	26	Whole body	MR detected 79% of major malformations. Multiple comparisons from each case made.	2x2 data not extractable
(Ezawa et al. 2003)	Adults with cancer	37	Whole body	In 7 cases MR less informative than the autopsy. MR + Conventional autopsy better than conventional autopsy alone in 30 cases. Blinding unclear. Only 29 cases had full autopsy	2x2 data not extractable
(Cohen et al. 2008)	Fetus	99	Brain	Complete correlation only in 60% of cases when only macroscopic autopsy findings compared with MR imaging. Retrospective series. Blinding unclear.	Duplicate publication, 2x2 data not extractable
(Cohen et al. 2007)	Fetus, Children	250 to 300	Brain and spinal cord	Complete agreement in 63% when only macroscopic autopsy findings compared with MR imaging. Autopsy superior to MR in 6%, MR superior to autopsy in 31%.	Duplicate publication, 2x2 data not extractable, published as a review
(Breeze et al. 2006)	Fetus	30	Whole body	87.5% sensitivity and 95.5% specificity for detecting brain malformation. 62.5% sensitivity and 87% specificity for lung lesions. Poor utility for other organs.	2x2 data not extractable
(Weustink et al. 2009)	Adults	30	Whole body	Complete agreement of minimally invasive autopsy (MR, CT imaging and percutaneous biopsy) with conventional autopsy in 77%. Multiple comparisons made from same case.	2x2 data not extractable

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Post-mortem MR imaging of brain in children had a sensitivity and specificity of 7% (95% CI–0%, 47%) and 89% (95% CI–33%, 100%) for detection of subarachnoid bleed, 86% (95% CI–23%, 100%) and 50% (95% CI–16%, 84%) for detection of sub dural bleed, and 80% (95% CI–12%, 100%) and 89% (95% CI–52%, 99%) for detection of cerebral contusion respectively (Figure 4).

4.5.2.3 Accuracy for identification of pathological lesions in other visceral organs

Post-mortem imaging had extremely poor sensitivity for detecting cardiac lesions (12% (95% CI–3.9%, 31.3%) including structural and ischaemic heart disease. For identification of gastrointestinal, pulmonary and renal lesion lesions, post-mortem MR imaging had sensitivities of 58%, 82% and 73% and specificities of 98%, 86% and 99% respectively, with wide confidence intervals. There was limited amount of data on musculoskeletal imaging, which again shows wide confidence intervals (Figures 5, 6).

All the studies included were of small size and poor to moderate quality and many studies did not have pathologist blinded MR results. None of the studies followed STARD guidelines. QUADAS assessment for study quality of included studies is given in Figure 7.

4.6 DISCUSSION

Overall pooled sensitivity and specificity of post-mortem MR imaging for identification of the most significant abnormality in fetuses was 69% (95% CI–56%, 80%) and 95% (95% CI–88%, 98%), and for identifying the cause of death in children and adults was 28% (95% CI–13%, 47%) and 64% (95% CI–23%, 94%), respectively. Specific meta-analysis on individual organ pathology showed that post-mortem MR imaging had a pooled sensitivity and specificity for fetal brain and spinal cord anomalies of 90% (95% CI–76%, 97%) and 94% (95% CI–86%, 98%), respectively. Sensitivity for detection of intracranial bleeds, cerebral contusions and most visceral organ pathology, except kidneys, was poor with wide confidence intervals. Some of the lesions may be of medicolegal significance and may be suggestive of an unnatural cause of death.

Therefore, the existing data, prior to 2009, is insufficient to recommend the use of post-mortem MR imaging as an alternative for conventional autopsy.

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4.7 MEANING AND INTERPRETATION OF THE RESULTS

I analysed data on fetuses separately to children and adults, as the purpose of autopsies are different in these groups. Current data on accuracy of post-mortem MR imaging is based on small, poor quality studies with major selection bias and inadequate blinding of MR and autopsy reports. Accuracy of MR imaging appears to be limited to detection of congenital abnormalities of fetal brain and spinal cord. Caution needs to be exercised in extrapolating these results to other age groups.

Of concern is the poor sensitivity of post-mortem MR imaging for detection of traumatic brain lesions and intracranial bleeding in infants, indicating that less invasive autopsy may fail to detect a non-accidental injury.

On initial examination, the accuracy of post-mortem MR imaging for detection of renal lesions appeared to be good. However, in a significant proportion of cases, although post-mortem MR imaging reported the kidneys as abnormal, definitive diagnosis could be made only by histological examination. Thus, if post-mortem MR imaging is offered as less invasive autopsy in such cases tissue biopsy would be also be required. Post-mortem MR imaging was least accurate for cardiac lesions, both for detection of malformations and ischaemic heart disease.

4.8 STRENGTHS AND LIMITATIONS OF THE STUDY

The major limitation of systematic reviews, particularly of diagnostic studies, is clinical heterogeneity. Autopsy is a complex procedure and comparing it with an imaging modality is not straightforward. Moreover, requirements from post-mortem examination and underlying pathologies in fetuses, children and adults are very much different. I therefore anticipated clinical heterogeneity and meta-analysis was performed after carefully examining individual case data from all studies at two levels that were decided a priori.

I have not included post-mortem MR imaging in forensic cases referred by the Police. This is because the objective of autopsy in such cases is often demonstration of the mode of death, rather than identifying the cause of death. Moreover, due to ethical and medicolegal implications, forensic studies cannot be performed in a blinded way, thus introducing major bias in the estimation of diagnostic accuracy.

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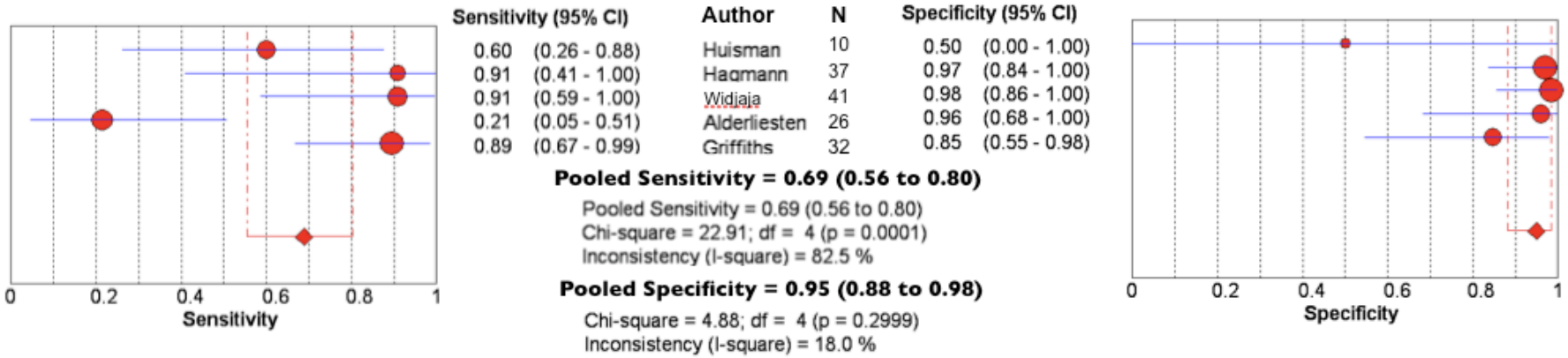
Figure 2. Diagnostic accuracy of less invasive autopsy in fetuses

Overall diagnostic accuracy of "less invasive autopsy" by post-mortem MR imaging for detection of the most significant abnormality in fetuses or cause of death in fetuses.



NB: Red circles = point estimates of the sensitivity/specificity weighted to the sample size, Blue lines = 95% confidence intervals (CI) of the sensitivity/specificity of the individual studies, Red diamond = Point estimates of the pooled sensitivity and specificity, Horizontal red lines=95% CI of the pooled sensitivity and specificity

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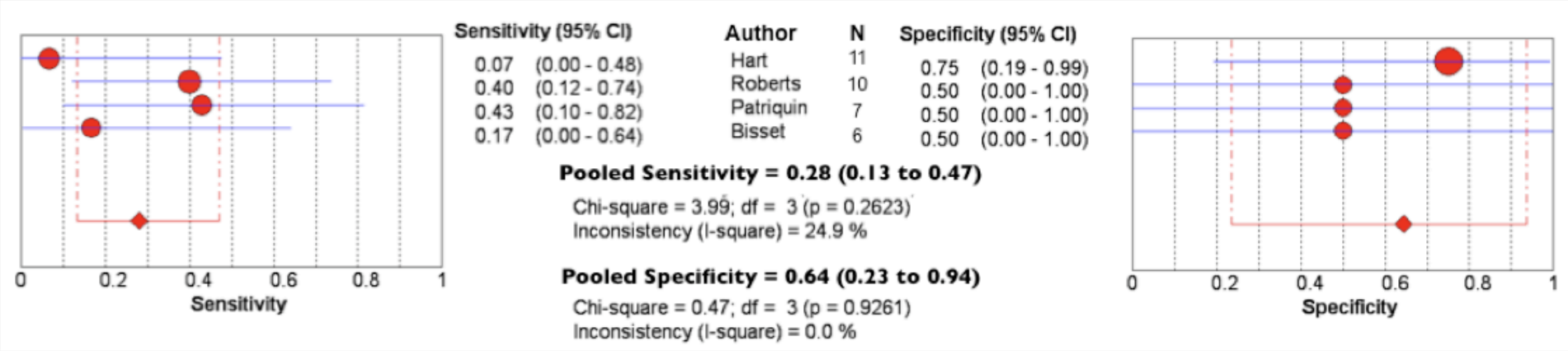
Figure 3: Diagnostic accuracy of less invasive autopsy in children and adults

Overall diagnostic accuracy of "less invasive autopsy" by post-mortem MR imaging for detection of cause of death in children and adults.



NB: Red circles = point estimates of the sensitivity/specificity weighted to the sample size, Blue lines = 95% confidence intervals (CI) of the sensitivity/specificity of the individual studies, Red diamond = Point estimates of the pooled sensitivity and specificity, Horizontal red lines=95% CI of the pooled sensitivity and specificity

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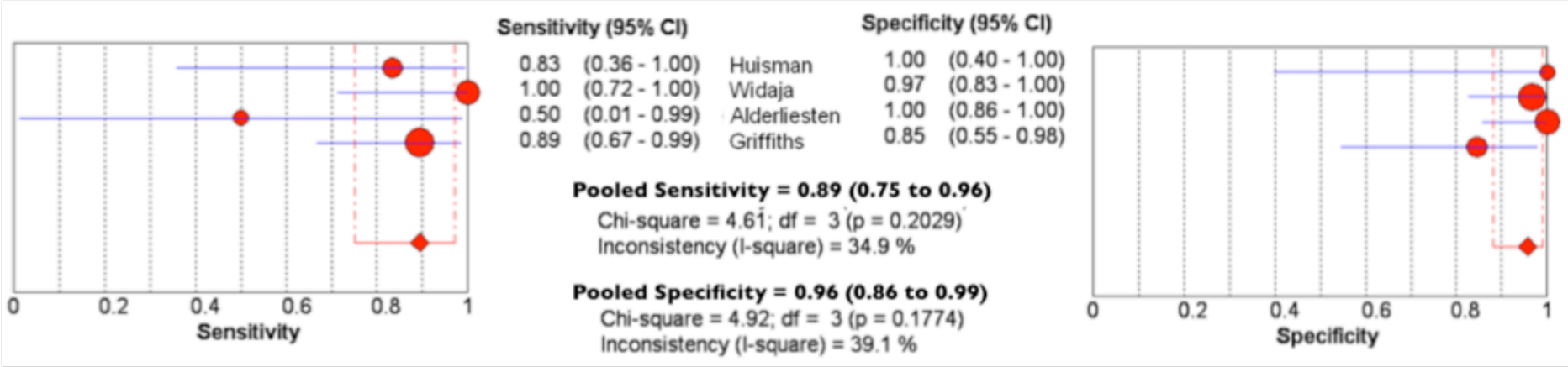
Figure 4: Sensitivity and specificity for detection of congenital malformations of fetal brain and spinal cord.



NB: Red circles = point estimates of the sensitivity/specificity weighted to the sample size, Blue lines = 95% confidence intervals (CI) of the sensitivity/specificity of the individual studies, Red diamond = Point estimates of the pooled sensitivity and specificity, Horizontal red lines=95% CI of the pooled sensitivity and specificity

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Figure 5. Sensitivity and specificity for detection of cardiac pathology (a) and lung pathology in fetuses, children and adults (b).

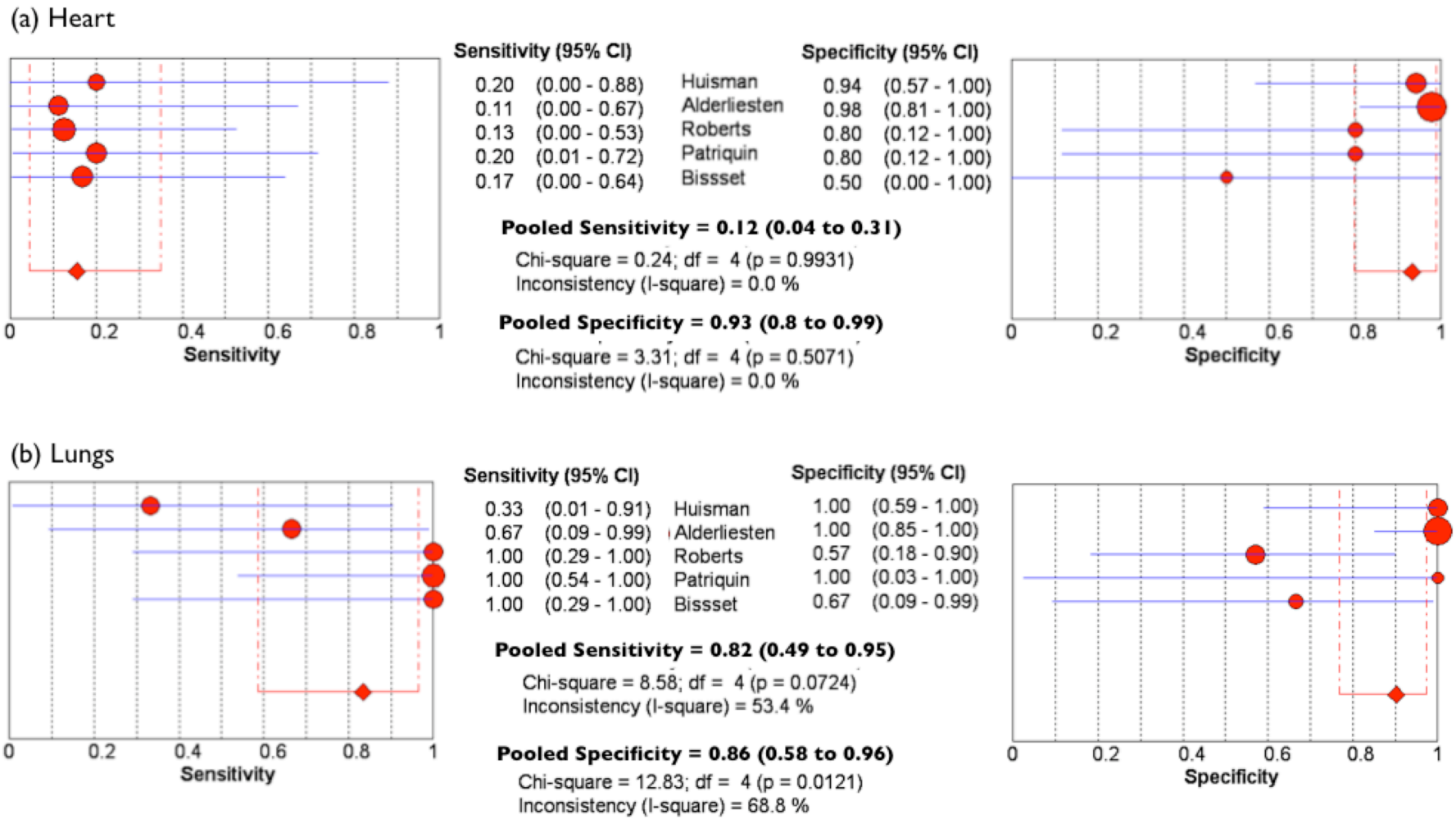

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Figure 6. Sensitivity and specificity for detection of gastrointestinal pathology (a) and renal pathology in fetuses, children and adults (b).

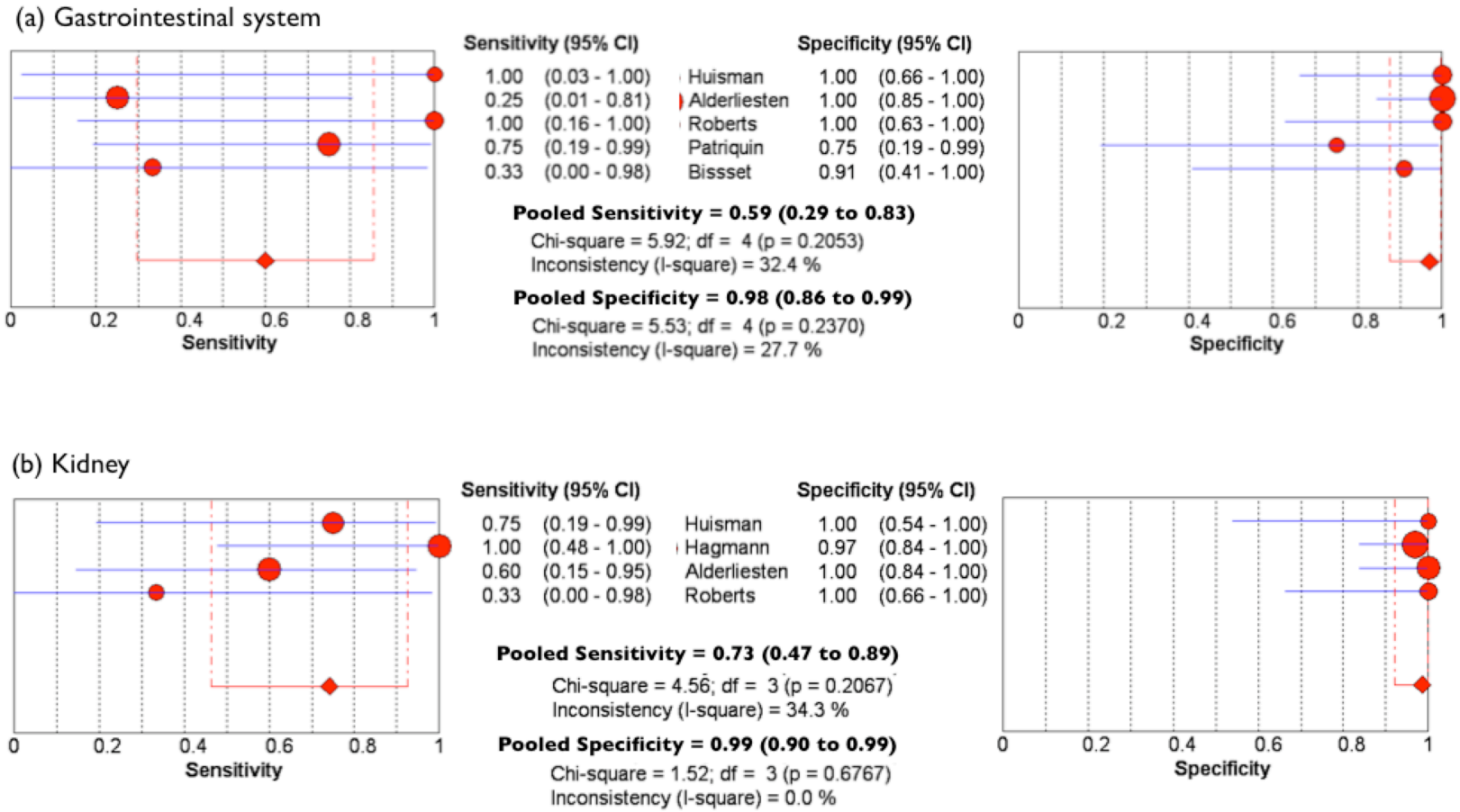

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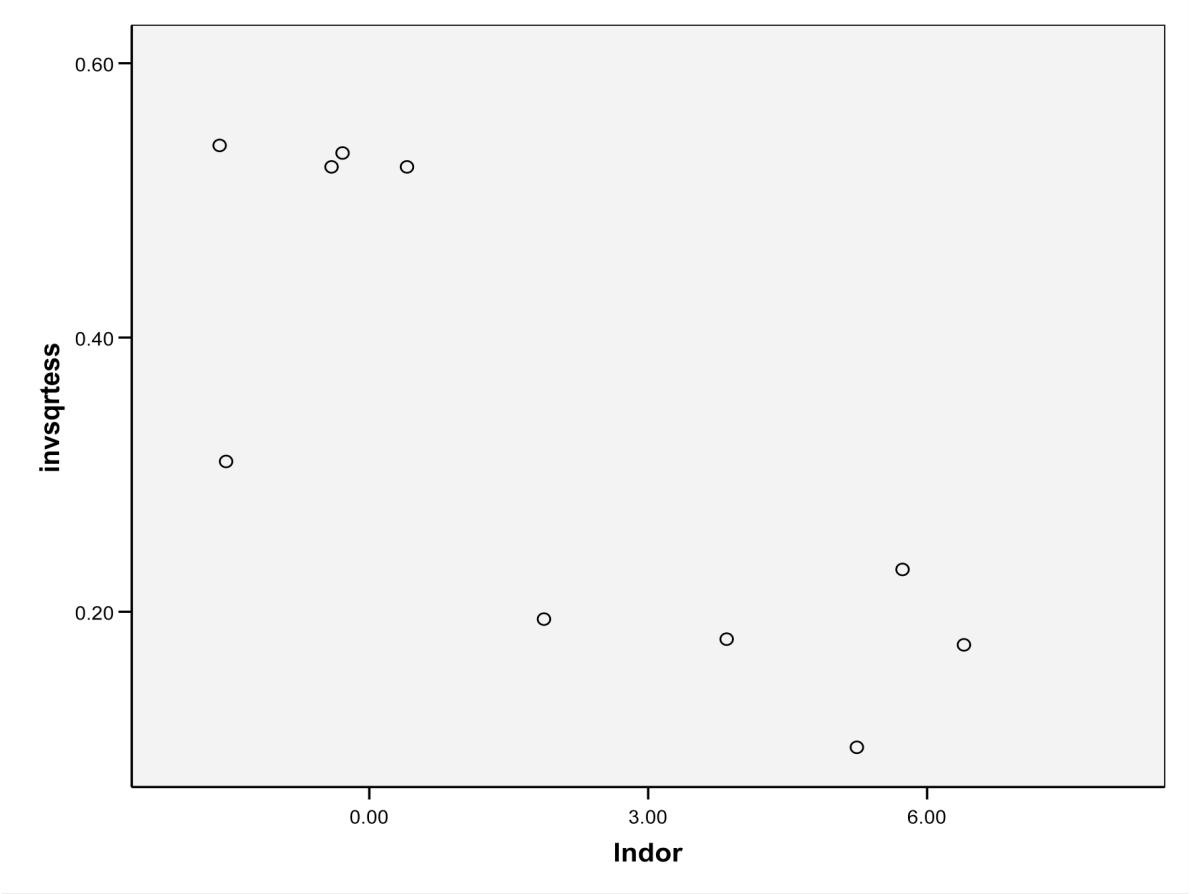
Figure 7. Examination of Publication bias

The p value for publication bias was >0.05 suggesting a lack of publication bias.

NB: Invsqrtess= Inverse of square root of effective sample size, Indor=inverse of diagnostic odds ratio.



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Poor accuracy of post-mortem MR imaging in the published studies could be due to a wide range of factors including:

(a) Inappropriate sequences – Most post-mortem studies have primarily used 2D data sets. Whilst this may be appropriate for brain imaging, 3D sequences may provide superior information on visceral organs, in particular heart.

(b) Low field strengths – All the studies have used field strength of 1.5 Tesla or below. High field MR imaging at 9.4 Tesla has been reported to rival light microscopy in animal models (Driehuys et al. 2008; Petiet et al. 2008), however, there is very little published data in humans.

(c) Lack of experience and expertise in reporting post-mortem MR images, particularly with regards to post-mortem cardiac imaging may be a major reason for the poor accuracy

(d) Poor quality study design and small sample size of the included studies – Confidence intervals for pooled diagnostic indices were wide reflecting small number of cases in the studies rather than statistical heterogeneity. Very few studies have used rigorous double-blinded evaluation of post-mortem MR in detecting clinical important lesions, rather than post-mortem artefacts.

Thus, the published data prior to 2009 do not support the use of post-mortem MR imaging as an alternative for conventional autopsy in fetuses, children or adults. The data suggests that, offering MR imaging as an alternative to autopsy, may not detect major pathologies, particularly with regards to unnatural causes of death, homicide and may give misleading information. Further development in post-mortem MR techniques and rigorous evaluation in large prospective studies are required, before this is used as a clinical tool.

However, there are substantial difficulties in undertaking prospective post-mortem research, particularly in obtaining consent from newly bereaved parents for such research. This has led to virtual disappearance of autopsy research in the UK. These issues and a model for effective prospective research are described in the next chapter.

CHAPTER 5: CONSENT FOR POST-MORTEM IMAGING

5.1 SUMMARY

Pessimism regarding prospectively consented paediatric autopsy research in the UK has resulted from organ retention controversies, recent changes in the Coroners' (Amendment) Rules 2005 and the Human Tissue Act. Here I examined the feasibility and acceptability of a prospective telephone consenting model for post-mortem magnetic resonance (MR) imaging research in HM Coroners' cases. Following each autopsy referral from HM Coroner, permission to contact the family for research was requested. A family liaison sister, with experience in dealing with bereaved families, then contacted the parents by telephone, explained the study and obtained oral, and then written consent for post-mortem imaging. Of the 76 eligible HM Coroner's cases referred during the study period, permission to contact parents (provided by HM Coroners' Office) was obtained for 32 cases (42%). The research sister contacted 32 parents during the study period of which 31 (96.8%) gave oral research consent. 'Helping other parents in the future' and 'the importance of post-mortem research' were the main reasons for parents wanting to participate in research. Prospective consenting for HM Coroners' cases for research is feasible in children, and can be done ethically by parental consenting via telephone contact, before autopsy by appropriately trained staff. However, close co-ordination between mortuary staff, HM Coroners, research staff and medical staff is required.

5.2 INTRODUCTION

The diagnostic accuracy of any new investigation can be adequately evaluated, only by comparing against an accepted gold standard; therefore it was important to compare the accuracy of post-mortem MR imaging with the independent gold standard i.e. conventional autopsy. This also meant that only the cases in which parents consented for a complete autopsy were eligible for the study. Obtaining research consent from newly bereaved parents is an extremely difficult and sensitive issue. This is even difficult in cases under the investigation of HM Coroner, as the access to these parents is challenging. However, as vast majority of autopsies in the UK are performed on the behalf of HM Coroner, the only way to have adequate number cases for this study was to recruit HM Coroners cases. Furthermore, HM Coroners cases are the population most likely to benefit from this research and the primary reason why the Department of Health funded this project.

Controversies relating to organ retention and recent changes to the Coroners Rules and the Human Tissue Act in the UK have added to the complexities of autopsy research in children (Thayyil et al. 2008a). Whilst HM Coroners do not require parental consent for retention of tissue for diagnostic investigation of the cause of death, no tissue or data (e.g. magnetic resonance (MR) imaging) can be collected or retained for research purposes alone, without explicit parental consent. According to the new regulations, it is the duty of the HM Coroner to inform the pathologist of the parents' wishes for the use of residual tissue in research within a specified time period. However, many HM Coroners' Officers are not trained to, nor have adequate resources to, obtain informed research consent from parents. Nevertheless, if parental wishes are not known within this period, the tissues taken at autopsy must be disposed of by the hospital. HM Coroners vary widely in their adoption and interpretation of the new rules (Delaney et al. 2007) and only in a minority of cases are appropriate parental consent information for the pathologist (Weber et al. 2007; Weber et al. 2008c).

Moreover, even in cases where HM Coroners' Officers do provide signed consent forms, these are usually not available at the time of autopsy (Weber et al. 2007). Most prospective autopsy research requires specimens to be taken soon after death or

during the autopsy; delay or improper processing will diminish or even abolish the value for research purposes (Krous et al. 2004; Millar et al. 2007).

The major issue affecting the organ retention discussions was lack of appropriate consent and involvement of the next of kin. Many affected families stated that they had no objection to post-mortem research and retention of tissues for the same; their complaint was primarily that they were neither aware nor involved in such a process (Millar et al. 2007). However, contacting newly bereaved parents for research consent has been perceived as unethical (Krous et al. 2004) and this complex ethical situation has lead to a virtual disappearance of prospective autopsy research in the UK.

In an attempt to improve this situation and to recruit cases into the current project, I initiated extensive consultations with several key people and experts in the Department of Health, Human tissue bank, ethics committee and HM Coroners. One very practical solution was to perform post-mortem MR imaging as a part of Coronial autopsy and to obtain parental consent 6 to 8 weeks later, when the case may be off subjudice. This time was optimal, as the HM Coroners officers normally meet up with parents at this time, to inform them about the autopsy results and to ask what parents would like to do with residual tissues, that may be collected at the time of autopsy as a part of the clinical work up.

However, the UK department of Health suggested this would not be in line with the Human Tissue Authority act, and upfront consenting was required before acquiring any data for research purposes. However, obtaining upfront consenting would be impracticable, as over 80 HM Coroners referred cases for autopsy to Great Ormond Street Hospital; therefore it was physically impossible for myself as the researcher to take consent from the parents directly. A pragmatic alternative would be for consent to be taken by the HM Coroners officer at the time of referral for autopsy. HM Coroners officers normally obtain the consent for use of tissue in research at this time, therefore, logistically it would much easier to obtain consent for MR imaging at the same time. Therefore, I initiated extensive discussion with the HM Coroners to explore this possibility. Unfortunately, such an option was not possible as the HM Coronial services were already overstretched and they did not wished to be involved in research. To further complicate things, in cases being investigated by HM Coroner, the hospital staff are not permitted to contact the parents directly, without the permission of the HM Coroner; A no win situation!

A final approach would be to obtain permission from the HM Coroners on a case-by-case basis to contact parents, and then to obtain telephone consent from parents. Given that the window of opportunity, between referral of a case for autopsy to Great Ormond Street Hospital and performing the autopsy might be a few hours only, the logistics of this model would be challenging. Furthermore, the ethical aspects and parental attitudes towards a 'cold call' was not known.

5.3 METHODS

Prior to the start of the study, 20 key HM Coroners from London and the surrounding areas (out of a total of 65 HM Coroners referring cases to Great Ormond Street Hospital for Children) were approached explaining the study and requesting assistance with this process. It was made clear that MR and CT scans would be performed without extra cost to HM Coroners and would not cause any delay to conventional autopsy. The study was approved by the local research ethics committee and was funded by the UK Department of Health.

This study was performed over a five-month period between February and July 2008. Following each referral for HM Coroner's autopsy, a researcher contacted the HM Coroner's Officer, requesting permission to contact the parents (Figure 8). If agreed, a senior family liaison sister (trained in family counselling and with extensive experience in dealing with recently bereaved families) then contacted the parents by telephone, explained the study and took oral consent for MR imaging.

A written consent form was then sent through the post. Responses of the parents and HM Coroners' Officers regarding participation in the research were recorded in a systematic way. MR and CT imaging were performed prior to a standard HMC autopsy, in cases where consent was obtained.

5.4 RESULTS

Of the 20 HM Coroners approached for assistance prior to the start of the study, 2 declined, 1 requested additional funding to participate, 2 agreed and 15 did not reply.

During the study period 76 cases from 38 different HM Coroners were eligible for recruitment. HM Coroners' officers were approached in all these cases requesting permission to contact the parents for research, except those where the Coroner had

requested no future contact, or if the autopsy had to be carried out within 4 hours of arrival. Permission to contact the parents was granted in 32 cases.

The research nurse contacted all of the 32 parents by telephone, of which 31 (96.8%) gave oral research consent. The median age at death was 2 months (range 1 day to 7 years) and median time after death at the time of consenting 2 days (1 to 7 days). Median time of telephone conversations was 25 minutes (5 to 90 minutes). In three cases, the parents requested more time to consider their options and requested a second telephone call some hours later; all three parents consented following the second telephone call.

Responses of the HM Coroners' Officers and parents are given in Table 8 and Table 9 respectively. All parents felt that telephone contact by the bereavement nurse was an appropriate way of requesting consent, including the parent who refused consent to participate in the research. All the parents wanted to know more about the post-mortem process, when it would take place and when the body would be returned to them. Most parents spontaneously talked about the circumstances that led to the death of their child. The script used by the family liaison sister for consenting is given in Table 10.

An information leaflet and a written consent form were sent out in the post and were received back between 1-8 weeks after telephone consenting in all cases, without any reminders. Two parents requested that the research papers published from the study be sent to them. Three parents contacted the research doctor directly at a later stage by telephone to make sure that the child had indeed been recruited into the study.

Table 8. Responses from the HM Coroners/Coroner's Officers regarding contacting parents for research

Those who granted permission to contact parents (n=32)	
Very important research/will give full support	6
No comments, but agree for research team to contact parents	25
Often have parents requesting that a full autopsy is not performed and this research would be beneficial for such parents	1
Those who declined permission to contact parents (n=44)	
HM Coroner too busy/not available/autopsy had to be done within 4 hours of arrival in hospital	34
Inappropriate to contact bereaved parents soon after death	5
Parents won't understand the research	3
Not appropriate to consent parents twice, as the HM Coroner's Officer will be taking tissue research consent later	2

Table 9. Reason given by parents for agreeing or declining to participate in the research

Parents who consented to research (n=31)	
Want to help other parents in similar situation	13
Research is important	4
Want to have as much information as possible	4
No specific reason given	10
Parents who declined to participate in MRI research (n=1)	
Want only one autopsy, either less invasive or normal, not both	**1**

Figure 8. Flow chart for the consenting process

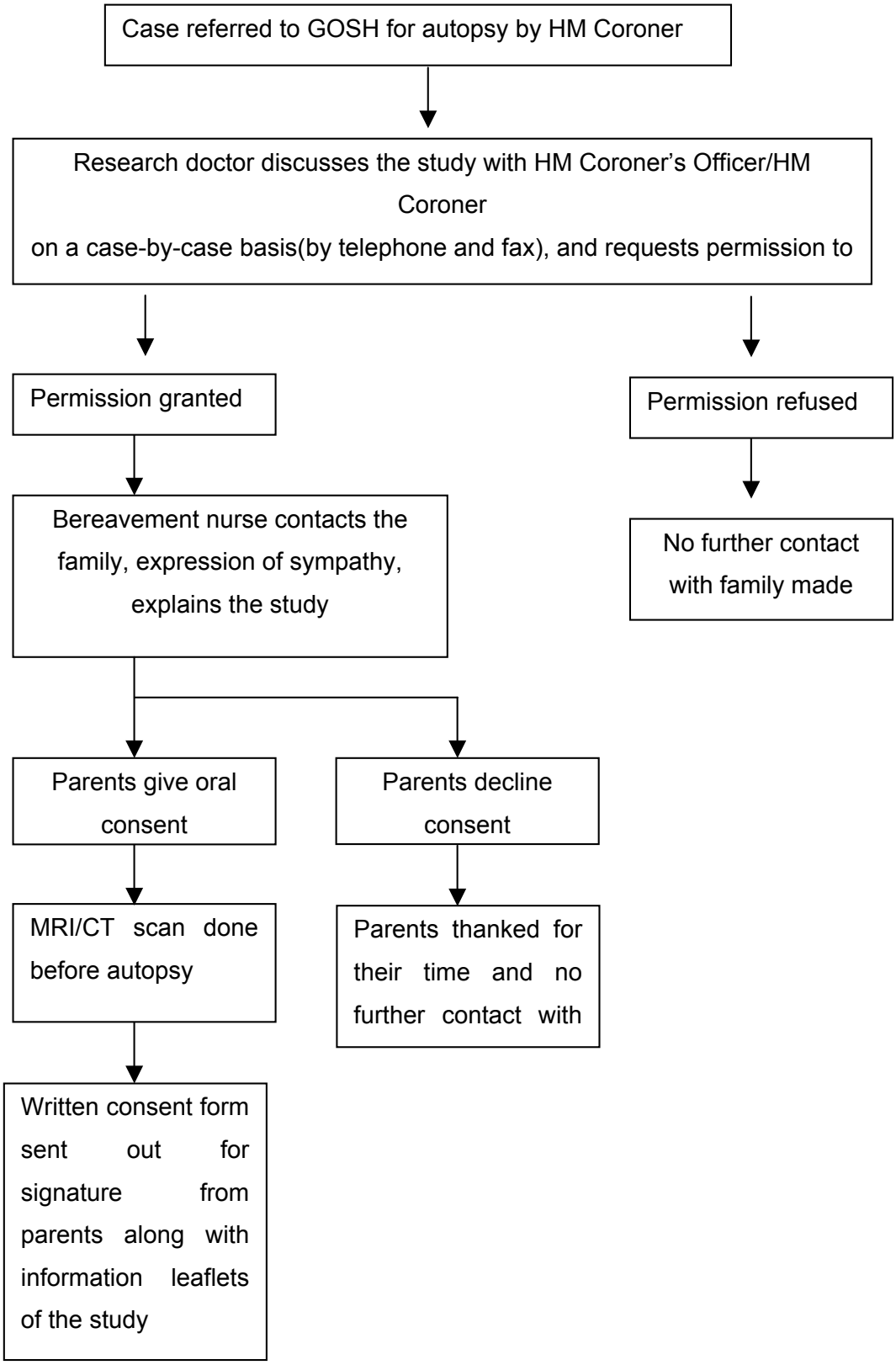


Table 10. Script used by the family liaison sister for consenting

1. General introductions
2. Confirm that parents are aware of the autopsy being performed at Great Ormond Street Hospital for Children.
3. Sympathetic listening to parents if they wish to talk about events around death, spontaneously.
4. Answer parental queries regarding post-mortem examination if any, time when it will be performed and when the child would be released for burial / cremation.
5. Explain regarding the research on post-mortem MR imaging.
6. Check if parents would like to have some time to think about it. (In which case a second telephone call is made, if parents wish)
7. Obtain oral consent, if parents to wish to participate
8. If parents do not wish to participate, thanks expressed and re-assured their wishes would be honoured.
9. Check if the parents considered the cold call obtrusive and apologise if that was the case.
10. Send the information leaflet and consent form by post for parents to sign and return.

5.5 DISCUSSION

This data demonstrates that prospective parental research consenting prior to autopsy is feasible following an unexpected death of a child and can be undertaken in an ethical manner that appears acceptable to the majority of parents. Contrary to popular belief that newly bereaved parents would react angrily to such an approach, I found that most parents viewed the process positively and did not have any objections to the telephone approach. Indeed, most parents spontaneously quoted their wish to help other parents in similar situations in the future as their main reason for participating in the study. This model has major implications for paediatric autopsy research in the UK.

The model in this study is very similar to the prospective telephone consenting model that was used to establish the MRC Sudden Death Brain and Tissue Bank in adults, in Edinburgh (Thali et al. 2003a; Millar et al. 2007). This MRC tissue bank was established by a joint effort with the area Procurator Fiscal Service (analogous to the HM Coroner in England and Wales) in Edinburgh, the police and forensic pathologists. The model was highly successful in contributing to a European network (BrainNet Europe) for brain tissue in adults (Millar et al. 2007; Millar et al. 2008). Though our initial approach with the HM Coroners' in London and surrounding areas were unsuccessful, as the study progressed the majority of HM Coroners' became supportive of the research.

It needs to be appreciated that the sudden unexplained death of a previously healthy child is a traumatic event which may have very different implications for the family compared to the death of an elderly relative. Therefore, it cannot be assumed that a model that works for adults and the elderly is directly applicable to paediatric cases. In California, legislation was recently amended for mandatory inclusion of SUDI cases into research without parental consent (Krous et al. 2004). Several experts have proposed a similar model for other countries, making research use of post-mortem tissue "opted in", for the greater benefits of society (Krous et al. 2004); however, this was rejected by the UK Parliament.

Consenting by telephone has been suggested to be intrusive as opposed to consent request by post. Again, in this small cohort, we did not find any parent who found this method inappropriate when specifically asked. In fact, it appears that the opportunity to discuss the post-mortem process soon after death with a bereavement nurse was beneficial to parents, although the nurse did not offer specific bereavement counselling. Clearly the experience and personality of the person taking research consent is of paramount importance.

The perceptions of professionals that parents are unwilling to consent to research in HM Coroner's post-mortem cases (only 42% of parents referred for telephone consenting by the Coroners) appears to differ from the parents' willingness to take part in research (97% of those subsequently asked). Though this may represent extremely good screening by the Coroners (only those who were referred would agree to take part in the study), it is more likely that the preconceived opinions of

professionals involved in the post-mortem process may be different from the reality of most parents regarding their choice to participate in research.

Snowdon e*t al.* have reported a sharp contrast between the attitudes of clinicians and bereaved parents to participation in post-mortem research (Snowdon et al. 2004c; Snowdon et al. 2004b). Post-mortem research was highly valued by parents who were deeply affected by the loss of their baby. Parents were keen to make a contribution to research, which was driven primarily by altruism and a feeling that the child's life was not wasted. Such participation is thought to be beneficial in the bereavement process. Conversely, clinicians may be reluctant to recruit children in trials related to post-mortem studies, for the fear of seemingly inappropriate and insensitive requests. The authors comment that the caution and selectivity exercised by the doctors may not be appropriate, at least for some parents.

Traditionally autopsy research in the UK has been performed by obtaining a waiver for explicit parental consent from the host institutional research ethics committees, or by obtaining retrospective parental consent at a later stage (Embleton et al. 2001; Kinoshita et al. 2001). In HM Coroners' cases, even though the Coroner has the authority to permit any post-mortem investigation into the cause of death, this cannot be used for post-mortem research studies, which involve additional investigations or sample taking. Prospective parental consent is required prior to performing any additional research investigation in such cases. Ethical concerns about such research have been raised even for non-invasive post-mortem research (e.g. MR imaging), when performed without explicit parental consent (Kinoshita et al. 2001; Lane et al. 2001).

Engagement of parents is the key to credible post-mortem research, ensuring that lessons are learnt from the organ retention discussions. The proposed "Less Invasive Autopsy" project by the Department of Health is based on such parental engagement, and is intended to eventually offer a modified autopsy protocol, which, along with continual involvement of parents, will hopefully improve the consented autopsy rates in hospital deaths of all ages as well.

The model we have proposed could also potentially be used to allow for the creation of post-mortem research tissue banks that could be used to address the continuing issues related to sudden death research. However, at present this system is far less

effective than the consenting system used by the MRC Sudden Death Brain and Tissue Bank, in collaboration with the Procurator Fiscals in Scotland (Thali et al. 2003a; Millar et al. 2007). This is due to the differences in Coroners rules in the regions, and an amendment to HM Coroners rules would be required to use a similar system in England (Delaney et al. 2007; Weber et al. 2007). From a pragmatic perspective, we would propose a model for discussing all areas of research in which the HM Coroner's Officer provides the hospital with parent contact details where appropriate at the time of referral and a bereavement nurse could then contact the family to explain the autopsy process, expected time frames and take oral consent for post-mortem research, such as imaging and tissue retention, prior to the autopsy, which would be followed up by written consent. This model may also be acceptable and beneficial to the under-resourced HM Coronial system in the long-term.

While the storage of tissues from consented cases would be covered by ethical approval of individual projects, tissue banks are required to store the tissue once the specific research projects have been completed. Such paediatric post-mortem tissue banks have not yet been established in the UK, and use of this model may be the first step towards such a process. It is likely that even in hospital cases, more parents may consent to use of post-mortem tissues for research, if an appropriate bereavement nurse is involved in such discussions.

In summary prospective parental consenting by telephone is feasible before paediatric autopsies in HM Coronial cases. However, the current system allows this to occur only in a minority of cases and therefore many parents may be denied the opportunity to participate in post-mortem research. I used this model in the prospective study to compare the accuracy of post-mortem MR imaging with conventional autopsy and this study is described in the next two chapters.

CHAPTER 6: STUDY PROTOCOL

In this chapter, I describe some of the common methods that have been used throughout this thesis for pre-autopsy MR imaging. Prior to the start of the study, a series of meetings with pathologists and radiologists were conducted to decide on the MR sequences and data collection in a systematic way, so that comparison between MR images and autopsy reports was possible.

To examine the accuracy of any diagnostic test, it is essential that it is compared with an external reference test i.e. the 'gold standard', in a blinded way. The index test and the gold standard should be completely independent of each other. Throughout this work, I have used conventional autopsy as an independent gold standard. All autopsies in this work have been conducted according to the guidelines of the Royal College of Pathologists by experienced paediatric or perinatal pathologists. Nevertheless, it is possible that conventional autopsy techniques may have some systematic errors.

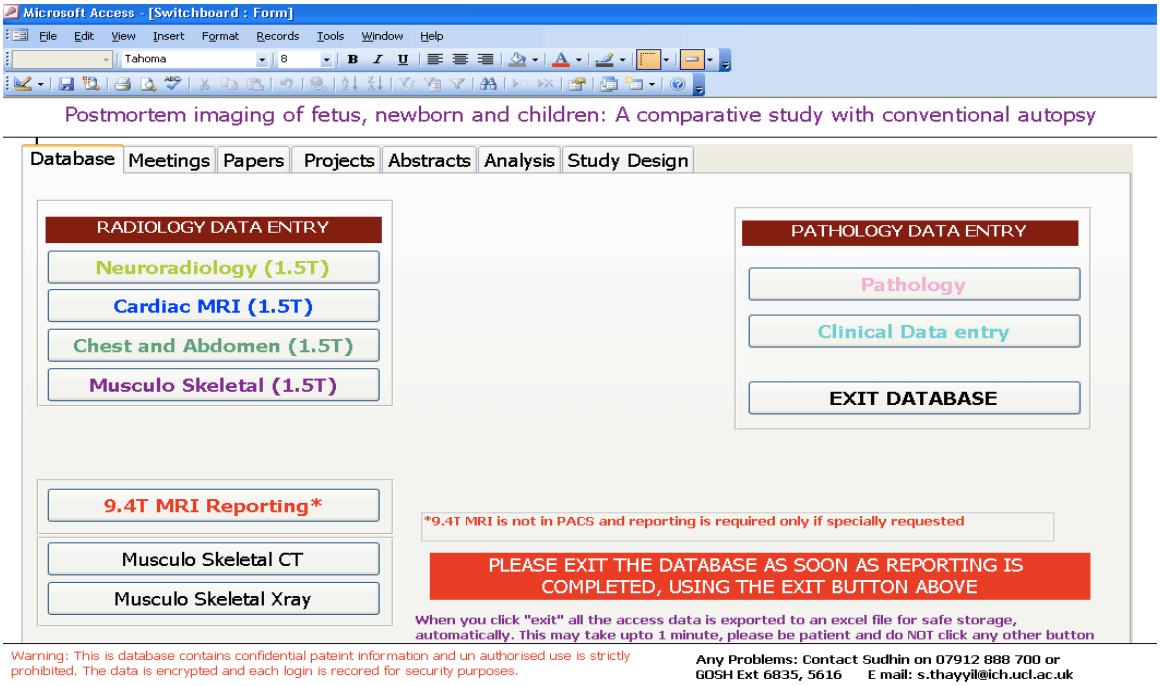
6.1 DATABASE

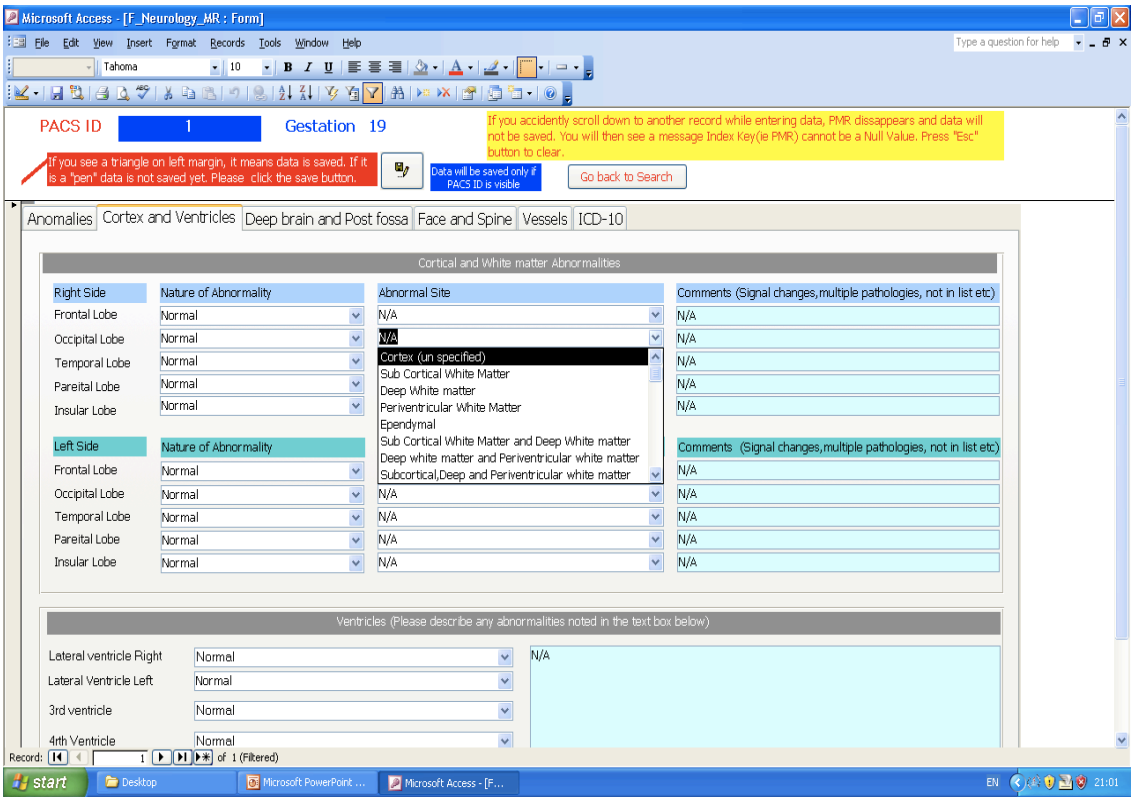
It was clear that a large number of variables needed to be collected using predefined variables and that a database was required. Therefore, during the first 3 months of this project, I developed a large Access database (Microsoft Inc, USA), with drop down options based on these terminologies, for each variable. The tables were linked to each other using a one to one relation. The data was collected into one back end database and was fed through separate frontend databases for the radiologists and pathologists, so that they remained blinded to each others reports

6.2 ETHICS APPROVAL AND CONSENT

Consent in HM Coroners cases was taken using the model described in chapter 5. Briefly this involved a rapid chain of events starting with the mortuary technician alerting me of a potential case being referred, I then contacted the HM Coroners officer to collect ask permission for contacting parents and to collect the contact details. Often this involved detailed explanations of the study each time and faxing the study sheets etc. Once the permission was obtained, I contacted the research nurse (Angie Scales) who rang the parents to take an oral consent for MR imaging via telephone. Following this, I performed the MR imaging immediately, so that autopsy could be performed without any delay.

Figure 9. Frontend of the database





Consent in hospital cases was obtained as a part of the standard NHS consent form, by the clinicians consenting for autopsy. The study was approved by the ICH/GOSH research ethics committee (Please see the Appendix).

6.3 TRANSPORT OF BODIES FOR MR IMAGING

The cases for post-mortem MR imaging were recruited from two hospitals, University College Hospital (UCH), London and the Great Ormond Street Hospital (GOSH). At GOSH, the MR scanner is situated on the same level as the mortuary, within 100 metres proximity (Figure 11). Therefore, I transferred these cases in special containers for MR scanning, avoiding other patients or children in the scanning area. I transported the bodies from UCH using a dedicated vehicle. Most cases at both these hospitals underwent detailed skeletal survey (or faxitron in fetal cases), either before or after the MR scan. Conventional autopsy was performed after the post-mortem MR scan.

6.4 PROTOCOL FOR MR IMAGING

All MR scans were performed on a 1.5 Tesla Siemens Avanto scanner (Erlangen, Germany) at GOSH, except where indicated other wise (please see chapter 12). Most scans were performed out of hours between 5 pm to 12 midnight by myself (Figure 10).

Daytime scans were performed by one of the two research radiographers. The bodies were examined to remove any metallic objects, for example intraosseus needles, prior to the scan. Detailed scanning protocol is given in Table 11.

Figure 10. MR Scanning facility at Great Ormond Street Hospital

Top panel shows the MR console and the magnet. Bottom panel shows the scanning table and fluoroscopy suite. Note the lead door between the magnet and fluoroscopy suite. The MR table can slide between MR and fluoroscopy suite for co-registration techniques, when the door is open.

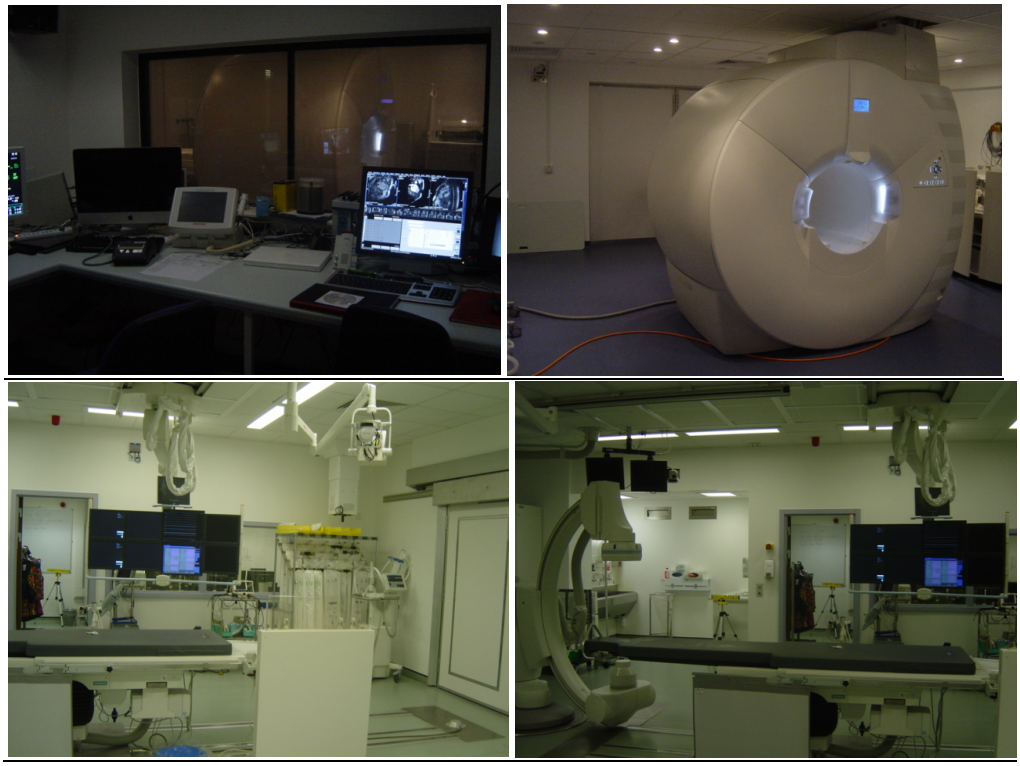


Figure 11: Mortuary at Great Ormond Street Hospital

Refrigerators for storing bodies at 4 ^{0}C and the transport containers used for infants and older children are shown in the top panel. Bottom panel shows the autopsy room.

Field strength of the scanner: I used a 1.5 Tesla scanner for this study and for the vast majority of the work described in this thesis. Increasingly 3 Tesla scanners are being used in clinical practice. The signal to noise ratio (SNR), and hence the resolutions that can be achieved increases with the field strength. Nevertheless, for a foreseeable future 1.5Tesla MR scanners are likely to be the workhorse in radiology. Furthermore, SNR can be increased by increasing the scan times (for example increasing number of acquisitions) and higher resolutions can be achieved with 1.5 Tesla scanners, if required, as the duration of scan is not a major issue in post-mortem imaging.

Coils: I used a multichannel (8 channels) standard head matrix coil for scanning head in newborns and children. Smaller fetuses were scanned entirely inside the head coil. A multichannel spine matrix coil in combination with one or two phased-array body matrix coils (6 channels)were used for scanning of the body.

MR sequences: I used standard sequences that were available in the MR scanner for use in live patients of the same age and organ (Table 11, Figure 12). No particular adaptations were made with regards to any of the MR parameters apart from using higher resolutions and longer scan times.

3D Constructive Interference Steady State (CISS): This Siemens specific gradient echo sequence is a stimulated T_2 echo and provides strong T_2-weighted 3D images. The advantage of this sequence is very high SNR and resolution that can be achieved (McRobbie et al. 2007: 66-67). I used a 3D isotropic CISS sequence for brain and whole body imaging, particularly heart.

T_1-weighted sequences: I used an isotropic 3D Fast Low Angle Shot (FLASH) sequence, which allowed heavy T_1-weighting for brain imaging and an isotropic 3D volumetric interpolated breath-hold sequence (VIBE) for T_1 weighted body imaging. VIBE is a modified fast 3D gradient-echo sequence (McRobbie et al. 2007: 66-67). A gradient echo (haem) sequence was used to increase sensitivity of haemorrhage detection.

T_2-weighted sequences: For T_2-weighted brain imaging, I used a dual echo-tau Short Inversion Recovery (De STIR) sequence; this sequence, particularly the shorter echo time (proton density) gives good contrast in presence of high brain water content as in paediatric and post-mortem imaging. 2D sequences with high in plane resolution were

acquired in sagital and coronal planes. For body imaging, I used 3D T_2-weighted turbo spin echo sequences.

The total scan duration was between 1 to 1 ½ hours for infants and children and between 1 ½ to 2 hours for fetuses. The next chapter describes a large prospective study on post-mortem MR imaging 200 cases of fetuses, newborns and children using these sequences, and comparing it with conventional autopsy.

Table 11. Details of the MR sequences

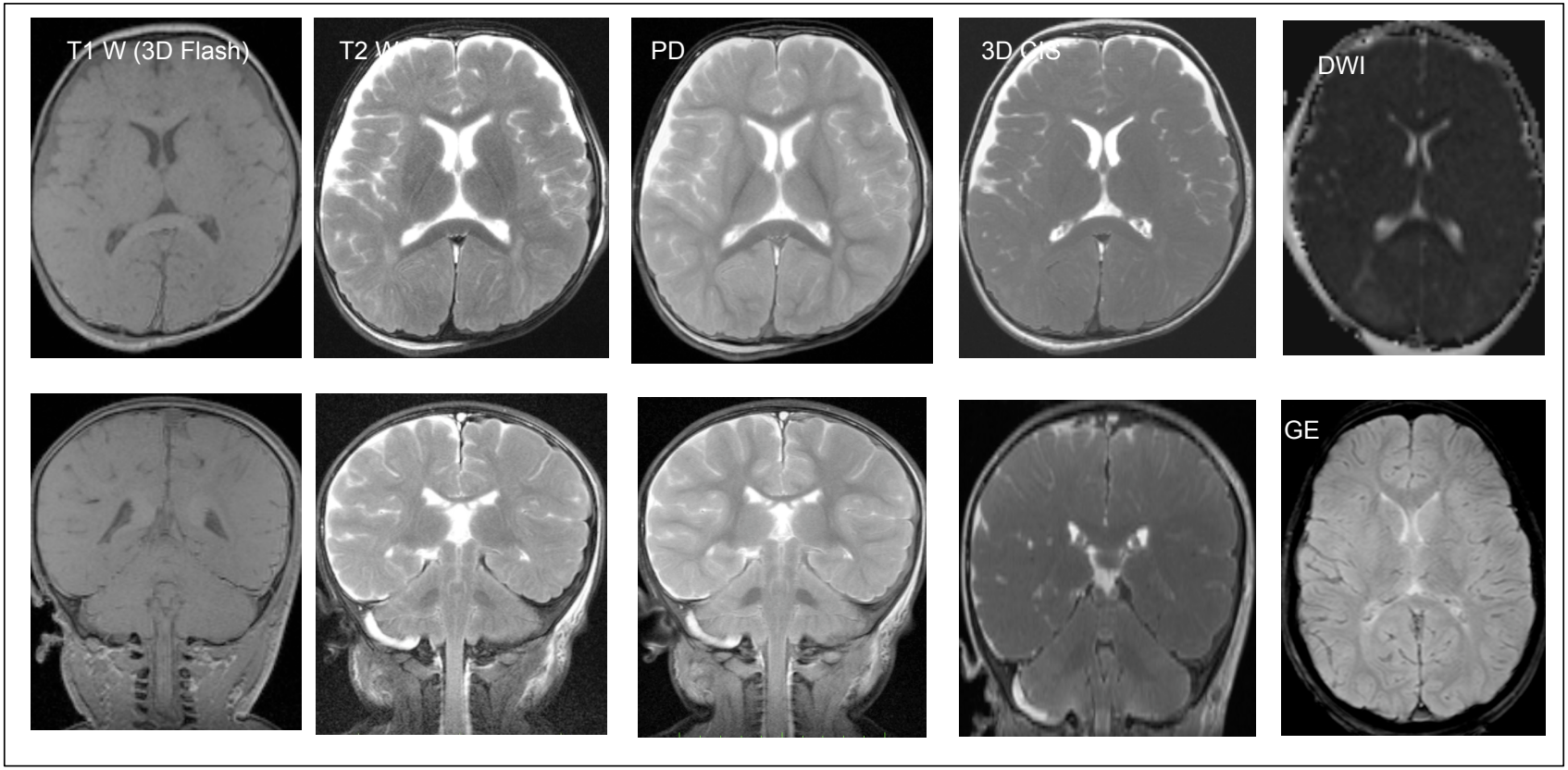
Sequence	Voxel size	TA (ms)	TR (ms)	TE (ms)	Flip angle^0	Averages
BRAIN IMAGING						
3 D CISS	0.6x0.6x.06 mm	13.5	9.2	4.6	70	4
3D Flash T_1W	1x1x1 mm	5.4	11	4.9	15	3
2D Destir T_2W	0.4x0.4x0.4 mm	13.5	5460	14,115	150	6
GE (Haem)	0.5x0.4x4 mm	6.3	800	26	20	4
DWI	B=0, b=500, b=1000					
SPINE IMAGING						
2D T_2–W TSE (children only)	1x1x3 mm	5.43	3050	109	170	3
3D CISS (fetus only)	0.6x0.6x1 mm	4.2	9.1	4.5	70	8
3D T_1–W Flash	0.6x0.6x1 mm	3.5	11	5.3	15	10
BODY IMAGING						
T_2 W TSE	0.8x0.8x0.8 mm	6.2	3500	276		2
3D CISS	0.8x0.8x0.8 mm	5.2	5.2	2.3	54	3
3D T_1W VIBE	0.8x0.8x0.8 mm	5.5	5.9	2.4	25	8
3D CISS (cardiac)	0.6x0.6x0.6 mm	29	5.6	2.5	54	10

TA: Time for acquisition, TR: Relaxation time, TE: Echo time

CISS: Constructive Interference Steady State, GE: Gradient Echo, TSE: Turbo spin echo, DWI: Diffusion weighted imaging

Figure 12. Post-mortem MR imaging of a normal brain in an infant

Axial and Coronal views are shown (PD: Proton density, GE: Gradient echo)



CHAPTER 7: WHOLE BODY POST-MORTEM MR IMAGING

7.1 INTRODUCTION

The systematic review described in chapter four, highlighted several knowledge gaps, in particular a lack of rigorously conducted prospective studies comparing post-mortem MR imaging with conventional autopsy. Having established a successful pathway for recruitment of cases, this study compares the accuracy of post-mortem MR imaging with conventional autopsy in fetuses, newborns and children.

Comparing post-mortem MR imaging with conventional autopsy is challenging due to a variety of reasons. First, the terminologies used by radiologists for reporting MR imaging are often different to that used by pathologists in describing autopsy findings. This makes analysis difficult. Second, while post-mortem artefacts at conventional autopsy are well described, there is little data on normal post-mortem changes seen on MR imaging. Many lesions that may be considered as pathological on MR imaging during life may have little significance after death. Hence many studies have mistakenly claimed higher accuracies of post-mortem MR imaging based on these changes, particularly air and fluid collections (Brookes et al. 1996; Cohen et al. 2008). Third, there is potential to introduce bias by multiple comparisons on the same case. Several published studies have used such an analysis, rendering confidence interval of diagnostic tests, falsely narrow (Weustink et al. 2009). Fourth, there may be systematic errors in conventional autopsy procedures, particularly with regards to fetal brain where brain is frequently autolysed, thus neuropathological examination may be sub optimal. Therefore the gold standard may not be infallible (Lavanya et al. 2008). Fifth, it may be inappropriate to compare post-mortem MR imaging with all components of autopsy. For example the information that can be obtained from examination of placenta cannot be obtained by post-mortem MR imaging or by undertaking open dissection of the body. Therefore a more pragmatic approach would be comparing the accuracy of post-mortem MR imaging with dissection and macroscopic and microscopic examination of visceral organs. Finally, the importance of index test (i.e. MR imaging) and gold standard (i.e. autopsy) being completely independent and blinded to each cannot be overemphasised; nevertheless this is the most common bias in the published literature on post-mortem MR imaging (Thayyil et al. 2009a).

7.2 HYPOTHESIS

Whole body magnetic resonance imaging can provide an accurate, detailed, three-dimensional post-mortem record of structural abnormalities and the disease processes of the whole body in the fetus, neonate and child, with comparable diagnostic information to a conventional autopsy.

This hypothesis will be explored in the remainder of this thesis, systematically and in a staged way. In the first stage, I undertook a prospective blinded comparative study of post-mortem MR imaging and conventional autopsy as described in this chapter.

7.3 AIM

- To examine common post-mortem artefacts on MR imaging
- To compare the accuracy of less invasive autopsy by post-mortem MR imaging with conventional autopsy for detecting the cause of the death
- To compare accuracy of post-mortem MR imaging for detection of pathological lesions in each organ system in fetuses, newborns and children with that of conventional autopsy.

7.4 METHODS

Post-mortem MR imaging was performed in consecutive fetuses, newborns and children referred for conventional autopsy to Great Ormond Street Hospital or University College London Hospital over a 2-year period. Details of methods used for consenting, transport and scanning are described in chapter 6. All bodies were kept in the mortuary at 4 ^{0}C until immediately prior to the MR scan.

Each MR image was reported by a minimum of four radiologists; one per organ system: (1) Brain and spinal cord; (2) Chest and abdomen; (3) Cardiovascular system and (4) Musculoskeletal system. The clinical data were available to the radiologists, however they were blinded to the autopsy data.

Autopsy was performed following the MR imaging by a perinatal or paediatric pathologist. Inputs were provided from paediatric cardiac pathologists or paediatric neuropathologist, as required. The pathologists were blinded to the MR imaging report.

7.4.1 Analysis

Less invasive autopsy by post-mortem MR imaging was defined as a post-mortem examination that included the information form all non-invasive (e.g. imaging, external examination, placental examination, genetic testing) and minimally invasive (e.g. blood test, skin or muscle biopsy) investigations. Conventional autopsy included the information obtained from open dissection and macroscopic and microscopic examination of visceral organs. In addition, all post-mortem information from non-invasive and minimally invasive investigations as above, apart from post-mortem MR imaging was considered as part of conventional autopsy. Therefore the net comparisons were between post-mortem MR imaging and information obtained from dissection and direct examination and microscopy of visceral organs, as the ancillary investigations were included in both these processes.

The analysis was performed in two stages. In the first stage accuracy of MR imaging in identifying the most important pathology (following termination of pregnancy) and/or cause of death (still births, non-fetal cases) was examined. Fetuses were analysed separately to newborns, infants and children. Cases were also sub grouped according to the nature of autopsy referral i.e. hospital autopsy versus HM coronial autopsy. In this analysis comparisons were between less invasive autopsy and conventional autopsy.

In the second phase, the accuracy of post-mortem MR imaging for detection of pathological lesions in each organ system was examined separately. Again the analysis was performed separately in fetuses and children. Only cases in which there were complete data sets of both index tests (MR imaging and the gold standard conventional autopsy) were included in the calculation of the diagnostic indices and confidence intervals. Here comparisons were between the data obtained from post-mortem MR imaging and conventional dissection, internal examination and histological examination of the particular organ.

7.4.2 Sample Size Calculation

Sample sizes were calculated according to the precision of estimate that could be generated for the diagnostic indices. Assuming sensitivity of post-mortem MR imaging to be 60%, 196 cases would be required for a 7% precision in the estimate. If

the sensitivity was in the region of 80%, the precision of estimate would be 5.7%. Likewise for a 90% sensitivity the precision would increase to 4.3%.

7.5 RESULTS

Post-mortem MR imaging was performed on 210 out of the 325 cases referred during the two-year study period. The cases recruited were unselected and consecutive apart from when the scanner or the research personnel was not available or if parental consent was not obtained (8 cases only). The first 10 cases were used for optimisation of the imaging protocol and as learning exercise for the radiologists and were not included in analysis. In one case, parents had consented only for partial autopsy (heart only); this case was therefore excluded. Of the remaining 199 cases, 160 were fetuses and 39 were infants and children. Mean (SD) age at post-mortem MR imaging was 3.5 (2.1) days and mean time at autopsy was 4.5 (3) days. Of the 160 fetal cases included in the study, 63 (39%) were performed following termination of pregnancy and 97 (61%) were unexplained intrauterine deaths. Mean (SD) gestation of the fetuses was 22.7 (6.6) weeks.

7.5.1 Post-mortem Artefacts

In the brain, small amounts of intraventricular bleed without dilation of the ventricles, blood clots or additional bleeds in the parenchyma were seen in 102 (64%) of fetuses with no autopsy correlate. These were considered to be related to the death process and not of any clinical significance. Homogenous appearance of brain occurred with maceration and streaky linear opacities in white matter were seen in 17 (11%) cases of fetal brain. In all these cases, the brain was severely autolysed and no useful information was obtained on histopathology. Abnormal appearance cortical blood vessels were seen in 185 (93%) of cases on gradient echo sequences resembling a crazy pavement. On autopsy no sub-arachnoid bleeds were seen in these cases, and again these probably represent clot in the cortical vessels, indicating such ‘crazy paved appearance’ is probably of no clinical significance (Figure 13, 14). Retinal detachment and collapsed eyeballs were commonly seen in fetuses and this was not considered as pathological on autopsy.

Small amounts of pleural effusions, pericardial effusions and ascites were seen in most cases on post-mortem MR imaging; however these have little clinical significance. Blood clots had varying signal intensities in the heart and again these

were considered as normal post-mortem change (Figure 13). Artefacts may also occur due to blood soaked clothes around the body (Figure 15).

Figure 13. Common post-mortem artefacts on MR imaging

a) Small intra-ventricular bleed in a 22-week fetus without ventricular dilation or clots. b) Crazy paving appearance of the cortex on gradient echo sequence may be due to clots in the superficial blood vessels of the brain in an infant, as these are not seen in proton density weighted MR images. This is a normal post-mortem finding and should not be mistaken for sub-arachnoid bleeds. c) Small pleural and pericardial effusions in an infant. Note thickened appearance of ventricles and variable signal changes in the heart due to blood clots. d) Retinal detachment in a newborn. This is a normal post-mortem change and should not mistaken for retinal bleeds.

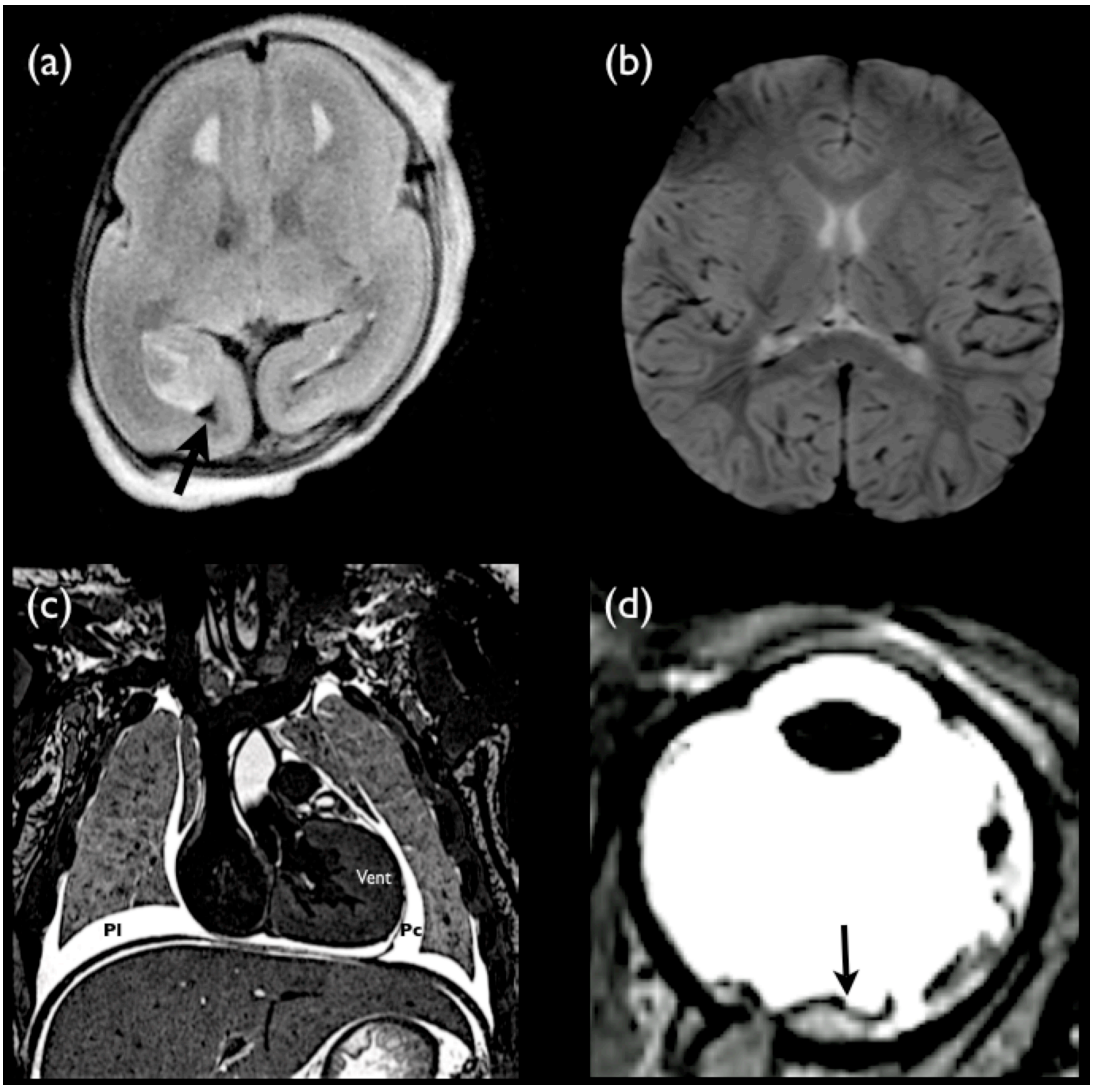
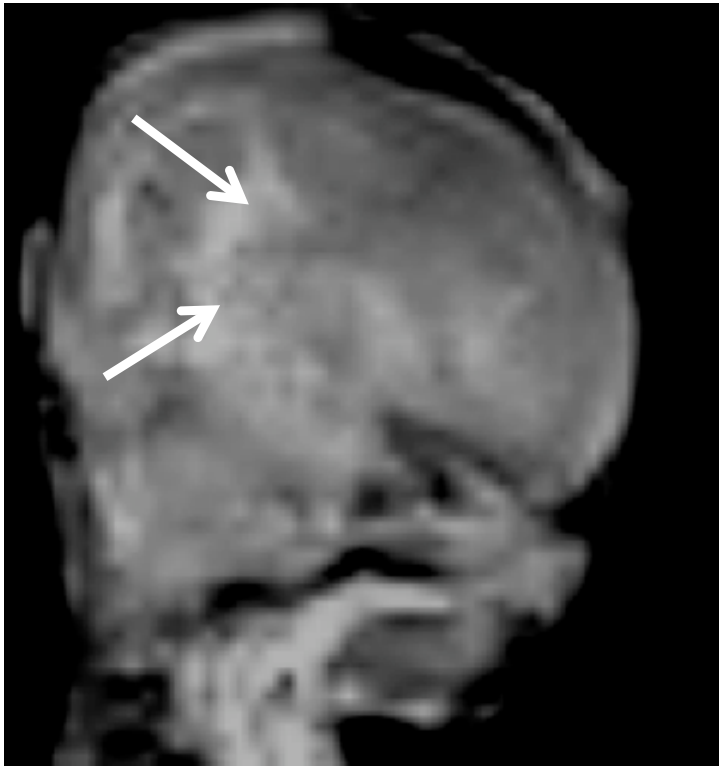


Figure 14. Other artefacts on post-mortem MR imaging

Severe autolysis of brain in a fetus showing homogenous appearance and streaky white matter changes shown on left (white arrow). The MR image was non-diagnostic. The brain was completely liquefied at autopsy and no useful information was obtained. High signal intensity from thyroid on VIBE sequence on a full term still born infant shown on right. Histopathology of thyroid was normal.



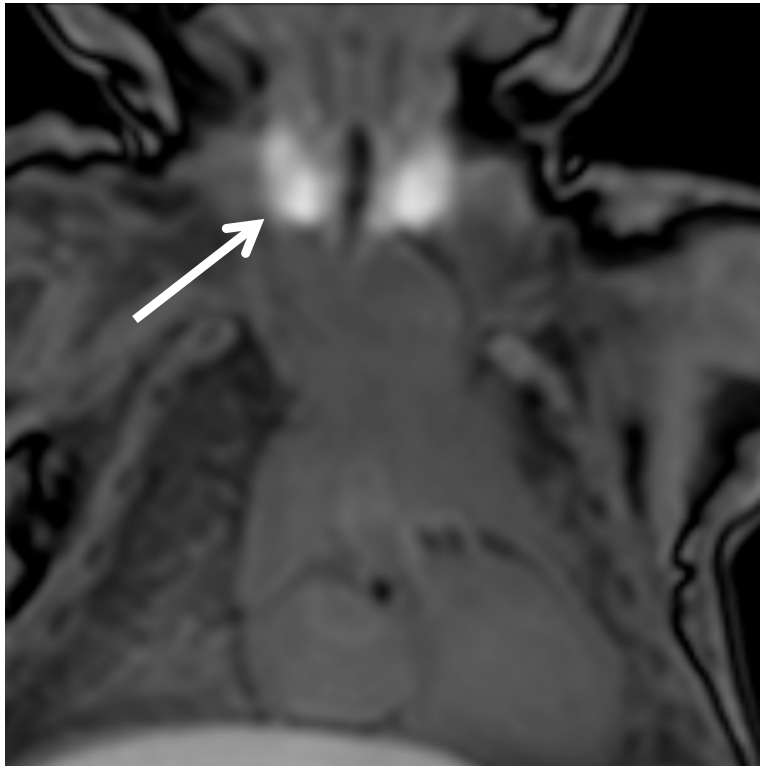
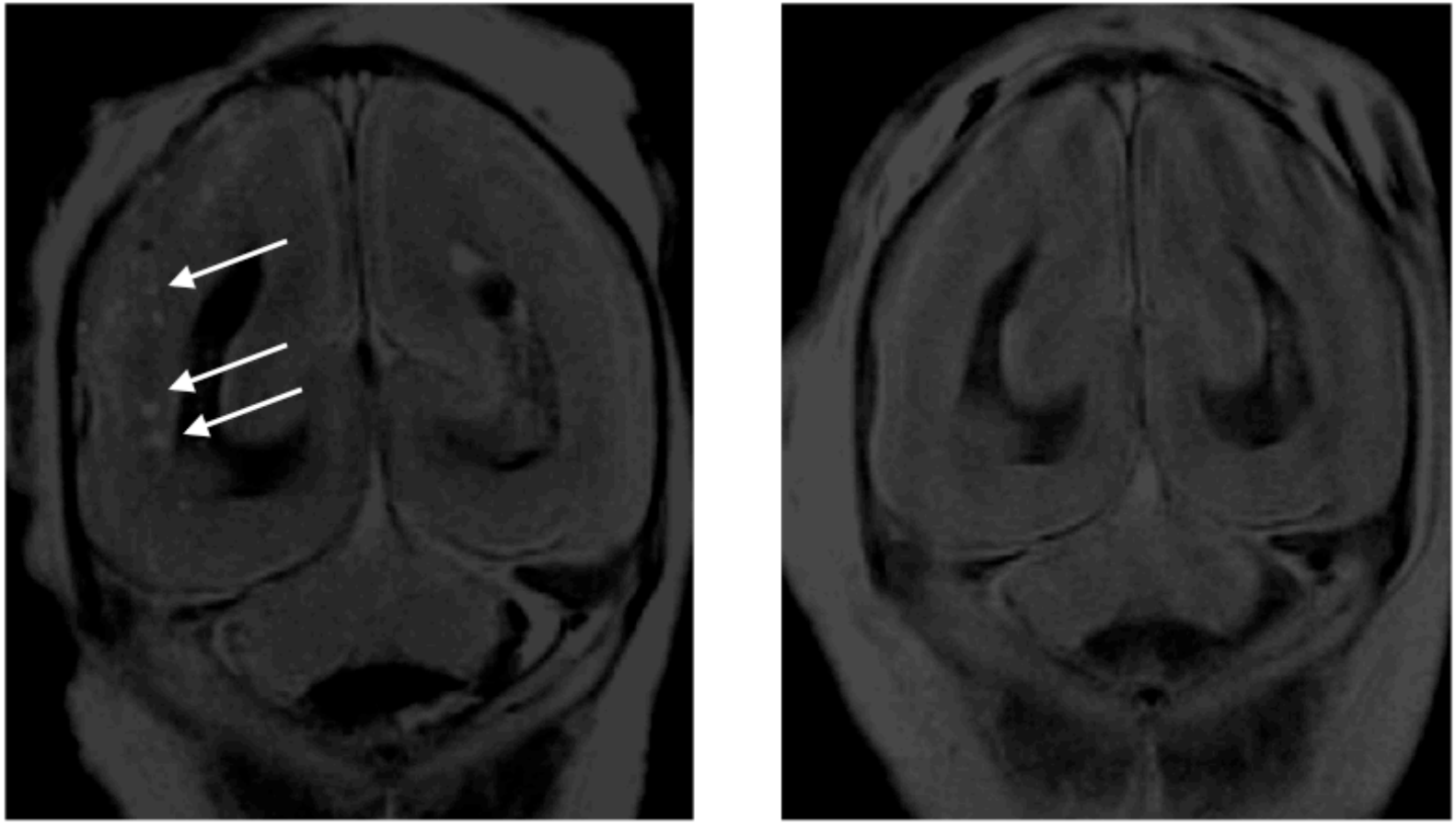


Figure 15. External artefacts

Speckled appearance on the brain (cortex) due to an artefact induced by a blood-stain cloth around fetal head (left). The spot disappeared after removing the cloth.



7.5.2 Fetuses

7.5.2.1 Detection of major pathology and/or cause of death in fetuses

Of the total 160 fetal cases, 97 (61%) were unexplained intrauterine deaths. Of these 97, the cause of death was explained by conventional autopsy in 26 (27%) cases and by less invasive autopsy in 25 (26%) (Table 14). The cause of death detected by conventional autopsy and less invasive autopsy was similar in 96% of the cases (25 out of 26). In the remaining cases (73%), the cause of death remained unexplained. Among the cases where a cause for death could be established, post-mortem MR imaging contributed in 7 (28%) of cases, placenta in 9 (35%) and other blood tests in 3 (12%) cases.

Among the 63 termination of pregnancy, the main reason (ante-mortem diagnosis) for termination of pregnancy was confirmed in 48 (76%) by conventional autopsy and 52 (82.6%) by less invasive autopsy. In 3 cases, the ante-mortem diagnosis was refuted by conventional and in 5 cases by less invasive autopsy. The higher utility of post-mortem MR imaging was related to neuroimaging and autolysis of brain (see below).

Over all, a complete agreement between MR imaging and conventional autopsy was seen in 123 (77%) of cases with regards to detection of all significant pathological lesions (i.e. including the pathologies that may not have caused death). Additional significant information was obtained from placental examination in 30 cases (19%), post-mortem X-ray in 8 (6%) cases, genetic tests in 6 cases (3%).

7.5.2.2 Organ-wise analysis in fetuses

Of the total 160 fetuses, 60 fetuses were less than 20 weeks of gestation. The cardiac MR imaging was non-diagnostic in 42 cases (70%) less than 20 weeks and 18 (18%) $\geq$ 20 weeks; neuroimaging was non-diagnostic in 26 (43%) cases less than 20 weeks and 9 (9%) in $\geq$20 weeks; chest and abdomen images were non-diagnostic in 28 (47%) cases less than 20 weeks and 8 (8%) cases $\geq$20 weeks. Musculoskeletal images were non-diagnostic in 12 (20%) fetuses less than 20 weeks and 18 (18%) in cases more $\geq$20 weeks. Non-diagnostic musculoskeletal images were related to suboptimal positioning of the limbs, rather than the gestation of the fetuses.

Table 12. Cause of death by less invasive autopsy and conventional autopsy in hospital cases

No	Age	Clinical Summary	Key MR findings	Key pathology findings	LIA COD	Autopsy COD	Agreement
1	3 y	Unexplained collapse, abdominal pain	Abnormal bowel dilation, abnormal appendix, No air fluid levels	Appendicitis-ruptured	Appendicitis (Figure 24)	Appendicitis	Yes-M,C
2	1 d	Preterm, gastroschisis	Mal rotation, mid gut volvulus, gastroschisis	Gastroschisis, Volvulus	Volvulus	Volvulus	Yes-M
3	2 d	Severe HIE	Ischemic changes in brain	Ischemic changes in brain	HIE	HIE	Yes-M,C
4	1 d	Severe HIE	Ischemic changes in brain	Ischemic changes in brain	HIE	HIE	Yes-M,C
5	2 d	Unexplained neonatal death	Global infarction	No abnormality	Unexplained	Unexplained	Yes-M,C
6	8 d	Preterm, post op PDA ligation death	SDH, IVH, parenchymal bleed	SDH, IVH, parenchymal bleed	Unexplained intracranial bleeds	Unexplained intracranial bleeds	Yes-M
7	2 d	Severe HIE	Ischemic changes in brain	Ischemic changes in brain	HIE	HIE	Yes-M
8	4 mo	Acute encephalopathy	Thalamic bleed/infarction/necrosis	Acute thalamic necrosis	Acute thalamic necrosis (Figure 22)	Acute thalamic necrosis	Yes-M
9	14 mo	Seizure disorder, liver failure ? Alperts syndrome	Abnormal WM and ventricle shape	Reduced WM, isolated insular polymicrogyria, passive congestion of liver	Likely liver failure	Liver failure	Yes-C
10	5 y	Leukodystrophy, immuno suppressants, sepsis	Calcification in parietal occipital lobes, abnormal and small pancreas.	ARDS and adenovirus infection, haemorrhagic pancreatitis, capillary haemangioma in occipito parietal region	Unexplained	Disseminated adeno virus infection, ARDS	No

Agreement: M=Cause of death based on MRI only, C=Cause of death based on ante-mortem clinical findings, M, X=Cause of death based on X-ray.

SUDI= Sudden and Unexplained Death of an Infant, RA=Right atrium, IVH= Intraventricular haemorrhage, RV=Right ventricle, WM=white matter, ARDS=Adult respiratory distress syndrome, SAH=sub arachnoid haemorrhage, HIE=Hypoxic ischemic encephalopathy, ASD=Atrial septal defect

Table 12. Cause of death by less invasive autopsy and conventional autopsy in hospital cases (continued)

No	Age	Clinical Summary	Key MR findings	Key pathology findings	LIA COD	Autopsy COD	Agreement
11	16 y	Retts syndrome, post op death, Multi organ failure	Microcephaly, diffuse CNS signal changes, pneumonia, ASD, deep collection in spine	Multi organ failure, sepsis, degenerative neuropathological changes	Sepsis, Multi organ failure	Sepsis, Multi organ failure	Yes-M, C
12	21 mo	Septo optic dysplasia, chicken pox pneumonitis, respiratory failure	Septo optic dysplasia, B/L schizencephaly, absent septum pellucidum, Big RA, RV	Septo-optic dysplasia, polymicrogyria, schizencephaly, chicken pox pneumonitis	Chicken pox pneumonitis	Chicken pox pneumonitis	Yes-C
13	1 d	Preterm, difficult to ventilate	GLH, thalamic and WM bleed, SAH, mildly dilated renal pelvis	SAH, cerebellar bleed, Pneumonia, pulmonary hypoplasia	Intracranial bleeds	Pulmonary hypoplasia, Pneumonia,	No
14	2 d	Severe HIE	HIE, dilated sigmoid	HIE, meconium aspiration	HIE	HIE	Yes-M
15	3 wks	Term HIE, cooled	Ischemic changes in brain	HIE, renal tubular necrosis, fat necrosis	HIE	HIE	Yes-M
16	2 d	Preterm, difficult to ventilate	Small aplastic kindeys, hydrocolpos, potters sequence, ischemic changes in brain, PDA, dilated RV	Small cystic kidneys, vesico-vaginal fistula, hydrocolpos, urethra atretic, pulmonary hypoplasia	Likely pulmonary hypoplasia in view of potters sequence	Pulmonary hypoplasia	Yes-M
17	2 d	Severe HIE	HIE, subdural bleed compressing brain stem	HIE, subdural bleed compressing brain stem, tentorial tear	HIE, brain stem compression (Figure 19)	HIE, brain stem compression	Yes-M
18	3 d	Severe HIE	Ischemic changes in brain, ASD	HIE, ASD	HIE	HIE	Yes-M
19	1 d	Unexplained neonatal death	Abdominal distension, low lying cord, HIE	Bifid uterus, urethrovaginal fistula, streak ovaries, cleft vertebrae	Unexplained	Unexplained	Yes-M
20	1 mo	Severe HIE	Old HIE, cystic lesions occipital region	Old HIE, cystic lesions occipital region, adherant jejenum	Unexplained	Unexplained	Yes-M

Table 13. Cause of death by less invasive autopsy and conventional autopsy in cases referred by HM Coroner

No	Age	History	Key MR findings	Key pathology findings	COD from LIA	COD from Autopsy	Agreement
21	7 mo	SUDI	Dilated ventricles and RA, swollen cerebellum	Dilated cardiomyopathy, swollen cerebellum	Cardiomyopathy/ myocarditis	Cardiomyopathy	Yes-M
22	2 mo	SUDI	Temporal bone fracture, IVH, uncal herniation, spinal cord injury	Temporal bone fracture, IVH, uncal herniation, spinal cord injury	Non accidental injury-head injury	Non accidental injury-head injury	Yes-M+X
23	1 mo	SUDI	No abnormality	No abnormality	Unexplained	Unexplained	Yes-M
24	1 mo	SUDI	No abnormality	No abnormality	Unexplained	Unexplained	Yes-M
25	10 mo	SUDI	No abnormality	No abnormality	Unexplained	Unexplained	Yes-M
26	4mo	SUDI	No abnormality	No abnormality	Unexplained	Unexplained	Yes-M
27	20 mo	SUDI	Dilated RV, abnormal lung shadow-pneumonia, HIE	Myocarditis, pneumonia	Cardiomyopathy/ myocarditis	Myocarditis	Yes-M
28	12 d	SUDI	Ischemic changes in brain	No abnormality	Unexplained	Unexplained	Yes-M
29	32 d	SUDI	No abnormality	Pulmonary bleed suggestive overlay	Unexplained	Unexplained	Yes-M
30	3 d	SUDI	Streaky white matter changes in brain	No abnormality, Staph auerus in blood culture	Unexplained	Unexplained	Yes-M
31	4 mo	SUDI	Global infarction, HIE	Pneumonia	Unexplained	Pneumonia	No
32	2 y	SUDI	Thalamic bleed ?AVM	Ruptured AVM-Thalamic bleed, Pneumonia	Ruptured AVM-Thalamic bleed	Ruptured AVM-Thalamic bleed	Yes-M
33	1 mo	Post op cardiac	VSD leak, dysplastic pulmonary valve, residual coA	VSD leak, residual coA, dysplastic pulmonary valve and tricuspid valve	Residual cardiac problems	Residual cardiac problems	Yes-M
34-39	1 mo-2 yrs	SUDI	Ischemic changes in brain	No abnormality	Unexplained	Unexplained	Yes-M

Table 14. Cause of death by less invasive autopsy and conventional autopsy following an unexplained stillbirth

No	Pathology	MR	Agreement
1-10	Chorioamnionitis	Chorioamnionitis	Yes-Placenta
11	Chorioamnionitis, pneumonia-E coli	Chorioamnionitis	Yes-Placenta
12	Chorioamnionitis, pneumonia, pulmonary hypoplasia	Chorioamnionitis, pneumonia, pulmonary hypoplasia	Yes-Placenta
13	Congenital leukaemia, chloromas in brain	Congenital leukaemia, chloromas in brain	Yes-MRI, bloods
14	Congenital toxoplasmosis-brain, lung, placenta	Congenital Toxoplasmosis-brain, placenta	Yes-MRI, Placenta
15	**Duodenal atresia with malrotation**	**Unexplained**	**No**
16	Extensive intracranial bleed	Extensive Intracranial bleed	Yes-MRI
17	Extensive intracranial bleed	Extensive Intracranial bleed	Yes-MRI
18	Fetomaternal bleed	Feto maternal bleed	Yes-Bloods
19	Hepatosplenomegaly: Rhesus isoimmunisation	Hepatosplenomegaly: Rhesus isoimmunisation	Yes-MRI, Bloods
20	Histiocytic inter villositis	Histiocytic intervillosits	Yes-Placenta
21	Hydrocephalus, migration disorder	Hydrocephalus, migration disorder	Yes-MRI
22	Large ompholocele	Large ompholocele	Yes-MRI
23	Placenta ischemia villous thrombosis	Placenta ischemia villous thrombosis	Yes-Placenta
24	Placenta ischemia villous thrombosis	Placenta ischemia villous thrombosis	Yes-Placenta
25	Placental infarction	Placental infarction	Yes-Placenta
26	Placental abruption	Placental abruption	Yes-Placenta
27-97	Unexplained	Unexplained	NA

Figure 16. Lissencephaly:

Post-mortem MR imaging of 22 week fetus terminated for ventriculomegaly, showing figure of 8 appearance and a smooth brain on T_2–weighted axial and coronal images, suggestive of lissencephaly. Brain was completely autolysed at autopsy and nueropathological examination was not possible. Genetic testing was requested based on the post-mortem MR imaging appearances, which showed 17p Unbalanced Translocation (Miller Dieker Syndrome), therefore altering the recurrence risk. This would have been missed if post-mortem MR imaging was not performed

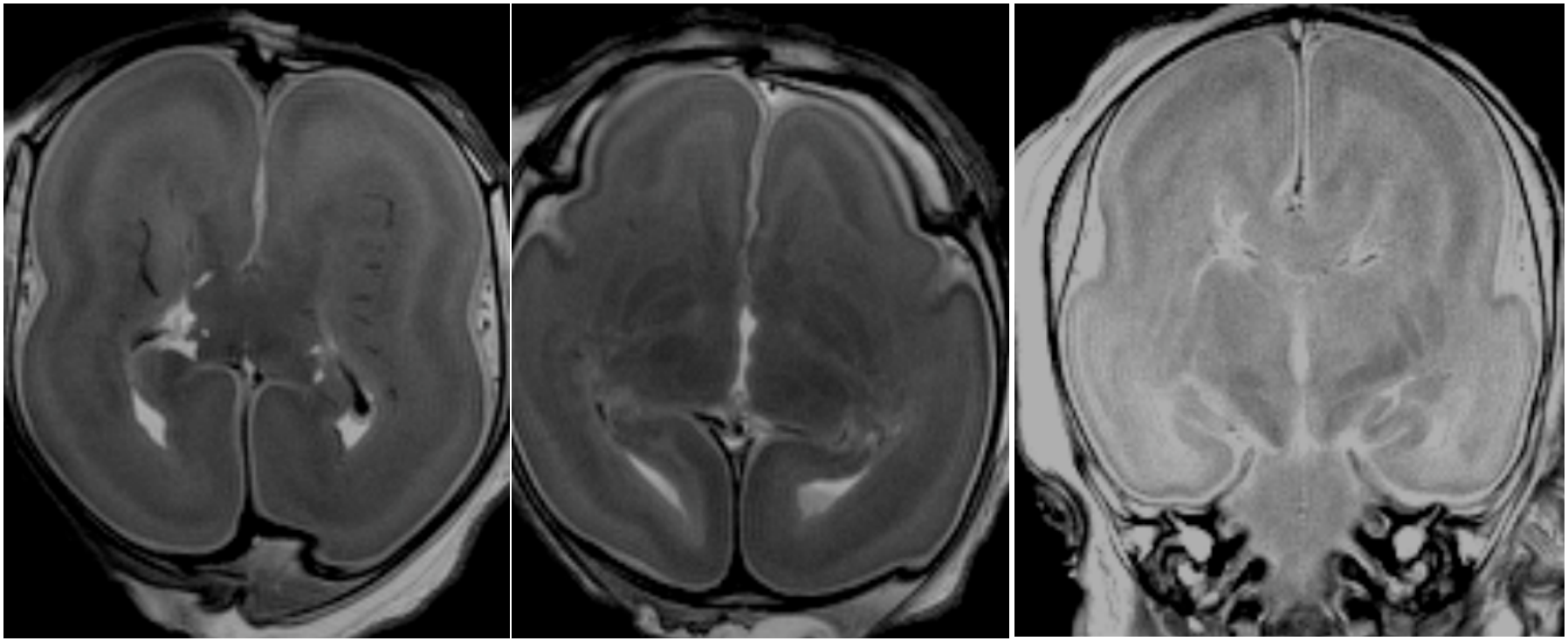


Figure 17. Ventriculomegaly and posterior fossa cyst

A 22-week fetus terminated for holoprosencephaly on antenatal US imaging. Ventriculomegaly and posterior fossa cyst was seen on post-mortem MR imaging. Axial T_2–weighted image (left), sagital T_1–weighted image (middle) and coronal T2-weighted image (right) shown. There was no evidence of holoprosencephaly. These findings were confirmed on autopsy.

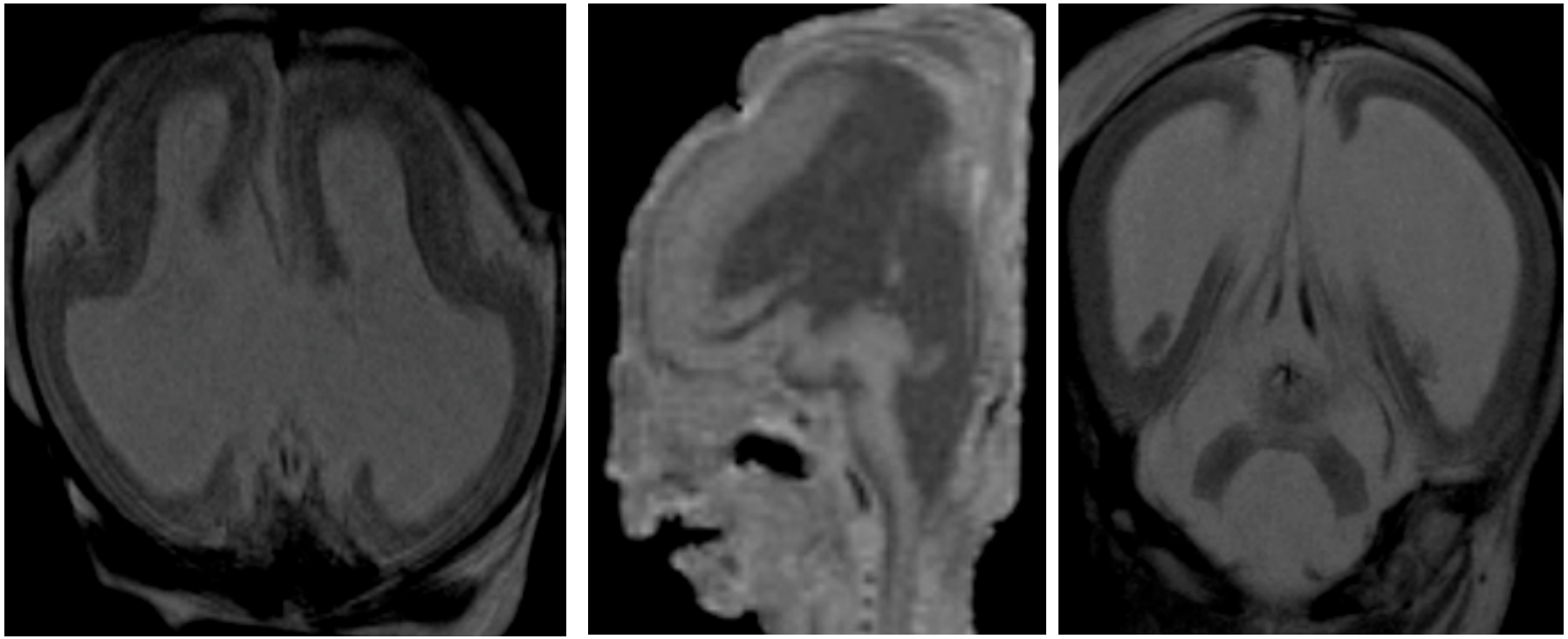


Figure 18. Comparison of antenatal and post-mortem MR imaging

Antenatal MR imaging (T_2-weighted) of a fetus at 20 weeks showing significant ventriculomegaly (top panel). Post-mortem MR imaging (3D CISS-1st three images, and sagittal T_1– weighted image-bottom right) confirmed ventriculomegaly, however the size of ventricles appeared smaller due to loss of CSF pressure after death. In addition, absent corpus callosum was noted on post-mortem MR imaging (bottom panel). These findings were confirmed at autopsy.

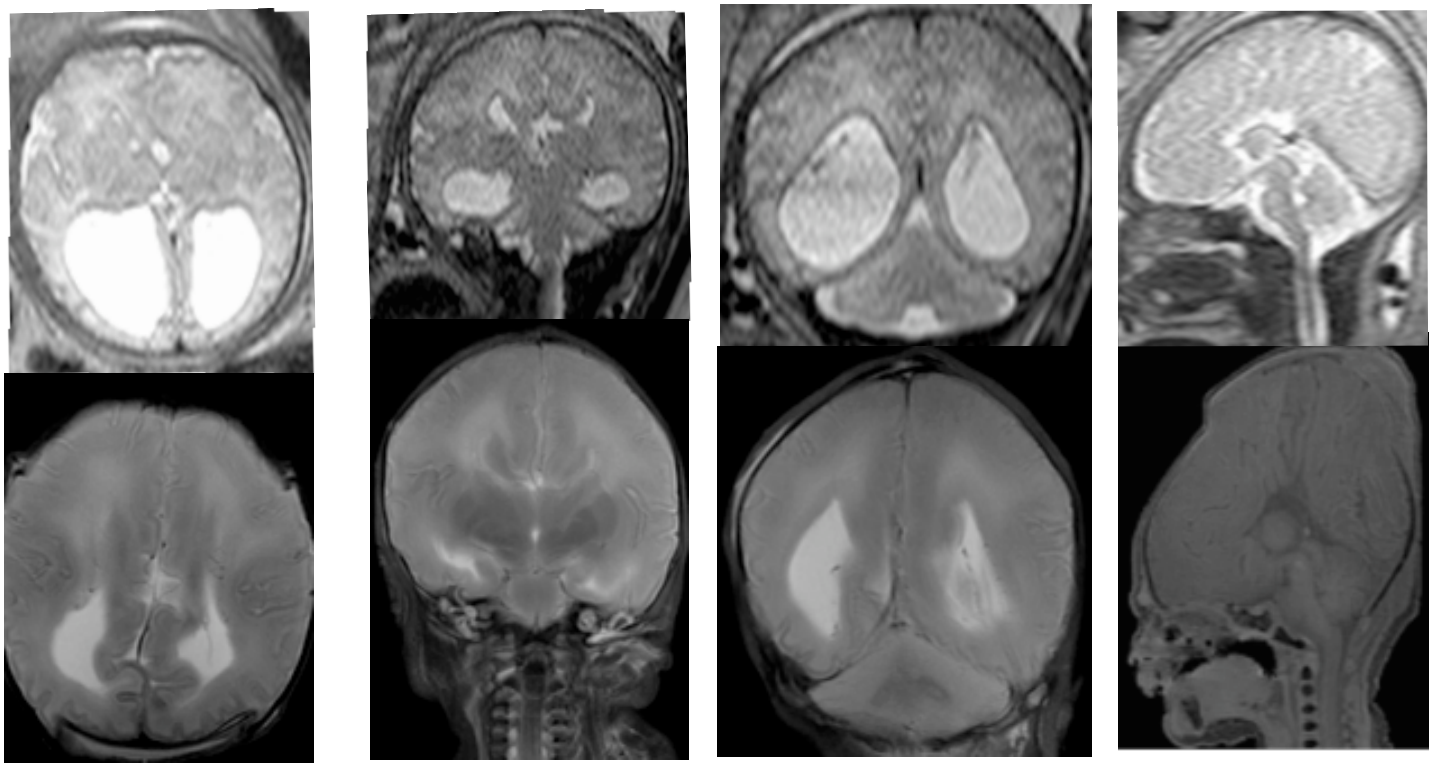


Figure 19. Ante-mortem and post-mortem MR imaging in neonatal encephalopathy

(a) Ante-mortem MR imaging (T_1–weighted sagittal) at in an infant with severe neonatal encephalopathy at 24 hours of age showing cerebral oedema and small amount of subdural bleed (white arrow), without any pressure effect. aEEG showed burst suppression. Care was withdrawn at 56 hours of age in view of extremely poor outcome (b) Post-mortem MR (3D CISS-sagittal) of the showed fatal brain stem compression from the subdural bleed (white arrow). Autopsy confirmed these findings and identified a tear in tentorium.

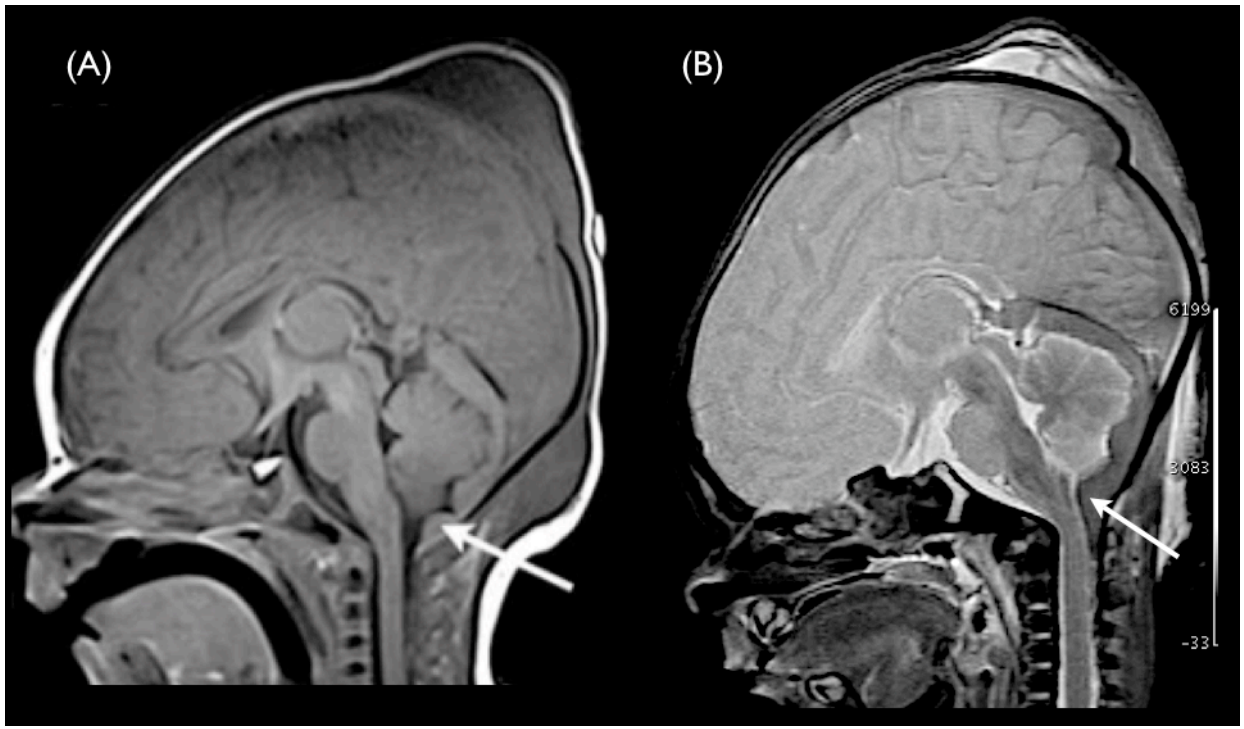


Figure 20. Capillary haemangioma of brain

Four year old child transferred to GOSH from Greece with multiorgan failure and sepsis. The child was on immunosuppressant and steroids for a subacute encephalopathy and ante-mortem MR imaging was reported as white matter disease (from Greece). Post-mortem MR imaging (T_2–weighted axial) was suggestive of calcification in parieto-occipital lobe and a capillary malformation (left). These findings were confirmed on histopathology (right).

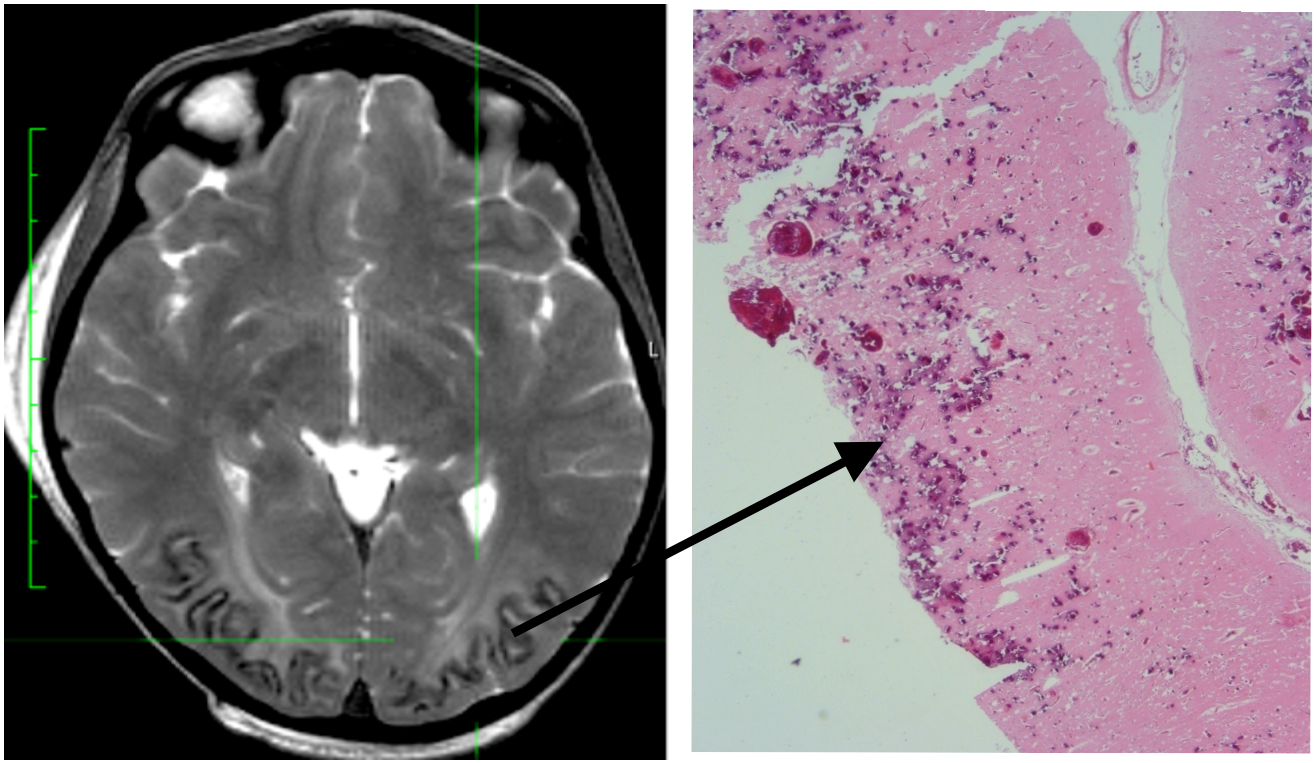


Figure 21. Thalamic bleed from rupture of a cerebral aneurysm

Thalamic and intraventricular bleed on T2–weighted axial MR image (a) in a two year old child who collapsed acutely. Autopsy confirmed these finding and showed a ruptured aneurysm of anterior cerebral artery. Post-mortem MR (T_2–weighted axial) of an infant following sudden unexpected death showing subdural and parenchymal bleeds suggestive of non accidental injury (b). Autopsy confirmed these findings and identified parietal skull fracture; Extensive parenchymal and ventricular bleeds (c) in a 34 week fetus on post-mortem MR imaging (3D CISS). This was confirmed at autopsy.

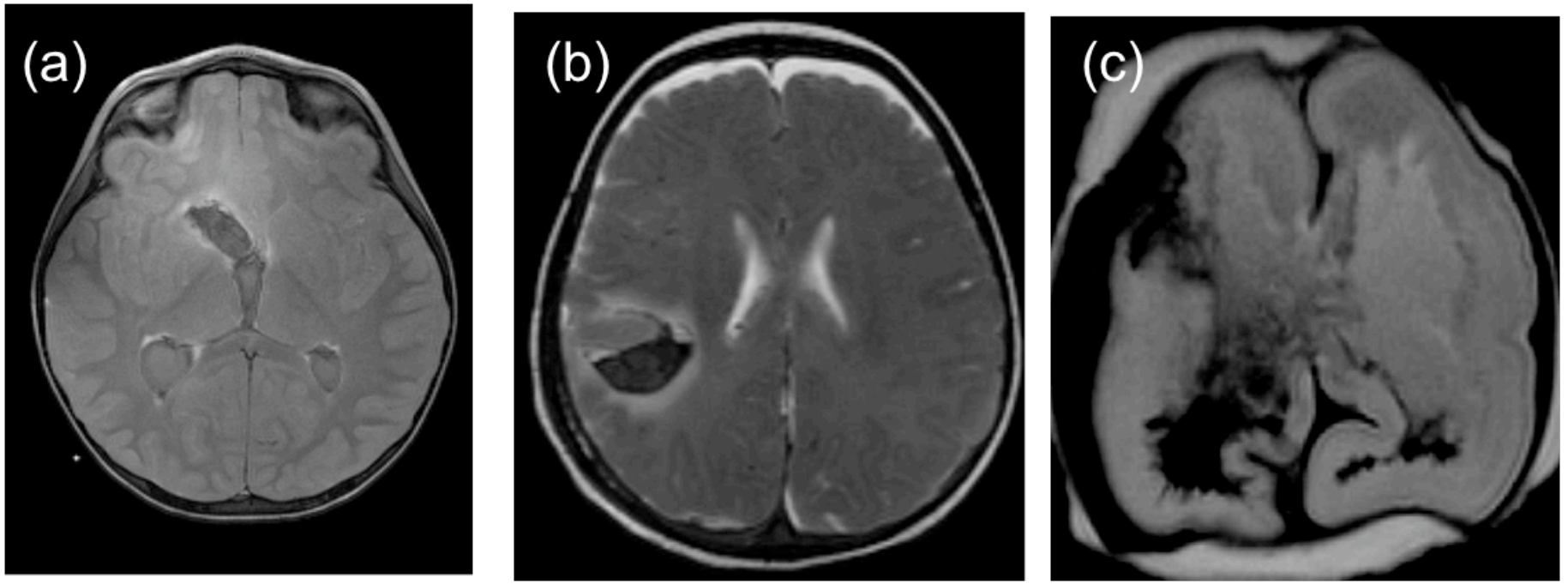
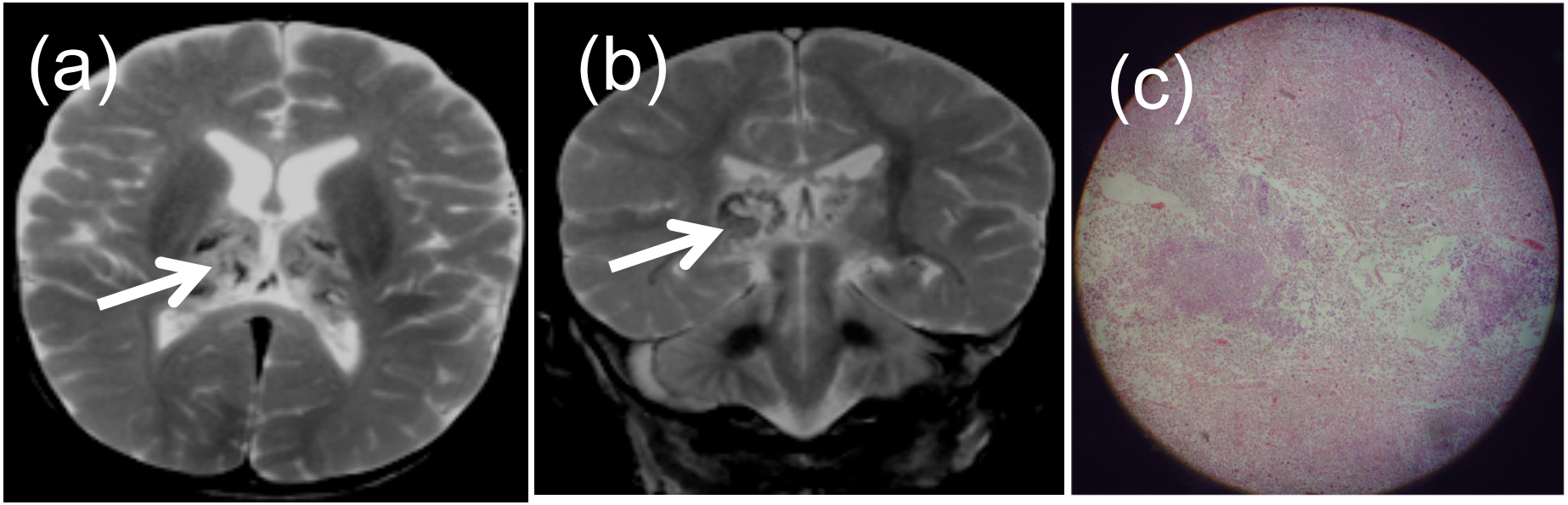


Figure 22. Acute infantile thalamic necrosis

Four-month old child presenting with acute encephalopathy. Post-mortem MR imaging (T_2–weighted axial and coronal images) shows extensive bilateral necrotic lesions in thalamus (white arrows) (a, b). This was confirmed at autopsy (acute thalamic necrosis). H&E stained right thalamus shown in (c)



The brain was completely autolysed and neuropathology was not possible in 39 (65%) cases less than 20 weeks. Of these 39 cases, MR imaging was non-diagnostic in 24, normal in 9 and abnormal in 6. The abnormalities in these cases were major bleeds in brain parenchyma and ventricles (2 cases), established lesions in white matter (1 case), small cerebellar vermis (1 case), agenesis of corpus callosum and interhemispheric cyst (1 case) and lissencephaly (1 case) (Figure 16, Figure 17, Figure 19, Figure 23) (Table 15); all were considered to be clinically significant, in particular the last case changed the recurrence risk offered to parents. This information would have been lost, if post-mortem MR imaging was not performed.

The cases in which either MR image or autopsy were non-diagnostic, were excluded in calculation of organ wise diagnostic accuracy of MR imaging. The overall diagnostic indices with 95% confidence intervals for each fetal organ is given are Table 15 and diagnostic indices according to gestational age are given in Table 16. Gestational age did not have a significant effect on positive and negative predictive values of the MR imaging, once the non-diagnostic cases were excluded.

Post-mortem MR imaging of the brain had a very high sensitivity (96.9 (95% – CI 83, 99) for detection of pathological lesions in the fetuses. The only lesions that were missed were minor ischemic lesions. No cases of intracranial bleeds were missed. On the other hand, 41 false positive cases were seen (specificity of 52.9%); i.e. abnormal post-mortem MR imaging but normal neuropathology. The false positive cases included porencephaly (1), migrational arrest (1), ventriculomegaly (2) and agenesis of corpus callosum (2), hypoxic ischemic injury (3) and varying degrees of intraventricular bleeds with clots and parenchymal bleeds (32).

The overall sensitivity of cardiac MR imaging was 53.8 (95% CI–25, 80) and specificity was 94.3 (95% CI–88, 97) for detecting clinically significant pathological lesions. The sensitivity of cardiac MR imaging in fetuses less than 20 weeks was low compared to older fetuses (>20 weeks). However, the prevalence of cardiac disease was higher in the smaller fetuses.

Chest MR imaging had very little discriminatory power and the overall sensitivity was only 4.2 % (95% CI– 0, 21). The pathologies that were missed on MR imaging included pnuemonias, pulmonary hypoplasias, meconium aspirations, pulmonary bleeds, lung cysts and tracheoesophagal fistulas.

Post-mortem MR imaging of the abdomen had a sensitivity of 63.6% (95% CI–40, 82) and specificity of 94.3% (95% CI–88, 97). The pathological lesions that were missed on post-mortem MR imaging included anal atresia and small adrenals (1 case), extramedullary hemopoiesis in liver (2 cases), liver infarct and capsular hematoma (2 cases), small benign cysts in the kidney (1 case) and duodenal atresia with malrotation (1 case). In the last case, the cause of death was related to the intra abdominal pathology. False positives on MR imaging included mild renal pelvic dilatations, small kidneys and bowel loop dilations.

Post-mortem MR imaging of the skeletal system showed an apparent high diagnostic utility. However, the prevalence of musculoskeletal abnormalities was low (4.4%) and MR imaging detected gross abnormalities like skeletal dysplasias, microcephaly and spinal defects.

7.5.3 Newborns, Infants and Children

7.5.3.1 Detection of cause of death in newborns, infants and children

Of the 39 newborns, infants and children, 20 were hospital cases and 19 were referred by HM Coroners following an unexpected death. Thirtyone cases were less than one year of age, five between 1 to 3 years and three cases more than three years of age. Of the 20 hospital cases, the cause of death reported by less invasive autopsy and conventional autopsy was same in 18 (90%) of cases (Table 12). The final cause of death (COD) in remaining two (no 10 and 13) were disseminated adenovirus infection and pulmonary hypoplasia with pneumonia; histological examination and conventional autopsy was required to make this diagnosis in these cases. Among the 18 cases where COD was accurately diagnosed by less invasive autopsy, the final diagnosis was based primarily on post-mortem MR imaging in 11 (61%) and on clinical information in seven (no: 1,3,4,5,9,11,12) (Table 12).

Figure 23. Pentology of cantrell

Termination of pregnancy at 18 weeks for neural tube defect. Post-mortem MR imaging (3D CISS) showed meningomylecle, absent pericardium, coarctation of aorta, and exompholos major (Pentology of Cantrell). These findings confirmed at autopsy.

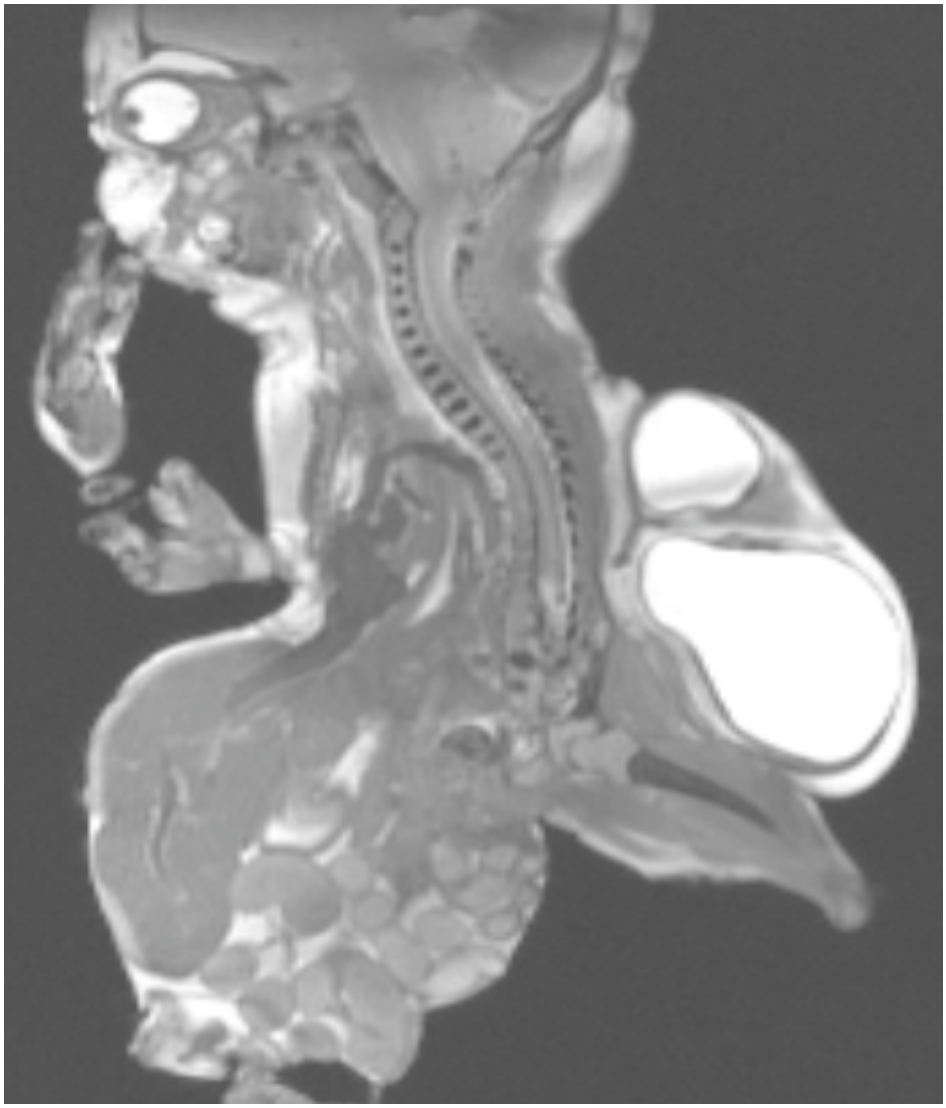


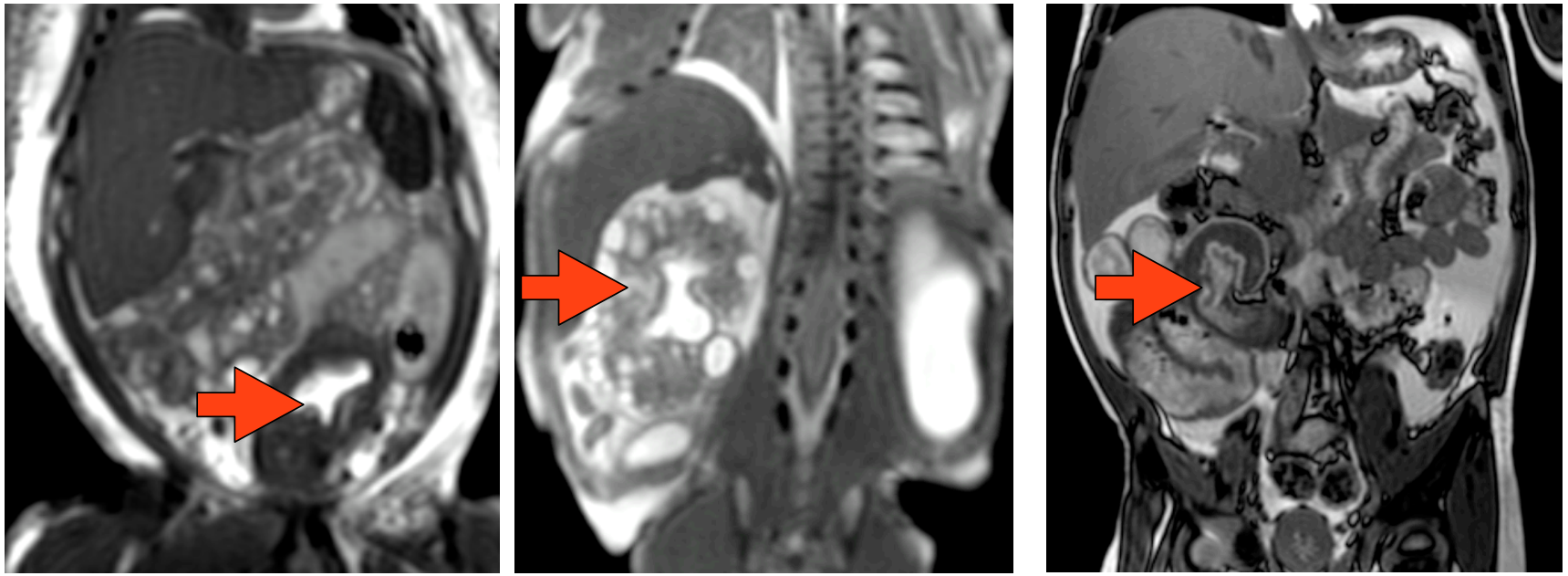
Figure 24. Abdominal pathology on post-mortem MR imaging

3D CISS post-mortem MR images of abdomen in coronal plane. Red arrows shows the pathological lesions.

A. Hydrocolpos in a 22 week fetus (left), confirmed at autopsy

B. Multiple renal cysts in 23 week fetus (middle), confirmed at autopsy

C. 4 year old child with abdominal pain and acute collapse, showing abnormal appendix (right). Autopsy showed appendicitis.



Of the 19 cases referred by HM Coroners, the cause of death by less invasive autopsy and conventional autopsy was the same in 18 (95%); one case of pneumonia (no: 31) was missed by less invasive autopsy (Table 13). Among the 18 cases correctly diagnosed by less invasive autopsy, 17 (94%) were based on the information from post-mortem MR imaging and one was based on MR imaging and X-rays for detecting skull fracture (no: 22). In two cases, the heart was diagnosed to be abnormal and likely cause of death; nevertheless definite diagnosis required histological examination (no: 21, 27)

7.5.3.2 Organ-wise analysis in newborns, infants and children

This analysis is based on direct comparison of post-mortem MR imaging with the information from open dissection of body and histological examination of the internal organ of interest (Table 17). For detection of a pathological lesion in the brain, post-mortem MR imaging had a sensitivity of 94.1% (95% CI–71, 99) and a specificity of 36.4% (95% CI–17, 59) (Figures 19-21). The only pathological lesion missed by MR imaging was scattered neuronal necrosis in pons, suggestive of subtle ischemic injury in a newborn infant (details not given in table). This was not considered as clinically significant. False positives (n=14) included small amounts of intraventricular bleed (2 cases), low-lying spinal cord with possible spinal dysraphism (1 case), and ischemic changes in brain (11 cases). Again none of these were considered to be clinically significant. Neuropathological examination was normal in all these cases.

Cardiac MR imaging had a high sensitivity 100% (95% CI–59, 100) and specificity 93.8% (95% CI–79, 99) for detection of pathological lesions; however the prevalence of heart lesions were low 17.9% and the confidence intervals for the estimates are rather wide.

The MR imaging of chest and abdomen had a very poor sensitivities 13.3% (95% CI–1, 40%) and 50% (95% CI–15, 84), respectively. The lung pathologies missed by MR imaging included pneumonia (5 cases), alveolar oedema with pulmonary bleed (1 case), congestion with interstitial bleed (1 case), congestive vasculopathy (1 case), meconium aspiration (2 cases), pulmonary interstitial emphysema (PIE) and pulmonary bleed (1 case) and hyaline membrane disease (1 case). Intra abdominal pathologies missed by MR imaging included passive venous congestion of liver, adrenal and testicular haemorrhage, erthema over transverse colon, narrowing of

small bowel and urethrovaginal fistula. Musculoskeletal imaging had poor sensitivity (40% (95% CI–5, 85) with wide confidence intervals. Skeletal abnormalities (fracture skull, cleft vertebrae) were present in two cases, both were missed on MR imaging. The skeletal lesions were easily detected on X-rays and at autopsy.

7.6 DISCUSSION

7.6.1 Post-mortem MR Imaging in Fetuses

Fetal post-mortems are performed with two very different aims; following an unexplained death the aim of autopsy is to detect a cause of death; on the other hand, the aim of autopsy following termination of pregnancy is to confirm or refute the ante-mortem diagnosis. The cause of death detected by less invasive autopsy and conventional autopsy was similar in 96 (99%) out the 97 cases. Whilst this is impressive, two important factors need to be considered: (a) In 73% of cases the cause of death remained unexplained even after a conventional or less invasive autopsy, (b) In the cases where a definite diagnosis was made, information from MR was contributory only in 7 (28%) of cases; in the remaining cases placental examination and other post-mortem blood tests were required to make the final diagnosis. Following termination of pregnancy, the proportion of cases in which the main diagnosis was confirmed or refuted was similar with less invasive autopsy and conventional autopsy.

On the other hand, fetal post-mortem MR and conventional invasive autopsy showed a complete agreement in 77% of cases, when all abnormalities detected at post-mortem were considered. MR images were non-diagnostic for most visceral organs, particularly for cardiac MR imaging in fetuses less than 20 weeks. This is likely to be due to the low resolution in proportion to body size that can be achieved in such small fetuses using conventional MR imaging. In addition, conventional neuropathological examination of brain was non-informative in the majority of the fetuses less than 20 weeks; MR imaging was still useful in many of these cases.

Brain MR imaging had a very high sensitivity, but lower specificity (52.9%) in fetuses. No major intracranial pathology or bleeds were missed on MR imaging. The lower specificity i.e. large number of false positives are more difficult to interpret some of these may to be related to fixation artefacts of conventional neuropathological examination, others, for example small amounts of bleeds and

hypoxic injury may be related to a death process, that may not have much clinical significance.

The sensitivity and specificity of post-mortem cardiac MR imaging was 53.8% (95% CI–25, 80) and 94.3% (95% CI–88, 97), respectively. Sensitivity of cardiac MR imaging was lower in fetuses less than 20 weeks. Post-mortem MR imaging of abdomen had sensitivity of 63.6% (95% CI–40, 82) and specificity of 94.3% (95% CI–88, 97). Post-mortem MR imaging of lungs had poor diagnostic utility. MR imaging accurately detected renal lesions, however histological examination was required to make a definitive diagnosis. The diagnostic utility of musculoskeletal system is unclear, even though the point estimates of diagnostic indices were high. No interpretation can be made on the utility of post-mortem MR imaging for musculoskeletal injuries due to low prevalence of such lesions in this population. Ancillary non-invasive post-mortem investigations like placental examination, genetic testing and radiology provided additional clinically significant information in 30% of the cases.

7.6.2 Post-mortem MR Imaging in Newborns and Children

The final cause of death by less invasive autopsy and conventional autopsy was similar in newborns, infants and children in 90% of cases following a hospital autopsy and 95% of cases following a coronial autopsy. However, in hospital cases, clinical history and investigations provided substantial information in upto 40% of cases, which could not have been obtained from post-mortem MR imaging. On the other hand, in HM Coronial cases, final cause of death was detected by post-mortem MR imaging alone in most cases (94%), even though definite diagnosis in two cases required cardiac histological examination. The causes of death missed by post-mortem MR imaging were related to lung infections.

Nevertheless, all pathological lesions detected at conventional autopsy were diagnosed by less invasive autopsy in 42% of cases only; in the remaining 58%, only a partial correlation of conventional autopsy and less invasive autopsy was seen. Post-mortem MR imaging of brain again had a high sensitivity, even though specificity was low; this was due to the large number of false positives related to an over diagnosis hypoxic injury on MR images. No major intracranial pathology or bleeds were missed on MR imaging. Post-mortem MR imaging of lungs again was of limited

utility. The point estimates for sensitivity and specificity of cardiac MR imaging was high, 100% (95% CI – 59, 100) and 93.8% (95% CI–79, 99) respectively; however the confidence intervals were wide and prevalence low. Post-mortem MR imaging of the abdomen had a sensitivity of 50% (95% CI –15, 84) and specificity of 83.8% (95% CI–66, 94). The number of cases with musculoskeletal lesions were small to make any definitive conclusions.

This data suggests if a post-mortem MR imaging of brain is normal, it is unlikely that the cause of death is inside the brain and major intra-cranial structural pathologies can be excluded. The reason for frequent appearance of global infarction on post-mortem MR imaging, where neuropathological examination is normal, is unclear. Conversely, post-mortem MR imaging cannot be relied upon to rule in or rule out lung pathologies, which sometime may cause sudden unexpected death in infancy. Poor sensitivity of gastrointestinal MR imaging relates to difficulties in detecting subtle pathologies and fistulas, however these lesions are unlikely to be fatal. From this small series, it cannot be concluded that a normal MR imaging of abdomen could exclude a cause of death in abdomen, a more prudent approach may be to combine the post-mortem imaging with laparoscopy. Utility with regards to skeletal injuries and pathologies appears to be low; thus MR should not be relied upon to exclude fractures and other non-accidental injury. X-rays and CT imaging have a definite role in such cases.

The analysis was performed by comparing post-mortem MR imaging directly with the information obtained from open dissection, macroscopic and microscopic examination of visceral organs, and not with ancillary post-mortem investigations like placental examination or genetic testing. Clearly, such investigations have an already proven role in perinatal autopsy. Furthermore, parents are unlikely to object to these tests as they are non-invasive. Indeed, the data presented suggests that such ancillary investigations provide crucial information in upto one third of perinatal autopsies. Therefore, in perinatal pathology practice, post-mortem MR imaging should not be performed in isolation, but only in conjunction with all other ancillary post-mortem investigations.

The sensitivity of post-mortem brain imaging is consistent with published literature and the results of the meta-analysis in chapter four. For example, Griffith et al reported 100% sensitivity and 92% specificity of post-mortem MR imaging of fetal

brain in a prospective cohort of 40 fetuses (Griffiths et al. 2003). However, in some cases the gold standard was post-mortem MR imaging, rather than conventional autopsy. In this cohort, the authors did not consider the influence of ancillary post-mortem investigations. Subsequently, the authors re-analysed the data from this cohort along with other retrospectively collected post-mortem imaging data (total 99 cases), including the information from ancillary post-mortem investigations including placental examination (Cohen et al. 2007; Cohen et al. 2008). They reported that useful information would have been lost in 71% of the cases, if MR imaging was the only post-mortem investigation undertaken, highlighting the importance of ancillary post-mortem investigations like placental examination.

Another major difference between the published literature on post-mortem MR imaging (Brookes et al. 1996; Cohen et al. 2007; Cohen et al. 2008) and current work is that small intraventricular bleeds without ventricular dilation or clots, small pleural effusions and pericardial effusions were excluded from analysis. Such lesions are often and clearly seen on post-mortem MR imaging; however often do not have autopsy correlates. Many previous studies have claimed superiority of post-mortem MR imaging based on these findings. Nevertheless, these findings are of limited clinical significance. Other common post-mortem changes seen on MR imaging include crazy paved appearance of brain on gradient echo sequences, retinal detachments and abnormal shape of eyeballs.

My study was not intended to address the issue of false positives, and conventional autopsy was considered as the gold standard. Neverthless, the interpretation of pathological lesions seen on MR imaging, but without any autopsy correlates can only be speculative, as to whether these represent ‘genuine false positives’ or lesions that were ‘missed on conventional autopsy’. The autopsy in all the cases were performed by experienced perinatal and paediatric pathologists in specialist centres; therefore it is unlikely that the lesions ‘missed’ on autopsy represent a lack of expertise. Nevertheless, post-mortem MR imaging of fetal brain did suggest major pathologies in certain cases, which were not seen on conventional autopsy. In some cases, but not always, this was due to complete liquefaction of the brain rendering neuropathological examination uninformative. An increasing number of case reports have been published recently suggesting that crucial information may be missed if MR imaging of brain is not performed as a part of conventional autopsy. For example, the

cerebellum in a fetus was noticed to be missing at autopsy, despite being clearly seen on post-mortem MR imaging (and abnormal); thus highlighting issues related to fixation artefacts (Lavanya et al. 2008).

On the other hand, post-mortem MR imaging detected all major intracranial pathology in fetuses, newborns and children, therefore the opening of head may be unnecessary if MR imaging is normal. Nevertheless, alternative imaging (X-rays or CT imaging) may be required to detect bony lesions and skull fractures, as MR imaging was inaccurate for detection of fractures. However, this issue is not explored in my thesis work, as there is already extensive data supporting post-mortem CT imaging from several forensic imaging groups, in such cases.

Table 15. Diagnostic accuracy of post-mortem MR imaging for detection of major pathological lesions in fetal organs

System	Sensitivity	Specificity	PPV	NPV	LR+	LR-	Prevalence
Brain	96.9 (83,99)	52.9 (41,63)	43 (31,55)	97.9 (88, 99)	2 (1.6, 2.6)	0.005 (0.008, 0.41)	26.9
Heart	53.8 (25,80)	94.3 (88,97)	53.8(25,80)	94.3 (88,97)	9.5 (3.8,24)	0.49 (0.27,0.88)	10.9
Lung	4.2 (0, 21)	98.5 (94, 99)	33.3 (0,90)	84.8 (78,90)	2.7 (0.25,28.9)	0.97 (0.89,1)	15.5
Abdomen	63.6 (40,82)	94.3 (88,97)	66.6 (43,85)	93.5(87,97)	11.2 (5.1, 24.5)	0.38 (0.2, 0.67)	15.2
Musculo-skeletal	100 (59,100)	100 (97,100)	100 (59, 100)	100 (97,100)			4.4

PPV=Positive predictive value, NPV=Negative predictive value, LR+ Positive likelihood ratio, LR- Negative likelihood ratio

Table 16. Diagnostic accuracy of post-mortem MR imaging for each fetal organs based on gestational age.

System/gestational age	Sensitivity	Specificity	PPV	NPV	LR+	LR-	Prevalence
Brain<20	87.5 (47, 99)	55.5 (35, 74)	36.8 (16, 61)	93.8 (69, 99)	1.96 (1.2, 3.2)	0.2 (0.03, 1.5)	22.9
Brain≥20	100 (85,100)	51.7 (38, 64)	45.3 (31, 59)	100 (88, 100)	2 (1.6, 2.7)	-	28.6
Heart <20	22.2 (2, 60)	96.4 (87, 99)	50 (6, 93)	88.3(77, 95)	6.1 (0.9, 38)	0.8 (0.6, 1.1)	14
Heart ≥20	83.3(35,99)	95.5 (88,98)	55.6 (21,86)	98.8 (93,99)	18.5 (88,98)	0.17 (0.03, 1)	6.3
Lungs <20	12.5 (0,52)	98.1(89, 99)	50 (1, 98)	87.9 (76, 95)	6.5 (0.4, 93.9)	0.8 (0.7, 1.2)	13.3
Lungs ≥20	5.6 (0, 27)	98.8 (93, 99)	50 (1, 98)	82.6 (73, 89)	4.6 (0.3, 69.5)	0.9 (0.85, 1)	18
Abdomen <20	41.7 (15,72)	94(83,98)	62.5(24,91)	87 (75,94)	6.9(1.9,25)	0.6 (0.38, 1)	19.4
Abdomen ≥20	81.8 (48,97)	95(87,98)	69(38,90)	97.5(91,99)	16.6(6.1,44.8)	0.19(0.05,0.7)	11.9
Musculoskeletal<20	100(29,100)	100(93,100)	100(29,100)	100(93,100)	-	-	5
Musculoskeletal≥20	100(39,100)	100(96,100)	100(39,100)	100(96,100)	-	-	4

Table 17. Diagnostic accuracy of post-mortem MR imaging for detection of major pathological lesions in newborn and children

System	Sensitivity	Specificity	PPV	NPV	LR+	LR-
Brain	94.1 (71,99)	36.4 (17,59)	53.3 (34,71)	88.8 (51,99)	1.47 (1,2)	0.16 (0.02,1.17)
Heart	100 (59,100)	93.8 (79,99)	77.8 (39,97)	100 (88,100)	16 (4.2, 61.2)	
Lung	13.3 (1, 40)	87.5 (66.6, 96.7)	40 (5,94)	60 (43,77)	1.1 (0.2,5.7)	0.99 (0.77, 1.3)
Abdomen	50 (15,84)	83.8 (66,94)	44.4 (13,78)	86.6 (69,96)	3.1(1.1, 8.9)	0.59 (0.29, 1.2)
Musculoskeletal	40 (5,85)	100 (89,100)	100 (15, 100)	91.9 (78,98)		0.6 (0.29, 1.2)

PPV: Positive predictive value, Negative predictive value, LR+: positive likelihood ratio, LR-: negative likelihood ratio

7.6.3 Limitations and Knowledge Gaps

Accuracy of post-mortem imaging was lower in smaller fetuses less than 20 weeks for all organs, particularly for cardiac MR imaging and many images were non-diagnostic. Higher resolutions required to scan such small fetuses are often challenging to achieve using conventional MR scanners. One potential solution to this problem is by using higher field strengths, thereby increasing the resolution and reducing partial volume effects. This issue will be addressed in 11 (high field imaging).

Another issue is the large number of ischemic lesions and appearance of global infarctions seen on post-mortem MR imaging that had no autopsy correlates. Neuropathological examination has well defined criteria for differentiating hypoxic ischemic injury or infarction from post-mortem related change. However, it is unclear if post-mortem MR imaging can differentiate between normal death process and ischemic lesions accurately. It is also possible that T_1 and T_2 relaxometry values after death are different to that during life, thus altering the tissue contrast on post-mortem MR images. These issues will be explored in chapter 7 and 8.

Some of the pathologies resulting in abnormal organ size were missed on post-mortem MR imaging, for example pulmonary hypoplasia, small kidneys, enlarged liver and spleen. One possible solution for this problem is accurate estimation of visceral organs volumes from MR imaging using 3D volume rendering techniques. Post-mortem cardiac MR imaging showed a variable accuracy; in addition the prevalence of cardiac lesions was low. It is also unclear if the bias from knowing the results of ante-mortem cardiac status would have influenced the interpretation of the cardiac MR images. Higher 3D resolution may also increase the accuracy of cardiac MR reporting. This issue will be addressed in chapter 9.

Accuracy for detection of pulmonary lesions, in particular pneumonia was extremely poor. MR imaging accurately detected renal lesions; however, definite diagnosis could be made only following histological examination. This highlights the need for percutaneous biopsies and tissue diagnosis. This will be discussed in chapter 12.

In summary, this study suggests good agreement of post-mortem MR imaging with conventional autopsy, particularly if one is interested only in the cause of death; however, there are several unresolved issues. This include differentiation of normal

death process and ischemic lesions in brain, cardiac imaging, imaging of small fetuses, need for estimation of visceral organ volumes and obtaining tissue diagnosis. These issues are dealt with in subsequent chapters.

CHAPTER 8: POST-MORTEM BRAIN IMAGING IN HYPOXIC ISCHEMIC ENCEPHALOPATHY

8.1 INTRODUCTION

In chapter 7, I noticed a very high incidence of false positive MR reports with regard to detection of hypoxic ischemic injury, i.e. in several cases where MR imaging showed global infarction or evidence of ischemic injury, neuropathological examination of the brain was normal. There are several clinical implications of these findings, particularly in the context of neonatal encephalopathy.

Neonatal encephalopathy is a catastrophic end to a pregnancy; 10-15% affected infants will die in the neonatal unit, 10-15% will develop cerebral palsy, and up to 40% will have other significant severe disabilities, including blindness, deafness, and problems with cognition and behaviour (Pierrat et al. 2005; Robertson et al. 2006). It is estimated that approximately 1 million deaths occur worldwide every year from neonatal encephalopathy (Lawn et al. 2005); these deaths are now referred to as "intrapartum related deaths" acknowledging the difficulties in establishing a clear ante-mortem aetiology.

Following a death related to neonatal encephalopathy, a detailed post-mortem examination is important for helping establishing the aetiology, timing and excluding underlying congenital or genetic defects; particularly the ones that may recur (Squier et al. 2004). In addition, understanding the reasons why a newborn infant has died allows parents to grieve and doctors to detect unexpected responses to treatments. Finally, birth asphyxia related brain injuries are of medicolegal significance and one of the commonest causes of litigations in obstetric practice; thus a diagnosis of hypoxic ischemic injury on post-mortem MR imaging may have a legal implication.

A recent study on encephalopathic infants by Rutherford and colleagues at the Hammersmith hospital suggested that ischemic brain injury which occurred before death can be accurately detected by post-mortem MR imaging (Nicholl et al. 2007); however my experience as described in chapter 7, was quite contrary to this. Therefore I wanted to examine whether post-mortem MR imaging can differentiate ante-mortem hypoxic-ischemic brain injury from a normal death, using a rigorous, blinded, case control study design.

8.2 AIMS

- To compare post-mortem T_1 and T_2-weighted MR images of infants who died from neonatal encephalopathy (NE) with infants who died unexpectedly i.e. sudden unexpected neonatal death (SUND), without a preceding history of encephalopathy.
- To compare the signal intensities on post-mortem T_1 and T_2-weighted MR images in areas commonly affected by a hypoxic injury process in infants with NE and SUND.
- To compare the signal intensities on post-mortem T_1 and T_2-weighted MR images in these areas on ante-mortem MR imaging with post-mortem MR imaging, in a sub group of infants with NE.

8.3 METHODS

I selected infants for this case control study from my post-mortem MR imaging database described in chapter 6. The scanner (1.5 Tesla) and MR sequence details are as given in chapter 6; T_1, T_2-weighted images of the brain were used for this study.

In the first phase, MR images of ten infants with perinatal asphyxial encephalopathy, who had evidence of hypoxic ischemic injury on neuropathological examination, were selected from this database. MR images from ten newborns with sudden unexpected neonatal death and normal neuropathological examination served as controls-all images were anonymised. The cases and controls were matched based on time since death, gestation and age. The pathologists were blinded to MR images. Each MR image was reported in a systematic way by two specialist paediatric neuroradiologists (Dr Kling Chong, Dr Rox Gunny), blinded to clinical history and autopsy findings. Consensus opinion was recorded.

In the second phase, ante-mortem MR imaging was performed in two infants with severe neonatal encephalopathy, followed by post-mortem MR imaging when these infants died. Exactly similar sequences were using for ante-mortem MR imaging (performed at a 1.5 Tesla Siemens Avanto MR scanner at University College Hospital, London) and post-mortem MR imaging (performed at Great Ormond Street Hospital, London).

In all cases, regions of interest (ROI) were drawn along eleven predefined areas to measure the signal intensity (SI) using image J and mean SI were recorded. Contrast to noise ratios were calculated by (SI A-SI B)/noise. Higher CNR increases perception of the distinct differences between two clinical areas of interest in the brain (McRobbie et al. 2007: 66-67).

These regions included frontal grey matter, frontal white matter, posterior grey matter, posterior white matter on left and right sides, putamen, globus pallidus and thalamus.

In each case the brain was fixed by immersion in 10% formal saline and macroscopic examination was undertaken at 1-2 weeks and then processed in paraffin blocks. This included anterior frontal lobe, posterior frontal (level of the pulvinar/motor cortex i.e. peri-rolandic cortex), thalamus including lateral geniculate nucleus, amygdala, hippocampus level at the level of lateral geniculate nucleus, basal ganglia at the level of putamen/globus pallidus, striatum, periventricular white matter, midbrain at level of inferior colliculus, pons and medulla and upper cervical cord. Paraffin sections were stained by haematoxylin and eosin. Neuronal eosinophilia and kayorrehexis, astrocyte gliosis, activated microglia, accumulation of macrophages and haemorrhage were considered as histopathological features of ischemic injury. A paediatric neuropathologist examined all brains.

8.4 RESULTS

Median (range) gestation, birth weight and time since death were similar in infants with neonatal encephalopathy (NE) and the sudden death group (SUND) (Table 18).

Evidence of ischemic injury and infarction was seen in post-mortem MR imaging in 90% of the cases (i.e. ischemic injury on neuropathological examination) and 80% of control infants (i.e. no ischemic injury on neuropathological examination). The negative and positive predictive values of post-mortem MR imaging were 67% (95% CI–9, 99) and 55.6 (30, 78%) respectively, suggesting poor discriminatory power of MR imaging in detecting ischemic lesions (Table 19). The sensitivity and specificity of post-mortem MR imaging for diagnosing ischemic injury was 91% (95% CI–58, 99), 20% (95% CI–2, 55) respectively.

The commonest ischemic change noticed on MR imaging was lack of grey / white matter contrast and diffuse high signal intensities (Figure 25). The normal high signal

intensity of the posterior limb of the internal capsule on T_1 weighted images (Rutherford et al. 1998) was absent in all cases, even when histopathological examination of brain was normal. Conversely some deep nuclear lesions, midbrain and pontine lesions diagnosed as normal on post-mortem MR imaging, had ischemic changes on histopathological examination. One case had long standing ischemic lesions with cystic change on neuropathological examination and this was accurately diagnosed on MR imaging. Diffusion weighted imaging (Rutherford et al. 2004) showed restricted diffusion in all infants and was not different between cases and controls.

Table 18. Baseline characteristics of HIE and SUND

Median (range)	HIE (n=10)	Control (n=10)	p
Gestation (weeks)	38.5 (37 to 40)	37.3 (36 to 39)	>0.05
Birth weight (kg)	3.4 (3.1 to 4.1)	3.3 (2.9 to 3.8)	>0.05
Age at death (d)	3 (2 to 22)	7 (3 to 28)	>0.05
Post-mortem interval (d)	3 (2 to 4)	4 (2 to 5)	>0.05

8.4.1 Comparison of Signal Intensities on Post-mortem MR Images

The mean signal intensities on T_1–weighted images in the cortical grey matter, deep nuclei or white matter in infants with perinatal asphyxial encephalopathy were similar to the control infants. No difference was seen in white matter to deep nuclei (thalamus and putamen) signal intensity ratios between cases and controls on T_1–weighted images ($p>0.05$) (Figure 27).

However, the mean signal intensity on T_2-weighted images showed a significantly higher signal intensity in the thalamus and putamen in the HIE group, when compared to the control group (Figure 28). Furthermore, the white matter to deep nuclei signal intensity ratios were significantly lower in the asphyxial encephalopathy group, when compared to the sudden death group ($p<0.05$).

Table 19. Comparison of brain imaging findings and neuropathology

No	Type	MR report	Pathology report
1	NE	Brain swelling and global oedema suggestive of global infarction	Extensive hypoxic ischemic changes in pons, medulla, hippocampus and cerebellum. White matter preserved
2	NE	Brain swelling and global oedema suggestive of global infarction, infratentorial subdural bleed pressing on brain stem	Hypoxic neuronal injury is seen in pons, olives, dentate and hippocampus, infratentorial subdural bleed pressing on brain stem, tear in tentorium
3	NE	Normal	Few scattered neuronal necrosis
4	NE	Bright dentate, diffuse swelling of cerebellum and deep grey matter	Selective neuronal necrosis in pons, hippocampus, dentate due to hypoxia. Small focus of white matter injury in left frontal lobe
5	NE	Global infarction, swollen deep grey matter, abnormal thalamus, lentiform, caudate	Global ischemia and hypoperfusion affecting pons, midbrain, thalamus and deep layers of cerebral cortex
6	NE	Global infarction, diffuse increase signal intensity	Extensive acute hypoxic change in many areas, neocortex, subependymal region, thalamus, midbrain, pons, medulla, spinal cord grey matter, pons, medullary tegmentum, occulomotor nuclei
7	NE	Diffuse increased signal intensity, no swelling	Diffuse hypoxic changes in dentate nucleus of cerebellum, basal ganglia, cortex and peri ventricular white matter
8	NE	Diffuse increased signal intensity, no swelling	Scattered hypoxic neurons in frontal and occipital lobes, spine, mid brain, pons, cerebellum
9	NE	Global cerebral and cerebellar infarction and thalamic changes	Global extensive hypoxic neuronal necrosis
10	NE	Thalamus and deep nuclei normal, cystic changes posterior paretio occiptal frontal and temporal lobe, oedema and swelling, sub acute injury older than few days	Cystic changes in posterior parietal and occipital lobes, cortical infarction with underlying white matter necrosis. Thalamus shows neuronal necrosis and astrocystosis. Consistent with HIE 3 weeks back
11-18	SUND	Global infarction, diffuse increase signal intensity	Normal
19,20	SUND	Normal	Normal

Figure 25. Post-mortem MR images in infants with normal neuropathological examination and hypoxic ischemic brain lesions

T_1 and T_2–weighted MR images in a neonate with normal histopathological examination at autopsy (top panel) and in a newborn where major ischemic changes are noted (bottom panel). Both images show lack of grey white matter contrast, global infarcted appearance and diffuse increase in signal intensities and are indistinguishable from each other.

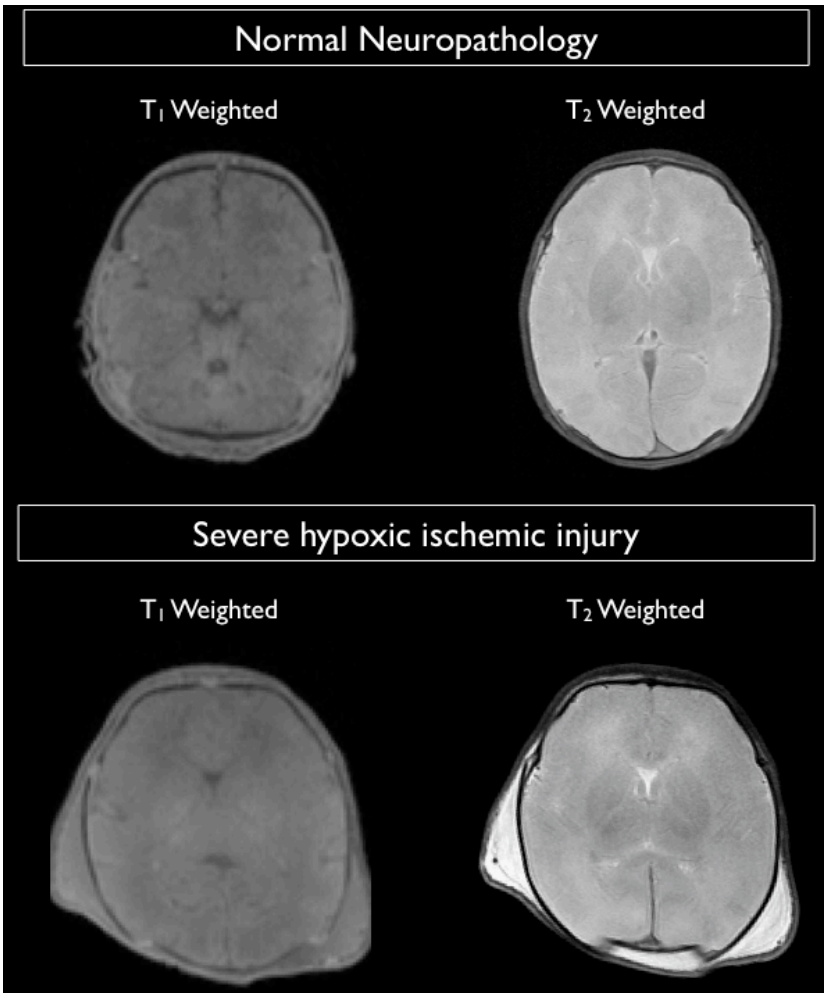
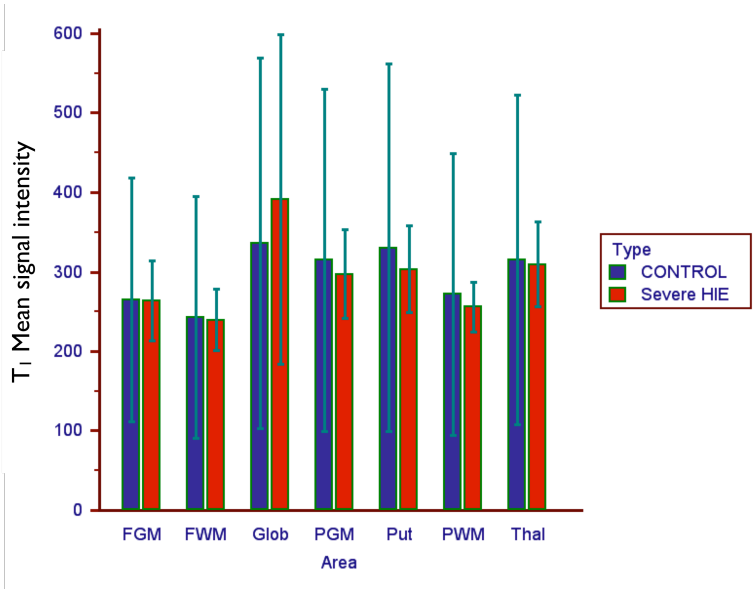


Figure 26. Mean signal intensities in infants with HIE and control infants on T_1–weighted images

Mean (SD) signal intensities in various brain regions on post-mortem MR imaging in top panel. FGM–frontal grey matter, FWM–frontal white matter, Glob–Globus pallidus, PGM–posterior grey matter, Put–putamen, PWM–posterior white matter, Thal–Thalamus. Mean (95% CI) signal intensity ratios of white matter and deep nuclei (grey matter) on T_1–weighted images shown in the bottom panel.



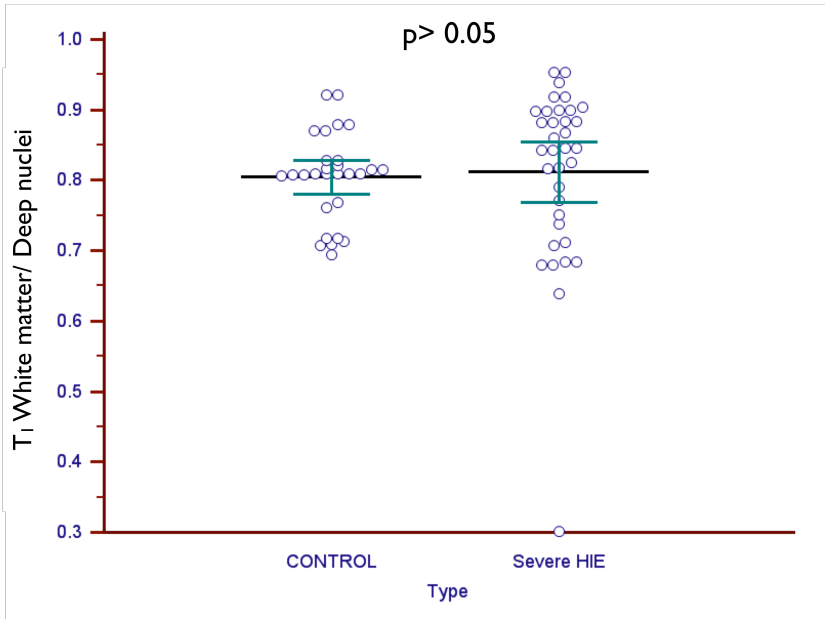
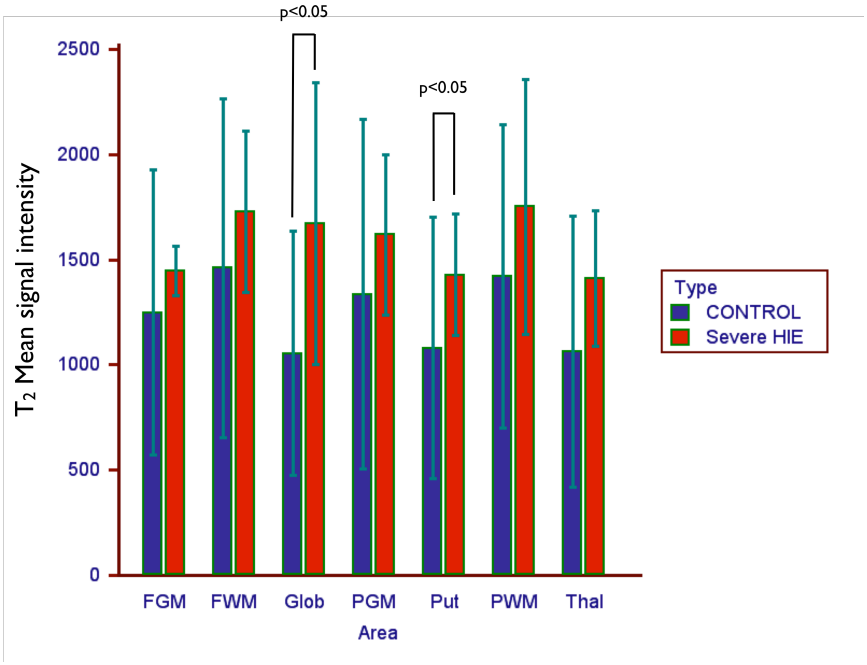


Figure 27. Mean signal intensities in infants with HIE and control infants on T_2–weighted images

Mean (SD) signal intensities in various brain regions shown in the top panel. FGM–frontal grey matter, FWM–frontal white matter, Glob–Globus pallidus, PGM–posterior grey matter, Put–putamen, PWM–posterior white matter, Thal–Thalamus. Mean (95% CI) signal intensity ratios of white matter and deep nuclei (grey matter) on T_2–weighted images shown in the bottom panel.



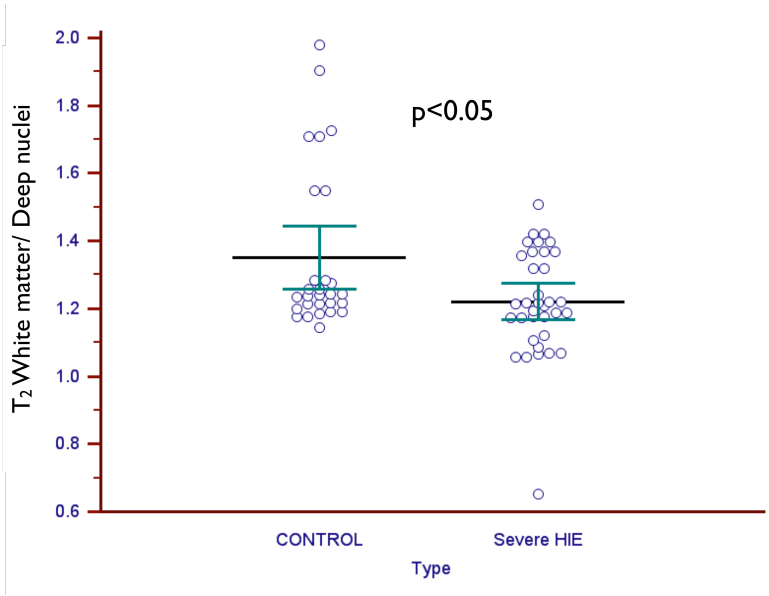


Figure 28. Ante-mortem and post-mortem MR images

Ante-mortem T_1–weighted (a) and T_2–weighted (c) of an infant with severe neonatal encephalopathy suggestive of global hypoxic injury and loss of high signal intensity in posterior limb of internal capsule on the T_1–weighted image. Post-mortem T_1 (b) and T_2–weighted (d) MR images of same infant showing evidence of global infarction.

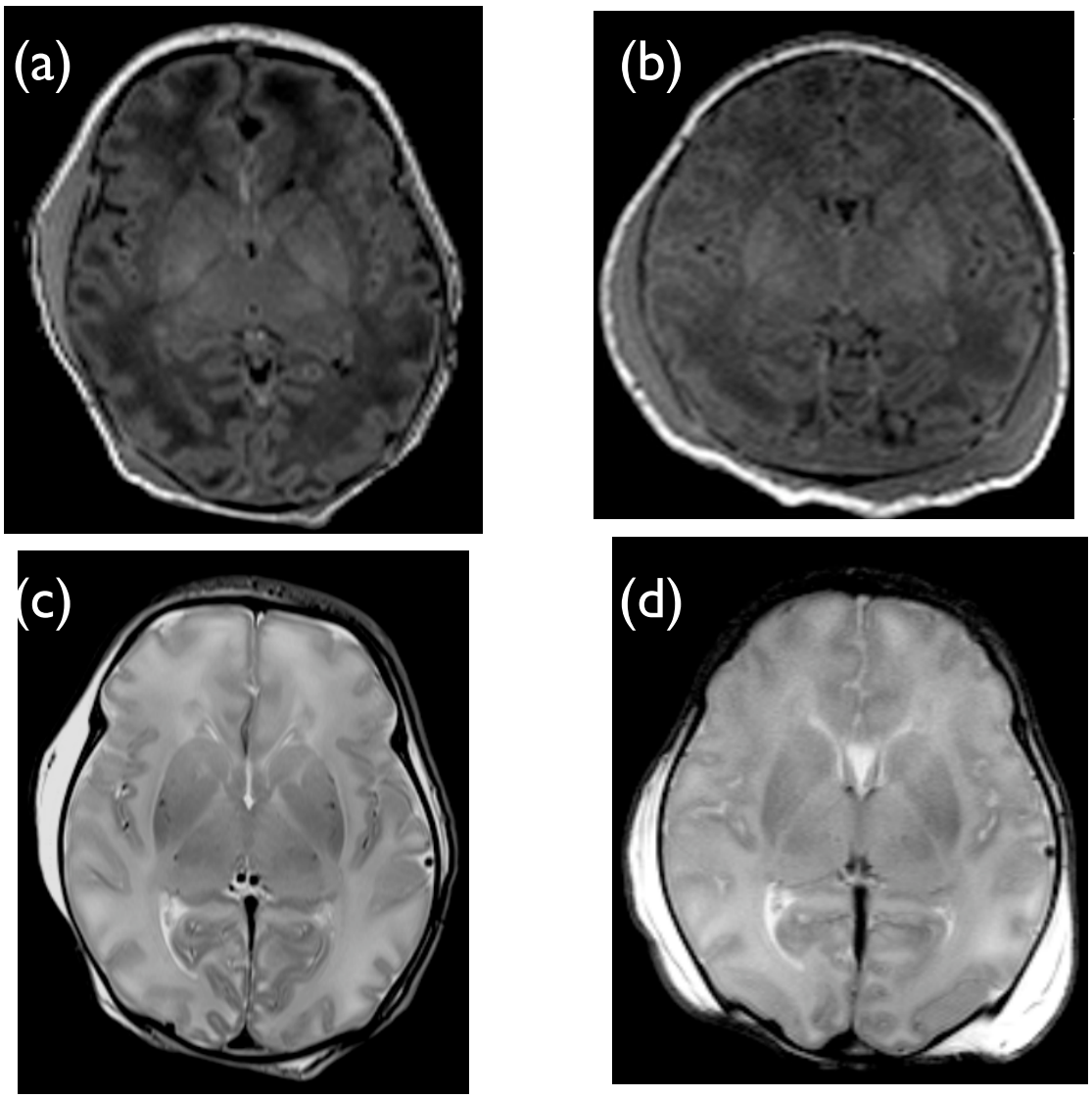
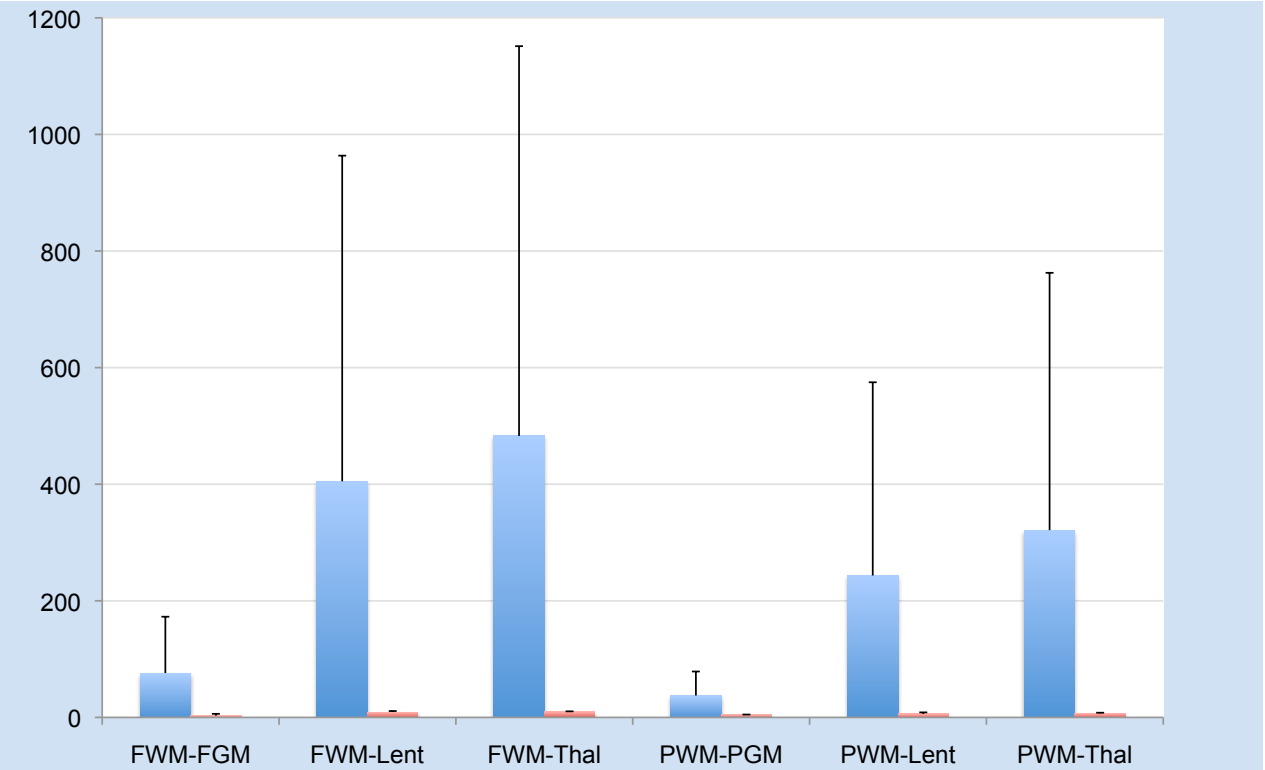
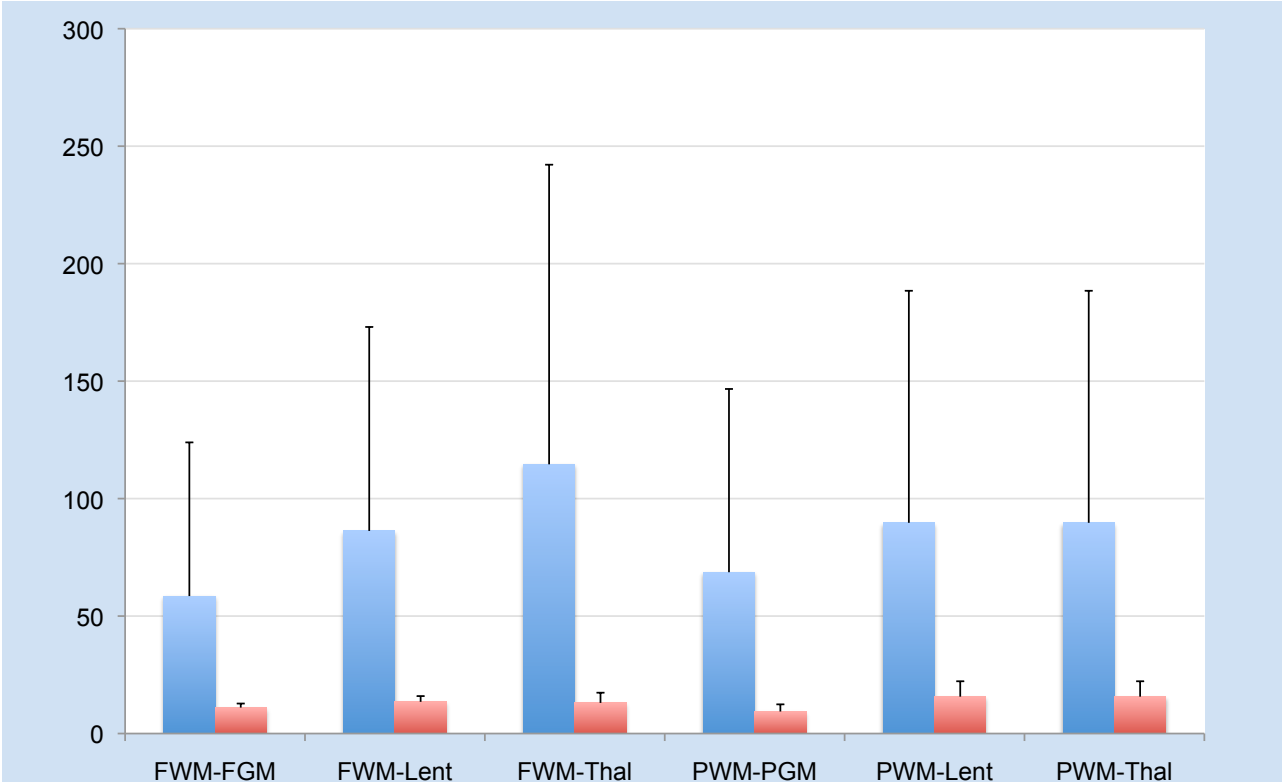


Figure 29. CNR in ante-mortem and post-mortem MR images on T_1-weighted images



Contrast to noise ratio on T_1–weighted images during life (blue bar) and after death (red bar). Error bars indicate SD. A trend towards reduced CNR was seen in all areas, even though the difference was not statistically significant. FWM: Frontal white matter, FGM: frontal grey matter, Lent: lenticular nucleus, Thal: Thalamus, PWM: Posterior white matter, PGM: Posterior grey matter

Figure 30. CNR in ante-mortem and post-mortem MR images on T_2-weighted images



Contrast to noise ratio on T_2–weighted images during life (blue bar) and after death (red bar). Error bars indicate SD. A trend towards reduced CNR was seen in all areas, even though the difference was not statistically significant. FWM: Frontal white matter, FGM: frontal grey matter, Lent: lenticular nucleus, Thal: Thalamus, PWM: Posterior white matter, PGM: Posterior grey matter

8.4.2 Comparison of Ante-mortem and Post-mortem MR Images

Two infants with severe NE had MR imaging before death at UCH; these infants were ventilated at the time of MR imaging. A decision was subsequently made to withdraw life support in these infants and the parents consented for recruitment into the post-mortem MR imaging study.

A loss in grey white matter contrast in both T_1 and T_2–weighted images was apparent on visual examination of these images (Figure 29). Signal intensities in the regions of interests drawn in the pre specified areas on T_1 and T_2–weighted images, before and after death in the same infants, are given in Figure 29 and Figure 30. Clearly, the numbers are inadequate to make a definite conclusions, however, a trend towards reduced tissue contrast in various brain regions was seen.

8.5 DISCUSSION

This study demonstrates that post-mortem conventional brain MR imaging at 1.5T cannot differentiate ante-mortem cerebral hypoxic-ischaemic injury due to perinatal asphyxia from age-matched cases with sudden death and no apparent preceding hypoxic-ischaemic insult. Both the perinatal asphyxial encephalopathy and sudden death infant groups were scanned at a similar time since death. The normal evolving post-mortem process itself leads to loss of grey/white matter differentiation, loss of normal high signal intensity in posterior limb of internal capsule and high signal intensities on white matter in T_2–weighted sequences. These changes therefore should not be mistaken as evidence of ante-mortem hypoxic-ischemic injury. I confirmed this observation using quantitative MR imaging methods (T_1 and T_2 signal intensities). There was no difference in the signal intensities in cortex, white matter and deep brain nuclei in babies with and without evidence of hypoxic damage on T_1–weighted images. The mean signal intensity on T_2–weighted MR imaging was higher in the thalamus and putamen in the asphyxia group, compared to the sudden death group. Although this observation requires further validation, an altered grey/ white matter T_2 ratio might be useful for defining hypoxic ischemic injury in post-mortem brain.

In a small subgroup of infants (n=2) in this study who had both ante-mortem and post-mortem MR imaging, a trend towards lower CNR in various brain areas was seen on post-mortem MR images, compared to ante-mortem MR images. This observation needs to be confirmed in larger cohort of infants.

A prolongation of T_1 and T_2 values and restricted diffusion are normally seen following a hypoxic ischemic injury in ante-mortem MR imaging (Liauw et al. 2007); T_1 values and diffusion tend to normalise earlier than T_2 values. A recent study on ante-mortem MR imaging in cooled infants with neonatal encephalopathy also noted that T_2 hyper intensities in basal ganglia and thalamus were the best predictors of adverse outcome (Massaro et al. 2010).

Nevertheless, these results contrast to some of the earlier published literature on post-mortem MR imaging. Rutherford and colleagues reported post-mortem MR imaging in six babies with neonatal encephalopathy and concluded that post-mortem MR imaging can accurately diagnose ante-mortem hypoxic ischemic injury. However, in this study, histological examination of brain was done only in one case. Moreover, there were no "control" cases i.e. babies without perinatal asphyxial encephalopathy and radiologists were not blinded to clinical reports (Nicholl et al. 2007). Cohen and colleagues also reported that post-mortem MR imaging can detect ante-mortem hypoxic-ischemic changes; again radiologists were not blinded to clinical history in these cases and no comparison was made with babies without ischemic injury (Cohen et al. 2007). With a more rigorous study design as in this study where the radiologists were blinded to the clinical and autopsy history, MR imaging was not useful in differentiating the normal evolution of post-mortem changes from hypoxic-ischaemic injury. Diffusion weighted imaging is difficult to interpret in a post-mortem setting due to restricted diffusion occurring both cases and controls after death.

In a more recent study comparing ante-mortem brain CT with post-mortem CT imaging in adults from Japan, a reduction in Hounsfield units (HUs) ratios of grey and white matter was seen following death (Takahashi et al. 2010).

This study has several limitations. I examined changes in signal intensity and have not attempted to quantify or validate this observation by T_1 and T_2 relaxometry. The latter would have been a more robust measure. There is no published data on in vivo post-mortem relaxometry of human brain. Future studies should explore how relaxometry is affected following death. Furthermore, I did not attempt to perform any co-registration of the MR images and histological data. However, often the ischemic lesions are more widespread in infants with severe perinatal asphyxial encephalopathy and co-registration may not be necessary. Finally, the placement of regions of interest

is subjective. However, I drew ROI after anonymisation of cases and controls, so as to minimise this bias.

Unlike post-mortem MR, well-defined neuropathological criteria exist for differentiating ante-mortem hypoxic-ischemic injury from post-mortem changes. Whilst the appearance of global infarction and loss of grey/ white contrast on post-mortem MR imaging may have little diagnostic utility, post-mortem MR imaging may still have important role in perinatal asphyxial encephalopathy for excluding other brain abnormalities such as migration defects, structural abnormalities and intracranial bleeds. The development MR abnormalities may be related to survival time after the hypoxic injury. Therefore, whilst acute fatal hypoxic events are indistinguishable from normal death process, if the infant survives for several days or weeks after the hypoxic injury, post-mortem MR imaging may show and well established ischemic injury with tissue loss or cystic changes that can be differentiated from death process (Figure 19) (Thayyil 2010).

In summary, this study suggests that caution is needed in the diagnosis of perinatal hypoxic ischemic brain injury using post-mortem brain MR as the normal death process cannot be differentiated from previous hypoxic-ischemic injury on post-mortem brain MR imaging. The physiological basis of these observations as to why the MR imaging was unable to differentiate between ischemic change and normal death process is unclear. In contrast there are well-defined criteria on neuropathological for differentiating ischemic injury from post-mortem changes. The first step in understanding how death process affects the MR images is to understand how changes in T_1 and T_2 values after death. This issue is therefore explored in the next chapter.

CHAPTER 9: POST-MORTEM T_1 AND T_2 RELAXOMETRY OF BRAIN

9.1 SUMMARY

Accurate interpretation of post-mortem MR imaging and optimisation of the MR sequences require an understanding of normal post-mortem relaxometry values, therefore I examined the contrast to noise ratio (CNR) and T_1 and T_2 relaxometry values in the brain of fetuses, newborns and infants, after death. I obtained T_1 and T_2-weighted MR images using a 1.5 Tesla Siemens Avanto scanner (Erlangen, Germany). In the first phase I calculated CNR in twelve brain regions of five newborn infants after death and compared this with CNR from five infants during life. In the second phase I performed T_1 and T_2 relaxometry in 18 fetuses and infants, prior to autopsy. I used an inversion-prepared turbo spin echo sequence with a repetition time (TR) of 7030 ms, echo time (TE) of 115 ms and six different inversion recovery times for T_1 relaxometry and a spin echo sequence (TR 2400 ms) with 16 different echo times for T_2 relaxometry. Pixel by pixel T_1 and T_2 maps and ROI were drawn on cortical and deep grey matter (GM) and white mater (WM). The MR images were reported by a paediatric neuroradiologist blinded to autopsy findings. Phase I: CNR on both T_1 and T_2–weighted images were significantly lower in most brain regions after death, when compared to that during life. Phase II: All the MR images were reported to have a lack of GM-WM contrast and high signal intensity in the WM on T_2-weighted images, that in a live brain would be suggestive of an ischemic change or global infarction. Neuropathological examination was normal in all cases. Mean (SD) T_1 values in white matter and deep grey were 1898 (327) ms and 1514 (202) ms in fetuses ($p>0.05$) and 1234 (180) ms and 1016 (161) ms in infants/newborns ($p>0.05$), respectively. T_1 values showed an inverse relation with increasing gestational age in fetuses, in both GM and WM. Mean (SD) T_2 values were 283 (11) ms in WM and 182 (18) ms in deep GM in infants and newborns ($p<0.001$). Convergence in the T_1 values resulting in lack of GM-WM contrast and increase higher signal intensity in the white matter due to T_2 prolongation may represent normal post-mortem changes, and should not be misinterpreted as ischemic lesions that were present before death. T_2–weighted sequences provide higher tissue contrast in the brain. Post-mortem T_1 and T_2 relaxometry values appear to be higher than the published data from ante-mortem brain.

9.2 INTRODUCTION

There is limited understanding of the effects of normal post-mortem changes over time on the brain; the well known T_1 and T_2 imaging characteristics seen in paediatric neuroradiology may be very different post-mortem. Following death, the T_1 and T_2 relaxometry values change in animal models and fixed ex vivo human brain due to a variety of reasons, including cell death, water logging, maceration, drop in body temperature and state of preservation of the body (Fagan et al. 2008; Shepherd et al. 2009; Widjaja et al. 2009). These changes in the relaxometry values may result in alterations in MR signal intensity that may be misinterpreted as a pathological process. In addition, post-mortem MR imaging may require difference MR parameters to obtain optimal tissue contrast. To date all studies on post-mortem MR imaging, including my study described in chapter 7, have used the same sequences that has been used in ante-mortem MR imaging (Thayyil et al. 2010). This is a pragmatic approach, considering the difficulty in optimisation for each specific setting after death.

However, the knowledge of normal post-mortem T_1 and T_2 relaxometry is therefore one of the first steps for understanding these post-mortem changes from an MR perspective and in optimising MR sequences to best image the post-mortem brain. The published data on T_1 and T_2 relaxometry values is limited to fixed human brain(Pfefferbaum et al. 2004) or animal data(Fagan et al. 2008; Shepherd et al. 2009), neither of which can be directly applied to in vivo human brain imaging.

9.3 AIM

- To compare the contrast to noise ratio (CNR) in normal post-mortem MR images with normal ante-mortem MR images of newborn infants
- To calculate the T_1 and T_2 relaxometry values following death in the brain of fetuses, newborns and infants.

9.4 METHODS

All MR images were acquired using a 1.5Tesla MR scanner as described in chapter 6 and 7. The study was conducted in two phases.

Phase I: This phase included five newborn infants in whom post-mortem MR imaging was performed before autopsy and where neuropathological examination was normal. Details of the MR sequences are given in chapter 6. I examined contrast to noise ratio in twelve different brain regions by drawing circular regions of interest (ROI) of 5 mm^2 on the cortical grey and white matter in frontal and posterior regions at the level of centrum semiovale and over the thalamus and lentiform nuclei to measure signal intensities (SI). CNR was calculated by the formula (SI A- SI B)/Noise (McRobbie et al. 2007: 66-67). I then compared this with contrast to noise ratio in identical regions in MR images of five full term normal control infants during life, using similar sequences for T_1 and T_2–weighted imaging from University College Hospital. The data was collected as a part of another research project.

In the second phase, I performed T_1 and T_2 relaxometry MR imaging using a 1.5 Tesla MR scanner (Siemens Avanto, Erlangen, Germany) in fetuses, newborns and infants, before conventional autopsy. To minimise the variations in T_1 and T_2 relaxometry data due to exogenous factors such as post-mortem interval and maceration, I used the following inclusion criteria – (a) absence of a brain pathology from ante-mortem investigations (b) post-mortem interval between 2 and 5 days (c) body well preserved without external signs of maceration.

I acquired T_1 relaxometry in all cases and T_2 relaxometry only in newborns and infants due to scanning time constraints. Cases in which neuropathological examination was abnormal or not possible because of autolysis were excluded from analysis. All bodies were kept in the mortuary at 4 ^{0}C until the MR imaging was performed. I measured the axillary temperature using an industrial thermometer, before and after the MR scan. The MR scanning was started when the axillary temperature was between 7 to 8^0 C. I acquired relaxometry at the beginning of the MR scan to minimise variations in temperature between cases. The bodies were kept covered in a thermal blanket to minimise the rise in temperature during scanning.

I used an inversion-prepared turbo spin echo sequence to acquire T_1 relaxometry. Following parameters were used: voxel size – 1.1 x 0.8 x 4.5 mm, field of view (FoV) – 200 mm, FoV phase – 76.6%, slice thickness – 4.5mm, recovery time (TR) – 7030 ms, echo time (TE) – 115 ms, averages – 2, flip angle – 150^0, orientation– coronal. I used six different inversion times – 3600 ms, 2200 ms, 1200 ms, 250 ms, 150 ms and 100 ms. Total time of acquisition was 14 min and 16 sec (2 min 36 sec x 6).

I used a multi spin echo sequence to acquire T_2 relaxometry using the following parameters: voxel size – 0.8 x 0.6 x 6 mm, field of view (FoV) read – 150 mm, FoV phase – 75%, slice thickness – 6 mm, recovery time (TR) – 2400 ms, averages – 2, flip angle – 180^0, orientation– coronal. Sixteen different echo times (TE) were used– 22 ms, 44 ms, 66 ms, 88 ms, 110 ms, 132 ms, 154 ms, 176 ms, 198 ms, 220 ms, 242 ms, 264 ms, 286 ms, 308 ms, 330 ms and 352ms. Total time of acquisition was 12 min 4 sec.

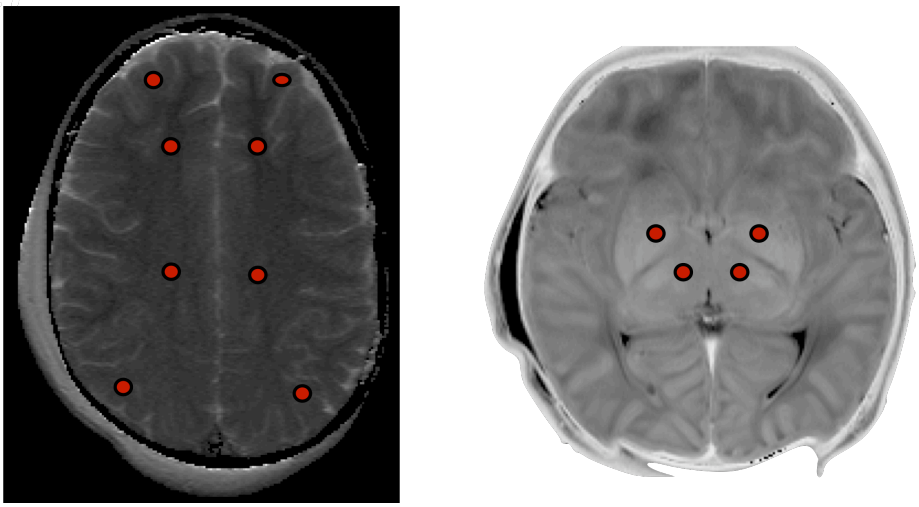
Pixel by pixel T_1 and T_2 maps were then created using a software developed in-house with Matlab version 6 (MathWorks, Cambridge, UK); T_2 by fitting a decreasing exponential to the signal intensity as a function of echo time (2-parameter fit); T_1 using a conventional inversion recovery equation (3-parameter fit including inversion efficiency). I drew circular regions of interest (ROI) of 5 mm^2 on the cortical grey and white matter in frontal and posterior regions at the level of centrum semiovale and over the thalamus and lentiform nuclei (Figure 31). Image J (Version 1.4, National Institute of Health, USA) was used for image analysis and the data were imported into an excel spreadsheet.

An experienced paediatric neuroradiologist (R Gunny) reported the MR images, blinded to autopsy findings. Autopsies were performed by specialist paediatric pathologists, with input from a paediatric neuropathologist if required. Pathologists were blinded to the MR imaging findings.

I used Wilcoxon rank test to examine differences between paired data and Mann Whitney U test for comparing unpaired data. SPSS Version 17 (SPSS Inc, City, Country) and Medicalc (Company, City, Country) were used for analysis.

Figure 31. ROI placements

T_1 and T_2 maps showing placement of regions of interest (ROI). 1a. ROI placed on left and right cortical grey and white matter, anteriorly and posteriorly at the level of centrum semi ovale. 1b. ROI in thalamus and lentiform nuclei



9.5 RESULTS

In the phase one of this study, CNR of twelve brain regions of five newborn infants during life was compared with CNR in the same regions following death. A significant reduction in CNR was seen was in most brain regions in both T_1 (Figure 32) and T_2-weighted images (Figure 33), in particular between cortical grey and white matter (Table 20). In the second phase, relaxometry data was acquired on a total of 26 cases, of which 2 were excluded from analysis due to autolysis and uninformative neuropathology. The remaining cases included 18 fetuses, two newborns and four infants.

The median (range) gestation of fetuses was 31 weeks (25, 39 weeks) and the median age of the infants were 8 months (2 weeks to 12 months). The median (range) time from death/delivery to MR imaging was 4 days (2 to 5) in fetuses and 2.5 days (2 to 4) in infants. The median axillary temperature was 7.9 ^{0}C (7.5, 8.2) in fetuses and 7.4 ^{0}C (7, 7.8) in infants at the beginning of the scan. By the end of the scan the temperatures increased to 10.8 ^{0}C (9.1, 12.1) in fetuses and 8.7 ^{0}C (8.4, 9.1) in infants.

The conventional T_1 and T_2-weighted MR images in all the cases showed reduced grey-white contrast on T_1-weighted image and increased signal intensity in the white matter on T_2-weighted images suggestive of an ischemic insult or global infarction. No other abnormality was seen on MR images. Neuropathology was normal in all cases.

Mean T_1 and T_2 relaxometry values in fetal and infant brain are given in Table 21. No significant difference between the T_1 relaxometry values in the cortical grey and white matter was seen (Figure 34). The T_1 relaxometry values in fetuses were significantly higher than in infants in corresponding regions. The T_2 relaxometry values in the white matter were also significantly higher than the grey matter in newborns and infants (Figure 35). Mean T_1 and T_2 values from left and right sides of the brain were similar ($p>0.05$), therefore average values were used. An inverse correlation of T_1 relaxometry values and gestational age was seen in the deep grey matter ($r=-0.69$, $p=<0.001$) and in the white matter ($r=-0.54$, $p<0.0001$) (Figure 36).

Table 20. Comparison of contrast to noise ratio (CNR) in T_1 weighted and T_2 weighted images, before and after death in newborn infants

Area	CNR on T_1–weighted images		p value	CNR on T_2–weighted images		p value
	Ante-mortem	Post-mortem		Ante-mortem	Post-mortem	
FWM-FGM	7.2 (3)	0.1 (1.6)	0.0059	16.3 (1.2)	9.0 (2.1)	0.0002
PWM-PGM	8.8 (3.4)	5.2 (1.1)	0.095	12.9 (1.2)	2.9 (3.6)	0.0004
FWM_Lent	13.1 (2.4)	6.0 (1.5)	0.0026	18.4 (2.3)	13.5 (5.5)	0.1033
FWM-Thal	9.6 (1.9)	6.2 (1.6)	0.0319	20.5 (2.9)	13.3 (6.1)	0.0453
PWM-Lent	10.3 (3.9)	5.5 (0.8)	0.0533	15.1 (3.8)	13.3 (5.0)	0.5338
PWM-Thal	6.8 (2.4)	5.7 (2.4)	0.4358	17.1 (3.7)	13.1 (5.6)	0.2124

FWM: Frontal white matter, FGM: frontal grey matter, Lent: lenticular nucleus, Thal: Thalamus, PWM: Posterior white matter, PGM: Posterior grey matter

Table 21. Post-mortem T_1 and T_2 relaxometry values in fetuses and infants.

Area	Mean (SD) T_1 Value (ms)	Mean (SD) T_2 values (ms)
FETUS		
Frontal grey matter	1898 (306)	
Frontal white matter	1898 (327)	
Posterior grey matter	1884 (264)	
Posterior white matter	1930 (367)	
Lentiform nuclei	1516 (200)	
Thalamus	1514 (202)	
NEWBORNS AND INFANTS		
Frontal grey matter	1229 (202)	225 (12)
Frontal white matter	1234 (180)	283 (11)
Posterior grey matter	1113 (206)	209 (14)
Posterior white matter	1286 (66)	281 (22)
Lentiform nuclei	1080 (101)	185 (22)
Thalamus	1016 (161)	182 (18)

Figure 32. CNR on T_1-weighted MR images

Mean contrast to noise ratio on T_1-weighted images during life (blue bar) and after death (red bar). Error bars indicate SD. * $p<0.05$. FWM: Frontal white matter, FGM: frontal grey matter, Lent: lenticular nucleus, Thal: Thalamus, PWM: Posterior white matter, PGM: Posterior grey matter.

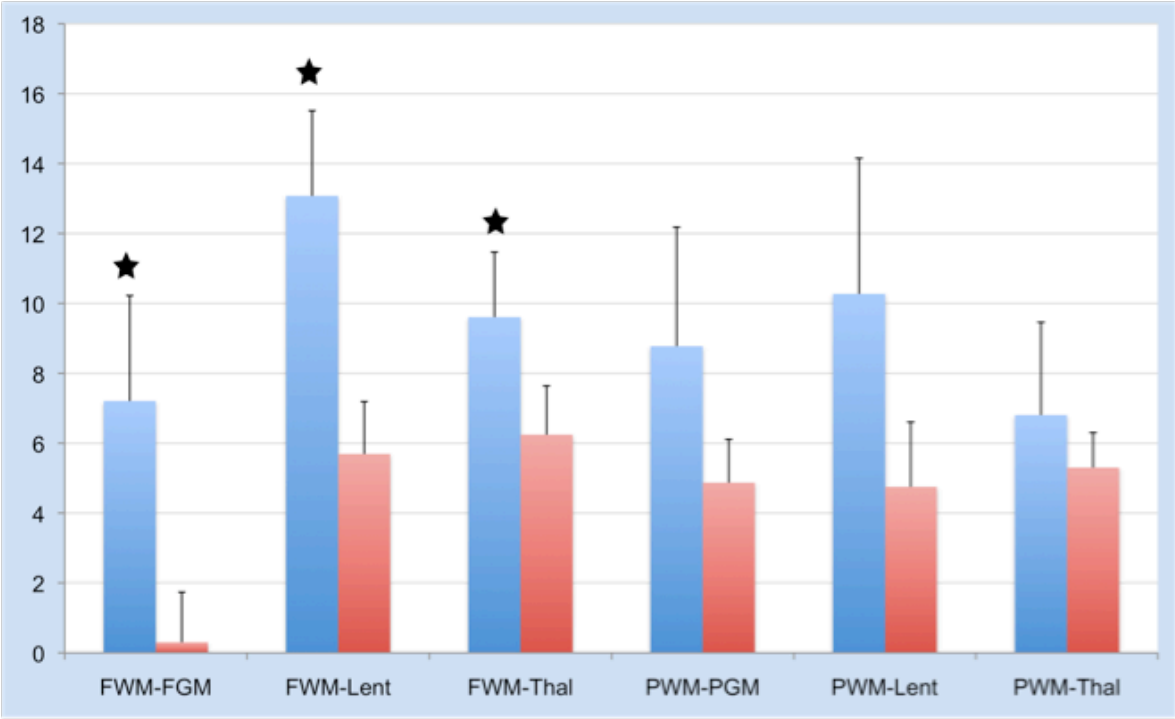


Figure 33. CNR on T_2-weighted MR images

Mean contrast to noise ratio on T_2-weighted images during life (blue bar) and after death (red bar). Error bars indicate SD. * $p<0.05$

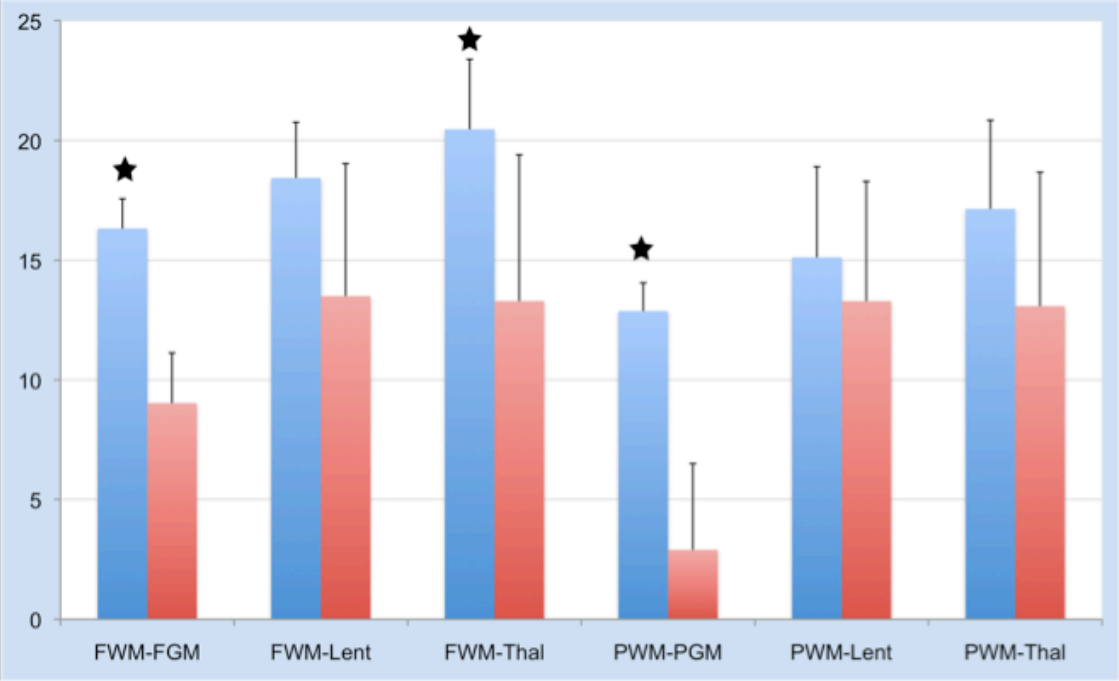


Figure 34. T_1 relaxometry

Mean (SD) T_1 relaxometry values in fetal and infant/newborn brain. FGM=frontal grey matter, FWM=frontal white matter, PGM=posterior grey matter, PWM=posterior white matter, Lent=Lentiform nuclei, Thal=Thalamus

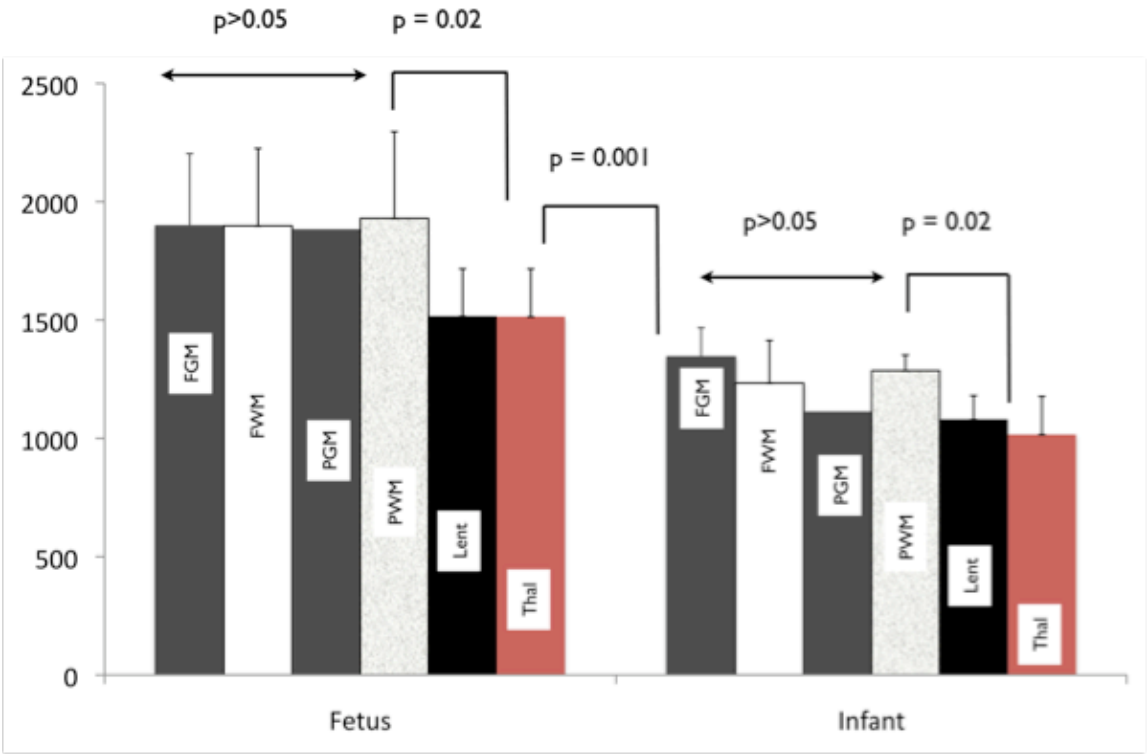


Figure 35. T_2 relaxometry

Mean (SD) T_2 relaxometry values in infant/newborn brain. GMF=frontal grey matter, WMF=frontal white matter, GMP=posterior grey matter, WMP=posterior white matter, Lent=Lentiform nuclei, Thal=Thalamus

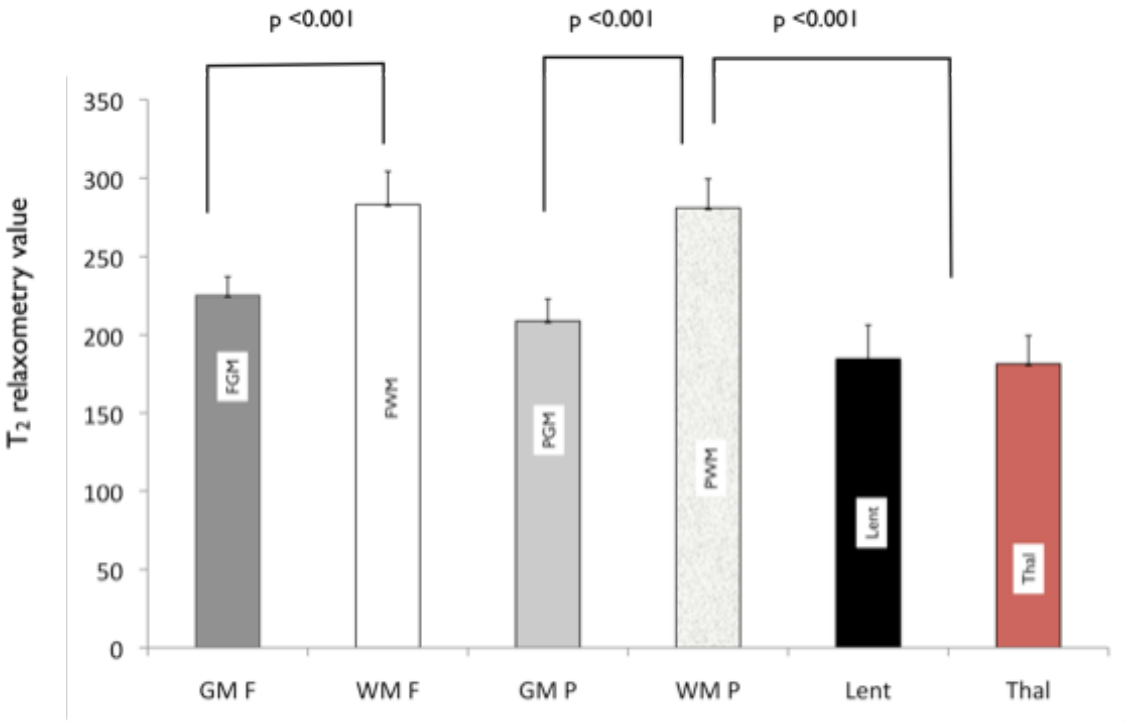


Figure 36. Gestational age and T_1 relaxometry

Correlation of white matter and thalamic T_1 values with gestational age in fetuses.

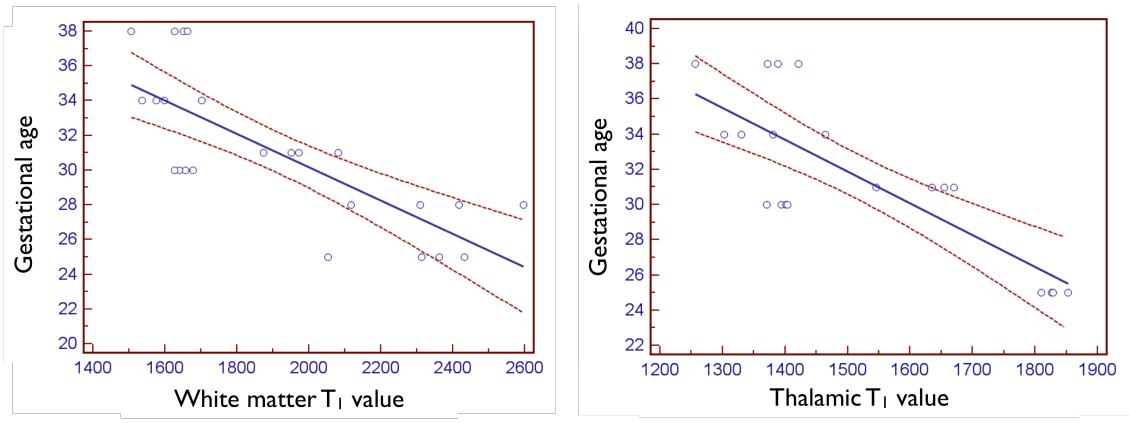
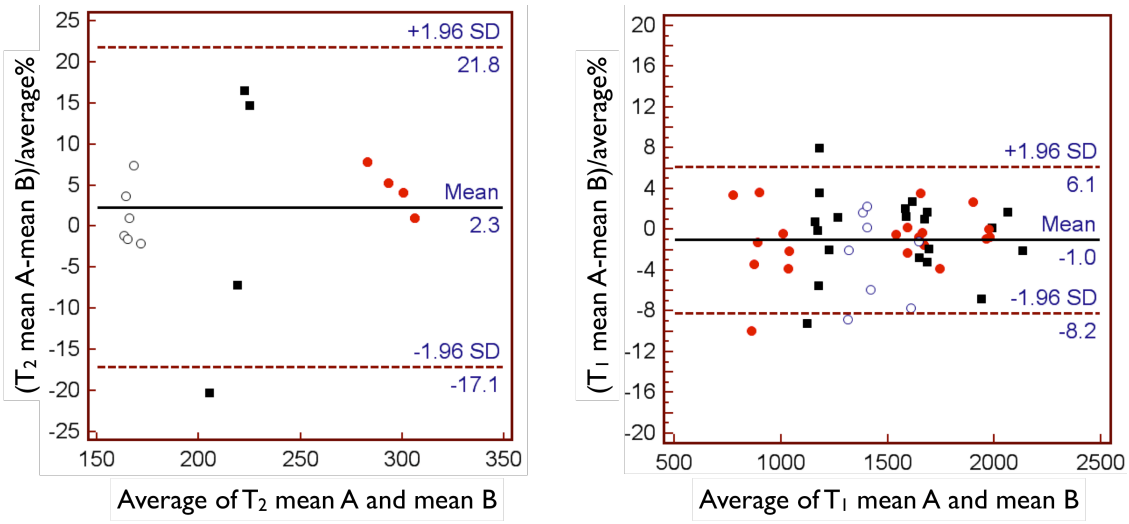


Figure 37. Bland Altman plots of relaxometry

Bland Altman plot exploring intra-observer variability of T_1 and T_2 relaxometry values.

NB: Black square=cortical grey matter, White circle=deep nuclei, Red circle=white matter



Intra-observer variability was judged to be good, since the 95% confidence intervals was within 10% of the mean values for white matter and deep grey matter. The agreement limits were wider (upto 20%) for cortical grey matter on T_2 maps (Figure 37). No relation of post-mortem interval and T_1 and T_1 relaxometry values were seen on regression analysis.

9.6 DISCUSSION

This study reports the first measurement of T_1 and T_2 relaxometry values in post-mortem human brain. No significant difference in T_1 values in the cortical grey and white matter was seen, suggesting a convergence of T_1 values may occur after death. The T_1 values in white matter and grey matter reduced with increasing gestation in fetuses. Prolongation of T_2 was seen in the white matter. Lack of grey white matter contrast and diffuse high signal intensity in the white matter on T_2-weighted images are normal post-mortem changes and does not necessarily indicate ischemic injury. The relaxometry data presented in this study may be useful for optimisation of the post-mortem MR imaging sequences.

The published data from premature infants between 24 to 35 weeks during life suggests that the mean T_1 values are 1771(227) ms in white matter and 1216 (275) ms in the deep grey matter(Counsell et al. 2003). I found T_1 relaxometry values of 1898 ms in frontal grey and white matter, and 1516 (200) in the deep grey matter, suggesting an increase in the T_1 values occurring following death. Moreover, the difference between the deep grey and the white matter T_1 value decreased from 555 ms before death to 382 ms after death. Similar T_1 values in the cortical grey and white matter does account for the lack of grey white contrast seen on T_1 weighted post-mortem MR images. Decreasing T_1 values with increasing gestation may be due to the increasing myelination (Counsell et al. 2003) or may be due to reduction in free water content.

The T_2 relaxometry values from healthy newborn infants are 222 (25) ms in the frontal WM and 221 (22) ms in the posterior white matter(Hagmann et al. 2009). The post-mortem values in my study are higher than these values, which account for the post-mortem high signal intensity seen in the white matter. The prolongation of the brain T_1

and T_2 relaxometry values in my study is contrary to the previously published data on fixed human and animal brains. In fact most published literature on fixed animal and human brain suggests a decrease in T_1 and T_2 values with post-mortem interval (Pfefferbaum et al. 2004; Fagan et al. 2008). This may be related to the excess tissue dehydration that may be occurring in fixed brain tissues; such dehydration would not occur in in-vivo human brain imaging. Indeed, when the brain tissues were stored in special chambers to prevent dehydration and washed overnight to remove fixatives, Shepherd and colleagues observed that the T_1 values increase by 22% and T_2 values increased by 65% in rat brain cortex (Shepherd et al. 2009).

9.6.1 Limitations of the Study

There are several limitations of this data. Firstly, I used a cross sectional study design for examining the post-mortem T_1 and T_2 relaxometry; the optimal study design would have been serial MR imaging before and after death at various time points. However, such study designs are unrealistic from an ethical and pragmatic point of view. Secondly, I did not find an association between post-mortem interval and the relaxometry values. However, this may be a biased observation and may merely reflect the homogeneity of my samples and narrow range of post-mortem interval. It is possible that the relaxometry values have already plateaued and the relaxometry changes occurred soon after death, before MR imaging was performed. I used a specific range (2 to 5 days) for the post-mortem interval, excluding very early and very late scans, as this is the time most post-mortem MR imaging can be realistically performed in a clinical setting.

Thirdly, the slice thickness of the MR images was high; therefore some degree of subjectivity and partial volume effects may have occurred in drawing regions of interest, particularly in cortical grey matter. However the intra-observer variability in our study was good. Fourth, there is well proven inverse relation of the temperature and the T_1 and T_2 values, which could have affected the interpretation. Fifth, normative data is difficult to define in a population that has died unexpectedly. Nevertheless, I selected cases that are as close to normality as possible, so that post-mortem changes can be accurately defined and used a pragmatic standardized approach reflecting the clinical scenario for scanning. Sixth, my data may be applicable only to post-mortem MR imaging at 1.5 Tesla

and not at higher field strengths (Thayyil et al. 2009b), as the relaxometry values are dependent on field strength. Finally, the lack of contrast between grey and white matter and flat T_1–weighted images, does not mean that that T_1–weighted imaging is not useful in post-mortem settings. My cohort did not include cases with intracranial bleeds or structural neuropathology; it is possible that T_1–weighted images are still useful in such settings.

In this study, I report in vivo post-mortem T_1 and T_2 relaxometry values for brain in fetuses and infants for the first time. The post-mortem T_1 relaxometry showed an inverse relation with increasing gestational age in the fetus. Convergence in the T_1 and T_2 values resulting in lack of grey white matter contrast and increase higher signal intensity in the white matter due to T_2 prolongation may represent a normal post-mortem changes, and should not be misinterpreted as ischemic lesions. Post-mortem T_1 and T_2 values appear to be higher than the published data from ante-mortem brain.

CHAPTER 10: POST-MORTEM CARDIAC IMAGING

10.1 SUMMARY

Here, I compared the accuracy of high-resolution 3D post-mortem MR imaging in fetuses, neonates and children with conventional autopsy, for identifying cardiac abnormalities. Post-mortem MR imaging was performed in 15 consecutive fetuses, newborns and children with a cardiac abnormality and 15 age matched controls, using a 1.5 Tesla MR scanner before conventional autopsy. A 3D T_2-weighted turbo spin echo (TSE), 3D T_1-weighted volumetric interpolated breath-hold examination (VIBE) sequence and a 3D constructive interference in the steady state (CISS) sequence were used for cardiac imaging. A paediatric cardiac MR radiologist reported the MR images, blinded to all clinical details, autopsy information and allocation group. The pathologists were blinded to the information from the MR imaging. The median (range) gestation and weight of fetuses (n=22) was 21 weeks (16 to 35 weeks) and 332g (21 to 2375g) respectively. The median age and weight of children (n=8) was 1 month (1 day to 7 months) and 3.7kg (2.1 to 7.3kg) respectively. Post-mortem cardiac MR imaging was non-diagnostic in all fetuses less than 20 weeks (n=6). In the remaining 24 cases, post-mortem cardiac MR imaging had a sensitivity of 100% (95% CI 63%, 100%) and specificity of 73% (95%CI 45%, 91%) for detecting a significant structural cardiac abnormality. High resolution post-mortem 3D cardiac MR imaging may be a useful alternative or adjunct to conventional cardiac autopsy in larger fetuses, newborns and children for detecting structural cardiac abnormalities.

10.2 INTRODUCTION

This study builds on the work described in chapter 7, where I noted a variable accuracy of post-mortem cardiac MR imaging with wide confidence intervals. The sensitivity of post-mortem MR imaging was poor in smaller fetuses. However, the prevalence of cardiac lesions in the population was low (11%) and the cardiologist was not blinded to the clinical information. Therefore the knowledge of ante-mortem cardiac scan results may have influenced the accuracy of cardiac MR imaging. Furthermore the 3D isotropic cardiac MR sequences used were at lower resolution (1 to 1.4 mm^3).

Cardiac abnormalities are found in up to 22% of fetal autopsies (Grant et al. 2008), of which 17 to 54% may be detected antenatally (Stoll et al. 2001; Tegnander et al. 2006a; Tegnander et al. 2006b). Cardiac defects are seen at autopsy in approximately 10% of sudden death in infants and could be the cause of death in 84% of these cases. Even though the vast majority of such abnormalities are structural, only 40% are detected before death (Tennstedt et al. 1999; Dancea et al. 2002). Thus, fetal and paediatric cardiac autopsies have a crucial role, not only for quality assurance of antenatal screening programmes and ante-mortem diagnostic procedures, but also in counselling parents regarding cause of death and implications for future pregnancies (Boldt et al. 2002; Piercecchi-Marti et al. 2004).

Cardiac autopsy is a labour intensive procedure, and the specialist paediatric cardiac pathology expertise necessary for such assessments is not widely available; thus children, or the heart, often need to be transported to specialist centres for cardiac autopsies, particularly in complex malformations (Grant et al. 2008). There is therefore a need for developing rapid, accurate and less invasive techniques for post-mortem examination of the heart, that can be easily performed in routine clinical practice (Parker 2004).

10.3 AIM

To examine the diagnostic accuracy of high-resolution, 3D post-mortem cardiac MR imaging (1.5 Tesla) with conventional autopsy in fetuses, newborns and children, with the radiologist blinded to the clinical data.

10.4 METHODS

Fifteen fetuses, newborns and children with a cardiac abnormality detected at autopsy and 15 age-matched controls, were selected from the post-mortem database described in chapter 6. All the cases had post-mortem cardiac MR imaging using a 1.5 Tesla MR scanner, before autopsy (please see chapter 6). A 3D T_2-weighted turbo spin echo (TSE), 3D T_1-weighted volumetric interpolated breath-hold examination (VIBE) sequence and a 3D constructive interference in the steady state (CISS) sequence were used for cardiac imaging (Table 22).

Table 22. Typical parameters for post-mortem cardiac MR imaging

Parameter	3D CISS	3D T_2-weighted TSE	3D T_1-weighted VIBE
Voxel dimension	0.6x0.6x0.6 mm	1.4x1.4x1.4mm	1.4x1.4x1.4mm
TE (milliseconds)	2	172	2.12
TR (milliseconds)	4.7	3500	5.87
Bandwidth	400	890	350
Averages	5-10	1	5
Acquisition time	Upto 60 minutes	8min	8 min

The MR images were then anonymised and were reported by a specialist paediatric cardiac MR radiologist (Prof Andrew Taylor), blinded to all clinical details and autopsy information and allocation to case or control group. Autopsy was performed by one of the four perinatal or paediatric pathologists (10 to 20 years experience) or a specialist paediatric cardiac pathologist (12 years experience), blinded to the cardiac MR reporting.

Statistical Analysis

All data is given as median values with ranges. Exact confidence intervals based on F distribution were used to calculate confidence intervals for sensitivity and specificity (Leemis et al. 1996). SPSS Version 16.0 for Macintosh (SPSS Inc, Chicago, Illinois),

Medcalc Version 10.0 MedCalc Software, Mariakerke, Belgium) and MetaDisc Version 1.4 (Ctr.de.Colmenar Viejo, Madrid) were used for statistical analysis.

10.5 RESULTS

At autopsy fetuses had a median gestation of 21 weeks (16 to 35 weeks) and median weight of 332g (21 to 2375g) respectively. Newborns and children had a median age of 1 month (1 day to 7 months) and median weight of 3.7kg (2.1 to 7.3kg). Post-mortem MR imaging was performed at a median interval of 3 days (1-5 days) after death.

Comparison of MR imaging and conventional autopsy

Cardiac MR imaging was non-diagnostic in all 6 fetuses less than 20 weeks gestation (cases 24-30) (Table 23). Of the remaining 24 cases, 9 had significant cardiac abnormalities detected at autopsy (cases 1-9). Cardiac MR imaging was abnormal in all 9 (Figure 38), but histological examination was required to confirm the diagnosis in 2 (Case 7: myocarditis, Case 9: dilated cardiomyopathy) (Figure 39). MR imaging failed to detect minor abnormalities in 2 fetuses (cases 10: petechial haemorrhage, Case 11: persistent left superior vena cava). There were 4 false positive cases from cardiac MR imaging (case 12-15) (Figure 40). A general paediatric or perinatal pathologist performed the cardiac examination in all these cases.

In fetuses $\geq$ 20 weeks, newborns and children, post-mortem cardiac MR imaging had a sensitivity of 100% (95% CI – 68%, 100%), specificity of 73% (95% CI – 51%, 90%), positive likelihood ratio of 3.6 (95% CI – 1.6, 8) and negative likelihood ratio of 0.07 (95% CI – 0.05, 1.1) for detecting a significant structural cardiac abnormality. Positive predictive value of cardiac MR imaging was 67% (95% – CI 39%, 86%) and negative predictive value was 100% (95% – CI 72%, 100%).

10.6 DISCUSSION

Contrary to previous reports, I have demonstrated good diagnostic utility of post-mortem 3D cardiac imaging in fetuses, newborns and children. Post-mortem cardiac MR imaging had a similar detection rate and diagnostic yield to that of conventional autopsy for all major structural abnormalities in larger fetuses, newborns and children. The negative

predictive value of post-mortem cardiac MR imaging was good–100% (95% – CI 72%, 100%) for detecting significant cardiac pathologies.

This report is in sharp contrast with the published literature on post-mortem cardiac MR imaging. Alderstein *et al.* compared post-mortem MR imaging with conventional autopsy in 26 fetuses (Alderliesten et al. 2003). Cardiac abnormalities were seen in 5 cases at autopsy, but none of these cases were detected by post-mortem cardiac MR imaging (sensitivity 0%). Breeze *et al.* have reported a comparison of post-mortem whole body imaging and autopsy in 36 fetuses . Of the eight fetuses who had cardiac lesions, only two were detected by post-mortem MR imaging (25% sensitivity); however, there were no false positives (100% specificity). Cohen *et al.* have reported 2 cases (no cardiac lesions) of post-mortem cardiac MR imaging in sudden infant death; however, both the images were non-diagnostic (Cohen et al. 2007). These three studies used 2D cardiac MR imaging with high slice thickness (2-3 mm), and the interpretation of cardiac images was done by general radiologists. Our group have previously reported feasibility of 3D post-mortem cardiac MR imaging and offline re-constructions in fetuses and newborns in small case series and case reports (Deng et al. 1996; Brookes et al. 1999; Taylor et al. 2006). In this study, I evaluated the accuracy and potential clinical utility of post-mortem cardiac MR imaging in a systematic way.

This study differs from the previously published data described above in several ways. Firstly, isotropic 3D images were acquired with good resolution in all direction, which enabled accurate identification of small complex structures in any imaging plane. Secondly, images were reported by an experienced academic congenital heart disease radiologist, who was blinded to all clinical and autopsy data. Though this may be a slightly artificial situation compared to the ‘real-life’ scenario, it reduced the potential bias from previous investigations and clinical history that may spuriously increase the diagnostic accuracy (Houssami et al. 2004). Thirdly, the high incidence of cardiac lesions in present sample population, i.e. 50% (low in previous reports) and matched controls, provides this study with the necessary discriminatory power to identify the accuracy of MR imaging for cardiac lesions.

Table 23: Comparison of post-mortem cardiac MR imaging and conventional autopsy findings

No	Age (weeks)	Clinical Summary	Post-mortem cardiac MR Imaging	Conventional Autopsy
1*	20	TOP, Tetrology of fallot	DORV, large VSD, narrow LVOT and RVOT	DORV, large VSD, narrow RVOT, Overriding of aorta
2	20	TOP for Dandy walker	Small RV, possible coarctation	Possible coarctation
3*	21	TOP for Dandy walker variant	Small outlet VSD (0.2 cm)	Small outlet VSD (0.2 cm)
4	21	TOP for NTD, Major abdominal wall defects	ASD, coarctation, infundibular pulmonary stenosis, prominent azygos vein	Possible Coarctation
5	32	Preterm difficult to ventilate ?Pulmonary hypoplasia	Hypertrophied RV, large PDA, possible pulmonary stenosis	Large PDA
6*	44	VSD, Interrupted aortic arch, DORV, PA banding, Post operative death	VSD, pulmonary stenosis	VSD (patch leak), Dysplastic pulmonary valve, Narrowing at origin of LPA
7*	68	SUDI	RV hypertrophy, dilated RA, RV, LA, LV	Dilated RA, RV, LV, Dilated cardiomyopathy
8	84	Septo optic dysplasia, ASD	Big RA, RV, ASD	ASD
9*	140	SUDI	Dilated RA	Focal myocarditis
10	34	IUFD	Normal	Normal apart from persistent left SVC
11	35	IUFD	Normal	Normal apart from petechial haemorrhages in pericardium
12	22	TOP for holoprosencephaly	PFO, VSD, Overriding Aorta	Normal
13	35	Downs syndrome, IUFD	VSD, Coarctation	Normal
14	38	IUFD	Coarctation	Normal
15	40	IUFD	Coarctation ?Aortic Interruption	Normal
16-24	21-52	SUDI (2), TOP (1), IUFD (7)	Normal (10)	Normal (10)
25-30	16-19	IUFD (6)	Non diagnostic (6)	Normal (2), Abnormal (4)

TOP: Termination of pregnancy, RA: Right atrium, RV: Right ventricle, LA: left atrium, LV: Left ventricle, DORV: Double outlet right ventricle, SVC: Superior vena cava, ASD: Atrial septal defect, LVOT: Left ventricular outflow tract, RVOT: Right ventricular outflow tract, IUFD: Intra uterine fetal death, PDA: Patent ductus arteriosus

*Post-mortem cardiac examination performed by paediatric cardiac pathologist

Figure 38: Pulmonary valvular stenosis

Pulmonary valvular stenosis (arrow) and residual muscular ventricular septal defect (VSD - *), in newborn operated on for double outlet right ventricle and interrupted aortic arch. Post-mortem MR findings confirmed at conventional autopsy. Pulmonary stenosis, dysplastic pulmonary valves and residual VSD likely to have resulted in the post operative death. A, sagittal view and B, coronal view (PT - pulmonary trunk, RV - right ventricle and LV - left ventricle).

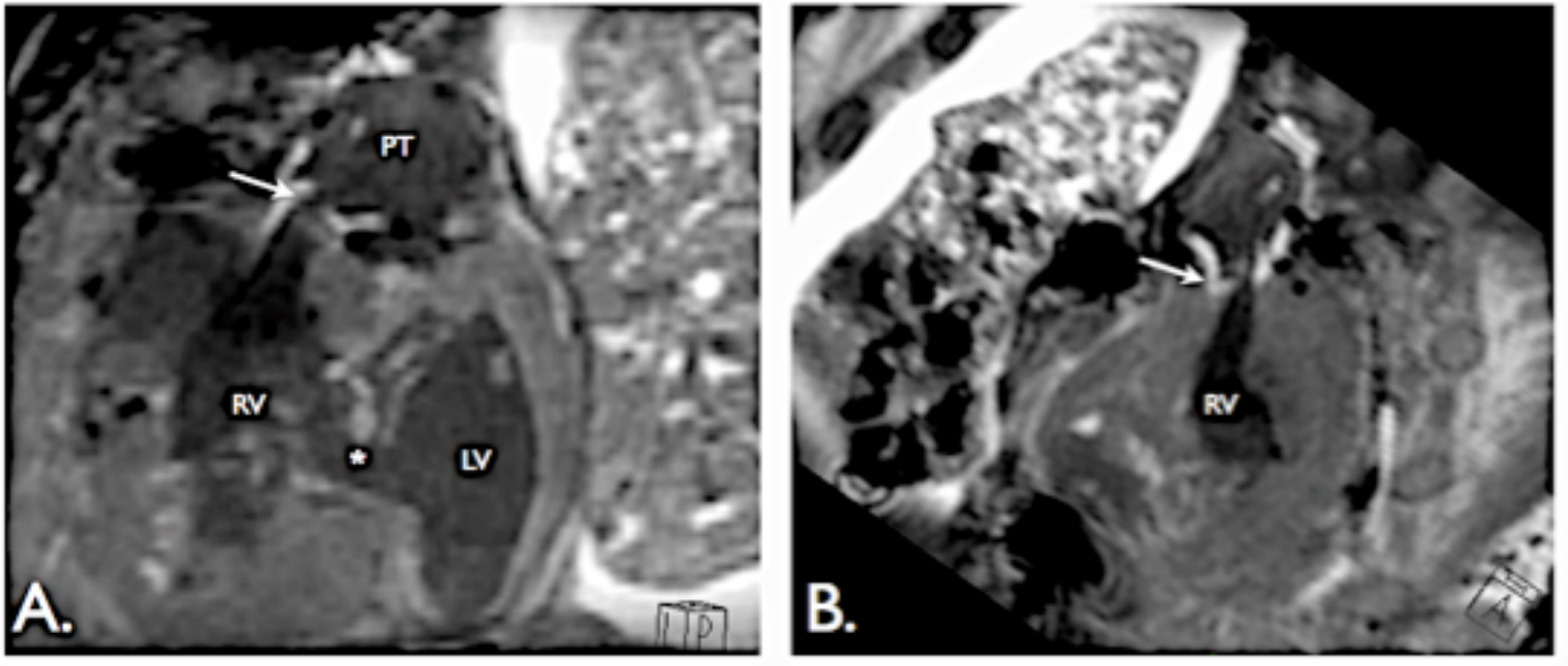


Figure 39: Dilated cardiomyopathy

Dilated cardiomyopathy in a previously healthy 7 month old infant, who had a sudden unexpected death. Note dilated RA - right atrium, RV - right ventricle, LA - left atrium and LV - left ventricle. Post-mortem MR findings confirmed at conventional autopsy. Cardiomyopathy presumed to be the cause of death.

A, 4-chamber view, B, RV vertical long axis and C, LV vertical long axis.

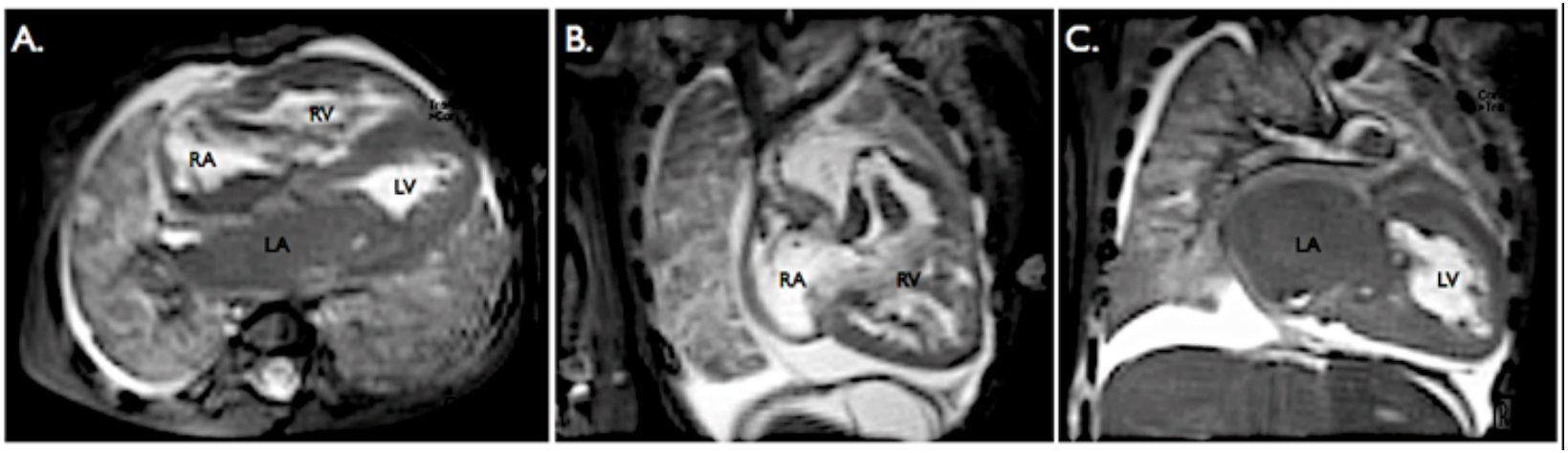
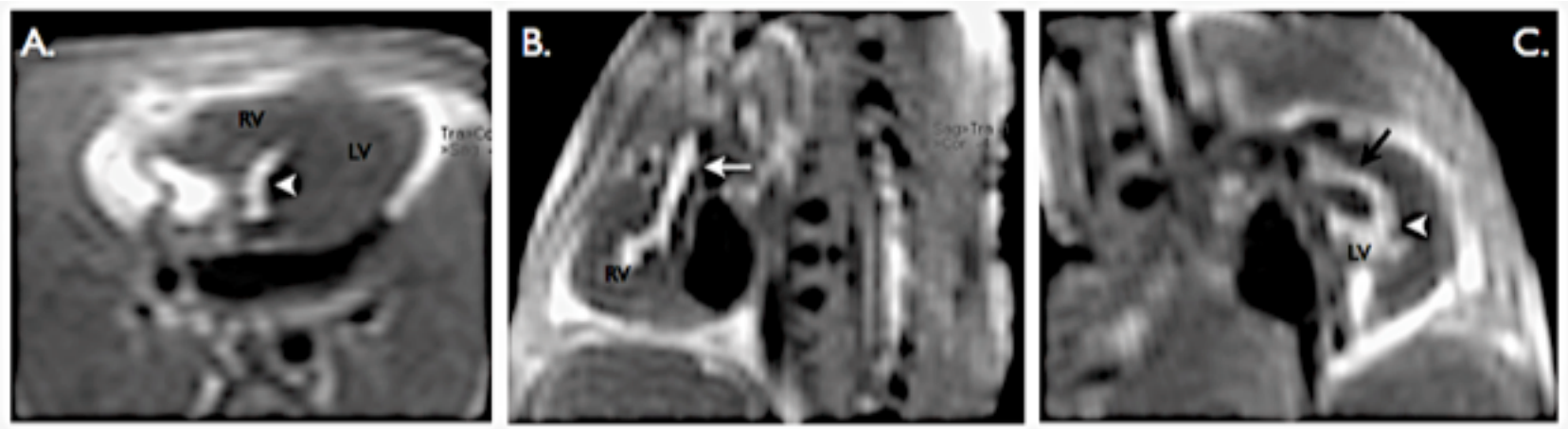


Figure 40: Ventricular septal defect

Isolated, small membranous ventricular septal defect (VSD) in a 22 week fetus, delivered following termination of pregnancy for holoprosencephaly (VSD - arrowhead in A and C). A, axial view, B, sagittal view and C, right ventricular outflow track view (RVOT - black arrow) (LV - left ventricle). Note how the right ventricle (RV) is directly connected to the ascending aorta (white arrow) in the sagittal plane (B). These post-mortem MR findings were not seen at conventional autopsy.



10.6.1 Potential Limitations

There were four false positives from cardiac MR imaging. These could either be due to inadequate resolution of cardiac MR imaging for accurate assessment of aorta arch, thus resulting in an over diagnosis of arch abnormalities, or to cardiac lesions, particularly ventricular septal defects not being detected at traditional autopsy. It was not possible to explore the issue of false positives, using the current study design.

In two of the cases histology was required for an accurate diagnosis. As myocarditis accounts for 1.3% of sudden infant deaths (SUDI) cardiac biopsy may be important in this sub-group (Weber et al. 2008b). Further investigation of percutaneous biopsy done under image guidance may be a satisfactory alternative to open dissection and biopsy; however there are no satisfactory techniques for this as yet. (Aghayev et al. 2008b; Breeze et al. 2008b). The further addition of genetic studies for cardiac ion channelopathy may also be useful in this subgroup as anecdotal evidence suggests that 10-35% of sudden infant and childhood deaths may be due to a defect in this pathway.

In smaller fetuses, resolution of conventional MR imaging was inadequate to obtain adequate quality cardiac images; however magnetic resonance microscopy at higher field strengths (9.4Tesla) may be useful in this group. High field MR imaging may be also useful in accurate assessment of valve leaflets and aortic arch, which is again difficult to examine at 1.5T conventional MR imaging. Estimation of cardiac weight is an important part of conventional autopsy; however it may be possible to estimate this from MR images. This issue will be explored in the next chapter.

In this study, high-resolution, 3D CISS sequences were most useful and T_1-weighted images least useful. Small pericardial, pleural effusions, intra-cardiac air and blood clots are post-mortem artefacts and are better visualised on post-mortem MR imaging, than at conventional autopsy. However, they are of little clinical utility and were therefore excluded from the analysis. Similarly ventricles may have a thickened appearance after death and this should not be mistaken for ventricular hypertrophy. Those reporting post-mortem MR images should be familiar with normal post-mortem artefacts in heart.

10.6.2 Conclusion

There are several clinical implications of this study. Post-mortem 3D cardiac imaging could be performed as a screening procedure before autopsy to guide the pathologist to look for specific lesions. The MR imaging may help in determining which cases should be referred to a specialist paediatric cardiac pathologist, with imaging performed locally and transferred electronically to a specialist cardiac radiologist for reporting. Furthermore, post-mortem MR imaging with guided biopsies, could be offered as a less invasive autopsy, to parents who refuse conventional autopsy.

This study shows high-resolution, 3D post-mortem cardiac MR imaging can provide equivalent structural information to that of conventional autopsy in the majority of larger fetuses, newborns and children. Moreover, routine use of post-mortem cardiac MR imaging as an adjuvant to conventional autopsy may increase the yield from conventional autopsy. However, the population in this cohort is primarily fetuses, newborns and infants, where structural cardiac lesions are more important. Caution need to be exercised in extrapolating these results to older children or adults, where fatal cardiac pathology may not have structural abnormalities, for example cardiac ion channelopathies or coronary heart disease. Further development of less invasive autopsy techniques, for example post-mortem genetic testing or angiography may have a role in detecting such cardiac pathologies.

CHAPTER 11: ESTIMATION OF VISCERAL ORGAN WEIGHTS

11.1 SUMMARY

Estimation of individual visceral organ weights is an important component of traditional autopsy. This study examined the feasibility and accuracy of non-invasive internal organ weight measurement using post-mortem MR imaging in fetuses, newborns and children. Phase 1: In-vitro scanning of 36 animal organs (heart, liver, kidneys) was performed to check the accuracy of volume reconstruction methodology. Real volumes were measured by the water displacement method. Phase 2: 65 whole body post-mortem MR scans were performed in fetuses (n=30), newborns (n=5) and children (n=30) at 1.5T using a 3D TSE T2 weighted sequence. These data were analysed offline using the image processing software Mimics 11.0. Phase 1: Mean difference (SD) between estimated and actual volumes were -0.3 (1.5) ml for kidney, -0.7 (1.3) ml for heart, -1.7 (3.6) ml for liver in animal experiments. Phase 2: In fetuses, newborns and children mean differences between estimated and actual weights (SD) were -0.6 (4.9) g for liver, -5.1 (1.2) g for spleen, -0.3 (0.6) g for adrenals, 0.4 (1.6) g for thymus, 0.9 (2.5) g for heart, -0.7 (2.4) g for kidneys and 2.7 (14) g for lungs. Excellent co-correlation was noted for estimated and actual weights (r^2=0.99, p<0.001). Accuracy was lower when fetuses were less than 20 weeks or less than 300g. Rapid, accurate and reproducible estimation of solid internal organ weights is feasible using the semi-automated 3D volume reconstruction method.

11.2 INTRODUCTION

A major drawback of MR imaging that precludes its use as a replacement for conventional autopsy is the inability to estimate organ weights directly. The Royal College of Pathologists (UK) recommends that organ weight estimation should be part of the minimum autopsy dataset. This is particularly important in fetal, perinatal and paediatric autopsies, where an abnormal organ weight may be diagnostic of, or may provide clues to, the underlying diagnoses. For example, fetal liver weights are very useful in post-mortem diagnosis of intra uterine growth restriction (IUGR) (Wilson et al. 1992). Diagnosis of pulmonary hypoplasia is made on fetal lung to body weight ratios alone or in combination with histology (Sun et al. 1999).

Even though liver and spleen weight estimations have been reported in adult cadavers (Aghayev et al. 2005) and liver, lung and brain weights in fetuses (Breeze et al. 2008a), there are no reports on non-invasive weight estimation of all internal organs that are normally weighed at autopsy in neonatal or paediatric population. More importantly, all currently available methods (Aghayev et al. 2005; Breeze et al. 2008a) involve drawing manual regions of interest around the organs, which are time consuming and there are no automated or semi-automated techniques at present. In this study, I aimed to explore the utility of this 3D volume reconstruction for non-invasive organ weight estimation by post-mortem MR imaging.

11.3 AIMS

- To examine the accuracy of 3D volume reconstruction software by *in-vitro* MR scanning of solid animal organs.
- To investigate whether this method can be used for rapid and accurate non-invasive estimation of visceral organ weights in fetuses, newborns and children by whole body post-mortem MR imaging.

11.4 METHODS

The MR imaging was performed using a 1.5T MR scanners as described in chapter 6. The study was carried out in 2 phases.

11.4.1 Phase One

To check the feasibility of the technique and accuracy of the volume rendering software, fresh animal (chicken and goat) kidneys and livers were scanned using a T_2-weighted 3D turbo spin echo sequence (TR=3500ms, TE=360ms, flip angle=360^o, ETL=169, slice thickness=1.2mm, Acquisition time 6.20 min). The organs were obtained from a local supplier. The real volumes of the organs were estimated by water displacement method following MR imaging.

I analysed the MR data offline using the image post-processing software Mimics 11.0 (Materialise Inc, Ann Arbor, MI, USA). This program uses pattern recognition techniques and interpolation algorithms to automatically extrapolate the volume of a region of interested, selected in two-dimensional (2D) image data. The raw DICOM datasets from MR imaging were imported into this software and individual organs were examined in the 2D coronal, sagittal and axial views.

The mask definition is performed off line, using the post-processing software Mimics. Masks are automatically generated by this software according to the grey values in the image dataset. Each organ is characterized by different grey values, due to the different tissue signal properties. The operator has to choose the most appropriate grey threshold values to select each individual organ. Some areas of different organs are at the same grey level as the selected organ, and therefore are initially part of the same mask. A region growing function creates a new mask on the basis of the initial mask, but made of those structures that are only connected to a pixel selected by the operator in the organ of interest. Therefore, those areas with same grey value as the selected region of interest, but belonging to different organs, are automatically deleted in the new mask, since naturally disconnected. After this, I examined the accuracy of the mask selection in all the 3 planes. If the image contrast is good, as often is the case with liver and spleen, no further corrections are required. If the contrast is not good and the mask is picking up noise or still some adjacent structures, manual editing of the mask is done. The post-processing software has tools to draw, erase or restore parts of structures by clicking on the single pixels of the images, thus refining the organ outlines. Once this operation was completed for all the 2D MR slices, the 3D structure of the organs was rendered and their volumes automatically calculated.

11.4.2 Phase Two

Sixty-five post-mortem cases including fetuses, newborns and children were recruited into the study over a 6-month period as described in chapter 6. A family liaison nurse experienced in bereavement counselling took prospective parental consenting in HM Coroners cases, as described in chapter 5.

All cases were kept in the mortuary at 4 ^{0}C prior to the MR imaging (chapter 5). Following the MR imaging, all main visceral organs –thymus, thyroid, liver, spleen, pancreas, kidneys, adrenals, heart and lungs were segmented. The organ volumes were reconstructed as described above (Figure 41). Manual organ segmentation was performed when automated or semi-automated segmentation was not possible due to poor tissue contrast. Autopsy was performed by 1 of the 4 paediatric pathologists after MR imaging as described in chapter 6. Organs were weighed on an electronic scale as part of the standard autopsy procedure.

I repeated the non-invasive weight estimation in 10 randomly selected cases to examine intra-observer variability. Inter-observer variability was examined by another observer (Silvia Schievano), with 4 years experience with Mimics in 5 randomly selected cases.

11.4.3 Statistical analysis

All data are given as mean (standard deviation). The relationship between volumes estimated by MR imaging and weight at autopsy for each organ was examined using regression modelling. Slope was taken as density of the organ. The organ volume was multiplied by the density to get the estimated weight. SPSS 14, SPSS inc, Illinois, Chicago and MedCalc 9.3.9 was used for statistical analysis.

11.5 RESULTS

11.5.1 Phase 1 Experiments (Animal Scans)

Automated estimation was possible with all *in-vitro* animal organ scans (12 livers, 12 hearts and 12 kidneys) organs. Total volume estimation time from the MR images using Mimics was 1-2 minutes per organ. There was excellent agreement between the actual volume and estimated volume for all the three organs: kidney (r^2=0.99, p< 0.001), heart (r^2=0.99, p <0.001) liver (r^2 =0.99, p<0.001) and there was no significant bias (Figure 44). Repeated measurements (4 times) using 4 different upper and lower

thresholds (1100/2700, 1300/2700, 1100/2600, 1100/2500) rendered exactly the same volume (Mean difference in volume = 0, SD=0) for all organs (Table 24).

11.5.2 Phase 2 Experiments (Human Scans)

Post-mortem MR imaging was performed 1-9 days after death on 30 fetuses (14-42 weeks), 5 newborns and 30 infants and children (1 month -16 years). Liver and spleen weights from conventional autopsy was available in all 65 cases; kidney and lung weights in 55 cases; adrenal, thymus and heart weights in 51 cases. Total time (mean) for estimation per case (i.e. for of all internal organs) was 54 minutes (SD 9.4).

Pancreas and thyroid were difficult to identify accurately in most cases, mainly due to poor MR contrast in the T_2–weighted sequences. The slope of the line was taken as the density. Estimated density of liver was 1.01, spleen 1.03, kidneys 1.05, heart 0.98, adrenals 0.87 and thymus 1.1. Lungs in fetuses were denser (density=1.05) compared to infants and children (density=0.9) (Table 25). Adjusted weight of each organ is shown in Table 3 and Figures 3-5. For all organs there was a good agreement between the MR and autopsy methods ($r^2 = 0.9$, $p<0.001$).

Table 24. Comparison of MR measured volume and water displaced volume for ex-vivo animal organs

Organ	MR Imaging	Water displacement	Absolute differences		P*
			Mean	SD	
Kidney (ml)	53.8	54.2	-0.3	1.5	>0.05
Heart (ml)	41	41.7	-0.7	1.3	>0.05
Liver (ml)	218	220	-1.7	3.6	>0.05

*paired t test

Table 25. Regression analysis comparing weight at autopsy (real weights) and estimated volume from post-mortem MR imaging in fetuses, newborns and children

Real weights plotted on Y-axis and estimated volume from MR imaging on X-axis.

Organs	Equation (intercept+slope x)	95% CI of slope	r^2	p
Liver	0.78 + 1.01x	(1.014, 1.006)	0.99	<0.001
Spleen	0.53 + 1.03x	(1.04, 1.02)	0.99	<0.001
Kidneys	1.07 + 1.05x	(1.06, 1.04)	0.99	<0.001
Heart	-0.27 + 0.98x	(0.99, 0.97)	0.99	<0.001
Adrenals	0.3 + 0.87x	(0.88, 0.86)	0.99	<0.001
Lungs Fetus	2.36 + 1.05x	(1.06, 1.04)	0.99	<0.001
Lungs Child	1.9 + 0.9x	(0.92, 0.88)	0.98	<0.001
Thymus	0.45+1.1x	(1.12, 1.08)	0.98	<0.001

Table 26. Estimated MR organ weights and autopsy organ weights from 65 post-mortem cases

Organ	Estimated Mean Weight	Mean Weight at Autopsy	Absolute difference		p value*
			Mean difference	SD	
Liver (g)	160	161	-0.6	4.9	>0.5
Spleen (g)	16.9	17.5	-5.1	1.2	>0.5
Adrenals (g)	5.97	5.7	-0.3	0.6	>0.5
Thymus (g)	10.5	10.4	0.4	1.6	>0.5
Heart (g)	31	30	0.9	2.5	>0.5
Kidneys (g)	45.5	46.3	-0.7	2.4	>0.5
Lungs (g)	111	114	2.7	14	>0.5

NB: MR volume of each organ was multiplied with density of the corresponding organ to obtain estimated weight. *paired t test

The measurements for the liver, spleen and kidneys were excellent, with no significant systematic bias between the 2 methods. Variability in estimations was higher for other organs, though very much within acceptable clinical limits (i.e. + 20%). As can be observed from all the Bland-Altman plots agreement between the methods for measurements in the smaller fetuses (<20 weeks and/or < 300 g) was less good. If these data are removed from the analysis (n=15), standard deviation of percentage difference between estimated and actual weights became less (Liver 4.5 % vs 2.7%, Spleen 9.2% vs 6%, kidney = 6.9 vs 6.7%, adrenal 14% vs 10.9%, thymus 20% vs 13%, heart 13.8% vs 9.2%, lungs 12% vs 7.6%) (Figures 45-47). Excellent intra-observer variability and inter-observer variability was seen for liver, spleen and kidney. For other organs the variations were higher, though still within acceptable clinical limits (Table 26). CT scans did not have sufficient contrast resolution for identifying internal organs accurately.

Table 27. Reproducibility of the non-invasive organ weight estimations in fetuses, newborns and children

Organ	Intra-observer variability (n=10)		Inter-observer variability (n=5)	
	Mean difference (ml)	SD (% SD)	Mean Difference (ml)	SD (%SD)
Liver	-0.5	5 (1.3%)	-0.98	5.4 (5.5%)
Spleen	0.2	2.1 (5.9%)	-0.3	1(7.5%)
Kidney	0.4	2.6 (5.3%)	1.9	2.9 (6.3%)
Adrenal	0.3	1 (6.6%)	-0.2	1.8 (19.2%)
Heart	0.6	3.5 (5.6%)	2.2	4.6 (13.5%)
Lung	4.6	14 (10.5%)	-6.2	7.5 (14.6%)
Thymus	0.7	1.9 (10.6%)	-0.5	1.5 (9%)

Mean difference is the absolute difference between estimated and actual weights in millilitres. SD is the standard deviation of mean difference in millilitres. % SD is the standard deviation of (estimated weight- actual weight) / Actual weight x 100.

Figure 41. Thresholding and region growing in Mimics

MR data of a fetus, where the internal organs are outlined by automatic thresholding in coronal and axial planes. 3D volume rendered images of the visceral organs liver on bottom left in semi transparent mode.

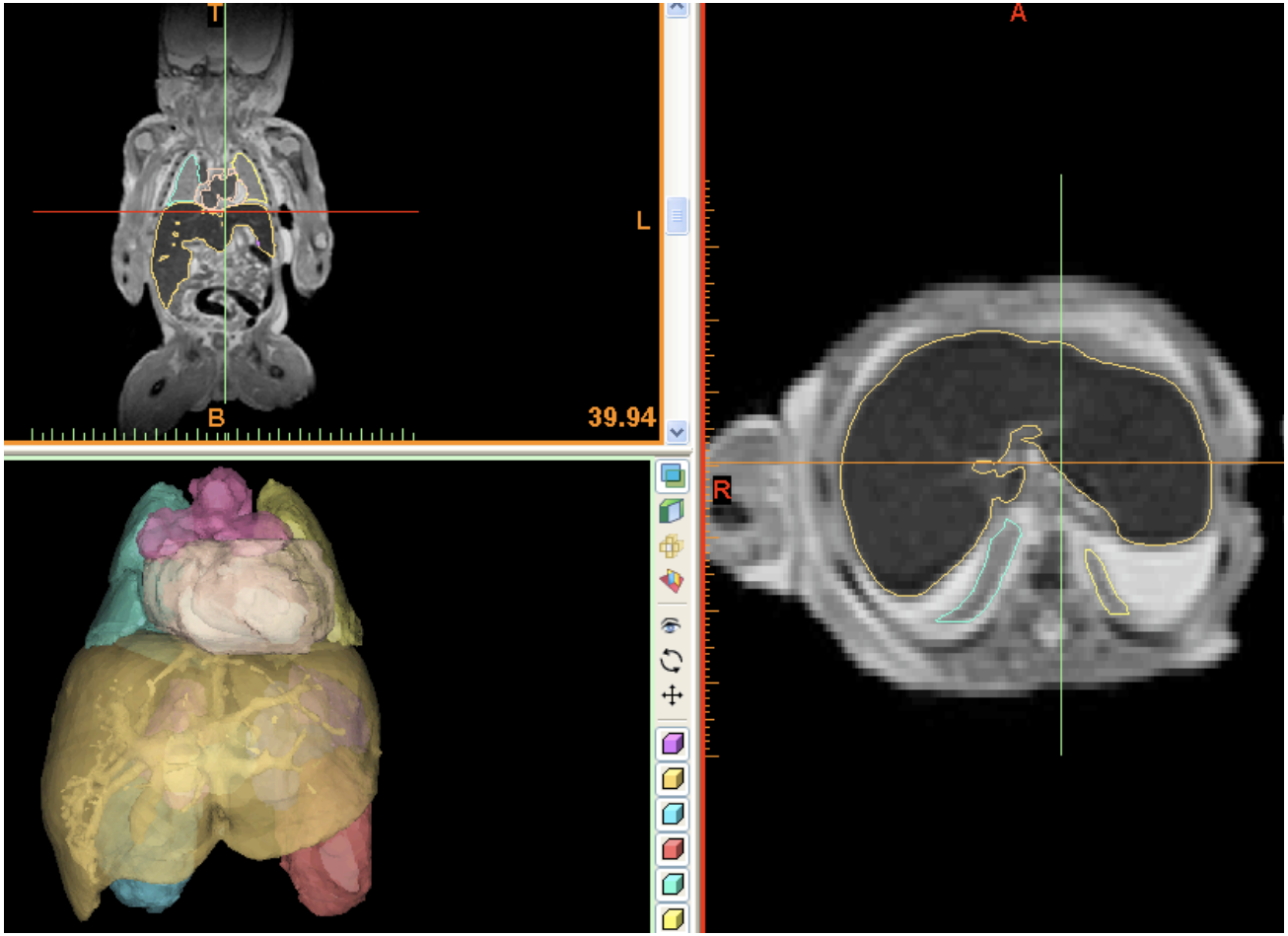


Figure 42. Bland Altman plots of organ volume estimations-animal organs

Bland Altman plots of estimated animal kidney, heart and liver volumes for animal organs. Percentage mean difference between estimated and actual volumes of each case is plotted on Y axis.

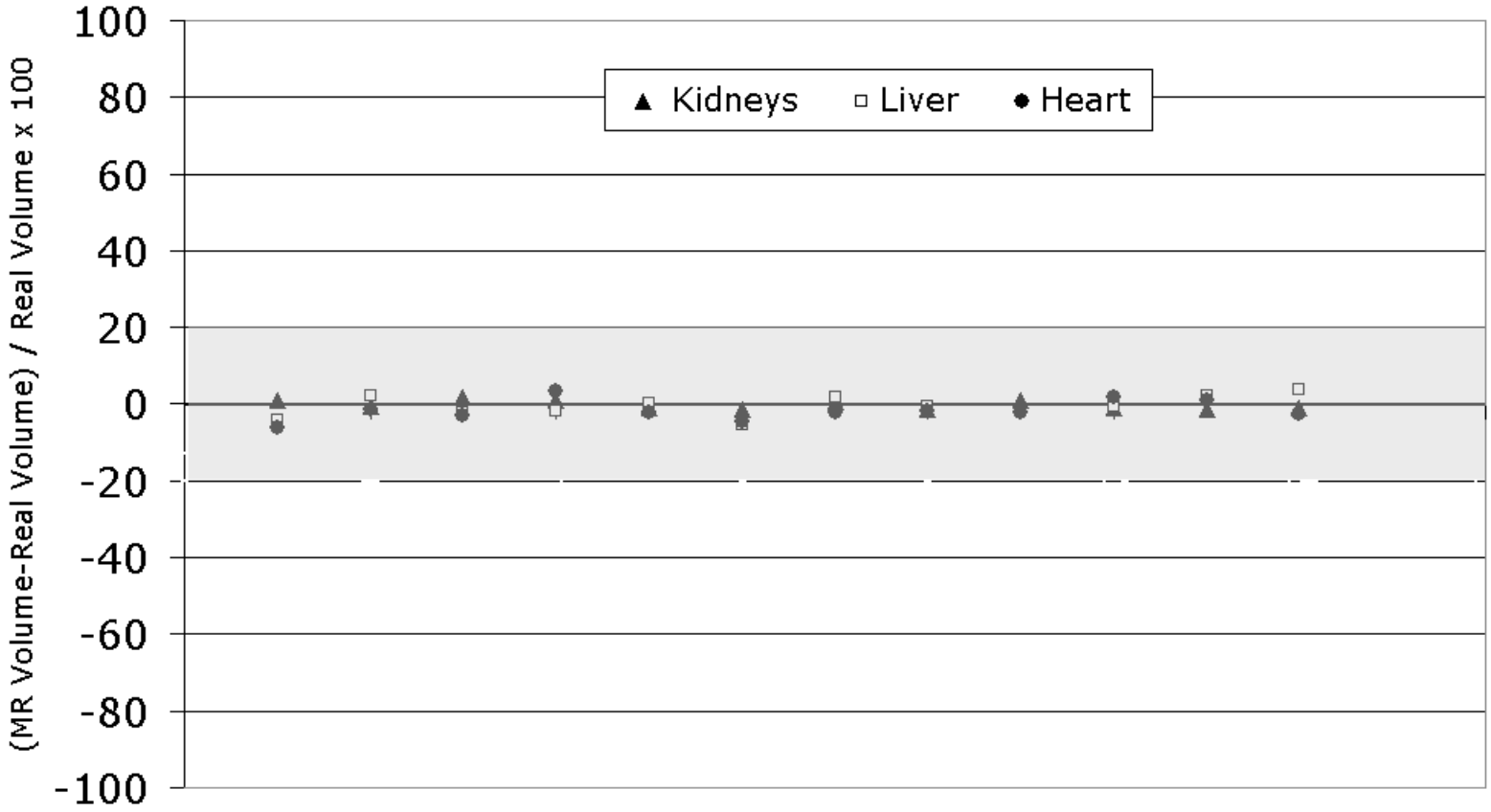


Figure 43. Actual and estimated liver and spleen weights

Estimated and actual weights of liver and spleen: Percentage mean difference between estimated and actual volumes of liver and spleen each case is plotted on Y axis. Cases are arranged in an ascending order of age (gestation) on X axes. Shaded area indicate clinically acceptable limits.

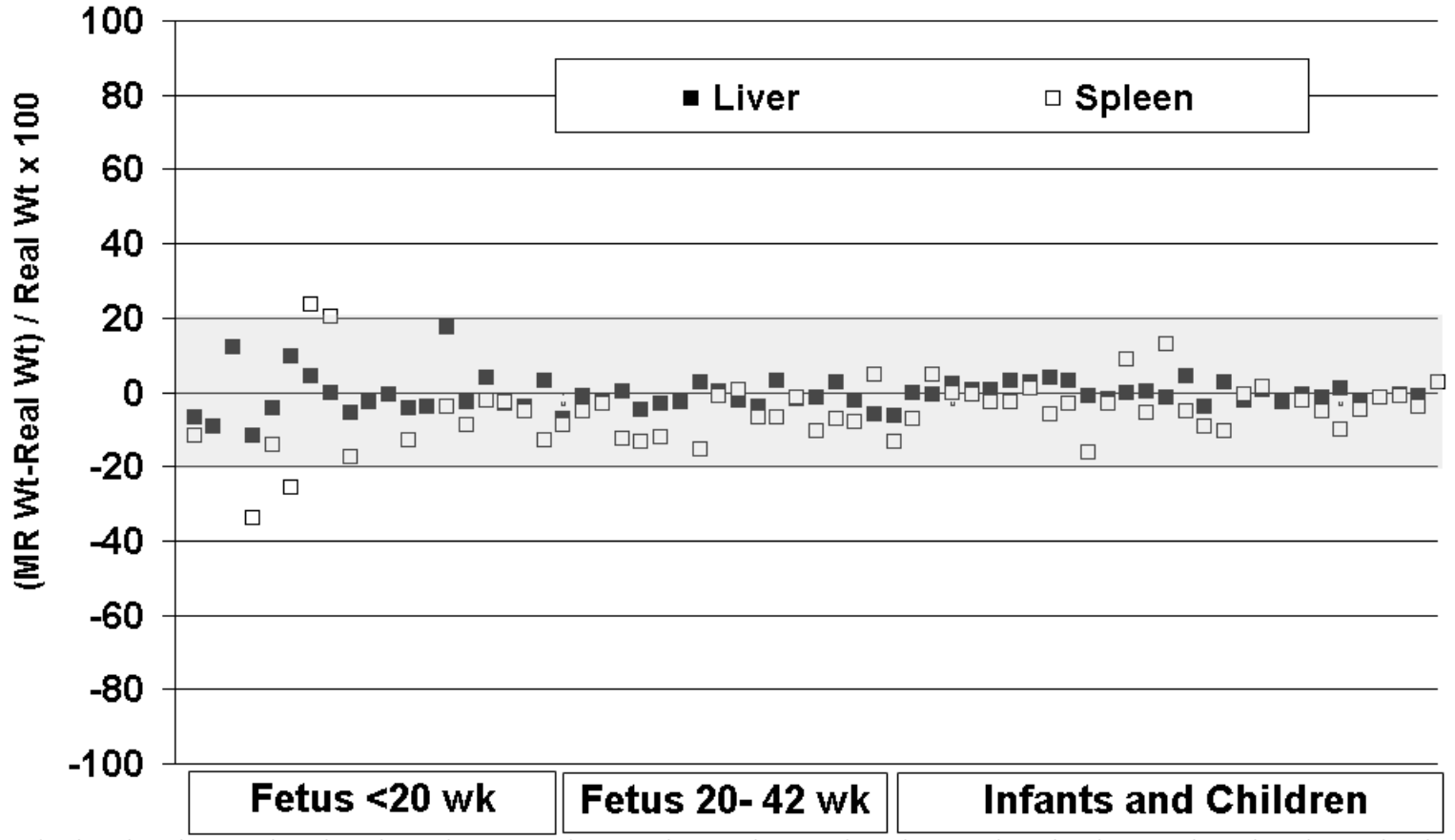


Figure 44. Actual and estimated lung and heart weights

Estimated and actual weights of heart and lung (combined): Percentage mean difference between estimated and actual volume in each case is plotted on Y axis. Cases are arranged in an ascending order of age (gestation) on X axes. Shaded area indicate clinically acceptable limits.

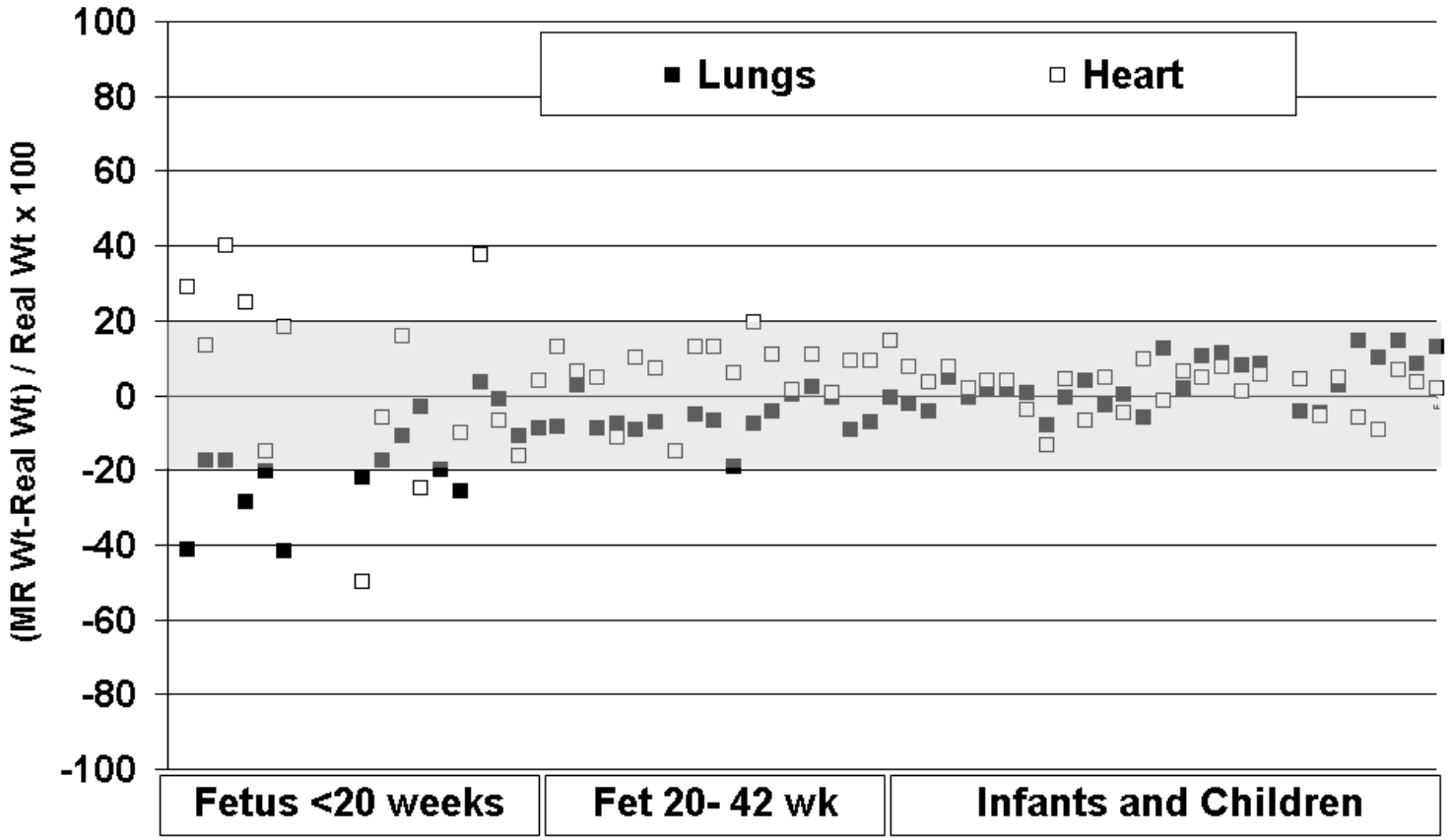
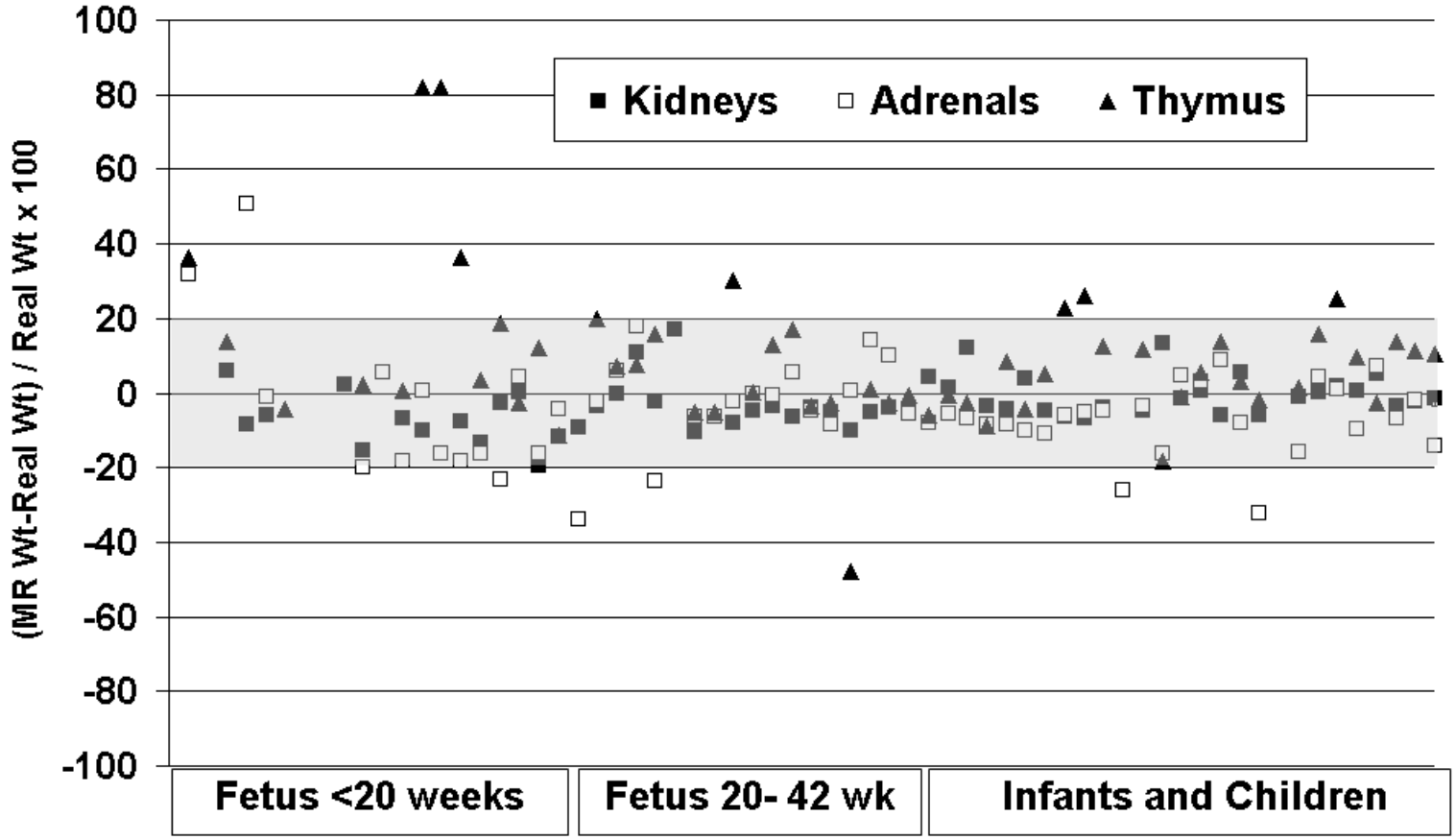


Figure 45. Actual and estimated weights of kidney, adrenal and thymus

Estimated and actual weights kidneys (combined), thymus and adrenals (combined): Percentage mean difference between estimated and actual weights of each case plotted on Y axis. Cases are arranged in an ascending order of age (gestation) on X axes. Shaded area indicate clinically acceptable limits.



11.6 DISCUSSION

The data presented suggests that semi-automated MR 3D volume reconstruction provides an accurate, reproducible, non-invasive method for measuring the weight of solid visceral organs in infants and children and fetuses greater than 20 weeks gestation. This study may have major implications for the utilisation of post-mortem MR imaging and can be considered as the first step towards less invasive autopsy. Moreover, this technique can be used for non-invasive estimation of liver and kidney weight in living patients before procedures such as organ transplantations and in fetal lung volume measurements.

Two separate groups have previously reported non-invasive estimation of organs weights by post-mortem MR imaging. Breeze et al., have described liver, lung and brain volume estimation in 20 fetuses using the ANALYZE v.5 software package (BIR, Mayo Foundation, MN, USA) (Breeze et al. 2008a). None of the other organ volumes were examined. A grid was superimposed on to the axial images and the volume was extrapolated from the number of grid points overlying the organ. Alternate slices were selected to save time. The authors reported a specific gravity of 1.15 for fetal lungs and significantly underestimated lung weights. Though this was also a trend in my study underestimation of lung weights was lesser in fetuses (density 1.05). Conversely, in infants and older children lung weights (density 0.9) were over estimated. This is very logical and consistent with the concept of increasing alveolar development and aeration occurring following birth.

Jackowiski et al., have reported non-invasive estimation of liver and spleen weight by MR and CT imaging in 44 adult cadavers (Aghayev et al. 2007). Accurate estimation of liver and spleen weights was possible both by MR and CT in this study. Even though I performed post-mortem multislice CT scan in many of our cases, I was unable to measure organs weights using CT images. This was due to the poor contrast of internal organs in post-mortem CT in children, unlike adults. Again Jackowiski et al., drew manual regions of interest around internal organs, which is labor intensive and unlikely to be scaled up for use in routine clinical practice. The semi-automated technique, I used, offers major advantages over manual techniques in this regard.

In current the study, liver, spleen and kidney weights correlated best with the actual weights and were most accurate. This could be explained in part by the sharp contrast

between these organs and neighbouring structures and these organs having a homogeneous specific gravity. In the heart, exclusion of clotted blood from the ventricles is important for cardiac weight assessment. Variability in accuracy in lung volume may be due to a variety of reasons including the changes in lung density during in-utero alveolar development at different gestations and aeration occurring after birth, post-mortem changes and underlying pathologies. A variety of changes occurring after death including post-mortem atelectasis, stasis in dependent areas and pleural effusions make lungs extremely heterogeneous. Mask applications are less accurate when the structures do not have uniform signal intensity. Defining lung margins accurately requires manual regions of interest to be drawn around lungs and could be time consuming. The accuracy is least and variability higher, when fetuses where less small, due to the poor definition of lung margins. However, it is likely that high field imaging at 9.4T would be useful in accurate lung volume estimations (please see chapter 12).

Though pancreas and thyroid were not visible using T_2-weighted sequences, good contrast of these organs was seen when 3D T_1-weighted volumetric interpolated breath hold examination (VIBE) sequences were used. It might be possible to do accurate estimation of thyroid and pancreatic volume using these sequences, in future studies.

Cannie et al have recently described a manual method for estimation of fetal total body volume by antenatal MR imaging. Reproducibility (i.e. inter and intra observer variability) has not been examined in this study (Cannie et al. 2006). The authors describe that the major limitation of fetal body volume estimation is that it is labor intensive and takes about 90 minutes, and no semi-automated techniques were available at the time. The semi-automated technique I described, takes about 3- 10 minutes per organ, depending on the contrast. It is possible that this method can estimate total fetal volume rapidly.

11.6.1 Limitations of the Study

This study is not without its limitations. The computer aided 3D reconstruction depends on thresholding, which can introduce errors in volume estimation. However, our group have previously reported very good accuracy of this method when carried out by 5 separate observers (Schievano et al. 2007).

Inaccuracies in estimation often occurred when the organ volumes were small as in case of fetuses less than 20 weeks. In small animal models remarkable tissue characterisation is possible by using high field imaging at 9.4T (MR Microscopy) in this population; however there is no human data as yet on whole body high field MR imaging. If indeed this technique is useful in small fetuses, accurate estimation of organ weight in this subgroup may be possible (please see chapter 12).

The reported densities for most human organs vary between 0.9-1.05, which is similar to the estimated organ densities that I obtained using regression modelling. Similar densities have been reported by Breeze et al., using regression modelling in post-mortem MR imaging of fetuses (Breeze et al. 2008a). In routine clinical practice, even if no such correction factor is used (i.e. assuming volume = weight (density=1), satisfactory estimations of most internal organs may be obtained.

Though I did not find any relation with post-mortem interval, this might be a spurious finding. Most of the cases did not have significant decomposition, as the bodies were kept at 4 ^{0}C soon after death. This temperature is known to reduce decomposition significantly. Advanced maceration was noted in a couple of fetal cases, where there was a significant delay between death and delivery following a missed abortion. However, autolysis was often seen in pancreas, which might have contributed to the difficulty in estimation of pancreatic volume. Neverthless, putrefaction has been rare in this series; this may be because most deaths occurred at home or hospital and the bodies were soon cooled down in the mortuary.

In summary, the automated 3D volume reconstruction methodology proposed in the study, allowed accurate and rapid non-invasive estimation of solid organ weights including liver, spleen, heart, kidneys, thymus, adrenals and lungs for “less invasive autopsy”, in fetuses more than 20 weeks. For pancreas and thyroid this method was inaccurate. Non-invasive methods can give only an estimate of the organ weights and the real weight may be marginally above or below the estimated weight. Organ weight estimations are inaccurate in small fetuses. One option for such cases is to use high resolution MR imaging at higher field strengths; this issue is explored in the next chapter.

CHAPTER 12: HIGH FIELD MR IMAGING

12.1 SUMMARY

Poor quality of conventional magnetic resonance (MR) imaging at 1.5T in small fetuses was high lighted in chapter 7. High-field whole body MR at 9.4T provides good imaging in small animal models, but has not been applied to human fetuses. I acquired whole body MR imaging at 9.4T and 1.5T in 18 fetuses less than 22 weeks, using 3D T2-weighted fast spin echo (FSE) sequences, before invasive autopsy. MR images for each system were compared with invasive autopsy in a blinded fashion. Tissue contrast measured in 14 different regions on 1.5T and 9.4T images were also compared and image quality scored on a 4-point scale. Spatial resolution, tissue contrast and image quality of high-field MR (9.4T) images for all organ systems were significantly higher than conventional MR (1.5T) images. All structural abnormalities detected by invasive autopsy and internal examination of visceral organs were detected by high-field MR imaging at 9.4T, whilst conventional MR imaging at 1.5T was non-diagnostic in most (78%) of the cases. Whole body high-field MR imaging is feasible in human fetuses and offers good tissue characterization even in fetuses as small as 5 grams.

12.2 INTRODUCTION

Difficulty in examination of smaller fetuses by conventional MR imaging has been consistently highlighted in chapters 7, 9 & 10. MR images, particularly for heart were often non-diagnostic quality in fetuses less than 20 weeks. However, post-mortem MR imaging extremely is important in this sub group of fetuses for a variety of reasons. Firstly, with advances in antenatal diagnosis an increase in number of termination of pregnancies occurring at earlier gestations. Secondly, conventional invasive autopsy is challenging in small fetuses owing to small size, maceration and autolysis, particularly with regards to adequate examination of the brain and heart. This is compounded by an increasing number of parents requesting rapid return of internal organs to the body for burial; thus prolonged fixation for optimal examination of brain tissue is often not possible (RCOG 2001). Finally, despite an increase in termination of pregnancy rates, a significant reduction in perinatal autopsies has been reported in the past decade (Dickinson et al. 2007a). The current rate of traditional autopsy following termination ranges from 50-67%, as a significant proportion of parents find traditional autopsy unacceptable. These factors all serve to make the development of less invasive methods of assessment increasingly important in smaller fetuses.

High-field MR imaging has opened up a new avenue of "virtual histology", particularly relating to heart and brain of transgenic small animal models (Schneider et al. 2003; Driehuys et al. 2008) and in excised human tissues (Beuls et al. 1993; Scholtes et al. 2006), where it may rival conventional light microscopy (Petiet et al. 2007). Moreover, being non-invasive, there is no risk of the tissue destruction or other artefacts that are produced during the process of tissue sectioning for histological examination (Driehuys et al. 2008).

12.3 AIMS

- To examine feasibility of post-mortem whole body high field (9.4T) imaging in human fetuses
- To compare the signal to noise, spatial resolution, tissue contrast for post-mortem imaging at 1.5 Tesla MR system with that of 9.4T MR system
- To compare the image quality and diagnostic utility of post-mortem MR imaging of fetuses at 1.5 Tesla MR system with that of 9.4T MR system.

12.4 METHODS

12.4.1 Study Population and Investigation

I prospectively studied 20 consecutive fetuses < 22 weeks referred for conventional autopsy to Great Ormond Street Hospital for Children or University College London Hospital, London. The details of consenting, transfer and storage of bodies and conventional MR sequences are given in chapter 6.

High field MR imaging was performed using one of two 9.4T Varian scanners (VNMRS, Varian Inc, Palo Alto, U.S.A) with bore diameters of 20 cm and 30 cm with the assistance from a high field MR physicist. The scanners were located at the Centre for Advanced Biomedical Imaging (CABI) and at Institute of Neurology (IoN). I used a 3D T_2-weighted fast spin echo (FSE) sequence for the whole body imaging, in both conventional and high-field scanners. Detailed protocol for conventional MR is given in chapter 6. The conventional MR parameters were– TE 360ms, TR 3500ms, Echo train length 169, FOV 125x200x64, Matrix 160x252x80, Voxel dimensions 0.8x0.8x0.8, Averages 10, flip angle $90/120^0$, TA upto 50min.

Sequences at high field strengths were optimised for image quality for by visual examination, whilst keeping the scan time less than 2 hours. For high field MR imaging, I used a rapid 39, 72 or 150 mm coil depending on the size of the fetus. Sequences for high field imaging were–TE 120ms, TR 500ms, Echo train length 8, FOV 100x50x50, Matrix 512x256x256, Voxel dimensions 0.2x0.2x0.2, Averages 1, flip angle 90/180. TA upto 70min.

The same group of radiologists as described in chapter 7, reported the 1.5T and 9.4T MR images separately and in random order, blinded to the unique identifiers of the cases. The data was entered into the Microsoft Access database (version 2003, Microsoft Corporation, USA) described in chapter 6. Each organ system was reported as abnormal (along with actual diagnosis), normal or non-diagnostic. In addition, the image quality of 1.5T and 9.4T images were also interpreted using a previously reported 4-point MR image quality rating scale categorised as: (1) Poor, (2) Moderate, (3) Good and (4) Excellent quality (Wyttenbach et al. 2003).

Mean signal intensity in 14 regions of interest (ROI) (2-3 regions per organ system) were calculated using Image J 1.40 (National Institute of Health, USA) by a single operator (ST) with 4 years experience in image processing. Size and position of ROI's

were equivalent for 1.5T and 9.4T images. Tissue contrast was calculated using the formula: (Signal Intensity area A – Signal Intensity area B) / (Signal Intensity area A + Signal Intensity area B) (McRobbie et al. 2007: 66-67)

Conventional autopsy was performed according to the guidelines of the Royal College of Pathologists (UK) as described in chapter 6 and 7. Duration of intra uterine retention after death was estimated from the difference between gestational age at delivery as predicted by early ultrasound or last menstrual period and the autopsy parameters (Maroun et al. 2005), in case of miscarriages. Autopsy data was entered into the same database, blinded to the MR imaging report. Again I defined 'less invasive autopsy' as an autopsy process that includes information from all non-invasive post-mortem investigations; including external examination of the fetus, placental histopathology, cytogenetic investigations, post-mortem radiography and high-field MR imaging), and excluding invasive dissection and macroscopic or microscopic examination of visceral organs; the definition used in chapter 7. Conventional autopsy included all invasive and non-invasive post-mortem investigations, except the information from MR imaging.

12.4.2 Statistical Analysis

Primary comparison was diagnostic accuracy and secondary comparison was image quality. All data were compared using the Mann Whitney U test. A p value of < 0.05 was taken to be statistically significant. Data were analysed using SPSS Version 16.0 for Mac (SPSS Inc, Chicago, Illinois).

12.5 RESULTS

12.5.1 Study Population

Two cases were used for optimising sequences and were not included in further analysis. In one case, T_2–weighted images could not be obtained at 9.4T. Data on the remaining 17 cases are shown in Table 2. Conventional MR at 1.5T could not be performed in 3 cases, due to small fetal size. A pathway for case selection is shown in Figure 46.

The median gestation of the fetuses was 16 weeks (11-22 weeks) and weight 59 g (5g-400 g). All scans were done within 24 hours of arrival in the mortuary. Median time between death and MR imaging was 4 days (2-8 days) and between MR imaging and

autopsy was 1 day (0-5 days). Five cases were termination of pregnancies and 12 were unexplained intrauterine death, of which 8 (67%) were retained in-utero for more than 1 week after death. Four (24%) fetuses were fresh (i.e. no evidence of maceration) and 13 (76%) were macerated or mummified on external examination at the time of autopsy.

12.5.2 Diagnostic Accuracy

At 9.4T, diagnostic images were obtained in all cases of termination of pregnancy and unexplained IUD, where the intra uterine retention period was less than 1 week (n=9). When the intra uterine retention period was more than 1 week (n=8), diagnostic images were obtained in 50% of cases for brain and 25% for other visceral organs. At 1.5T, MR imaging provided diagnostic images for the brain and spinal cord in only 2/14 (14.3%) cases, with non-diagnostic images for all other internal organs. For conventional autopsy, diagnostic information for the brain was obtained in 4/17 (24%) cases, and all of the remaining internal organs. Comparison between the diagnostic yield for the 3 methods of assessment is shown in Table 31.

For all cases, routine conventional autopsy of the brain provided no additional information over and above that obtained by less invasive autopsy by 9.4T MR imaging (Table 32). In the 13 cases in which formal autopsy of the brain was not possible, high-field 9.4T MR imaging provided diagnostic information on brain and spinal cord in 9 (70%) fetuses. Furthermore, for all cases where the intra uterine retention period was less than 1 week, conventional autopsy provided no additional information over and above that obtained by less invasive autopsy by 9.4T MR imaging.

Figure 46. Flow chart of the study

Ab=Abnormal, N=Normal, N/D=Non Diagnostic, TOP=Termination of pregnancy

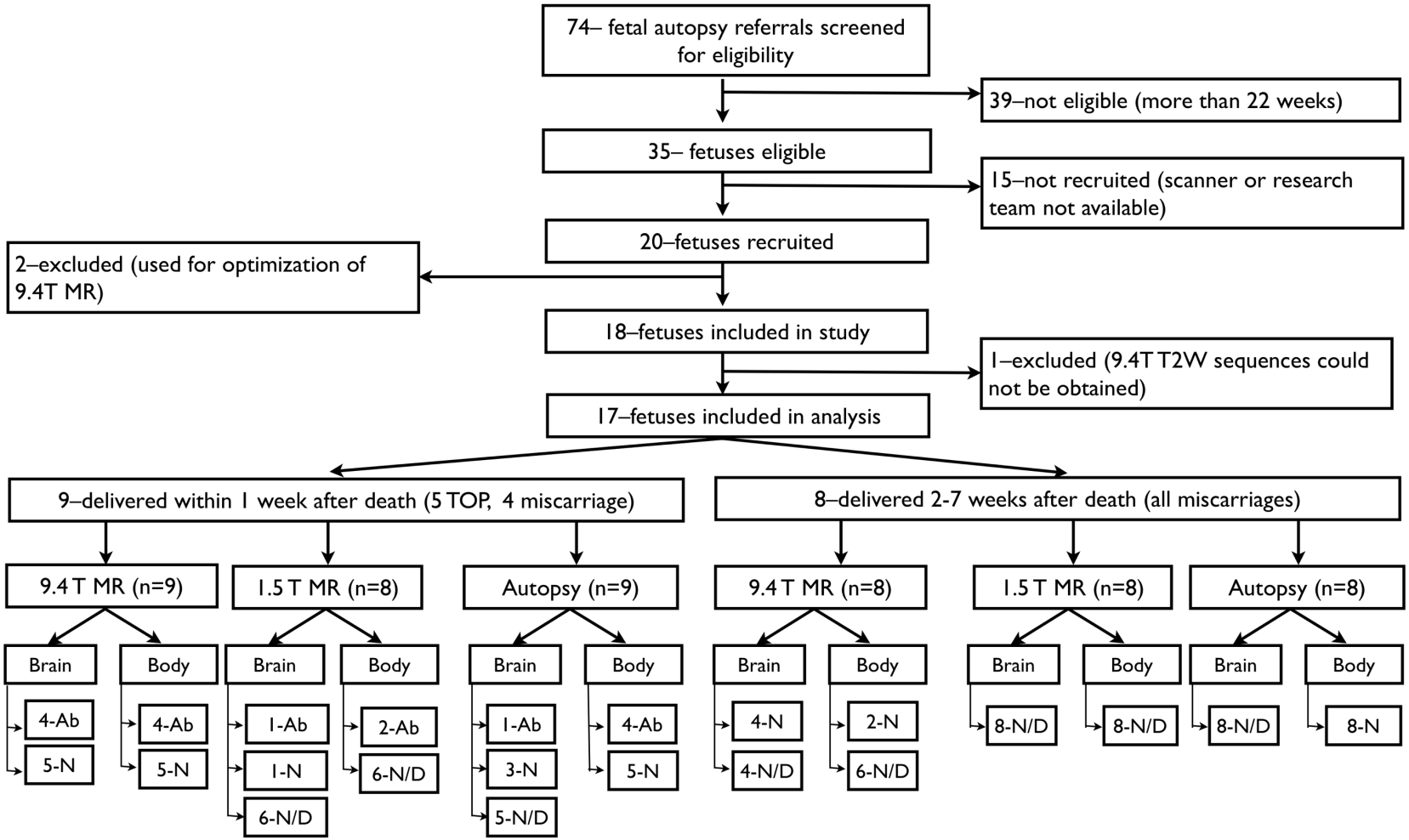


Figure 47: 1.5T MR, 9.4T MR and histopathological examination of a 19 week fetus terminated for myelomeningocele.

(a) and (d) 3D are T2-weighted TSE imaging of head and body in coronal plane at 1.5T MR. (b) and (e) 3D T2-weighted TSE imaging of head and body in coronal plane at 9.4T. (c) Histopathological examination of brain showing normal multilayered fetal cortex (f) 9.4T MR Sagittal view showing the cerebellar descent and Chiari 2 malformation. Features of Chiari-2 and myelomeningocele was seen with both 1.5T and 9.4T MR imaging and at autopsy. (1: Cortical plate, 1=subplate, 3=intermediate zone, 4=ventricular zone, 5=Ganglionic eminence and ventricular zone

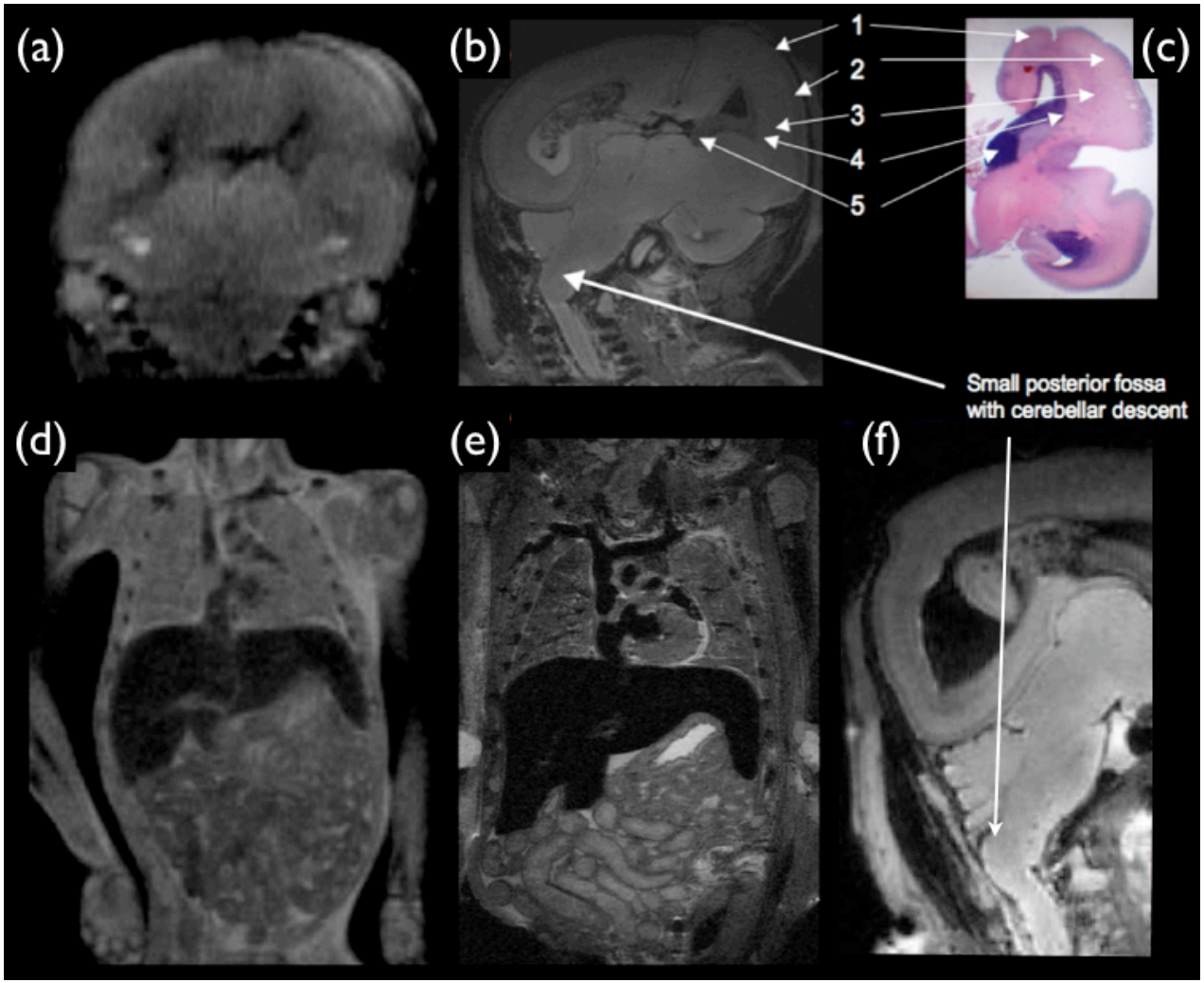


Figure 48. High-field 9.4T MR imaging of a fetus terminated for congenital diaphragmatic hernia and coarctation of aorta at 16 weeks (weight 64 grams). Figure (a) Coronal view of chest and abdomen demonstrating left congenital diaphragmatic hernia with stomach, intestine and spleen in right chest. Right ventricle is markedly hypertrophied and lungs hypoplastic. Left ventricle was very small. No coarctation was seen, however arch was small. Figure (b) and (c): Axial views of brain showing a pontine hematoma (PH). (d) Coronal view showing early normal development of corpus callosum (CC). (e) and (f) shows multilayered fetal cortex seen on high-field MR and histology. (CP: Cortical plate, SP=subplate, IZ=intermediate zone, SVZ=ventricular zone, G=Ganglionic eminence and ventricular zone). Corpus callosum could not be assessed at autopsy due to poor preservation of brain, otherwise formal neuropathology done by a paediatric neuropathologist was normal and no hematoma was seen. Cardiac examination by a paediatric cardiac pathologist was in complete agreement with MR findings

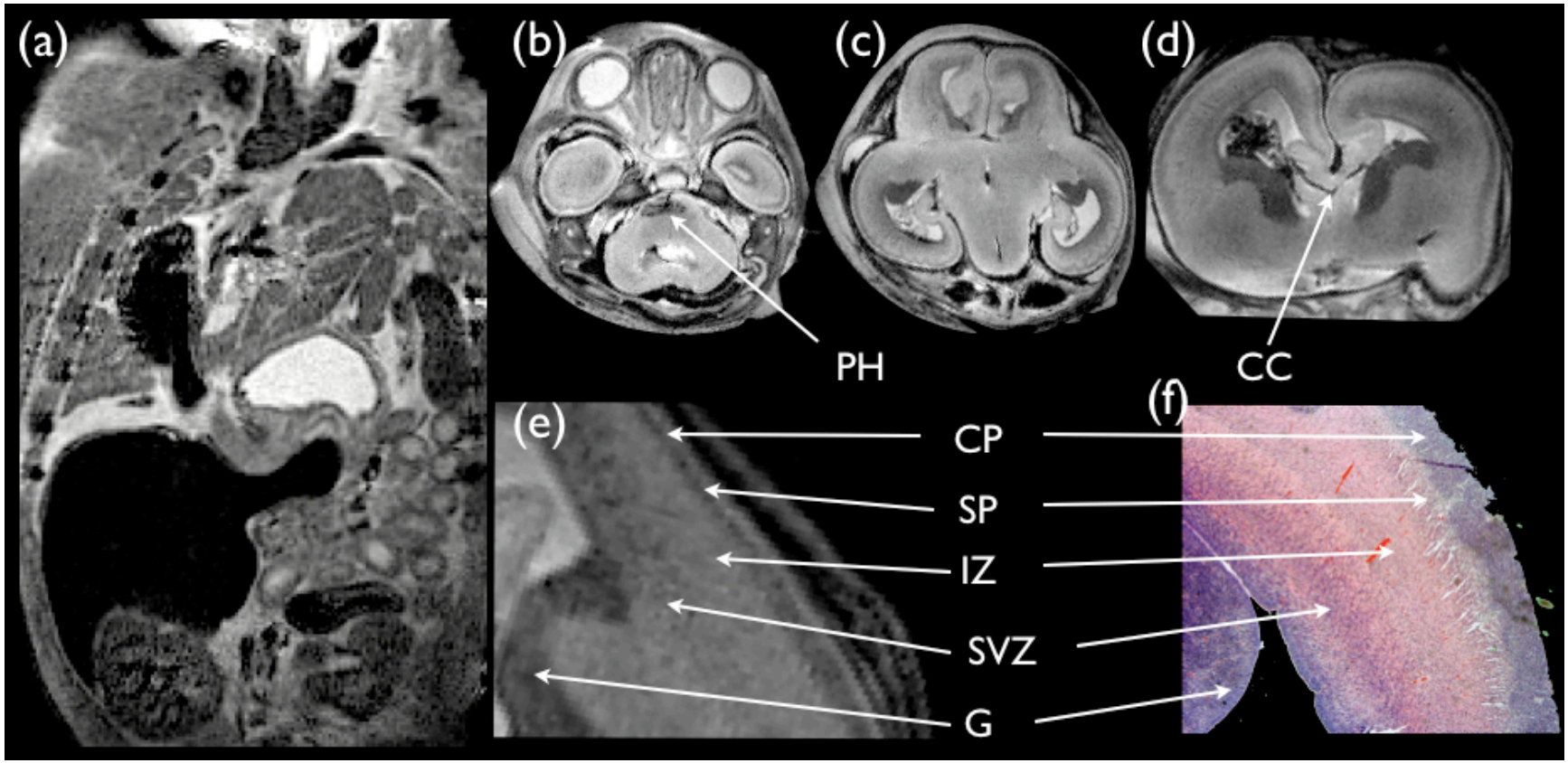


Figure 49. 22-week fetus terminated for skeletal dysplasia.

(a), (b) and (c): Coronal, sagital and axial images from 3D T2-weighted imaging at 9.4T. (d), (e) and (f) shows Coronal, sagital and axial images from 3D T2-weighted imaging at 1.5T. Cartilage and internal organs were normal on 9.4T MR imaging. Though 1.5T MR imaging showed diagnostic brain images (not shown), it was non-diagnostic for other systems.

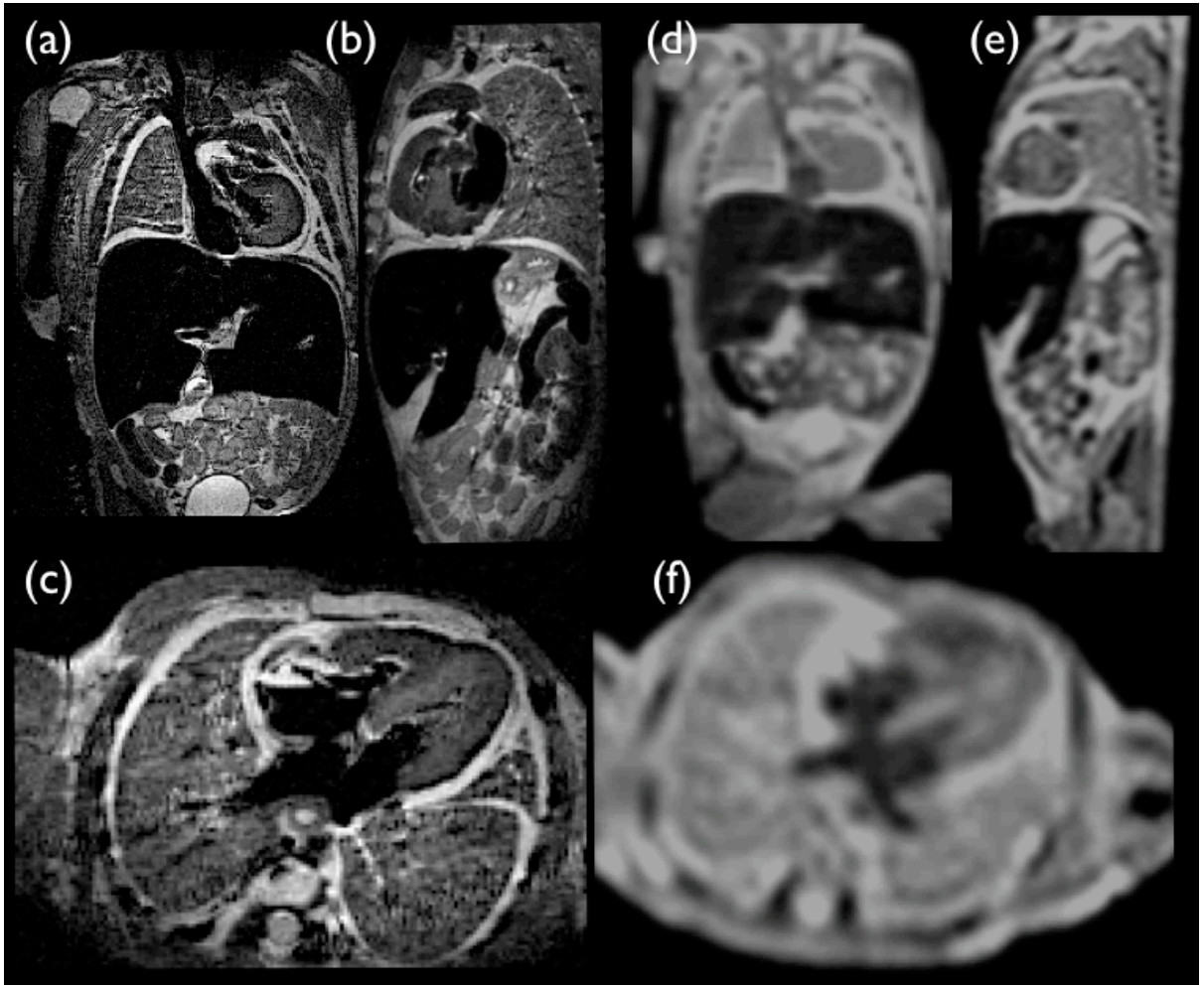
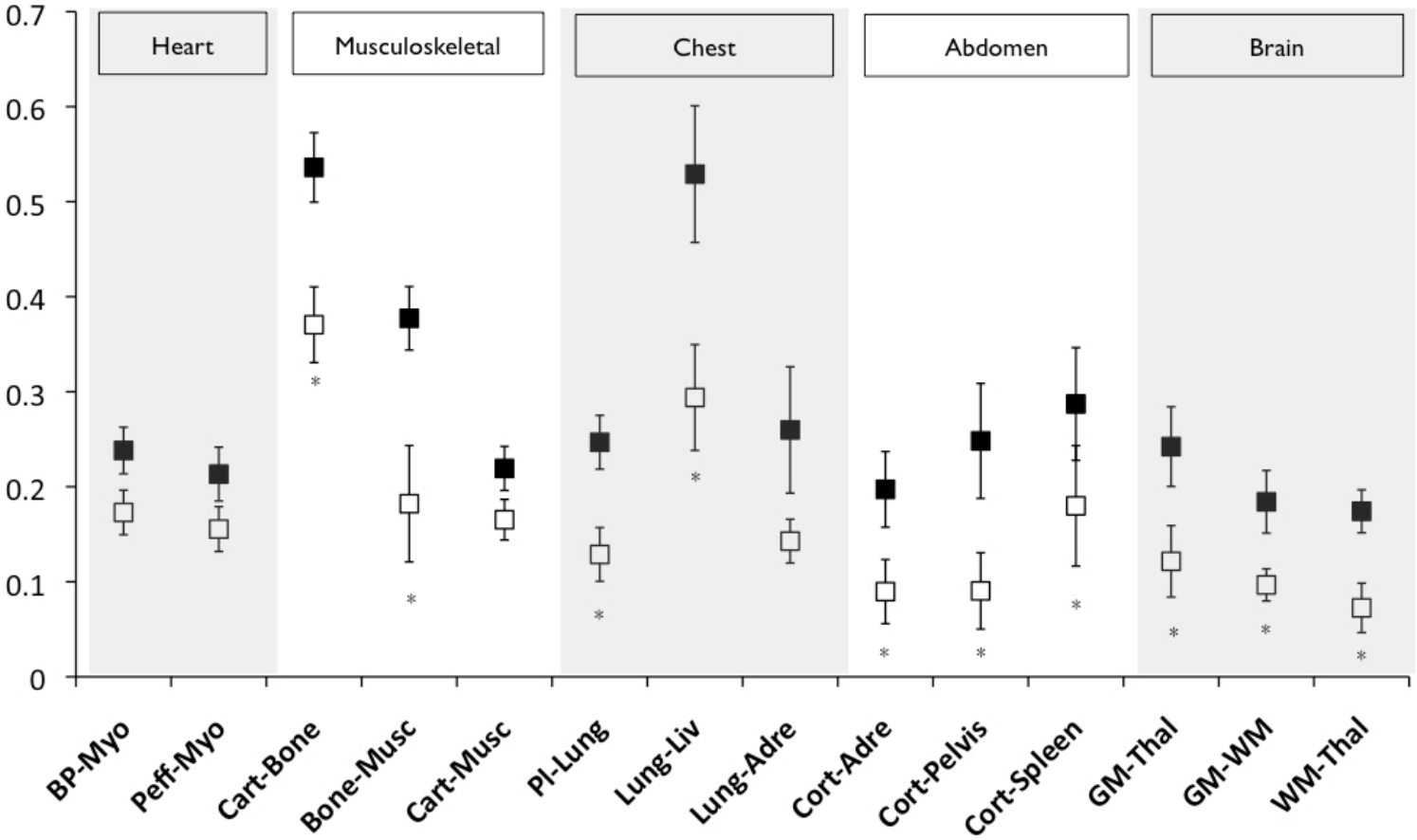


Figure 50. Mean (SE) Tissue contrast at 9.4T and 1.5T MR imaging of visceral organs

BP: Blood pool in right ventricle, Myo: Myocardium, Peff: Pericardial effusion, Cart: Cartilage (head of humerus), Bone: Humeral metaphysis, Musc: Muscle (upper arm), Pl: Pleural effusion, Liv: Liver, Adre: Adrenal, Cort: Renal cortex, Pelvis: Renal pelvis, GM: Germinal matrix, WM: White matter (Periventricular region), Thal: Thalamus. Asterisks indicate $p<0.05$ (Mann Whitney U test). Black box indicates mean tissue contrast at 9.4T and white box at indicates mean tissue contrast at 1.5T MR imaging.



12.5.3 Image Quality Assessment

For all cases, median (inter-quartile range) imaging scores for 9.4T MR imaging were significantly better than for conventional MR imaging at 1.5T (Table 29). Tissue contrast measured between different organs and tissues was significantly higher with 9.4T MR imaging than 1.5T MR (Figure 52, Table 30).

Table 28. Comparison of median image quality scores (inter-quartile range) at 9.4T and 1.5T MR Imaging

Organ	9.4T MR Imaging	1.5T MR Imaging	p value*
Brain	3 (1.6, 3.8)	2 (1, 1.5)	0.004
Heart	3.5 (2.8, 4)	1 (1, 1)	0.001
Chest/Abdomen	4 (2.7, 4)	1 (1, 1)	0.001
Musculoskeletal	4 (2.7, 4)	1 (1, 1)	0.001

*Mann-Whitney U test

On 9.4T high-field imaging, median (inter-quartile range) image quality scores were significantly better in the fresh fetuses (n=4) compared with the macerated stillbirths (n=13): 4(0) vs. 2.1 (0.9) [p= 0.001] for neuroimaging, 4(0) vs. 2.7 (1.2) [p= 0.045] for cardiac imaging, and 4(0) vs. 2.7 (1.2) [p= 0.045] for chest and abdomen imaging, respectively. For musculoskeletal imaging there was no significant difference – 3.5 (0.6) and 3.2 (1.1) [p=0.962], reflecting the lack of relevance of maceration in this system.

Table 29. Tissue *Contrast on MR imaging at 1.5T and 9.4T using 3D TSE (Turbo spin echo) sequences.

Region of interest	Mean (SE) Contrast at 1.5T	Mean (SE) Contrast at 9.4T	**p value
CARDIAC MR IMAGING			
Right ventricular blood pool and ventricular wall	0.17 (0.02)	0.24 (0.02)	0.051
Pericardial effusion and ventricular wall	0.15 (0.02)	0.21 (0.03)	0.254
MUSCULOSKELETAL MR IMAGING			
Cartilage and Bone	0.37 (0.09)	0.54 (0.04)	0.002
Bone and Muscle	0.18 (0.06)	0.38 (0.03)	0.006
Cartilage and Muscle	0.17 (0.02)	0.22 (0.02)	0.051
CHEST & ABDOMEN MR IMAGING			
Pleural effusion and Lung parenchyma	0.12 (0.02)	0.25 (0.03)	0.001
Lung and Liver	0.29 (0.05)	0.53 (0.07)	0.029
Lung and Adrenal	0.14 (0.02)	0.26 (0.07)	0.491
Renal Cortex and Adrenal	0.09 (0.03)	0.20 (0.04)	0.012
Renal Cortex and Renal Pelvis	0.09 (0.04)	0.25 (0.06)	0.011
Renal Cortex and Spleen	0.17 (0.06)	0.29 (0.06)	0.012
NEURO MR IMAGING			
Germinal matrix and thalamus	0.12 (0.03)	0.24 (0.04)	0.001
Germinal Matrix and periventricular white matter	0.09 (0.02)	0.18 (0.03)	0.051
Periventricular white matter and thalamus	0.07 (0.02)	0.17 (0.02)	0.003

*Contrast was calculated from (Signal Intensity of Region A – Signal Intensity of Region B) / (Signal Intensity of Region A + Signal Intensity of Region B)

**Mann Whitney U test

Table 30: Comparison of 9.4T MRI, 1.5T MRI and conventional autopsy

No	Clinical details	GA	Weight	High-field MR (9.4 T)	Conventional MR (1.5T)	Invasive Autopsy findings	Final Diagnosis
Group A: Fetuses following termination of pregnancies							
1	Skeletal dysplasia (Figure 3)	22	400g	PM IVH, Normal brain, All internal organs normal. Normal cartilaginous junction	PM IVH, Normal brain, liver, spleen, kidneys. Non-diagnostic for other organs. Normal cartilaginous junction.	All internal organs normal. Normal cartilaginous junction.	Skeletal dysplasia-NOS
2	Myelomeningocele (Figure 1)	19	270g	PM IVH, Lumbosacral Myelomeningocele, Chiari 2, Fronto parietal germinal matrix bleed, All other internal organs normal.	PM IVH, Lumbosacral Myelomeningocele, Chiari 2, Fronto parietal germinal matrix bleed. Normal liver, spleen, kidneys. Non-diagnostic for other organs.	Lumbosacral Myelomeningocele, Chiari 2, Normal internal organs.	Lumbosacral Myelomeningocele, Chiari 2
3	Right Congenital Diaphragmatic Hernia, Coarctation of aorta (Figure 2)	16	64g	Post-mortem IVH, Hematoma in choroid plexus and pons, Large PDA, LV<<RV, Small aortic arch, No coarctation, Pulmonary hypoplasia	Not done	Formal neuropathology normal, however unable to assess corpus callosum Large PDA, LV<<RV, Small aortic arch, No coarctation, Pulmonary hypoplasia	Right Congenital Diaphragmatic Hernia, Small Aortic arch, No Coarctation of aorta. LV<<RV, Pulmonary Hypoplasia
4	Fixed flexion deformity of limbs. Likely progressive genetic disorder	12	16g	Normal brain and internal organs	Too small to generate MR signal	Normal brain and internal organs	Fixed flexion deformity of limbs: Undetermined
5	Cystic hygroma	15+4	20g	No gross brain abnormalities, Pleural and pericardial effusion, pulmonary hypoplasia, No cystic hygroma.	Non diagnostic for all organs	Severely macerated fetus. Brain autolysed. Formal neuropathology not possible. Pulmonary hypoplasia, No cystic hygroma	Turner syndrome Pulmonary hypoplasia No cystic hygroma
Group B: Spontaneous miscarriages and unexplained Intra uterine fetal deaths							
Time from death to delivery		**9.4T MR Imaging**			**1.5T MR Imaging**	**Invasive autopsy**	**Final diagnosis**
<1 week (n=4)		Normal brain and internal organs in 4(100%)			Non-diagnostic images in all 11 cases. Too small to generate MR signal at 1.5T in 1 case	Formal neuropathology not possible due to autolysis. Other organs normal.	Unexplained IUD-3 Chorioamnionitis-1
2-7 weeks (n=8)		Normal brain 4(50%), Normal internal organs in 2(25%). Non diagnostic images in remaining cases.				Formal neuropathology not possible due to autolysis. Other organs normal.	Un explained IUD-8

Table 31. Comparison of diagnosis from conventional autopsy, 9.4 T MR imaging and 1.5 T MR imaging

Case	Death to delivery time	Brain			Body		
		Autopsy	**9.4 T**	**1.5 T**	**Autopsy**	**9.4 T**	**1.5 T**
1	≤1 week	Normal	Normal	Normal	Abnormal	Abnormal	Abnormal
2	≤1 week	Abnormal	Abnormal	Abnormal	Abnormal	Abnormal	Abnormal
3	≤1 week	Normal	Normal	Not done	Abnormal	Abnormal	Not done
4	≤1 week	Normal	Normal	Non Diagnostic	Normal	Normal	Non Diagnostic
5	≤1 week	Non Diagnostic	Normal	Non Diagnostic	Abnormal	Abnormal	Non Diagnostic
6	≤1 week	Non Diagnostic	Normal	Non Diagnostic	Normal	Normal	Non Diagnostic
7	≤1 week	Non Diagnostic	Normal	Non Diagnostic	Normal	Normal	Non Diagnostic
8	≤1 week	Non Diagnostic	Normal	Non Diagnostic	Normal	Normal	Non Diagnostic
9	≤1 week	Non Diagnostic	Normal	Non Diagnostic	Normal	Normal	Non Diagnostic
10	> 1week	Non Diagnostic	Normal	Non Diagnostic	Normal	Normal	Non Diagnostic
11	> 1week	Non Diagnostic	Normal	Non Diagnostic	Normal	Normal	Non Diagnostic
12	> 1week	Non Diagnostic	Normal	Non Diagnostic	Normal	Non Diagnostic	Non Diagnostic
13	> 1week	Non Diagnostic	Normal	Non Diagnostic	Normal	Non Diagnostic	Non Diagnostic
14	> 1week	Non Diagnostic	Non Diagnostic	Non Diagnostic	Normal	Non Diagnostic	Non Diagnostic
15	> 1week	Non Diagnostic	Non Diagnostic	Non Diagnostic	Normal	Non Diagnostic	Non Diagnostic
16	> 1week	Non Diagnostic	Non Diagnostic	Non Diagnostic	Normal	Non Diagnostic	Non Diagnostic
17	> 1week	Non Diagnostic	Non Diagnostic	Non Diagnostic	Normal	Non Diagnostic	Non Diagnostic

12.5.4 Comparison of Pre- and Post-mortem Diagnostic Information

Seven structural abnormalities were diagnosed on antenatal ultrasonography in the five fetuses (case 1-5) who subsequently had termination of pregnancy (Figures 49-51). Both less invasive autopsy at high-field 9.4T MR imaging and conventional autopsy confirmed 5 of these abnormalities and refuted 2 (Table 31) (cases 3 & 5). Five additional malformations, which were not detected on antenatal ultrasonography, were diagnosed in 3 fetuses, from pregnancy terminations (cases 2, 3 & 5). These abnormalities were detected by both high-field 9.4T MR imaging and by conventional autopsy.

12.6 DISCUSSION

This is the first study to demonstrate the feasibility and utility of non-invasive 3D, high-field MR imaging at 9.4T for whole body imaging of human fetuses. High-field MR imaging provided greater spatial resolution, increased tissue contrast and superior diagnostic information than conventional MR imaging at 1.5T. High-field MR imaging (and ancillary non-invasive post-mortem investigations) provided all the information that could be obtained by invasive autopsy for all internal organs in this series where the intra uterine retention period was less than 1 week. Moreover, clinically useful information on the brain could be obtained even in cases where maceration and autolysis prevented formal neuropathological examination. Conversely, conventional MR imaging at 1.5T was non-diagnostic in the vast majority of these small fetuses.

Several researchers have reported the poor diagnostic utility of conventional MR imaging in smaller fetus, particularly for organs other than brain (Alderliesten et al. 2003), including my observations described in chapter 6. This study again supports this observation. Conventional MR images can be acquired with an in-plane resolution of 0.4x0.4mm ; however, the slice thickness may be 1 or 2mm, thus introducing partial volume effects and limiting accurate analysis, particularly for organs systems other than the brain. It has been suggested that imaging fetuses after weighting the coil with bags of saline may improve image quality (Whitby et al. 2005) possibily by increasing the MR signal; however, this does not change the fundamental necessity for increased image resolution. Theoretically, it is be possible to achieve resolutions up to 0.4x0.4x0.4mm by sequence optimization for signal to noise and the

acquisition of multiple averages using conventional MR imaging at 1.5T. However, this would require extremely long scanning times (up to 24 hours) and would not be acceptable in clinical settings. The increased diagnostic utility of 9.4T MR imaging is primarily due to the ability to achieve high resolution isotropic 3D images, within clinically acceptable scan times. This reduces the partial volume effects, and therefore gives higher image quality.

High-field MR imaging at 9.4T is currently in an experimental stage, and is an area of active research. Even though resolution up to 18 microns is achievable by high-field MR imaging, this may require scan times of more than 18 hours at present and therefore limits applicability to human fetuses for routine examination. Several rapid acquisition methods and high-field MR staining techniques to increase image quality (e.g. addition of Gd-DTPA) have been developed for virtual autopsy of small animal embryos in the past few years (Driehuys et al. 2008). Once this is translated for use in human fetuses, an avenue for 3D virtual MR imaging may open up, which would greatly enhance the current understanding about early normal and abnormal organ development in human fetuses.

12.6.1 Limitations

I have not attempted formal optimisation of high field MR images. This needs to be performed systematically by measurement of T_1 and T_2 relaxometry values at higher field strengths. It is also possible that immersion of fetuses in MR stains (for example gadolinium) may increase the tissue contrast. Another major limitation of using MR imaging alone, regardless of field strength, is its lack of tissue availability for histological or ancillary diagnosis. Therefore a normal MR image may not always equate to normal microscopy performed at autopsy. Nevertheless, the utility of routine sampling of all visceral organs at perinatal autopsy, particularly when autopsy is performed following termination of pregnancy, is unclear. The published data and the data presented in chapter 7 suggests that lung, liver, kidney and cardiac biopsies are likely to have the highest yield. Therefore it is important to establish effective and accurate methods for percutaneous sampling of visceral organs.

CHAPTER 13: RAPID PROTOTYPING

13.1 SUMMARY

The recent decline in autopsy rates and lack of human anatomical material donated for research and training has resulted in issues for medical training in the UK. This study aims to examine the feasibility of making accurate 3D models of the human body and visceral organs using post-mortem MR imaging and rapid prototyping. I performed post-mortem MR imaging using 3D T2–weighted sequence in 11 fetuses and infants, before autopsy, using either a 1.5 T MR or 9.4 T MR scanner. Internal organs were reconstructed in silico and 3D models were creating by rapid prototyping. The median (range) gestation of fetuses was 20 (19 to 30) weeks and infants was 12 (8 to 16) weeks. The models created by rapid prototyping accurately depicted structural abnormalities and allowed clear visualization of 3D relationships. Accurate 3D modelling of anatomical features from post-mortem imaging in fetuses and infants is feasible. These models could have a large number of medical applications, including improved parental counselling, invaluable teaching resources and significant medico-legal applications to demonstrate disease or injury, without the need to show actual autopsy photographs.

13.2 INTRODUCTION

Learning anatomy and pathology from dissection of human bodies and examination of internal organs has traditionally played an important role in training of doctors, particularly surgeons and pathologists; hence the human body is often referred to as the best medical textbook. However, as stated throughout in my thesis organ retention issues and the subsequent introduction of the Human Tissue Act have led to a substantial decline in autopsy rates and therefore opportunities for research and training, in the past decade, in the UK (CMO 2006).

In order to meet this teaching role, I wanted to establish the feasibility of building 3D models of the post-mortem MR datasets collected for studies described in chapter 7 and 12. This would combine the 3D data with a technique known as rapid prototyping (3D printing).

In the current study, I examine the feasibility and potential applicability of combining 3D post-mortem MR data from fetuses and infants with rapid prototyping to create models of post-mortem features, with the aim of improving understanding and teaching of pathological conditions. In addition, such models could be clinically useful and highly acceptable for explaining post-mortem findings to parents and demonstrating pathological findings in the medico-legal setting.

13.3 METHODS

I acquired post-mortem MR images in 11 fetuses and children, prior to autopsy using a 1.5 T MR scanner (Avanto, Siemens Medical Solutions, Erlangen, Germany (n=10)) or a 9.4 T MR scanner (VNMRS, Varian, Palo Alto, CA, USA (n=1)) for fetuses less than 20 weeks. These cases were selected from a post-mortem MR imaging database, to reflect common structural abnormalities and/or injuries that are encountered in routine clinical practice, and to demonstrate the use of the technique across a wide variety of organs. I used T_2–weighted 3D turbo spin echo sequence for MR imaging (please see chapter 6, 7). In one case, CT data of the skull was also acquired (SOMATOM Definition, Siemens, Forchheim, Germany – parameters: collimation 2x32x0.6mm, rotation time 0.33s, pitch 0.2, kVp 120, mAs per rotation 375).

A biomedical engineer (Silvia Schievano) with 6 years experience in image processing reconstructed digital 3D volumes of the body and internal organs using

image post-processing software (Mimics 12.1, Materialise Inc, Leuven, Belgium), as previously described (Schievano et al. 2007; Thayyil et al. 2008b)]. This technique involved a series of steps and has been used by our group in creating of RP models of pulmonary outflow tracts in live patients. The steps involved are: (1) Three-dimensional MR data reconstruction—This can be performed using any 3D volume rendering software, I used the Mimics software (Materialise, Ann Arbor, Mich). The details of creating the 3D MR data sets is given in chapter 11; (2) RP protocol—RP is the name given to a wide range of related technologies used to fabricate physical objects directly from virtual or computer-aided-design data sources. Materials are added layer by layer and bonded in layers to form objects. The models can be of any geometric complexity and can be formed without the need for elaborate machine setup or final assembly. Thus in essence, a prototyping machine can be thought of as a 3D printer.

The digital 3D datasets (.stl format) were imported into a rapid prototyping system where the volumes were converted into solid objects, layer by layer. A Z Corp printer (Z Corporation, Burlington, MA, USA), that utilises inkjet print heads to deposit a binder into plaster powder was used for opaque and colour parts. An iPro SLA® system (3D Systems Corporation, Rock Hill, SC, USA) that employs an ultraviolet laser to cure liquid resin was used to build transparent models. Our group have demonstrated excellent accuracy of the proposed methodology in the previous study, with operator error <3.4% (Schievano et al. 2007).

Figure 51. Rapid prototyping of the brain

Rapid prototyping of brain from high field (9.4 T) MR images of a 16-week fetus. (a) external appearance of the brain. Note the smooth brain without sulci and gyri. (b) shows brain cut open in axial plane. G=germinal matrix. C=cerebrospinal fluid inside the lateral ventricles. Black arrows show the choroid plexus and bleeding into choroid plexus and ventricular cavity. (c) shows corresponding 9.4 T MR images in axial plane. The rapid prototyping model has been scaled to twice the original size. Conventional autopsy was difficult in this case due to autolysis of the brain.

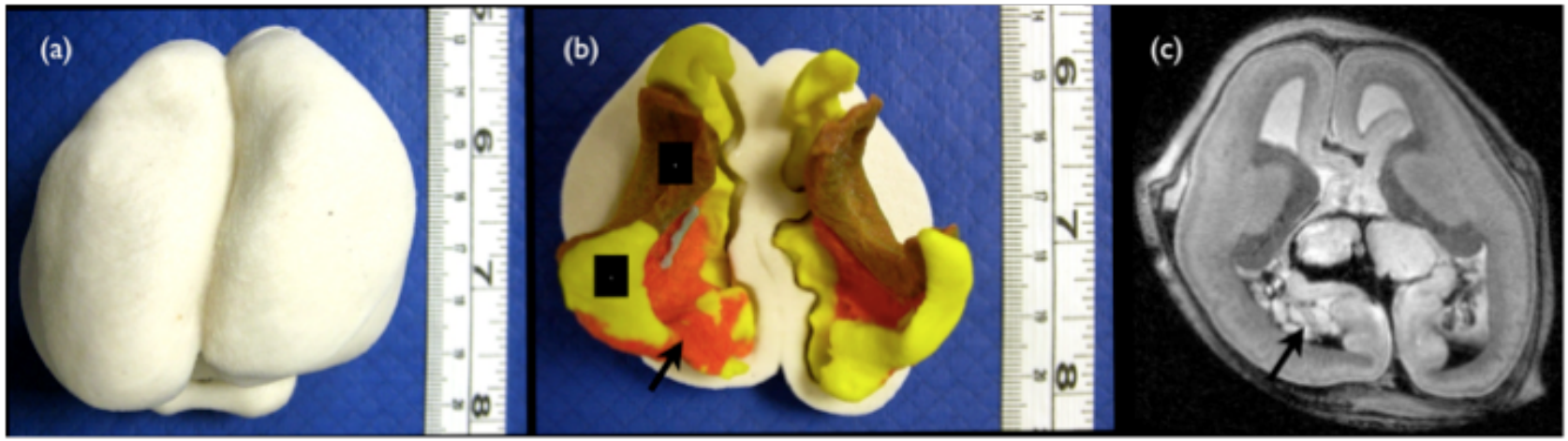


Figure 52. Rapid prototyping of visceral organs

(a) External rapid prototyping of a 30-week fetus with sacrococcygeal teratoma. (b) Posterior and (d) anterior view after opening the body model. (c) Post-mortem MR image in coronal plane. Teratoma is occupying most of the abdomen, displacing the intestines and other visceral organs. H=heart, S=spleen, A=adrenals, I=intestine, LK=left kidney, RK=right kidney, L=liver.

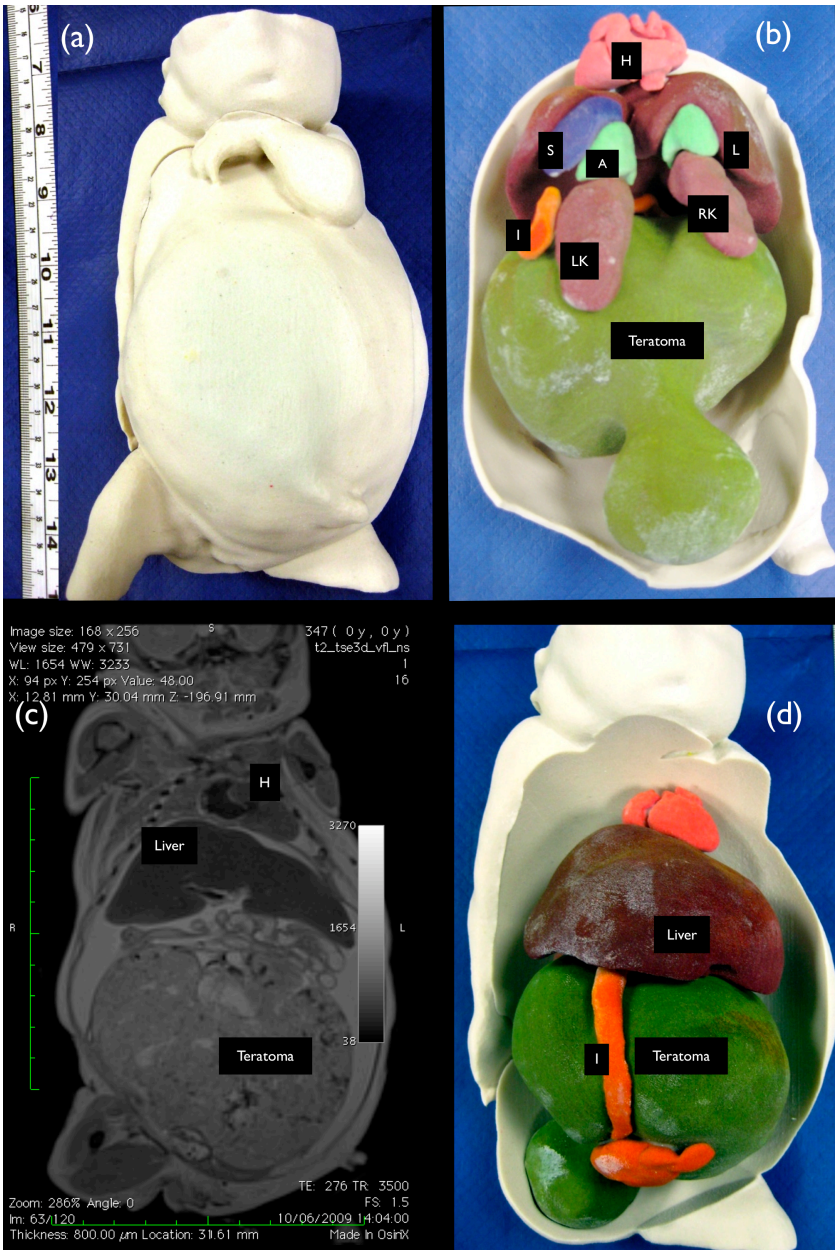


Figure 53. Rapid prototyping of the skull

(a-c) Fractured skull and underling parenchymal bleed in the brain in an infant. (d) Corresponding post-mortem MR image in the sagittal plane. Black arrows indicate the bleed. These findings were confirmed at autopsy. This model was built by combining and registering CT images, which provided the 3D skull structure, with MR data that were used to reconstruct the bleed volumes and positions.

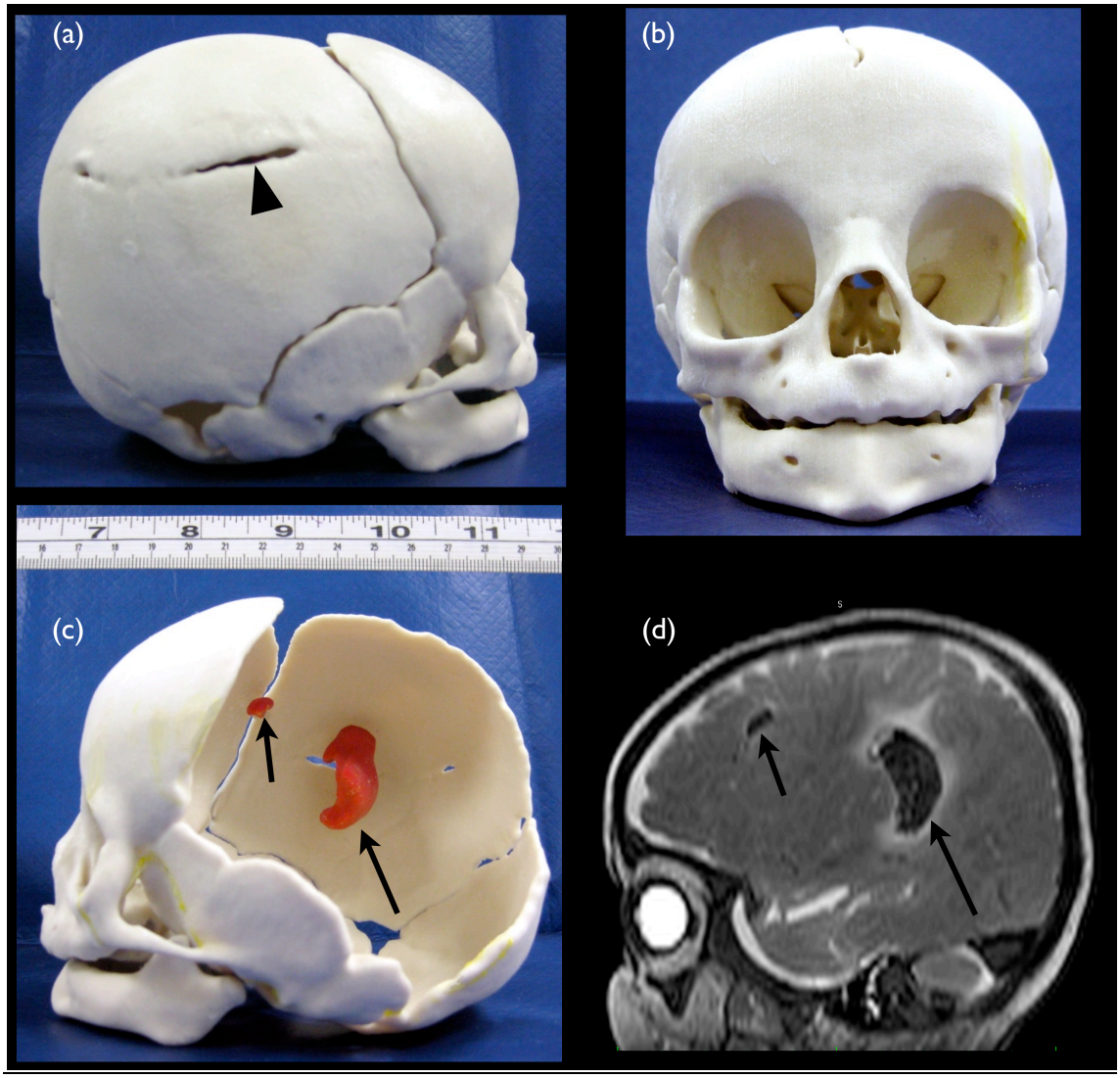
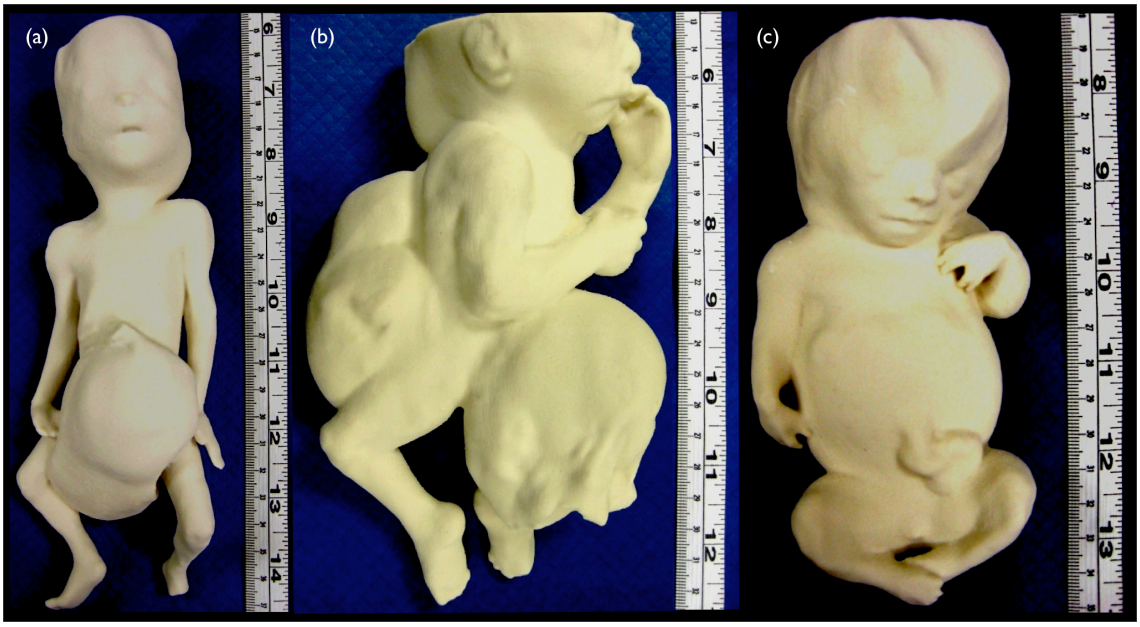


Figure 54. External rapid prototyping

External body rapid prototyping of (a) 19-week fetus with a large exompholos, (b) 18-week fetus terminated for a large myelemenigocele and exompholos (pentalogy of Cantrell) and (c) 20-week fetus with short limb skeletal dysplasia.



13.4 RESULTS

Cases included eight fetuses [(median (range) gestation 20 (16 to 30) weeks and weight 337 (65 to 1800) g] and three infants [(median (range) age 12 (8 to 16) weeks and weight 14 (13.2 to 16.5) kg]. Post-mortem MR imaging was done at a median (range) time of 4 (2 to 6) days after death. 3D reconstruction from the post-mortem MR images and rapid prototyping was feasible in all cases. Time for image elaboration was between 1 and 20 hours and varied according to MR image quality and number of organs reconstructed. Rapid prototyping time was between 1 and 12 hours according to volumes of the object, and costs were between £10 and £240 depending on the final model size. Structural anatomy and significant pathologies were easily identifiable by visual examination of the 3D models (Figures 53-56).

13.5 DISCUSSION

The data presented show that accurate 3D models of fetal and infant anatomy, including internal organs, can be created by rapid prototyping of whole body post-mortem MR images. Structural abnormalities of visceral organs and their relations to each other can be easily demonstrated, with a permanent record of the exact

anatomical features for each individual case created. Future application of such models may be useful in several ways; nevertheless all these potential applications are speculative.

- ***Improved understanding of complex congenital abnormalities*** – Congenital abnormalities often occur in small fetuses where the exact relations may be difficult to appreciate because of the small size of internal organs and autolysis. Furthermore, whilst the pathologist performing the autopsy may delineate the features during dissection, demonstration of such findings to others at a later date, for counselling or teaching, has traditionally been difficult. Not only does rapid prototyping allow for a permanent 3D record of the features and relative position, but also simple magnification enables larger replicates of the pathological features (Figure 51), making it easier to appreciate abnormal anatomy.
- ***Continued medical training from post-mortem data*** – Models of organs prepared by this technique may have important applications in medical training, particularly in anatomy and pathology. The rapid prototyping models can be stored long term without degradation or specific storage requirements. Such models can be annotated and since they are entirely non-identifiable, provide an ongoing unique teaching resource. For research purposes in the setting of rare congenital abnormalities this may also be important, since uncommon anomalies can be modelled, observed and categorised, without needing to retain either the fetus or organ, and precise replicas of rare findings could be produced multiple times and therefore shared across many training centres.
- ***Parental counselling*** – Clinicians may find these models particularly useful for explaining the abnormalities to parents, such as following a termination of pregnancy. Traditional post-mortem photographs are inappropriate for discussion with many parents, since the body is visible and blood will be present making the images unsuitable. However, this technique allows construction of a dissociated 'clean' model of the abnormal organ without requiring any autopsy photographs to be used. Some parents may indeed want to keep artificial replicas of their baby (Figure 54) or organ, which could help in bereavement process.
- ***Demonstration of significant pathologies in medicolegal cases*** – In many medicolegal cases relating to deaths of infants and children, juries and judges often depend heavily on evidence provided by pathologists, including post-mortem

photographs and/or drawings, to understanding the nature and mode of injury. However, in many cases, for the reasons stated above, autopsy photographs are deemed inappropriate for use in court due to the presence of blood or other non-specific post-mortem features. Rapid prototyping models allow demonstration of only the significant pathological findings with clear annotations and may be useful in demonstrating the mode of injury in such cases in the context of legal evidence (Figure 53). Reconstruction of the anatomical features may also be valuable in other settings such as mass disasters or cases in which a body is decomposed, so that conventional autopsy is difficult.

- ***Use of rapid prototyping for in-utero fetal diagnosis and management*** –

With development of rapid multi-slice snapshot MR sequences, 3D volume reconstruction of fetal organs has become possible (Rutherford et al. 2008). Therefore, rapid prototyping may have significant implications for in-utero fetal MR imaging, particularly in understanding the exact relations of the internal organs in cases of complex anomalies such as fetal diaphragmatic hernias and conjoined twins (Christensen et al. 2004), which may allow planning of subsequent surgical management in such fetuses, in future. In addition, facial models created by rapid prototyping of in-utero MR, may be useful in identifying facial dysmorphism in cases of genetic syndromes and allow clear demonstration of the extent of facial clefting. Modelling of the skeleton would be useful for diagnosing cases of skeletal dysplasia.

- ***A replacement for plastination*** – Over recent years, there has been controversy and public outcry related to the use of plastination to exhibit the human body and aid in medical training (BBC 2007). For the process of plastination, body parts are immersed in acetone chilled to 13^{o}F, and the water removed from every cell. The water is then replaced with a molten plastic material that later hardens. The parts retain their colour and shape, and many organs have a plastic appearance (BBC 2007). However, plastination requires the use of human bodies and therefore raises issues of acceptability; the rapid prototyping technique, we describe, does not involve use of any human tissue.

Many of these described applications, for the use of the rapid prototyping methods, are speculative and the utility of these applications will need to be explored with future studies.

At present, preparation of rapid prototyping models require specialist skills, expertise and equipment, which is likely to be available only in few selected centres in the UK. However, it would be possible to perform post-mortem MR imaging in most large hospitals and these images could be easily transmitted to a specialist centre for subsequent analysis and preparation of the models. Furthermore, with continued refinement of the rapid prototyping technique it will be possible to create future models that have the similar consistency, feel and colour to native human organs.

In summary, post-mortem MR imaging and rapid prototyping can create accurate 3D models of fetal and infant anatomical features and these models may have many potentially important medical and medicolegal applications.

CHAPTER 14: POST-MORTEM PERCUTANEOUS MR GUIDED BIOPSY

14.1 SUMMARY

Blinded needle biopsies have been extensively reported in the literature; however they are of limited utility in perinatal autopsies. The feasibility of US and CT guided biopsies has also been reported, again the utility in perinatal and paediatric settings may be poor due to poor tissue resolution. MR imaging offers the highest tissue contrast in post-mortem settings; however MR guided biopsies are technically challenging. In this work, I explore feasibility of two different MR guided biopsy techniques (a) Biopsy with steel needles by MR and fluoroscopy co-registration technique, (b) Biopsy under intermittent MR guidance using MR compatible needles, in a newborn piglet model. Whilst both techniques are feasible, the latter appears to be more promising and less technically challenging. Future work needs to be undertaken in humans using automatic MR compatible biopsy guns under intermittent MR guidance.

14.2 INTRODUCTION

Clearly, MR is not a microscope and cannot offer tissue diagnosis. High field MR imaging can offer very high-resolution virtual anatomical images; nevertheless, this is not comparable with histological examination and use of a variety of appropriate staining and immunohistological techniques for making the diagnosis. Thus, a normal MR image of an organ does not equate to a normal conventional autopsy; the latter includes detailed histological examination as a part of establishment of normality. Poor correlation of autopsy findings and MR findings, particularly for lung pathology (chapter 7), relates to lack of tissue diagnosis. In addition, cardiac and renal biopsies are important in making definitive diagnosis in many cases, even though MR may be a useful screening tool to detect these lesions.

Needle autopsies and percutaneous post-mortem sampling have been used in pathology practice for a while (Terry 1955); only few had autopsy comparisons and almost all the studies reached a predictable conclusion–needle autopsies are less accurate than conventional autopsy; however it may be useful when conventional autopsy is refused (Underwood et al. 1983; Benbow et al. 2003).

Aghayev et al have reported feasibility of CT guided biopsy in adults (Aghayev et al. 2008a). The authors obtained adequate samples from cerebrum, cerebellum, heart, liver, spleen and kidney. However, it is difficult to interpret the utility of these observations due to a number of major limitations in the study design and reporting. For example the authors did not provide the exact number of cases included in the study, neither did they perform conventional autopsy in these cases. No pathological lesions were seen in the biopsied samples, and so it is difficult to know if these represent true negatives or false negatives, in the absence of conventional autopsy.

CT scanning may indeed have an important role in percutaneous tissue sampling in adults. However, in my experience, the tissue contrast with CT imaging in infants and fetuses is extremely poor despite using higher doses. Therefore, it is often not possible to identify the visceral organs separately from each other on CT images, let alone identification of any pathology in this group. Hence, the utility of CT guided biopsy in fetuses and infants may be limited. However, I have not explored the use of CT in older children.

MR imaging offers the best contrast for internal organs and may identify the regions of interest, i.e. pathological lesions accurately. Such biopsies are used for brain, breast and prostate biopsies in clinical medicine routinely. Therefore theoretically MR guided biopsies may be the most accurate method of post-mortem tissue sampling.

However, there are several technical challenges and pragmatic issues that need to be overcome before post-mortem MR guided biopsy can be used for all visceral organs. In particular the access to the visceral organs is difficult in closed MR systems due to the small-bore size of the magnets. The hand of the operator will not be able to reach the biopsy equipment during the MR scan, thus biopsy under direct visualisation may be challenging. In addition, stainless steel needles cannot be used with MR guidance; therefore MR compatible needles (e.g. titanium) are required. An alternative is suction based MR guided systems used in breast biopsy; however such systems are very expensive.

14.3 AIMS

In this study I wanted to develop accurate methods for post-mortem biopsy using two different MR guided approaches, in a newborn piglet model.

- Post-mortem biopsy with stainless steel needles by MR and fluoroscopy co-registration technique.
- Post-mortem biopsy under intermittent MR guidance using MR compatible needles.

14.4 METHODS

14.4.1 MR-Fluoroscopy Co-registration Biopsy

All animals used in this study were already sacrificed (humanely) as a part of another research project. Therefore, Home office licence was not required for this work.

Post-mortem 3D MR imaging of four newborn piglets was performed on a 1.5 T MR scanner as described in chapter 6. Four custom made fiducial markers (made by injecting omnipaque into cod-liver oil capsules) were placed over the chest of the piglet. Coronal and sagital images were obtained using a 3D T_2-weighted turbo spin echo sequence (TR=3500ms, TE=360ms, flip angle 120^o, ETL=169, slice thickness=1.2 mm). Anteroposterior (AP) and lateral images were then obtained using

fluoroscopy. MR images were analysed offline using a semi-automated volume rendering and co-registration software (Mimics 9.0, Materialise Inc, Ann Arbor, MI, USA). A 3D volume of the liver and the fiducial markers was reconstructed (Figure E). Calibration for co-registration was done using the marker dimension (0.5 cm diameter). The 3D MR volumes were then co-registered on to fluoroscopy images and re-loaded into the fluoroscopy system. Percutaneous liver biopsy using a 10 G steel needle was then performed under fluoroscopy guidance on the AP and lateral views. Histological analysis was performed to confirm if the correct organ had been biopsied (Figure 57).

14.4.2 Direct MR Guided Biopsy

Three piglets were studied using this method. The piglet carcasses were kept in a stereotactic frame made of MR compatible material. MR imaging was performed using a 1.5T Siemens Avanto scanner using a 3D CISS (Constructive Interference Steady State) sequence. Once an axial view on the organ to biopsy was obtained, an MR detectable marker sliding on the top of frame was gradually introduced into the field of view, using a cine MR sequence. Once the marker was detected over the organ of interest imaging was performed again. The vertical distance from the marker to the organ was then measured in the axial MR image. The MR table was then withdrawn without moving the position of the piglet and an MR compatible needle was inserted to the calculated length. The position of the needle tip inside the organ was confirmed by rescanning. Once the position of needle tip was confirmed the MR table was retracted again, without disturbing relative positions and biopsy was performed (Figure 58). This process was then repeated for liver, heart and kidney biopsies.

Figure 55. MR fluoroscopy co-registration

Coronal (A) and sagital (B) T2 weighted MR images, and fluoroscopy images in AP (C) and lateral (D) view. Note the MR and X-ray visible fiducial markers. E) 3D volume rendered image of the reconstructed liver and fiducial markers using Mimics software. F) Registered MR and fluoroscopy images used to guide the biopsy needle during fluoroscopy. G) Liver histopathology.

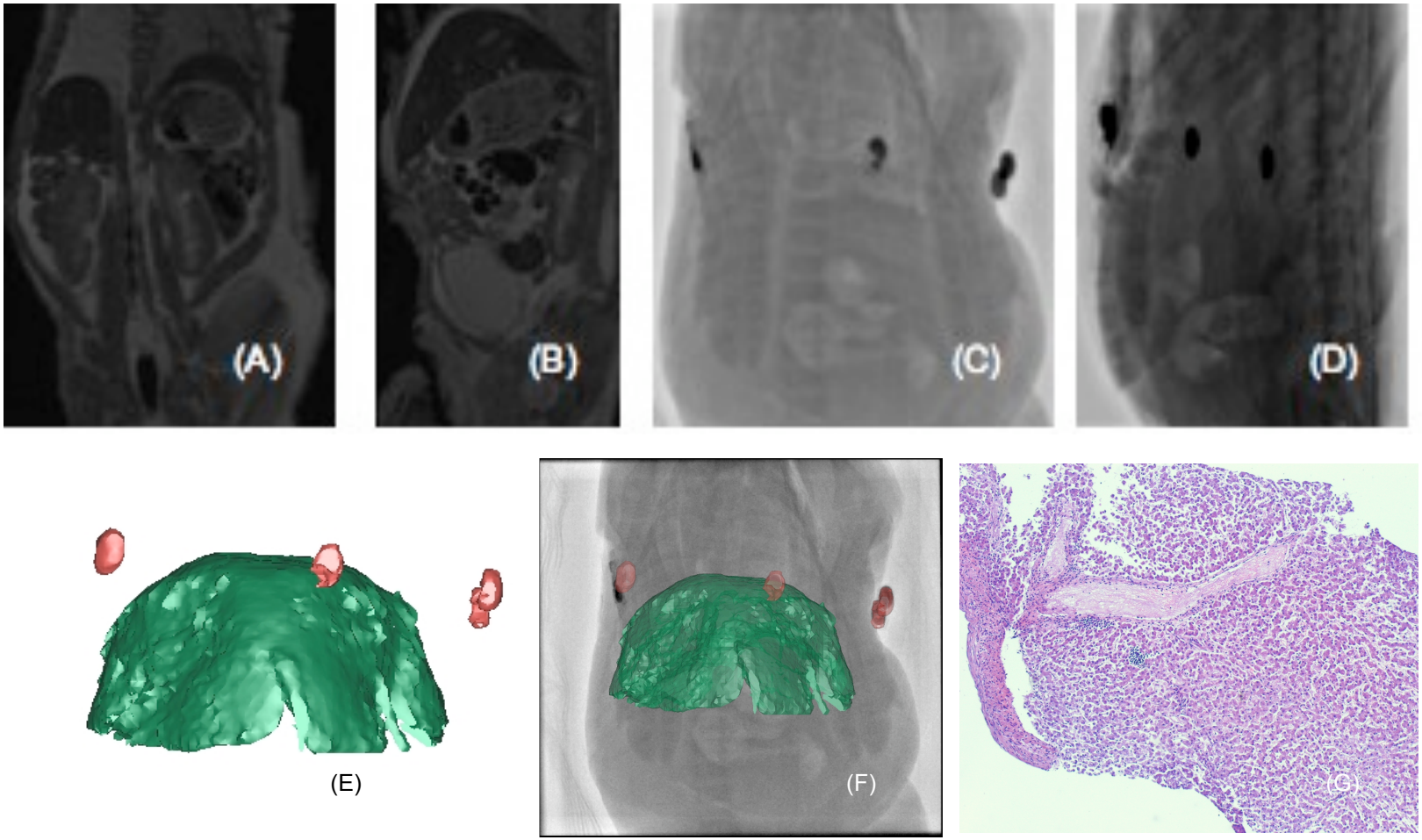
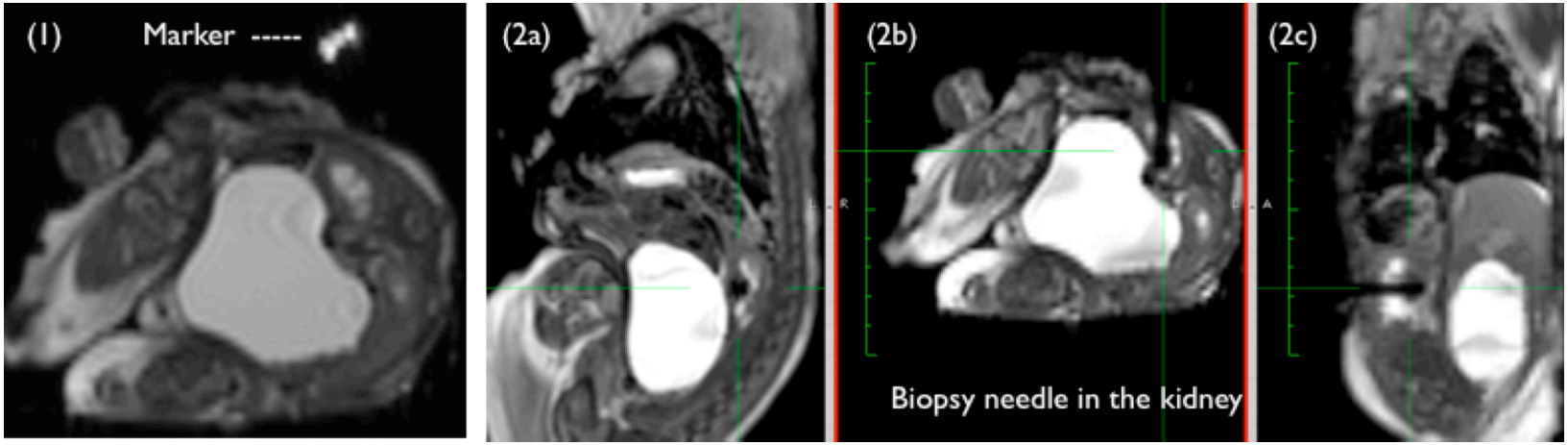
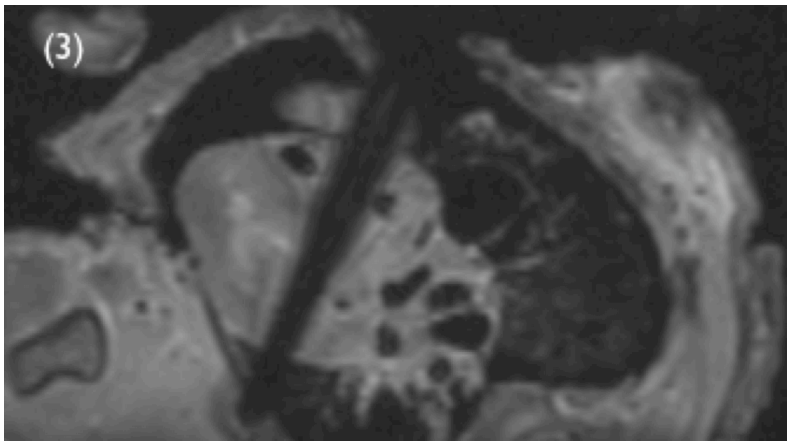


Figure 56. Direct MR guided biopsy

Top panel (1 to 2) shows renal biopsy and bottom panel shows cardiac biopsy (3) in a newborn piglet under MR guidance.





14.5 RESULTS

14.5.1 MR-Fluoroscopy Co-registration Biopsy

Four piglets were included in the study. Co-registration was successfully done in all cases (Time taken 1 ½ hours to 3 hours). Liver biopsies could be obtained successfully in all cases and histological examination showed normal liver tissue. Biopsies of kidneys, spleen, heart and adrenals showed only connective tissue on histological examination. The total time taken was between 3 to 6 hours for co-registration and biopsy.

14.5.2 Direct MR Guided Biopsy

In all three cases, heart and kidneys were hit and sampled accurately. However the sample volume was adequate only in one case, which confirmed cardiac and renal tissue. The total time taken was between 3 to 5 hours.

14.6 DISCUSSION

This work demonstrates the feasibility of MR-fluoroscopy co-registration and direct MR guided biopsy techniques for obtaining post-mortem biopsy. However, MR-fluoroscopy co-registration biopsy is technically challenging and inaccurate in targeting organs adequately. Biopsies under direct MR guidance are more realistic for clinical use and should be more accurate; however larger bore and sharper MR guided biopsy guns are required to obtain adequate sample.

Future work using automated MR guided biopsy guns or suction equipments with the direct MR guided technique described here may be required to establish the clinical utility of this technique. We used a prototype of a home made stereotactic frame using plastic material in this study. More user-friendly frames building upon this work may reduce the total scan and biopsy times. It may be possible for a pathologist or radiologist to perform such biopsies in principle, using any MR scanner.

Another alternative for MR guided biopsy is endoscopic biopsy. Several feasibility studies using an endoscopic approach for less invasive autopsy have been reported in recent years in adults. Such techniques may be useful in perinatal and paediatric autopsy and need to be explored in future studies.

CHAPTER 15: CONCLUSIONS AND FUTURE WORK

My thesis is focused on exploring the utility of post-mortem MR imaging as an alternative to conventional non-forensic autopsy in fetuses, newborns and children. In the introductory chapters 2 and 3, I have described the evolution & utility of autopsy, the causes for decline in the autopsy rates in recent years and various options currently available for less invasive autopsy. Of these, post-mortem MR imaging was most promising.

15.1 EXISTING PUBLISHED DATA ON POST-MORTEM MR IMAGING

The existing data prior to 2009 was insufficient to recommend the use of post-mortem MR imaging as an alternative for conventional autopsy (systematic review in chapter 4). The existing data is limited to small, retrospective and/or of poor quality studies, often with multiple sampling of the same datasets. The MR sequences used were sub-optimal, with none of the studies using high-resolution, 3D MR sequences. The vast majority of the published data were limited to fetal brain imaging. On the other hand, whole body MR imaging, particularly cardiac MR imaging had very poor diagnostic accuracy. Therefore, a clear need for further work on post-mortem MR imaging was established.

15.2 OVERCOMING CHALLENGES IN RECRUITMENT

My next major challenge was to recruit HM Coroners cases into the study; this was precisely the population where there was significant public demand for development of less invasive autopsy techniques. However, the recent changes in Human Tissue Authority Act and Coroners rules have lead to the virtual disappearance of any prospectively consented autopsy research in the UK, due to logical difficulties in contacting parents for post-mortem research. In addition, there have been concerns about the ethical aspects of contacting newly bereaved parents for research. Following extensive discussions with Department of Health, ethics experts, legal advisors and parental support groups, I decided to examine the feasibility of a telephone consenting model for obtaining research consent (Chapter 5). This work suggested that prospective parental research consenting prior to autopsy is feasible following an unexpected death of a child and can be undertaken in an ethical manner that appears acceptable to majority of the parents. Contrary to the popular belief that newly bereaved parents would react angrily to such an approach, I found that the majority of

parents viewed the process positively and did not have any objections to a 'cold' telephone call from a senior nurse experienced in bereavement counselling. Indeed, most parents spontaneously quoted their wish to help other parents in similar situations in the future as their main reason for participating in the study.

15.3 LESS INVASIVE AUTOPSY FOR CAUSE OF DEATH

Following this, I conducted a prospective study comparing whole body MR imaging with conventional autopsy in 200 fetuses, newborns and infants, which is described in chapter 6 and 7. Interestingly, the utility of less invasive autopsy by post-mortem MR imaging was dependent on the expectation from the post-mortem examination.

If the only purpose of post-mortem examination was to identify the cause of death, less invasive autopsy (i.e. post-mortem MR imaging along with other non invasive post-mortem investigations) was very accurate. The cause of death identified by conventional autopsy and less invasive autopsy was similar in over 90% of the cases following a hospital death, unexplained infant or childhood death referred by HM Coroners or an unexplained still birth.

However, reducing the wealth of the information provided by an autopsy to an exercise for identification of one, main single pathology or cause of death may not be entirely appropriate from a scientific point of view. The role of ancillary post-mortem investigations should not be underestimated, for example placental examination provided crucial information in upto one third of fetal autopsies. The study population in this work was predominantly fetuses, newborns and infants and caution need to be exercised in extrapolating these results to older children. Therefore, I examined the accuracy of post-mortem MR imaging in more detail, for each organ system.

15.4 POST-MORTEM BRAIN IMAGING

Post-mortem MR imaging appears to have a definite role and certain advantages over conventional autopsy for brain imaging. For example if the brain MR imaging was normal, it is unlikely that the cause of death was in brain. MR imaging had a very high sensitivity for detection of all major intracranial pathologies in fetuses, infants and children. The lesions missed on MR imaging were subtle abnormalities, without major clinical implications. However, abnormal MR imaging of brain does not necessarily mean there is an abnormal neuropathology; caution need to be exercised

in diagnosing hypoxic brain injury by MR imaging. Ante-mortem hypoxic brain injury cannot be differentiated from a death process (normal post-mortem change) on post-mortem MR imaging, and radiologists are likely to over diagnose hypoxic lesions and global infarctions. This is because T_1 and T_2 relaxometry values increase and converge in brain grey and white matter following death, thus giving an appearance of ischemic injury.

Small amounts of intraventricular bleeds are common on fetal post-mortem brain imaging, and has resulted in considerable controversy in the published literature. Such bleeds without any clots or ventricular dilatation on MR imaging do not usually have autopsy correlates; they are of very little clinical significance.

However, not all lesions detected on post-mortem fetal brain MR imaging without autopsy correlates should be considered as false positives; some of these (in fetuses only) may be due to systematic errors of conventional neuropathological examination and fixation artefacts exaggerated by autolysis. Therefore if post-mortem MR imaging of brain is not performed as a routine in fetuses, clinically important information may be lost. However, this does not appear to be an issue in newborns and older children, where autolysis was rare (in this cohort).

In summary, there is a strong case for routine post-mortem MR imaging of brain in all fetuses whether or not conventional autopsy is performed; it can also be used as a screening test to decide whether or not the head needs to opened in fetuses, newborns and children.

15.5 POST-MORTEM MR IMAGING FOR OTHER ORGANS

Most disappointing was the use of post-mortem MR imaging for detection of lung lesions. A normal MR image does not exclude lung pathologies like pneumonia, pulmonary bleed, meconium aspiration; alternatively a consolidation very well seen on MR imaging often had complete normal histopathology of lung. The only way for detection of lung pathologies appears to be by histological examination.

For abdomen, post-mortem MR imaging had a high negative predictive value for detection of major pathological lesions in this series. However, lesions like fistulas or bowel perforations can be missed by MR imaging. It is possible that a laparoscopic examination may be a better screening test than MR imaging for detecting abdominal

pathology; indeed MR imaging can be used to guide the endoscopist (this issue is not explored in my thesis). MR imaging had a very high negative predictive value and sensitivity for detection of renal lesions; nevertheless definitive diagnosis required histological examination. Again such tissues may be obtained by endoscopic or percutaneous biopsy.

On the other hand, post-mortem cardiac MR imaging had a very high sensitivity and negative predictive value for detecting a significant cardiac abnormality in larger fetuses, newborns and children. It was reassuring to note that cardiac MR even detected cases of myocarditis; a not uncommon cause of sudden infant death. Nevertheless a tissue biopsy was crucial to establish definite diagnosis in such cases and MR imaging only showed non-specific ventricular dilatation. Systematic errors of cardiac MR imaging included difficulty in assessing aortic arch in fetuses and evaluating heart valves. High resolution isotropic 3D cardiac MR imaging is essential for accurate diagnosis of cardiac lesions.

The utility for musculoskeletal imaging could not be explored in detail in this work, due to a low incidence of these lesions (i.e. forensic cases were excluded). The utility appears to be poor for detection of fractures and other traumatic injury; a CT scan may be a better alternative in such cases. I have not explored this issue in my thesis as the use of CT scan is well established in forensic practice.

15.6 POST-MORTEM MR IMAGING OF SMALL FETUSES

One major difficulty I encountered was in obtaining good diagnostic quality MR images from small fetuses, due to low MR signal at 1.5 Tesla. Therefore, I used high field (9.4 Tesla) imaging for this sub group; Magnetic resonance microscopy at high field strengths has opened an avenue of 3D virtual microscopy in animal models and ex vivo tissue imaging in humans. I acquired the first human in vivo whole body MR imaging at 9.4 Tesla (Chapter 10). All structural malformations detected at conventional autopsy could be identified using this technique, whilst conventional MR imaging at 1.5 Tesla was non-diagnostic in most of these cases. The increased accuracy of high field MR imaging appears to be due to higher resolutions and a reduction in partial volume effects. However, such scanners are not widely available, require high level of expertise and extensive infrastructure to maintain and are still in an experimental phase; thus clinical utility at present may be limited.

15.7 OTHER USES OF POST-MORTEM MR IMAGING

15.7.1 Visceral Organ Weight Estimation

Examination of visceral organ weights is an integral part of the autopsy; therefore I developed and validated a rapid and semi automated technique for this from post-mortem MR imaging. For most visceral organs accurate estimation of organ volumes was possible, nevertheless the accuracy and ease of estimation depended on the contrast between the concerned organ and surrounding tissues. These techniques do involve a substantial amount of time (2-4 hours per case) of a person experienced in MR image handling and manipulation. Conversion of volume to weight involves use of an unknown variable, i.e. density, which may vary with different gestations and age for some organs. Development of normograms based on MR volumes rather than converting to weights may be useful in clinical practice of less invasive autopsy.

15.7.2 Rapid Prototyping

In this work, I explored another interesting use of post-mortem imaging. I re-constructed accurate models of internal organs and skeletal system from high-resolution, 3D MR datasets using rapid prototyping methods. Essentially, this technique involved importing the digital, volume-rendered reconstructions from MR datasets into a 3D printer, which converts the volumes into solid objects, layer by layer; thus accurately reproducing the organ structure. Internal organs can also be magnified to display subtle pathologies. There are several potential applications of this technique, including medico-legal use for demonstrating injury in the courtroom, training and teaching purposes, parental counselling etc.

15.7.3 Post-mortem Biopsy

A major limitation of less invasive autopsy is the lack of tissue diagnosis. There are no accurate methods for post-mortem tissue sampling. I developed two novel methods for MR guided post-mortem biopsy using a newborn piglet model: (1) Biopsy with steel needles by MR and fluoroscopy co-registration technique, (2) Biopsy under intermittent MR guidance using MR compatible needles. Whilst both techniques are feasible, the latter appears to be more promising and less technically challenging. Clearly this work only establishes the proof of principle; future studies are required to examine the accuracy and clinical utility of these methods.

15.8 CLINICAL IMPLICATIONS

Even though less invasive autopsy had very good accuracy for detecting the cause of death, routine use of MR imaging as an alternative to conventional autopsy may not be advisable at present. Less invasive autopsy is likely to be more expensive than conventional autopsy; thus an option of a more expensive and less accurate test may not be appropriate. Further improvements in less invasive autopsy techniques are required to ensure that most clinically significant pathologies are detected by this technique, before it is offered in routine clinical practice. Nevertheless, the promise of post-mortem MR imaging-based, less invasive autopsy in a perinatal and paediatric setting appears to be more promising than in adults, where coronary heart disease is one of the commonest causes of sudden death (Oxford Less invasive autopsy group-personal communications).

A more selective approach is needed to ensure post-mortem data remains accurate and credible for the time being. Clearly, post-mortem MR imaging on its own is unlikely to be a satisfactory alternative to conventional autopsy; it is important to undertake ancillary non-invasive investigations like genetic testing, metabolic testing, placental examination and other post-mortem imaging. Failure to undertake such investigation may result in genetic, metabolic, infective or skeletal pathologies being overlooked. Thus, post-mortem MR imaging should be performed only with involvement of pathologists and not directly as a radiology service. I suggest the following approach based on the current work.

<u>A. Case where post-mortem MR imaging is likely to be most accurate and have the highest yield</u>

1. Fetuses terminated for a structural malformation, particularly of the brain
2. Brain imaging in sudden unexpected deaths in infancy or childhood (SUDI): A normal MR imaging is likely to exclude a cause of death in the brain, particularly an unnatural cause. Therefore opening of skull may be avoided in such cases
3. Suspected intracranial bleeds

<u>B. Cases where post-mortem MR imaging may have some role</u>

1. Suspected structural cardiac and renal malformations
2. Major intra-abdominal pathologies

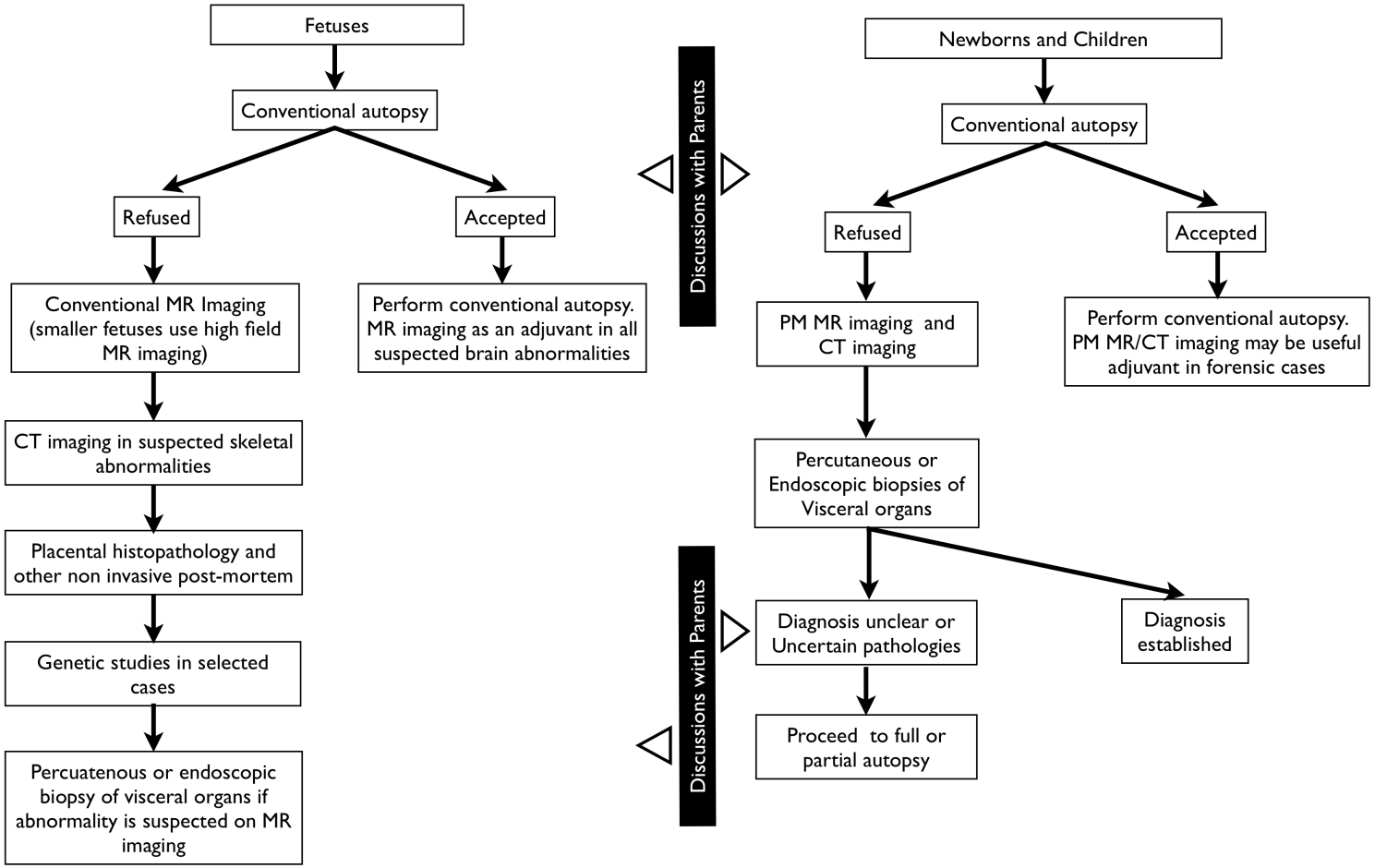
C. Situation where post-mortem MR imaging may be inaccurate or have poor yield

1. Pulmonary lesions including pneumonia, bleeds, meconium aspiration, adult respiratory distress syndrome (ARDS), hyaline membrane disease, small cysts
2. Liver or spleen infarctions, adrenal haemorrhage
3. Small sinus, fistulas
4. Fetuses less than 20 weeks (high-field MR imaging may be useful in this group)
5. Ischemic brain lesions
6. Musculoskeletal pathologies including fractures (X–ray and CT mandatory in this group)

15.9 FUTURE DIRECTIONS

The most crucial next step is the establishment of accurate post-mortem biopsy techniques. I have described two such methods in this thesis. An alternative approach is endoscopic examination of visceral organs following gas insufflation; however, the current data is limited to small case series (Fan et al. 2010). Furthermore, an endoscopic approach cannot be used for brain examination and may be of limited utility in detecting retroperitoneal lesions. Nevertheless, it is possible that the information from post-mortem MR imaging may guide the endoscopist to specific areas of interest, thus increasing the yield.

Figure 57. Flow chart for less invasive autopsy



It may be possible to optimize post-mortem MR sequences further; however, this would require more detailed serial MR relaxometry studies examining the effect of a wide range of potential confounding factors. Considering the fact that most pathology may be unexpected, specific optimisation for any pathology may not be realistic.

There are several fatal pathologies that can missed by conventional autopsy techniques and post-mortem MR imaging, for example cardiac ion channelopathies. These conditions are perfect 'assassins' and do not leave any detectable trace at autopsy; nevertheless it may be possible to identify these conditions by post-mortem genetic testing.

I have not evaluated the use of CT imaging in this thesis, as my work is focused on non-forensic cases. CT imaging has a well-established and definite role in forensic autopsy. The other issues that need to be explored in future work are cost-effectiveness and parental and professional attitudes towards less invasive autopsy.

Considering the infrastructure and technical expertise required to perform high quality less invasive autopsy, it is likely that such autopsies will be limited to few specialised centres in the UK. A network of such centres, national protocols and registries for less invasive autopsy need to be established, so that such a service can be adequately audited for quality assurance and effect on mortality statistics. An effective and evidenced based use of less invasive autopsy in routine clinical practice in the NHS, requires a joint effort from pathologists, radiologists, clinicians, parent groups and funding bodies and should be supported by adequate resources. Premature implementation may have major medicolegal implications and may result in loss of credibility of the HM Coronial death investigations in the UK.

CHAPTER 16:ORIGINAL RESEARCH FINDINGS

Original research findings from this work

1. Systematic review and meta-analysis of the published data prior to 2009, suggests that there is insufficient evidence to support the use of post-mortem MR imaging as an alternative to conventional autopsy in fetuses, newborns, children or adults.
2. Consenting for post-mortem research can be obtained from newly bereaved parents in an ethically acceptable way over the telephone by research nurses experienced in bereavement counselling; contrary to the popular belief most parents view such participation positively (http://www.ucl.ac.uk/news/news-articles/0908/09080701).
3. Cause of death can be accurately identified in more than 90% of cases by less invasive autopsy following a hospital death, unexplained stillbirth or an unexpected death under HM Coronial investigation.
4. Post-mortem MR imaging of brain has a very high negative predictive value in fetuses, newborn and children; opening of head can be avoided if post-mortem MR imaging of brain is normal.
5. High-resolution, 3D post-mortem cardiac MR imaging can accurately detect structural heart diseases in larger infants, newborns and children. Accuracy is poor in smaller fetuses.
6. Post-mortem MR imaging of lungs has poor utility; it can neither detect nor exclude pulmonary lesions. Normal post-mortem MR imaging of abdomen can excluded major intra-abdominal pathology.
7. Brain intra-ventricular bleeds without clots and crazy paved appearance of brain on gradient echo sequences are normal post-mortem MR artefacts.
8. Post-mortem MR imaging cannot differentiate between normal death process and ante-mortem hypoxic brain injury using conventional reporting methods. Loss of grey-white contrast, and loss of normal high signal intensity in posterior limb of internal capsule are part of the normal death process and should not be mistaken for an ischemic injury. However, signal intensities in deep nuclei are higher in infants who died from neonatal encephalopathy as

opposed to those who died unexpectedly and where neuropathological examination of brain was normal.

9. T_1 and T_2 relaxometry values in the brain are higher than values reported from ante-mortem brain; a convergence occurs in these values, in particular T_1 values following death. Hence T_1–weighted images have very little tissue contrast.
10. Visceral organs weights can be rapidly and accurately calculated from 3D post-mortem MR images using semi-automated techniques.
11. High field MR imaging is superior to conventional MR imaging for imaging smaller fetuses, in terms of image quality and diagnostic utility; this is due to the higher resolution achieved at higher field strengths, thus reducing the partial volume effects.
12. Reconstruction of anatomy is possible using rapid prototyping of 3D MR data sets. These models may have several clinical implications in future.
13. Post-mortem biopsies using MR-fluoroscopy co-registration or direct MR guidance are feasible in animal models. Utility in humans needs to be explored.

CHAPTER 17: REFERENCES

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CHAPTER 18: APPENDIX

VERSION 1.0
28.4.2007

Dr Andrew Taylor
Dr Sudhin Thayyil
Dr Nikki Robertson
Dr Neil Sebire

Great Ormond Street Hospital for Children NHS Trust

Great Ormond Street
London WC1N 3JH

Tel: 020 7405 9200

Post-mortem Magnetic Resonance Imaging in fetuses, newborn and Children: A comparative study with conventional autopsy.

Information leaflet for parents

Thank you for taking the time to read this leaflet. We know that this is a difficult time for you and appreciate the time you are taking to read this leaflet.

Background to the study.
MRI (Magnetic resonance imaging) and CT scans, as you may be aware are special techniques to get images of the body. An MRI scan can examine internal organs in detail and may be able to identify some of the problems that can be detected by a post-mortem examination. In some cases, we believe that MRI may even be better than a post-mortem examination. Many parents are understandably upset about the thought of their baby undergoing a post-mortem. We are doing this study to find out if an MRI scan of the whole body can give similar information to that of post-mortem, so that in future we might be able to offer an MRI scan instead of post-mortem.

What will happen if we agree to take part?
If you agree to take part we will arrange for your baby to have an MRI scan (and in some cases a CT scan as well) as soon as possible at Great Ormond Street Hospital for Children. This involves taking a series of pictures using a special machine. We may take biopsy using small needles under MRI guidance, for examination under microscope. The whole process will take about 2 hours. As soon as the scan is done we will arrange for your baby to be taken for the traditional post-mortem. We will ensure that at all times your baby will be treated with due respect and reverence.
The MRI scan will not delay the post mortem or the timing of burial or cremation. Taking part would not involve you in any extra hospital visits. Any additional information, if any from MRI/CT scan will be included in autopsy report to the coroner. We will also need to have access to the post-mortem results and the results of any other tests that were done before or after birth. This is so that we can compare the results of tests which are done traditionally with the results from the MRI and work out which combination of tests give the most accurate results overall.

Will my taking part in this study be kept confidential?
All information that is collected about you or you baby or during the course of the research will be kept strictly confidential. Any information we collect will only be used by the research team for the purpose of the study.

Who will have access to the case/research records?
All the data and images collected as part of this study will be stored on a secure computer. Only the researchers involved in this study will have access to the data collected in the course of this study. A representative of the hospital's Research Ethics Committee will also have access to data. The 1988 Data Protection Act safeguards the use of some types of personal information. This places an obligation on those who record or use personal information, but also gives rights to people about whom information is held. If you have any questions about data protection, please contact the Data Protection officer via the switchboard on 0845 155 5000. The results from our project will be published as papers in medical journals. No data will be published that allows for individuals to be identified in any way. If requested, we will be able to send you copies of any papers published when we have completed the study in 3-4 years time.

Do you have to take part?
It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and will be asked to sign a consent form. You will be given a copy of the signed consent form for your records. If you do not feel able to take part it will not in any way affect the care your family receives.

Who do I speak to if I have further questions or worries?
In the first instance please contact Dr. Sudhin Thayyil who is coordinating this project. His contact details are given below. Drs Sebire, Taylor and Robertson can also be contacted if you need any further information or if Dr Thayyil is not available. If you wish to speak to someone not directly involved in the study then please contact Hilary Brukner or Nicola Baxter (Mortuary Managers, GOSH) on 0207 405 9200 Ext 7906. If you have any complaints about the way in which the project is being or has been conducted, in the first instance please discuss them with any of the doctors listed below. If the problems are not resolved, or you wish to comment in any other way please contact Alistair Parker, Upton Farm, Gate House Lane, Framfield, East Sussex. TN22 5RS

Who is organising and funding the research?
This study is being organised by the Cardiothoracic, Radiology and Pathology Departments at Great Ormond Street Hospital for Children (Dr Taylor) and by the Foetal and Neonatal Medicine Units and Pathology Department at University College London Hospital (Dr Robertson). Funding is provided by the Department of Health. This study has been reviewed and approved by the Great Ormond Street Hospital Research Ethics Committee.
Thank you once again for all your time and trouble.

Contacts for further information:
Research Coordinator- Steven Kimberley- Tel 0207 405 9200 Ext 5616
Research Doctor- Dr. Sudhin Thayyil - Tel 0207 405 9200 Ext 6835 Mob: 07912 888 700 or s.thayyil@ich.ucl.ac.uk

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Version 1.0
REC reference number: **04/Q0508/41**
Study R & D Number: **04CC20**

Patient Name ……..
Unit Number ……..
Date of Birth ……..
Patient Identification Number for this trial:……..

CONSENT FORM

Title of Project: Post mortem magnetic resonance imaging in the fetus, infant and child: A comparative study with conventional autopsy

Researchers: Dr. Andrew Taylor, Dr Nikki Robertson, Dr Neil Sebire

1. I confirm that I have read and understand the information sheet dated 28/9/07 (version 1.0) for the above study, and have had the opportunity to ask any questions. ☐
2. I understand that my participation is voluntary, and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that sections of baby's medical notes may be looked at by responsible individuals named in the study or by from regulatory authorities from the Trusts. I give permission for these individuals to have access to baby's records. ☐
4. I agree to take part in the above study. ☐

Name of Parent/Legal Guardian	Date	Signature
Name of Person taking consent (if different from Researcher)	Date	Signature
Researcher	Date	Signature

1 for Patient; 1 for Researcher; 1 to be kept with Hospital Notes

6.2.08

To
Dr Vic Larcher
Ethics Chair
GOSH-ICH REC

Dear Dr Larcher,

Research on Dead piglets at GOSH

We are planning to do some MR/CT scanning and MR guided biopsy on dead piglets at GOSH (Principal Investigator: Andrew Taylor). The piglets will be coming from Dr Nikki Robertson lab in UCL and are sacrificed after the experiments according home office regulations (Ethics approval-UCL)

We have clearance from the medical director, chief executive and Infection control team at GOSH to do these experiments. Risk assessment has been done and appropriate systems, SOP are in place. Histological examination will be done on biopsied tissue at GOSH.

Could you please clarify if we need to get any formal approval from GOSH-ICH Ethics committee for the same.

Yours truly,

Andrew Taylor (Cardiac MRI, GOSH)
Sudhin Thayyil (Cardiac MRI, GOSH)
Neil Sebire (Pathology, GOSH)
Nikki Robertson (Neonatal Medicine, UCLH)

Institute of Child Health/Great Ormond Street Hospital Research Ethics Committee

The Institute of Child Health
30 Guilford Street
London WC1N 1EH

03 December 2007

Dr Andrew M. Taylor
Institute of Child Health & Great Ormond Street Hospital
Senior Clinical Lecturer in Cardiovascular MR

Dear Dr Taylor

Full title of study:
Post mortem magnetic resonance imaging in the fetus, infant and child: A comparative study with conventional autopsy REC reference number:
04/Q0508/41

The REC gave a favourable ethical opinion to this study on 31 August 2004.

Further notification has been received from a local site assessor following site-specific assessment. On behalf of the Committee, I am pleased to confirm the extension of the favourable opinion to the new site. I attach an updated version of the site approval form, listing all sites with a favourable ethical opinion to conduct the research.

R&D approval

The Chief Investigator or sponsor should inform the local Principal Investigator at each site of the favourable opinion by sending a copy of this letter and the attached form. The research should not commence at any NHS site until approval from the R&D office for the relevant NHS care organisation has been confirmed.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

Yours sincerely

Taki Austin
Research Ethics Co-ordinator

Email: t.austin@ich.ucl.ac.uk
Enclosure:
Site approval form

Copy to: Dr Tracy Assari, Institute of Child Health/ Great Ormond Street Hospital R&D Department