Development of the minimally invasive paediatric & perinatal autopsy

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A thesis submitted for the degree of Doctor of Philosophy, PhD

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

Signed:

Date:
Abstract

Introduction

Perinatal autopsy contributes useful clinical information to patient management in approximately 40% of cases but remains poorly accepted due to parental concerns regarding disfigurement. Post-mortem imaging is an alternative, but 1.5 T MRI lacks resolution below 18 gestational weeks. Additionally, the Royal College of Pathologists autopsy guidelines recommend extensive tissue sampling as part of the investigation of fetal loss, which imaging alone cannot provide.

Possible mitigating strategies include micro-CT for phenotyping small fetuses and laparoscopic techniques to obtain tissue samples. Interrogation of the evidence base for tissue sampling in different clinical scenarios is necessary to develop evidence-based practice and recommendations.

Methods

Minimally Invasive Autopsy with Laparoscopy (MinImAL) was performed in 103 cases.

Micro-CT was optimised in extracted organs and the diagnostic accuracy evaluated in 20 fetuses.

The Great Ormond Street Autopsy Database was retrospectively interrogated to investigate the yield of internal examination and visceral histology to the cause of death in 5,311 cases.
Results

MinImAL examination is reliable (97.8% successfully completed, 91/93) with good tissue sampling success rates (100% in lung, kidney, heart).

Micro-CT offers an accurate method of scanning small fetuses (97.5% agreement with autopsy, 95% CI, 96.6-98.4) with fewer non-diagnostic indices than standard autopsy in < 14 weeks gestation (22/440 vs 48/348 respectively; p<0.001).

Histology of macroscopically normal viscera is valuable in the investigation of infant and childhood deaths. However, it provides almost no useful information relevant to cause of death or main diagnosis (<1%) in fetal cases.

Conclusions

MinImAL examination offers a reliable method of internal examination and tissue sampling, which may be acceptable when standard autopsy is declined.

Micro-CT provides an accurate, non-invasive method for phenotyping early gestation fetal anatomy.

Histological sampling of macroscopically normal visceral organs is valuable when investigating infant or child deaths but of limited value in fetal loss and hence should not be routinely performed.
Impact Statement

It is increasingly apparent that standard perinatal autopsy is not acceptable to most parents; however, up to 90-95% of parents would still accept some (less invasive) form of investigation after fetal loss. This study provides an evidence base that could be used to change the way perinatal autopsy services are offered. A number of less invasive options have been evaluated in this thesis and have been shown to be feasible for use in clinical service. The methods presented are already in use as part of the clinical autopsy service at Great Ormond Street Hospital. In particular:

1. **Minimally Invasive Autopsy with Laparoscopy (MinImAL)** can be performed as part of a perinatal autopsy service, with high procedure completion rates (91/93, 97.8%) and good tissue sampling success rates (100% in lung, kidney, heart), across a wide range of gestational ages and in most clinical scenarios.

2. **Micro-CT** can provide non-invasive, high-resolution, 3-dimensional volumes of human fetal anatomy, which can be stored as part of a patient record for re-examination at a later date. It is an especially good imaging modality for fetuses <20gw.

Chapter three (regarding the yield of invasive autopsy and tissue sampling) is likely to be controversial among my pathologist colleagues. I hope that it will be useful to clinicians and parents, when considering what kind of
investigation after death may be most useful (and most acceptable) in answering underlying clinical questions. I also hope that this chapter will stimulate pathologists and fetal medicine specialists to consider developing novel approaches to the investigation of intrauterine fetal death beyond the perinatal autopsy. The findings of this chapter urgently reinforce the need for further placental research and highlight the diminishing returns of invasive autopsy in the context of intrauterine demise.

3. Interrogation of the GOSH autopsy database demonstrated that organ histology is valuable in the investigation of infant and childhood deaths. However, histological sampling of macroscopically normal organs in fetal cases provides almost no (<1%) useful information, yet consumes resources, and hence should not be routinely performed.

Although acceptability to parents is yet to be formally evaluated, there has been a high demand for MinImAL procedures and micro-CT scans from both parents and clinicians. Economic cost-benefit analysis would be useful in evaluating wider implications for training and resource allocation to take this work forward.
Key academic achievements from this thesis

Royal College of Pathologists Silver Research Medal

Rapid translation of MinImAL and micro-CT autopsy into full clinical services at Great Ormond Street Hospital.

Successful academic collaboration with Nikon Metrology, leveraging over £800,000 of funding for micro-CT technology at Great Ormond Street Hospital and UCL GOS-ICH.

36 published peer-reviewed manuscripts arising from work undertaken during the production of this thesis (nine as first author or joint first author)

Three articles featured on the cover of journals

Four prizes for best oral presentation relating to this work

Speaker at eight international or national conferences / meetings
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Part one: General introduction

The introductory chapters contained within part one of this thesis will explore the perinatal autopsy and its current status, the rationale for minimally invasive perinatal autopsy, the relevant research questions raised by current practice and evidence, and present the experimental techniques employed throughout the remainder of the thesis.
Chapter one: The perinatal autopsy

1.1.1 Introduction

*Perinatal:* Greek: ‘peri’ (around, about, near) + ‘natal’ (birth, origin)

*Autopsy:* Greek: ‘autos’ (self) + ‘optos’ (seen); or ‘autoptēs’ (eyewitness)

The perinatal time period is defined differently by various agencies and may be applied from the perspective of either the fetus / infant (for example, referring to growth and development) or the mother (for example, referring to maternal mental health). This thesis will consider the perinatal period to extend to cover the entire gestational period of pregnancy up to and including 28 days after parturition.

Perinatal morbidity and mortality exert a considerable toll on the health of populations globally, with people in developing countries most at risk of adverse outcomes. In the UK, one in five pregnancies ends before 24 weeks (miscarriage or early intra-uterine fetal death (IUFD)). Historically, these events are not formally registered, and detailed statistics regarding miscarriages often remain estimations, as many women may not present to medical practitioners following an early miscarriage. In addition to this burden, around 1/80 pregnancies end in termination for fetal abnormality, stillbirth (fetal death over 24 weeks gestation, with no signs of life at delivery) or neonatal death (death up to 28 days after parturition).\(^1\)\(^3\) Fetal loss can have profound implications for maternal mental health extending beyond the affected pregnancy, with women who experience fetal loss at risk of mental
health sequelae such as depression,\textsuperscript{4} in addition to the stigma of the event itself, which may lead to societal isolation, both at a family and community level, as fetal deaths may be viewed as a maternal failure.\textsuperscript{5,6} Opportunities to limit or prevent the medical and psychological fallout of fetal loss therefore represent a substantial challenge to the medical community, being complex in aetiology, difficult to predict, and profound in effect.

Following fetal demise, an autopsy may be performed in order to elucidate contributing factors in the death. It is estimated that information gleaned from the perinatal autopsy process contributes to the management of future pregnancies or the healthcare of a living sibling in approximately 30–40\% of cases.\textsuperscript{7} Unlike in adult practice, the majority of autopsies within the perinatal age spectrum are consented procedures; however, overall, the procedure remains poorly accepted by parents,\textsuperscript{8} especially among certain ethnic and religious groups,\textsuperscript{9–11} with autopsy rates falling globally. The All Wales Perinatal Survey Commentary published in 2013 revealed a fall in the proportion of perinatal deaths in which consent was granted for autopsy from 58.9\% in 2003 to 49.6\% in 2013, though the number of deaths and the percentage in which consent for autopsy was sought remained approximately the same.\textsuperscript{12} Although Judaism and Islam do not prohibit the autopsy process per se,\textsuperscript{10,11} especially if a living relative may benefit from the investigation, the autopsy process may be construed to interfere with societal norms and practices that follow death, including timely burial (usually before sunset), ritual washing of the body and the belief that the body of the deceased should not be left alone. Additionally, the autopsy procedure itself may be viewed as mutilating.\textsuperscript{10,13}
Aside from moral or religious reasons, published evidence demonstrates that bereaved parents have an aversion to large incisions due to perceptions that the fetus or infant has “suffered enough”.\textsuperscript{14} From a clinical perspective, there is also a perception that autopsy reports vary in adequacy, alongside perceived difficulties in negotiating a highly specific informed consent process at an emotionally charged moment.\textsuperscript{15}

In contrast with medico-legal death investigation (discussed later), the overarching aim of a “parentally consented” perinatal autopsy is to provide parents and their clinicians with as much clinically useful information as possible within the limitations of parental consent. This could include:

- Any underlying diagnosis / ‘positive’ or abnormal findings
- Important ‘negative’ findings / to exclude certain diagnoses
- The likelihood of recurrence
- Implications for the medical management of future pregnancies
- Implications for living siblings, or parents themselves.

Historically, the perinatal autopsy has provided important insights into normal developmental processes and pathogenesis of congenital anomalies (Figure 1). However, the introduction of widespread first and second trimester antenatal ultrasound screening and the advent of cell-free DNA analysis has resulted in accurate antenatal detection of a wide range of fetal abnormalities.\textsuperscript{16-18} Consequently, the role of the autopsy is also changing, with detection of unexpected major fetal anomalies now less frequent, but the range of antenatal
fetal interventions and complexity of associated pathologies increasing. In addition, mechanistic data derived from many years of autopsy practice, which have provided improved understanding of numerous obstetric complications, is becoming increasingly less likely to generate new non-hypothesis based insights in the absence of the introduction of new and novel approaches.

The concept of the autopsy examination as a means of medical audit and governance remains important (for example, for terminations of pregnancy (ToP) or after complex medical treatment). However, where there are no specific clinical questions to be answered the additional clinical benefits of autopsy examination are unclear.
In addition to consented autopsies, (i.e. those performed with the consent of the parents), some autopsies are mandated by the legal framework within which an infant or child has died. Medico-legal autopsy investigations in the perinatal age range are relatively rare and usually occur in neonates that die suddenly and unexpectedly. The legal system governing the extent of investigation after death varies by country (e.g. Coronial system in England, Wales and Northern Ireland, and a more European (Procurator Fiscal) system in Scotland). Although there are procedural differences between regions, the
The overarching purpose of medico-legal death investigation within the UK is broadly comparable with systems in other Western countries. Depending on the scenario, the aim of a medico legal autopsy may be very different to those of a consented autopsy. Consider the example of HM Coroner, whose interest in a death is governed by laws to a strict legal remit, including:

- Who the deceased was
- How, when and where the deceased came by his or her death
- Whether the death was natural or unnatural

By investigating and establishing the facts around these tenets, the Coroner fulfils their legal duty, and their interest in the case ends. The scope of a post-mortem examination directed by a Coroner in order to fulfil the aforementioned Coronial remit will generally be extensive, and may involve multiple ancillary tests, including review of the scene of a death, toxicological analysis, and microbiological investigations. In some cases, to investigate possible criminality, full forensic autopsy techniques (including flaying of skin, removal of eyes, spinal cord and cervical spine) may be undertaken as part of a police investigation.

There is some evidence that the medico-legal system is changing to adapt to new technological possibilities and public attitudes. Although HM Coroners are legally required to direct an investigation into certain deaths, full autopsy may not be required in all cases. The Coroners and Justice Act 2009 permits “a registered medical practitioner” to “undertake a post-mortem examination” upon direction of the investigating Coroner; this legal phraseology could, in
future, encompass post-mortem imaging interpreted solely by a radiologist, although excluding pathologists from the chain of investigation entirely would be extremely controversial. Nevertheless, the 2009 Coroners and Justice Act recognises that medical methods of investigation after death are evolving by specifically providing the option for radiology-based investigations (either reported solely by a radiologist, or, more likely, interpreted in conjunction with a pathologist) as a viable option for future practice. In the context of perinatal and paediatric autopsy, this could indicate a willingness to shift towards the use of PMMRI (either as a primary method of investigation or as an adjunct), however, evidence of scenarios where invasive tissue sampling is likely to be useful (and how it can be optimally performed in cases where autopsy is strongly opposed by the family) is still emerging. It is the author’s hope that the methods and results presented within this thesis will help to clarify how minimally invasive methods of examination in the context of perinatal and paediatric autopsy can be applied in practice, with potential future applications in both consented and Coronial autopsies.
1.1.2 Elements of the perinatal autopsy

The traditional perinatal autopsy consists of several important components, all of which are incorporated into an overall autopsy report. Evidence suggests that the yield of clinically useful information is greater when perinatal autopsies are performed by specialist paediatric and perinatal pathologists (when compared to general pathologists), and it is therefore recommended that where possible all perinatal autopsies should be performed in specialist centres. An overview of the processes involved and the types of pathologies that may be detected at each stage is described below.

1. Clinical review

Following receipt of appropriate authorisation for the autopsy examination (either via parental consent or Coronial Authority), the pathologist reviews the medical notes, including the findings of antenatal imaging, other investigations and care provided; the maternal medical, obstetric and gynaecological history, and the circumstances of delivery / death.

This information enables the pathologist to target the examination appropriately to better address important clinical questions such as identification of potential genetic conditions and may influence the performance of subsequent aspects of the investigation. This step remains essential for the less invasive autopsy, as the choice of post-mortem imaging is dependent on the age/weight of the fetus. Additionally, review of the notes often raises clinical questions that may not be immediately apparent from the initial referral.
2. External examination

A detailed external examination of the fetus should be carried out to include identification of subtle dysmorphic features that may be difficult or impossible to identify sonographically, such as some types of facial dysmorphism, posterior cleft palate and abnormalities of the external genitalia. This step remains essential for the less invasive autopsy, as some external abnormalities may be difficult to pick up from imaging alone and may greatly influence diagnoses where present (e.g. post-axial polydactyly may be difficult to detect in an early gestation termination for skeletal dysplasia from imaging alone).

3. Post mortem imaging

Either before or following external examination (but prior to internal examination), a range of post-mortem imaging investigations may be undertaken. Typically, this included a single or series of radiographs of the whole body, to identify skeletal abnormalities. More recently, cross-sectional imaging (CT & MRI) and ultrasound examination has become available. The choice of an appropriate imaging modality is an important factor when considering the overall LIA process, as some modalities provide more information in certain clinical circumstances than others. Imaging should be performed prior to any internal examination, as the results of imaging should be correlated with the antenatal history and used by the pathologist to inform and refine the approach to internal examination. Moreover, the anatomical disruption caused by internal examination would be likely to
render post-autopsy imaging useless in terms of anatomical visualisation and introduce a myriad of additional artefacts due to tissue disruption.

4. Internal examination

Traditional autopsy includes systematic examination of all internal organs, within the limits of the authority provided to the pathologist by the consent. Parents may wish to limit the examination to a specific organ(s) or body cavities if there is a query about a particular diagnosis or clinical issue. Standard open internal examination is performed via a large midline incision from manubrium to pelvis (Figure 2). Following removal of the ribcage, internal organs are then inspected, examined and removed to be weighed and dissected.
Figure 2 Reconstructions of a standard autopsy incision in a neonate (A) and a mid-trimester fetus (B). The incision line in B is highlighted, as the skin has been apposed using tissue glue. Figure adapted from Sebire et al. with permission.
Minimally invasive approaches, such as laparoscope-assisted techniques, may be used at this point, in conjunction with post-mortem imaging, to obtain tissue samples via a much smaller incision (a 1-2cm incision permits sampling of all abdominal and thoracic organs). A laparoscopic approach allows direct visualisation of fetal organs, and permits photography, video recording and sampling but its accuracy for specific diagnoses compared to standard autopsy across a range of clinical scenarios remains to be established. Regardless of approach, in-situ sampling for microbiology, genetic studies, virology and histology can be performed. Organs are then returned to the body according to the wishes of the parents.

If formal neuropathological examination is required, for example following termination of pregnancy for a central nervous system abnormality, then the standard approach is to remove the brain for a period of fixation prior to dissection and sampling. This is especially required for fetal cases in whom the brain contains relatively little myelin and is therefore extremely friable making examination of the unfixed brain effectively impossible. However, for many structural central nervous system abnormalities, post-mortem imaging approaches provide excellent anatomical detail and it is likely that the requirements for brain removal and formal neuropathological examination in this setting may reduce in future.
5. Histological examination

The primary purpose of histological sampling (Figure 3) is to provide a morphological diagnosis of pathology and to exclude or confirm the presence of disease. Published autopsy guidelines recommend histological sampling of most major internal organs for perinatal post-mortem examinations regardless of clinical indication.\textsuperscript{33} While tissue diagnosis remains critically important in some scenarios, (for example diagnosis of subtypes of cystic kidney diseases and infant death), it may be of less value in IUFD, where the antenatal imaging and macroscopic examination of the organ is normal. It is therefore possible that more individualised and limited sampling protocols may be indicated cases according to the clinical features present, without reduction in diagnostic accuracy of the overall procedure.

Figure 3. A standard tissue block and slide, illustrating a typical tissue sample.
With technological laboratory advances it is however likely that in future such samples will undergo a range of genomic, proteomic, metabolomics (Figure 4) and other investigations, resulting in improved diagnostic accuracy from smaller tissue samples, reducing further the sampling requirements.\textsuperscript{21,34}

Figure 4. In sepsis-related deaths, proteomic analysis reveals a change in the glycosylation profile of alpha-1-antitrypsin (red arrows) when compared with non-infective deaths (black arrows). Image adapted from Hutchinson et al.\textsuperscript{21} with permission.
6. Placental examination

The placenta is the single most important investigation to determine the cause of intrauterine fetal death\textsuperscript{35,36} and it should always be submitted for examination with the fetus or infant for perinatal post-mortem examination. A number of pathologies with significant recurrence risks may also be identified on placental examination, such as villitis (Figure 5), chronic histiocytic intervillositis and fetal thrombotic vasculopathy. These may or may not be apparent at macroscopic examination.

The pathologist examines the gross placental specimen for abnormalities prior to slicing it for assessment of parenchymal lesions and to obtain placental tissue samples for histological examination. Samples may also be taken whilst fresh for fetal DNA analysis.

Figure 5. Villitis of unknown aetiology on light microscopy (Haematoxylin & eosin stain, 40x magnification, asterisk = affected region)
7. Ancillary investigations

Samples may be required for genetic studies / fibroblast culture; these should be acquired as soon as possible following delivery to maximise the chance of a successful result.

8. Retention of Organs

In some circumstances, it may be necessary for the pathologist to temporarily retain an organ for fixation and further detailed examination. This is particularly the case when considering pathology involving the central nervous system, since the fetal brain is extremely soft and difficult to assess when fresh, and important diagnostic information may be lost if it is not thoroughly formalin-fixed prior to dissection. Temporarily retaining the organ will allow for fixation to occur over a period (typically around 1-2 weeks) and following the examination and sampling, the retained organ can be reunited with the body prior to funeral arrangements. If the delay in burial is seen as a problem, the organ can be released to parents via an undertaker for burial after the initial funeral arrangements if required or parents may request that the organ is sensitively disposed of by the hospital, or may donate it for audit, teaching and research.

9. The Post-mortem Report

A complete perinatal post-mortem report which describes all of the significant macroscopic and microscopic findings, the results of ancillary investigations and a specific summary of the findings with clinicopathological correlation is
usually issued to the clinical lead approximately 6-8 weeks after the autopsy takes place.

Following autopsy, the body may be viewed or can be immediately released to the family, depending on funeral arrangements. Fetuses may receive a service, or can be respectfully disposed of by the hospital, according to parental wishes.
1.1.3 Effectiveness of the perinatal autopsy

The clinical indications for perinatal autopsy vary depending upon the gestation of the fetus, the clinical circumstances of the death and the previous medical and obstetric history of the parents. As such, defining the likely value or effectiveness of an autopsy is difficult, as an underlying clinical question (when not provided directly within a referral) must be interpreted by the pathologist performing the examination from the available information. However, a prospective assessment of the value / effectiveness of an autopsy procedure seems like a critical piece of information that would be of potential benefit to parents considering whether to consent to a full autopsy. In the clinical care of live patients, such information would be considered essential as part of the process of obtaining informed consent from the patient, for example, prior to an elective operation.

An effective autopsy could therefore be one which, in the case of a suspected fetal anomaly, either confirms the clinical impression or changes the diagnosis; seemingly contradictory outcomes, which both have value. In addition, an entirely negative autopsy examination may have the effect of reassuring parents and clinicians that nothing further could have been done to prevent the death or ruling out a potential genetic cause that may be known to occur within the family. The somewhat nebulous nature of the ‘effectiveness’ of the perinatal autopsy in this is not aided by clinical studies demonstrating a wide variation in value, with one review demonstrating that autopsy resulted in a change in diagnosis or additional findings in between 22-76% of cases.
1.1.4 Rationale for the development of less invasive autopsy

Mirroring the trend in consented adult autopsy, there has been a general decline in the proportion of perinatal deaths in which parental consent is granted for autopsy (Table 1).\textsuperscript{23,37} This trend has not been as dramatic as the decline seen in adult practice, where the consented autopsy is now a rarity.\textsuperscript{38} This decline has coincided with numerous tissue retention scandals and the subsequent introduction of the Human Tissue Act 2004.\textsuperscript{39} Further pressure was heaped onto adult autopsy practice following the National Confidential Enquiry into Patient Outcome and Death (NCEPOD) in 2008,\textsuperscript{40} which focussed on the Coroner’s Autopsy, and found that one in four autopsy reports was of unacceptable quality when judged by peers, with “the fact that there is no [public] outcry...a manifestation of the fact that families are unaware of the variable quality of the autopsy procedure”. In general, the report also found that “SpRs and paediatric pathologists produced better quality reports than other consultant histopathologists and Home Office pathologists”. Moreover, due to major variations in the laws governing investigation of deaths between England/Wales and Scotland, there exists a gross disparity in the number of deaths sent for invasive autopsy via the Coronal system (in England, Wales and Northern Ireland) and the Procurator Fiscal system in Scotland, with many of the autopsies in England being viewed as excessive, particularly by Jewish and Muslim families, who have actively lobbied for the introduction of alternative methods of death investigation.\textsuperscript{41}
It is likely that these factors and events have contributed to heightened public awareness of tissue sampling and retention as part of the post-mortem examination. Negative perceptions of pathology and the autopsy may therefore be complicating already difficult discussions between clinicians and relatives regarding the consent process for autopsy.\textsuperscript{38,42} Additionally, many medical professionals now no longer attend autopsies as part of their routine medical training,\textsuperscript{43,44} potentially leading to the portrayal of the autopsy as an opaque, outmoded procedure guarded by eccentric and paternalistic doctors.

Some evidence of these perceptions was described in a study on attitudes to the adult autopsy by Loughrey et al in 2000, shortly after the investigation into the Alder Hey organ retention scandal was established.\textsuperscript{42} The most important barriers in the minds of clinicians in this study were “difficulty obtaining consent from relatives because of their perceptions of the autopsy”, “advances in modern diagnostic techniques reducing the need for autopsy” and “unavailability of reports in clinically relevant time”. It is possible that by adopting imaging techniques as part of the autopsy examination, professional attitudes towards the process will soften, as the autopsy process could be seen to be more transparent and co-operative in nature, thus reflecting more general changes in modern medical practice by clinical practitioners (e.g. increasing use of imaging, collaborative decisions based on multiple professional opinions).
<table>
<thead>
<tr>
<th>Post-mortem status</th>
<th>Stillbirths§</th>
<th>Neonatal deaths§</th>
<th>Extended perinatal deaths§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not offered</td>
<td>50 (1.6%)</td>
<td>137 (10.0%)</td>
<td>187 (4.1%)</td>
</tr>
<tr>
<td>Not known if offered</td>
<td>67 (2.1%)</td>
<td>155 (11.3%)</td>
<td>222 (4.8%)</td>
</tr>
<tr>
<td>Offered but no consent</td>
<td>1503 (46.6%)</td>
<td>628 (45.7%)</td>
<td>2131 (46.3%)</td>
</tr>
<tr>
<td>Offered but unknown consent</td>
<td>83 (2.6%)</td>
<td>54 (3.9%)</td>
<td>137 (3.0%)</td>
</tr>
<tr>
<td>Offered and limited consent</td>
<td>120 (3.7%)</td>
<td>28 (2.0%)</td>
<td>148 (3.2%)</td>
</tr>
<tr>
<td>Offered and full consent</td>
<td>1402 (43.5%)</td>
<td>372 (27.1%)</td>
<td>1774 (38.6%)</td>
</tr>
</tbody>
</table>

Table 1. Number of post-mortems offered and consented to by type of death (stillbirth, neonatal death, extended perinatal death): United Kingdom & Crown Dependencies, for births in 2014. Reproduced from Manktelow et al MBRRACE-UK Perinatal Mortality Surveillance Report.\textsuperscript{37}

§ excluding termination of pregnancy and births <24+0 weeks gestational age
That the perinatal autopsy rate has not declined as severely as the adult equivalent may be related to the strong support that perinatal pathologists have received from fetal medicine specialists, obstetricians, clinical geneticists, and multiple charities. A joint working group between The Royal College of Pathologists and The Royal College of Obstetricians and Gynaecologists recommended that autopsy rates should remain above 75%.45 In 2010, Green Top Guidelines were issued by the Royal College of Obstetricians and Gynaecologists to ensure that clinical staff remained aware of the indications for autopsy and that suitable numbers of clinical staff were trained in consent taking.46

Research into parental attitudes to autopsy has revealed that traditional post-mortem examination is becoming less acceptable, especially among certain ethnic and religious groups.9,11,47 This has been seen worldwide, with autopsy rates falling in most countries. It is possible that negative perceptions of the autopsy are being accentuated by recent advances in medical imaging, increasing use of ‘Omic’ technologies, shifting population demographics and political pressure to encourage of development of potentially more acceptable, contemporary approaches.48

In addition to moral or religious reasons (Table 2), parents may experience emotional distress at the thought of an autopsy procedure, or the wait for the results.8,11 “The concept of “Investigation After Death” may therefore more accurately reflect the future of this approach, with personalised investigations
performed targeted to address the specific issues of particular cases, to improve the quality of information gained and increase parental acceptability.

<table>
<thead>
<tr>
<th>Religion</th>
<th>Autopsy</th>
<th>Tissue retention</th>
<th>Disposal of the body</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atheism</td>
<td>No prohibition</td>
<td>No prohibition</td>
<td>Burial or cremation</td>
</tr>
<tr>
<td>Christianity</td>
<td>No religious prohibition</td>
<td>No religious prohibition</td>
<td>Burial or cremation</td>
</tr>
<tr>
<td>Hinduism</td>
<td>No religious prohibition</td>
<td>No religious prohibition</td>
<td>Cremation without delay</td>
</tr>
<tr>
<td>Islam</td>
<td>Only if required by law</td>
<td>Only if required by law</td>
<td>Burial without delay</td>
</tr>
<tr>
<td>Judaism</td>
<td>Only if required by law</td>
<td>Only if required by law</td>
<td>Burial without delay</td>
</tr>
<tr>
<td>Sikhism</td>
<td>No religious prohibition</td>
<td>No religious prohibition</td>
<td>Cremation without delay</td>
</tr>
</tbody>
</table>

Table 2. Religious attitudes to autopsy within the UK. Adapted from Rutty et al.¹¹
1.1.5 Laparoscopic autopsy: Reconciling minimally invasive autopsy techniques with invasive examination

Currently, in most centres practicing paediatric and perinatal autopsies within the UK, the alternatives to a full autopsy examination are either no autopsy examination, or an external examination of the body with examination of the placenta only. Arthurs et al make the case that “it is implicit in the idea of ‘investigation after death’ that the level of invasiveness of the examination will vary from case to case”, depending on a combination of the clinical question to be addressed and the level of consent provided by the parents. A spectrum of investigations that reflect both the complexity of fetal medicine and the need to take parents’ wishes into account would arguably be more appropriate than the binary choice of ‘full autopsy with histology’ or ‘placenta only / external examination only’ currently offered. For example, in some cases, post-mortem imaging providing confirmation of an antenatally known abnormality may be sufficient to answer the clinical question whilst minimising parental distress. In other cases, histological sampling may be important to obtain a definitive diagnosis and counsel parents appropriately (e.g. presentation with bright, enlarged kidneys on fetal ultrasound suspicious of polycystic kidney disease). Furthermore, as histological sampling of major organs has been shown to be of paramount importance in the investigation of infant deaths, the clinical paradigm of ‘full autopsy’ or ‘no autopsy’ seems unacceptable to the majority of parents who suffer a neonatal death, where autopsy acceptance rates are approximately 25% (Table I). In practice, the act of organ sampling
can be performed via a 1-2cm incision in the abdomen if indicated in the clinical history or if an abnormality was shown on imaging. In the aforementioned example of suspected polycystic kidney disease, the kidneys could be sampled via a laparoscopic approach in order to obtain a tissue diagnosis and inform the clinical team and parents of the risk to future pregnancies. If the parents consented, other organs could also be sampled through this “key-hole” approach, which is termed the MinImAL examination (Minimally Invasive Autopsy with Laparoscopy) in this thesis. Approaching cases in such a manner should ensure that diagnostic yield is maximised, with minimal cosmetic effects, given that removal of entire organs can be performed via a 2cm incision in the upper abdomen. Laparoscopic approaches also facilitate visualisation of organs in situ.
Figure 6. Post-procedure (A, C) and post-reconstruction (B, D) images of a neonate (A, B) and a term stillbirth (C, D) following the MinImAL technique, presented in part 2 of this thesis. Adapted from Hutchinson et al. with permission.115

A recent retrospective study examining the utility of routine organ histology within the context of intrauterine fetal death demonstrated only 1 case from a cohort of >1,000 where the cause of death was demonstrated within organs that were antenatally, macroscopically and radiologically of normal
morphology (where placental examination was available).\textsuperscript{54} This finding is in direct contrast with that of Weber et al, who demonstrated the importance of routine histology within the context of SUDI investigations.\textsuperscript{51} This serves to highlight that the importance of histology (previously considered to be a lynchpin investigation as part of an ‘invasive’ traditional autopsy) is likely to vary with the context of the death and the clinical history provided to the pathologist. Although this logic appeals to common sense, there is little published literature regarding the overall diagnostic yield of histology in perinatal autopsy, and therefore a general approach of ‘sampling everything’ has prevailed until very recently, when evidence has begun to emerge of the diagnostic yield of macroscopic examination, routine placental histology and routine organ histology in the context of IUFD.\textsuperscript{54-57} There remains no published evidence regarding the diagnostic yield of routine visceral histology in terminations of pregnancy (either for organs thought to be abnormal, or otherwise) and this represents a major challenge to the concept of less invasive autopsies, as the ‘sample everything’ paradigm sits uneasily alongside a minimally invasive approach to autopsy. To this end, it is intended to utilise the Great Ormond Street Hospital autopsy database (see chapter three of this introduction for a more detailed description of the database) to evaluate the utility of routine organ histology in the context of terminations of pregnancy.
Chapter two: Post-mortem imaging

1.2.1 Introduction

In response to declining autopsy rates, improvements in imaging technology and objections to Coronial autopsies from Muslim and Jewish communities, there has been interest in whether alternative methods can provide a more acceptable method of investigation after death. As a result, multiple modalities have been investigated for use either as part of the autopsy examination, or as an outright replacement. These include post-mortem ultrasound (PMUS), post-mortem magnetic resonance imaging (PMMRI), and computed tomography (PMCT). The following chapter will explore the advantages and disadvantages of these methods, alongside the published literature on each methodology. Although post-mortem imaging with plain X-rays has been performed for many years, it has been generally regarded as an ancillary investigation to the autopsy itself, with particular benefits where there are known skeletal anomalies. As such, this modality will not be considered in greater detail.
1.2.2 Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) reflects changes in the way hydrogen nuclei within a magnetic field interact with radio waves. By changing the magnetic field applied across a subject, an MRI scanner affects the way that hydrogen nuclei emit and absorb radio waves, these changes are detected by receiver coils within the scanner, and information about the state of the nuclei is built up into images following a series of computations. Typically, images of soft tissues are of excellent quality due to the variable water content of different organs.

![Figure 7. Normal appearances on PMMRI. Coronal T2-weighted PMMRI in a late gestation stillbirth, demonstrating normal physiological post mortem imaging appearances. There is intracardiac gas, pleural and pericardial effusions in the chest (black arrows), bowel dilatation and widespread subcutaneous oedema (white arrows), all of which can be misinterpreted as pathological to radiologists unfamiliar with autopsy imaging. Reproduced from Arthurs et al.\textsuperscript{63} under Creative Commons CC BY license.](image-url)
Over the last 10 years, a considerable evidence base has developed regarding the use of PMMRI for investigation of neonatal, infant and perinatal deaths, both as a stand-alone investigation, and in conjunction with ancillary investigations, which range from blood sampling to laparoscopy-assisted visualisation and sampling of internal organs. The lack of uniformity in approaches taken to investigation after death using PMMRI potentially raises complex questions regarding the ‘optimal method’ of investigation utilising PMMRI and makes comparisons between papers evaluating the diagnostic accuracy rates of forms of less invasive autopsy (LIA) and minimally invasive autopsy (MIA) involving MRI difficult to compare. These problems lie beyond the scope of this thesis; however, this section will attempt to summarise the evidence for the use of PMMRI, along with its advantages and disadvantages, along with how it will be utilised within this thesis.

Several studies have now reported that PMMRI, together with standard, non-invasive ancillary investigations (e.g. microbiology, clinical genetics and placental examination) shows high levels of agreement with full diagnostic autopsy for the overall diagnosis reached / cause of death. The largest prospective trial of PMMRI versus standard traditional autopsy (Magnetic Resonance Imaging in Autopsy: MARIAS study) reported >90% concordance in fetuses and stillbirths, and 75% concordance in children when comparing the overall cause of death provided by “minimally invasive autopsy” (this was defined in the study as: PMMRI with genetics, microbiology, virology, biochemistry and placental examination) and full diagnostic autopsy. PMMRI
alone performed worse, with concordance rates with full diagnostic autopsy falling to 42.7% in fetuses and 69.1% in children.
Figure 8. PMMRI of congenital abnormalities. PMMRI is particularly good for congenital anatomical abnormalities, such as intracranial hemorrhage, brain malformations, renal anomalies, congenital heart disease and skeletal dysplasias. This example shows bilateral enlarged high signal kidneys at coronal T2-weighted PMMR in a 27-week gestation fetus, which are classical features of autosomal recessive polycystic kidney disease (a), confirmed at microscopy (b) Adapted from Arthurs et al. under Creative Commons CC BY license.
Most diagnoses missed by PMMRI alone varied according to the age of the deceased, with placenta-related causes of death representing a major source of ‘misses’ or ‘non-discovers’ in fetuses, and occult infection (pneumonia, sepsis, meningitis) representing the majority of ‘misses’ or ‘non-discovers’ by MRI in older children.

In addition to a high concordance rate, formal evaluation of the acceptability of LIA demonstrates that this an acceptable approach for many clinical staff, parents, Coroners and religious groups.\textsuperscript{14,64,65} In some cases, parents may not agree to any form of tissue sampling, but post-mortem imaging combined with ancillary investigations, such as placental histological examination, can still provide useful clinical information. For example, post-mortem scans can be compared with clinical imaging (either antenatally or during life if the death is neonatal) to refine diagnoses; additionally, ‘negative’ results may help to rule out specific entities or provide reassurance to parents of the absence of anomalies. In cases where a structural organ abnormality is detected, PMMRI may be used to inform less invasive organ examination and tissue sampling in conjunction with PMUS or laparoscopic examination.\textsuperscript{31,66} Where 3D volumetric imaging sequences have been obtained, the images can be reconstructed in non-anatomical planes.

In imaging childhood deaths, PM imaging can be particularly useful to delineate traumatic injuries prior to autopsy, including any internal haemorrhage, visceral or mesenteric injury associated with blunt or penetrating trauma to the body. It can give precise details about soft tissue
injuries associated with rib fractures, the severity of intracranial injury, and soft tissue injuries. PMMRI can be useful to show complications from inaccurate intraosseous needle placements, including unilateral soft tissue edema or gas tracking up the lower limbs.

PMMRI has some specific advantages over PMCT when considering perinatal and childhood deaths. PMMRI sequences facilitate soft tissue differentiation and are therefore particularly useful to clinicians when seeking to confirm or rule out potential congenital or birth-related abnormalities, including intracranial haemorrhage, brain malformations, renal anomalies, congenital heart disease and skeletal dysplasia.29

The limitations of PMMRI include reaching the limits of diagnostic accuracy due to lower spatial resolution (at 1.5 T / 3 T field strengths) for smaller fetuses and poor ossified bony detail, which can be of particular interest when a known musculoskeletal disorder is present.67 While PMMRI provides excellent anatomical detail in third trimester fetuses and infants, due to fetal size and limits of resolution, such methods are less effective below 18 weeks of gestation / fetal weight of 400g.28 PMMRI is also poor at delineating microscopic changes, such as renal dysplasia and disseminated sepsis, which are ultimately beyond the capacity of 1.5 T MRI to resolve, and which may have no imaging correlate in any other imaging modality. Even with the increasing availability of 3 T MRI, initial evidence suggests that there is little improvement in the diagnostic limit of around 18gw in fetal imaging.68 Additionally, as expected for a relatively new technique, there is a learning
curve for both PMMRI acquisition and reporting. Recognition of normal post-mortem changes, such as fluid redistribution / post-mortem lividity (in the form of subcutaneous oedema, pleural and pericardial effusions and ascites) can be challenging to radiologists unfamiliar with autopsy work, and determination of normal PM changes from pathologies (e.g. pulmonary lividity vs. pneumonia) also remains challenging in some areas.\textsuperscript{69,70} On a practical level, MRI slots are often in high demand, and post-mortem work will inevitably be lower priority than work in living patients. As a result, it can be difficult to obtain slots, which may lead to delays in post-mortem imaging that could be unacceptable to Jewish or Muslim families.\textsuperscript{65,71}
Figure 9. PMMRI from a one month old, reported as patchy consolidation suggestive of infection (A) but with histologically normal lungs (including post-mortem artefact, B). Adapted from Arthurs et al.\textsuperscript{112} under Creative Commons license (Attribution-Noncommercial).
1.2.3 Computed Tomography

Computed Tomography (CT) utilises differences in X-ray attenuation within the subject under examination to produce a cross-sectional image. Multiple X-rays are taken from different angles, and the resulting projections are reconstructed using a computer algorithm to provide a cross-sectional image of X-ray attenuation within the subject. As soft-tissues naturally exhibit only small differences in X-ray absorption, CT provides poor soft-tissue detail, with minimal contrast between most organs, though calcification, haemorrhage and bony pathologies are all well visualised using CT.

PMCT has been increasing in popularity as an alternative to post-mortem examination in adults, particularly with the addition of vascular contrast medium to create PMCT angiography. The main advantages of PMCT over PMMRI are speed of acquisition, availability in most hospitals, and the better bone detail that is achieved using unenhanced CT. Volume rendering techniques enable a 3D skeleton of the deceased to be reconstructed and can allow for improved assessment of some fractures (particularly useful when differentiating skull fractures from sutures in paediatric cases). Whilst intravascular contrast enhanced PMCT methods exist, this is not currently widely employed in the perinatal paediatric setting. In children and perinatal cases, PMCT has several disadvantages, including reduced soft tissue contrast due to reduced abdominal and subcutaneous fat, poor soft tissue contrast in the brain, and without vascular contrast medium, assessment of the thoracic and abdominal cavity is particularly challenging.
Unenhanced PMCT has been shown to perform worse than PMMRI in children.\textsuperscript{29,63} The main problem with PMCT is that it is mostly non-diagnostic in the smaller bodies which are often referred for perinatal autopsy. Without the addition of intravenous contrast (via femoral, umbilical vessels or direct intracardiac injection) for angiography or ventilating the lungs to improve lung imaging,\textsuperscript{73} PMCT typically performs worse than PMMRI apart from for bone imaging. PMCT is becoming particularly useful at detecting rib fractures.\textsuperscript{74} As both PMCT and PMMRI become more widely used in perinatal autopsy and the forensic setting, their diagnostic utility will become established as both radiologists and pathologists gain experience.

These alternative imaging-based investigations could potentially greatly expand the range of post-mortem approaches available to pathologists, clinicians and families and ‘investigation after death’ will better serve to represent this possible future.
1.2.4 Ultrasound imaging

Ultrasound generates an image by dynamic analysis of high frequency sound waves (typically 5 – 10 kHz) that are generated via a probe applied to the skin or mucosal surface of the subject. The sound waves emitted by the probe are transmitted and absorbed through various tissues differently (due to differences in acoustic impedance), with some waves reflected back to the probe as a result of this process. The resultant images are a dynamic analysis of the reflected waves. This allows for real-time, non-invasive, gross evaluation of solid organs, including the fetal brain, along with the potential for guided sampling.

The success rates of this style of sampling are currently poor, with studies demonstrating a large number of inadequate samples.\textsuperscript{75,76} Due to differences in acoustic impedance between solid organs and liquids, ultrasound can spot free fluid that may be indicative of internal bleeding, or perforation of an organ, however, due to the high acoustic reflectivity of air, ultrasound cannot visualise structures lying beneath a few millimetres of air. This can be problematic with some structures, such as bowel loops within the abdomen, or in decomposing bodies.

One recent study considered the diagnostic accuracy of PMUS examination (without sampling) in 123 cases performed across two specialist centres. It found overall sensitivity and specificity values of 74.7% (CI: 64.8 – 84.5) and 83.3% (CI: 70.0 – 92.5%) respectively, with 78% concordance (CI: 70.7 – 85.4%) with full autopsy.\textsuperscript{77}
Figure 10. (A, B) Post-mortem imaging in a fetus terminated at 29 weeks due to polycystic kidneys with severe oligohydramnios: (A) sagittal ultrasound image of fetal abdomen at level of left kidney showing enlarged kidney with loss of corticomedullar junction (> 90th percentile), measuring 41.1 x 23.2 mm, compatible with polycystic kidney; (B) coronal T2-WI magnetic resonance image showing enlarged hyperintense appearance of both kidneys. (C) Imaging in 26-week fetus with multicystic renal dysplasia. (D) Patient at 29 weeks of gestation showing normal appearance of kidney. Adapted from Kang et al. with permission.
1.2.5 Microfocus computed tomography

One potential solution for imaging smaller fetuses is microfocus computed tomography (micro-CT). Micro-CT can provide images with sub-micron voxel resolutions. To achieve this, micro-CT utilises several techniques that differ from clinical CT acquisitions (geometric magnification of the object, pinhole camera effect, and prolonged exposure times); these techniques are described in more detail in part four of this thesis. Although these techniques result in improved geometric resolution in the final imaging volume, they also limit the use of micro-CT to non-living patients unless substantial modifications are made to limit radiation exposure, as they require the subject to remain immobile for relatively long periods and increase the amount of ionising radiation received by the object of interest. As a result, applications of micro-CT within the medical field have been relatively limited to date.

Figure II. Exterior view of a Nikon Med-X micro-CT system with an adjacent image reconstruction station.
Micro-CT has been used extensively in industry for quality assurance and non-destructive testing, particularly where strict tolerances of parts are required, or where performance of a part is vital to the safe function of a system (e.g. aircraft engine turbine blade inspection). Micro-CT also plays an important role in archaeology, where historical artefacts can be analysed and ‘virtually dissected’ to investigate their contents, thereby minimising disruption and degradation due to handling. Use of micro-CT has also been well described in small animal phenotyping, bone morphology analysis, and plant biology.

The quality of images achievable using micro-CT makes it an ideal tool for examination of ex-vivo and post-mortem human specimens where conventional clinical concerns regarding minimising radiation exposure and long periods of inactivity are largely irrelevant. Resolutions comparable to high-power histology can be achieved with adequate magnification, alongside the additional benefit of an isotropic three-dimensional dataset, without requiring destruction of the specimen or labour-intensive practices, as necessary for High Resolution Episcopic Microscopy (HREM) and Episcopic Fluorescence Image Capture (EFIC).
Within the clinical environment, X-Ray based investigations are typically used to delineate bone pathologies (e.g. fractures, tumours), pathologies involving abnormal calcification (e.g. nephrolithiasis, breast pathologies including ductal carcinoma in situ) or where there are significant changes in the density of a particular soft tissue associated with a disease process (e.g. changes in lung density in organising pneumonia, pleural effusions and pneumothorax). For imaging of solid organs or soft tissue using clinical CT or plain X-ray radiography, a contrast medium is usually administered; this is because even though soft tissue components have very different structural properties, they differ little in terms of X-Ray attenuation. This is reflected by the National
Institute of Science and Technology (NIST) X-ray attenuation database, which provides a single attenuation graph for soft tissue X-Ray attenuation for modelling purposes, encompassing skin, fibrous tissue, muscle and fat. In the clinical environment, intravenous or oral contrast agents used typically contain iodine or barium ligands. These contrast agents can also be used in micro-CT acquisitions, however, alternative routes of administration (not requiring an intact circulatory system) may be required in post-mortem specimens. Initial proof of principle has been demonstrated in human fetuses, though technical considerations and diagnostic accuracy remain to be addressed and will be considered within this thesis in part four.
1.3.1 Introduction

Macroscopic examination of internal organs and histology could be described as the cornerstones of the traditional autopsy. They are also the investigations that currently require a major incision to perform and are therefore often objected to by parents. As part of developing clinical guidelines for minimally invasive autopsy, it will be necessary to establish the evidence base for histological sampling of organs under different clinical circumstances. If an evidence base were established that would inform the likely yield of internal examination/organ histology, it may help clinicians to better counsel parents regarding potential benefits of invasive sampling, and help to guide ancillary investigations such as genetic, microbiological or molecular analysis of tissue.

Currently, the Royal College of Pathologists recommends that blocks of all major organs be taken as routine in all circumstances (including terminations of pregnancy and intrauterine fetal deaths at any gestation). However, this guidance is based on expert opinion, rather than evidence of the efficacy of sampling, and recently published evidence suggests this approach has a low yield in the context of IUFD. In practice, the organ sampling strategy employed by pathologists at autopsy is heavily influenced by the clinical history and macroscopic appearance of the organs, with additional samples taken from organs either clinically suspected to be abnormal or those that are
evidently abnormal at macroscopic examination, in addition to the routine samples recommended by practice guidelines.

To establish the evidence-base for invasive examination, a large cohort of retrospective cases will be interrogated. The Great Ormond Street Hospital histopathology department uses an Access (Microsoft, Seattle, WA) database to document over 400 objective fields for each autopsy performed within the department. Named the Rapid Study Database, it was originally designed for the input of post-mortem data in cases of Sudden Unexplained Death in Infancy.\textsuperscript{50-52,90,91} Initially, the database was populated using retrospective analysis of previous cases; from 2015 onwards, the cases have been entered as they have been performed. Furthermore, the database has been adapted to include:

- Terminations of pregnancy
- Intrauterine fetal deaths and stillbirths
- Neonatal deaths (sudden and non-sudden)
- Childhood deaths (SUDC and non-sudden deaths)
- Other Coronal cases (forensic, referred brains & referred hearts)

As for SUDI deaths, IUFD, ToP, childhood and neonatal cases prior to 2015 were entered retrospectively using autopsy reports and clinical information sent at the time of referral. Cases subsequent to 2015 have been entered contemporaneously as performed. The database was previously used to collect and compare data in a cohort of more than 1,000 cases of intra-uterine fetal
death.\textsuperscript{54,57,92,93} Part three of this thesis will assess the yield of macroscopic examination of the organs and organ histology, in order to better identify the circumstances under which invasive examination and sampling is likely to be useful (or not useful) in providing a cause of death.
Chapter four: The project

1.4.1 Research questions identified

Although feasibility of a laparoscopic approach to autopsy has been demonstrated, this technique has yet to be performed in an unselected cohort of cases (it is unknown whether this method can be applied in this context) and the sampling adequacy of internal organs using a laparoscopic approach is yet to be assessed in this context.

1. Can laparoscopic autopsy be applied to an unselected cohort of cases?

2. What is the sampling adequacy of laparoscopic autopsy in perinatal cases?

Currently, no clinical imaging technique (PMCT, PMMRI, PMUS) can provide sufficient spatial resolution or adequate anatomical details of the organs in early fetal losses (approx. 5 – 15 cm total body size / <400g bodyweight). Conventional PMCT is typically non-diagnostic below 24 gestational weeks, and PMMRI tends to give non-diagnostic results <18gw / 500g bodyweight. Higher field strength magnets (3 T and above) may give some additional yield, particularly for congenital cardiac abnormalities and can also reduce the number of non-diagnostic studies and interpretive error rates for most body systems. The greatest improvements with imaging at 3 T (over 1.5 T) are seen for fetuses aged 20 weeks or less, however despite the higher signal to noise
ratio and spatial resolution, approximately 30% of cases still remain non-diagnostic (reduced from 53.8% at 1.5 T imaging).68

3. Can micro-CT be used in small fetuses for whole body autopsy?

Counselling parents regarding appropriate investigations after death is an extremely difficult process undertaken at a time of potential parental stress. Current evidence regarding autopsy practice adopts a blunderbuss approach to internal examination and organ histology based on expert evidence, without considering likely diagnostic yield in each scenario.

4. In what proportion of cases does macroscopic examination of any major organ contribute to the cause of death in perinatal and paediatric deaths?

5. How often does histological examination of any major organ contribute to the cause of death in perinatal and paediatric deaths?

6. How often histological examination of a macroscopically normal organ reveal a cause of death in perinatal and paediatric deaths?

The above questions form the basis for the aims of this thesis and will help to inform evidence-based clinical guidelines presented within the discussion section (part five).
1.4.2 Aims of this thesis

Overall aims:

1. Test the feasibility of laparoscopic autopsy by performing this procedure in a cohort of cases (estimated cohort size = 100), including an assessment of sampling adequacy.

2. Evaluate technical issues around the use of micro-CT, including choice of contrast medium and the effect of contrast on subsequent sampling and histology.

3. Demonstrate proof of principle for whole body fetal imaging using micro-CT and test diagnostic accuracy.

4. Interrogate the evidence base for macroscopic examination of organs and histological sampling in perinatal and paediatric deaths using the GOSH autopsy database.

5. Establish clinical guidelines for less invasive autopsy given the above findings.
1.4.3 Ethical approval

This thesis investigating minimally invasive autopsy techniques and novel methods of post-mortem imaging was commenced under pre-existing ethical approval, which was obtained prior to the author of this thesis commencing their fellowship (REC approval: 13/LO/1494 and CE2015/81).

Subsequent additional ethical approvals were obtained for the retrospective database analysis presented in part 3 (REC approval: 16/LO/0909) and micro-CT analysis of surgical (non-autopsy) human specimens (partly presented in part 4) (REC approval: 17/WS/0089). The author contributed substantially to both latter ethical approval applications.

No animals were harmed as part of the production of this thesis. Where animal material was used, the tissue was obtained following completion of ethically approved studies by an affiliated research group and had been earmarked as “spare tissue” or “for disposal” prior to these experiments.

All human samples presented in the following experiments were handled in accordance with the Human Tissue Act (2004). Fully informed, written parental consent for conventional autopsy, imaging and the use of tissue for research was obtained in all cases. Furthermore, all material was handled in accordance with specific parental instructions, where present.
Part one summary

Although autopsy examination has played a role in medical practice since the 1600s, it has recently come under increasing scrutiny from its end users; legal and medical professionals, and the public. Advances in medical imaging, increasing use of antenatal genetic testing and controversies associated with human tissue retention have combined with shifting population demographics and changing public attitudes, resulting in a reduction in acceptability of traditional autopsy and the encouragement of development of potentially more acceptable, contemporary approaches, both in adult and paediatric/perinatal populations.
Part two: Less invasive Autopsy

Pre-existing evidence

The acceptability of standard autopsy is poor, both in high and low-middle income countries. Post-mortem imaging shows poor sensitivity for the detection of occult infection and sepsis, so evaluation of organ histology remains an important component of investigation after death.

Several studies have reported the utility of needle autopsy sampling, predominantly in low or middle-income countries as a method of detecting deaths related to infection, though sample sizes have generally been small. Needle autopsy sampling studies from high income countries have shown poor sampling adequacy across small cohorts; it is therefore questionable as to whether this approach meets rigorous national guidelines that recommend extensive organ sampling in the context of perinatal and paediatric death.

Although proof of principle of laparoscopic sampling of internal organs has been published, to date, there has been no study reporting the experience, feasibility and sampling adequacy of laparoscopic autopsy as part of routine clinical practice, which may act as a bridge between acceptable autopsy and adequate sampling.

Added value of this work

Laparoscopically assisted autopsy was performed in 103 cases between 15 gestational weeks of age and 14 years old, where standard autopsy had been declined (or where a minimally invasive option was preferred by HM Coroner).
The procedure was completed successfully in 97.8% (91 / 93) of cases where the extent of the examination was not limited by parental consent. We found satisfactory rates of adequate histological sampling in most major organs; heart, lung, kidney (all 100%), liver (96.7%), spleen (94.5%), adrenal glands (89%), pancreas (82.4%) and thymus (56%), which compare favourably with published literature on needle autopsy. Procedure duration was similar to that of a standard autopsy. There was no statistically significant increase in the ‘unexplained’ rate for IUFDs that received laparoscopically assisted autopsy when compared with a previously published cohort of >1,000 cases.

Implications of all available evidence

Laparoscopically assisted autopsy can be learned by an autopsy practitioner and applied to current clinical practice. It provides reliable sampling of major organs with minimal disruption to the body and may provide an acceptable alternative to those who decline standard autopsy.

To assist the reader, a summary of the terminology used in the field of less invasive autopsy is contained in Appendix 1.
Chapter one: The rationale for less invasive autopsy

2.1.1 Introduction

Post-mortem examination can provide families with a cause of death following fetal demise or the death of a child or infant, with information obtained from the autopsy contributory to management of future pregnancies in up to 40% of cases, can provide tissue for research into childhood and congenital disease, acts as a comprehensive audit of antenatal findings or obstetric practice, and may be useful as a method of infectious disease surveillance in low or middle-income countries.\textsuperscript{7,21,26,94,95} In the United Kingdom (UK), the majority of perinatal autopsies are performed with parental consent rather than for medicolegal reasons. There has however, been a marked reduction in the proportion of parents willing to consent to traditional paediatric and perinatal autopsy, with fewer than half of parents who experience a stillbirth providing consent in the UK; for neonatal deaths, only around one quarter of parents agree to autopsy.\textsuperscript{37} Contributing factors to this parental lack of acceptability include the invasive nature of the procedure, ambivalence regarding the value of autopsy (both from parents and clinicians), and religious objections, particularly within Jewish and Muslim communities.\textsuperscript{9,14,47} Indeed, standard autopsy incisions are relatively large when compared with the size of the fetal body (and are large even when compared with open surgery incisions).
In low-income countries, performance of autopsy is highly variable, both due to cultural & religious reasons and due to lack of suitable infrastructure and availability of trained professionals, despite a strong interest in establishing a cause death within some communities (up to 75% of respondents).\textsuperscript{96,97}

In response to these factors, Less Invasive Autopsy (LIA) approaches to both adult\textsuperscript{98} and paediatric/perinatal autopsy\textsuperscript{60} have been suggested. Such strategies have mainly focused on the development post-mortem cross-sectional imaging (e.g. Post-mortem Magnetic Resonance Imaging (PMMRI) or Computed Tomography (PMCT)) to provide anatomical detail, with growing evidence to suggest that these investigations are acceptable to parents.\textsuperscript{47,65,71} Despite parental preference for LIA procedures, in some clinical scenarios, tissue-based investigations (e.g. histological examination) remain an important component of the autopsy. When considering childhood and infant deaths, PMMRI alone has a poor detection rate for some infectious pathologies (MARIAS), particularly in the lungs. To evaluate novel approaches to post-mortem organ sampling, PubMed databases were searched (between 1 January 2000 - 2018) using the MeSH term “Autopsy/methods” in combination with keywords “needle”, “perinatal” and “laparoscopic” with no language restrictions.
2.1.2 Needle biopsy autopsy

Percutaneous needle biopsy may prove more acceptable to parents as a method of investigation after death than standard autopsy due to perceived lack of invasiveness. This form of investigation could also potentially be offered by radiologists or clinicians with experience of clinical biopsy, improving the availability of investigation where no pathologists are present. Needle biopsies may be taken percutaneously (Figure 13) or through a single small incision in the abdominal cavity. Additionally, they can either be performed blindly in low-resource settings or performed with ultrasound assistance. In low to middle income countries, needle puncture autopsy could also theoretically reduce the risk of aerosolisation of pathogens; conferring a health and safety benefit to operators and mortuary staff. Drawbacks include the need for a skilled operator with adequate equipment, the inability to inspect organs directly, and technical difficulties involved in targeting bony structures and targets in the retroperitoneum.

Figure 13. Diagram illustrating needle biopsy autopsy. Image credit: ISGlobal (Reproduced under Creative Commons Licence CC-BY).
Although this technique offers a potential advantage over standard autopsy with regards to invasiveness and therefore acceptability, the evidence regarding the sampling adequacy of needle biopsy autopsy demonstrates a potential flaw with this method, with rates ranging from 8% in some organs to 100% in others.\textsuperscript{75,76,94,95,99} One study performed in the UK found the sampling adequacy rates to be unacceptable and concluded that needle autopsy “cannot be considered to provide useful clinical information as part of a ‘minimally invasive’ perinatal autopsy.”\textsuperscript{75} Further, authors of another study considered that although microscopic abnormalities may be detected, needle autopsy missed diagnosis of various congenital malformations, which can be discerned only after meticulous gross examination”.\textsuperscript{76} It may therefore be difficult for clinicians to counsel parents accurately and to manage parental expectations from an examination after death with such poor yields; in cases where a malformation is present, but the significance is unclear or requires clarification, this approach may be unsuitable.

However, in some contexts, needle autopsy may prove to be useful. More recent data using percutaneous USS guided sampling for histology and microbiology (liver, lungs, bone marrow, CNS) in disseminated infection (HIV-infected adults) showed concordance with autopsy in 89% (100 of 112) of patients.\textsuperscript{100} The value of needle autopsy in perinatal cases is less clear, with a recent study performed in Mozambique examining 59 cases (18 stillbirths and 41 neonatal deaths) reporting >75% concordance between MIA and standard autopsy for cause of death (Table 3).\textsuperscript{101}
Table 3. Sensitivity and specificity data for needle-core MIA reported by Menendez et al. 101 in the context of stillbirth and neonatal deaths. Adapted for use under Creative Commons License CC-BY.
At first glance these data appear promising, however, the high concordance rate published in this study relies on classification of small numbers of cases into broad modes of death (e.g. fetal growth restriction, intrauterine hypoxia), without identifying specific aetiologies, except for deaths relating to infectious diseases (the infectious agents responsible are identified in a further table within the same study). The presentation of some statistics in the study also appears to be problematic, for example, both cases of ‘other disease’ in neonates were missed (0/2), however, the overall accuracy for this subcategory is presented as 93% (95% CI: 80 - 98). Therefore, the problem remains that while needle autopsy is promising for identifying disseminated infectious diseases (more commonly seen in low or middle-income settings), focal infections, pathologies and anatomical malformations requiring anatomical dissection (more common causes of death in high income countries) may go undetected.
2.1.3 Laparoscopically assisted autopsy

In clinical medicine, laparoscopy (the use of telescopic instruments by a clinician to diagnose or treat conditions within the abdomen or pelvis, using several small incisions) can be used to avoid large incisions. One potential method to improve tissue yield that also permits visualisation of internal anatomy is to utilise laparoscopically assisted “keyhole” inspection and sampling of internal organs via a small incision, following adequate imaging. Although proof of principle of such an approach has been reported, the yield of such a technique in a larger cohort of cases remains undetermined.

During clinical laparoscopy, the operator makes one or more incisions in the abdominal wall to permit the passage of a laparoscope, inflator device and surgical / biopsy tools into the abdomen. Given that insufflation is not required for post-mortem laparoscopic examination, the number of incisions could be reduced to a single 1-2cm incision. Furthermore, the incision across the top of the head (necessary to remove the brain) may be unnecessary in the context of normal antenatal neurological imaging and a normal PMMRI of the CNS, due to the high negative predictive value of PMMRI for significant neuropathologies shown in an unselected cohort of 400 cases (NPV = 92.2%, 87.4 – 95.3). In general, for the practical element of the autopsy examination, following creation of a port site, the operating pathologist will then need to clear the abdominal cavity prior to visualisation of organs. This involves removal of the small bowel, large bowel and most of the liver. This can be achieved by
identifying the descending colon and incising it transversely. By applying tension to the proximal cut end with forceps, the mesentery can be followed with a scalpel or pair of sharp scissors, allowing visual inspection of the bowel for volvulus, atresia or malrotation during removal. Following removal of the bowel, the liver can then be removed piecemeal and the laparoscope introduced into the abdominal / pelvic cavity to begin visualisation and sampling. Once examination of the abdominopelvic cavity is complete, the inferior surface of the diaphragm can then be incised, and the camera introduced to the thoracic cavity. Following visualisation of an organ using the laparoscope, the organ can be dissected under direct vision or with laparoscopic assistance and removed via the primary incision for further inspection or can be left in situ as required. Following dissection outside of the body, organs can then be returned to the interior of the body via the primary incision and the body reconstructed for release to the family. In practice, although the paper by Sebire et al.\textsuperscript{31} serves to provide proof of principle on the use of the laparoscopic route in perinatal autopsy, technical aspects remain to be addressed, which will be explored over the following chapters. A comparison between clinical and autopsy laparoscopy is provided in Table 4. Table 5 summarises studies published to date on the use of laparoscopic autopsy.
Table 4. Comparison of consideration for laparoscopic techniques at surgery and autopsy.

<table>
<thead>
<tr>
<th>Study</th>
<th>Surgical</th>
<th>Autopsy</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of port sites</td>
<td>Multiple</td>
<td>One</td>
<td>Insufflation not required for autopsy, therefore port sites minimised</td>
</tr>
<tr>
<td>Location of port sites</td>
<td>Varies according to indication</td>
<td>Abdominal, periumbilical or left hypochondrium</td>
<td>Site can be chosen at autopsy to make extraction easier</td>
</tr>
<tr>
<td>Scope size</td>
<td>Varies according to patient and indication</td>
<td>Varies according to patient and indication</td>
<td>Fetoscopy scope = 2mm</td>
</tr>
<tr>
<td>Insufflation</td>
<td>Used to prevent damage to body anatomy and improve visualisation</td>
<td>Not required</td>
<td>Risk of tearing skin if used at autopsy due to post-mortem loss of elasticity</td>
</tr>
<tr>
<td>Biopsy equipment</td>
<td>Fine bore needle (14-16G)</td>
<td>Forceps, scissors, scalpel</td>
<td>Use of scalpel and scissors should help to ensure better sampling adequacy</td>
</tr>
<tr>
<td>Study</td>
<td>n</td>
<td>Age range</td>
<td>Method</td>
</tr>
<tr>
<td>------------------</td>
<td>----</td>
<td>-------------</td>
<td>-----------------------------------------------------------------------</td>
</tr>
<tr>
<td>Avrahami (1995)</td>
<td>20</td>
<td>Adults (not stated)</td>
<td>Thoracoscopy and laparoscopy with subsequent autopsy</td>
</tr>
<tr>
<td>Catheline (1999)</td>
<td>1</td>
<td>Adult (not stated)</td>
<td>Abdominal laparoscopy only</td>
</tr>
<tr>
<td>Damore (2000)</td>
<td>1</td>
<td>Adult (not stated)</td>
<td>Abdominal laparoscopy only</td>
</tr>
<tr>
<td>Cacchione (2001)</td>
<td>25</td>
<td>44 - 94</td>
<td>Abdominal laparoscopy with thoracoscopy or piercing of diaphragm</td>
</tr>
<tr>
<td>Fan (2010)</td>
<td>22</td>
<td>32 - 96</td>
<td>Laparoscopy and thoracoscopy</td>
</tr>
</tbody>
</table>

Table 5. Comparison of published studies and case reports documenting the use of laparoscopic autopsy techniques.
2.1.4 Current limitations of the less invasive autopsy

In adults, post-mortem CT (PMCT) is well established for investigation after death. PMCT may readily provide evidence of catastrophic haemorrhage, or pathologies associated with calcification, however, the range of pathologies that affect infants, children and perinates is different, both compared to adult cases and between age groups. Additionally, due to variability in the size of the body to be examined in paediatric and perinatal practice, a more nuanced approach to imaging protocols and technology is required to optimise resolution and maximise yield, and the approach must carefully consider the clinical history provided. Successful cadaveric imaging methods in adult pathology will therefore not suffice in younger age groups. While PMMRI provides excellent anatomical detail in third trimester fetuses and infants, due to fetal size and limits of resolution, such methods are less effective below 18 weeks / fetal weight of 400g. In this setting, it is possible that alternative methods, such as microfocus computed tomography (micro-CT) may provide high-quality fetal imaging,\textsuperscript{87} which may even be superior to traditional approaches, especially for early gestation fetuses. Micro-CT could be a particularly important imaging development in early gestation fetuses, as initial evidence suggests that 3 T MRI provides no significant increase in diagnostic accuracy when compared with standard 1.5 T MRI,\textsuperscript{68} while high-field MRI remains expensive and hard to access for clinical radiology.\textsuperscript{107}
In perinatal autopsy practice, post-mortem imaging with plain X-rays has been performed for many years but has been generally regarded as an ancillary investigation to the autopsy itself. Post-mortem cross-sectional imaging has shown promise, especially when used as part of a minimally invasive approach. In the MaRiAS study, minimally invasive autopsy demonstrated overall concordance with standard autopsy of around 95% in fetuses, when considering a combination of genetic analysis, microbiology, placental examination, external examination and imaging findings against the final autopsy diagnosis. Concordance dropped substantially when considering the performance of imaging alone against conventional autopsy. It can therefore be argued that tissue-based investigations remain critical in the overall process of investigation after death (although the relative importance of tissue investigations is likely to vary given the age of the case and the clinical context). If a minimally invasive strategy can be shown to reliably provide tissue in a large cohort of unselected cases, it should be more likely to gain acceptance into mainstream pathology practice, as clinicians may have more confidence in counselling patients for the examination, and pathologists may be more likely to accept it as a viable alternative strategy to standard autopsy. Additionally, a minimally invasive approach must be able to incorporate reliable acquisition of visceral histology, to satisfy national guidelines on autopsy practice from the Royal College of Pathologists.
Minimally invasive autopsy investigations could greatly expand the range of post-mortem approaches available to pathologists, clinicians and families. This project represents a potential step towards refocussing the perinatal autopsy process towards ‘investigation after death’.
2.1.5 Part two aims

1. Demonstrate that Minimally Invasive Autopsy with Laparoscopy (MinImAL examination) is feasible and reliable in a cohort of cases, including considerations of:

   a. Procedure duration
   
   b. Sampling adequacy
   
   c. Failure rate (% conversion to full autopsy)
   
   d. Cosmetic result

2. Comparison of sampling adequacy with existing studies.

3. Obtain data from sampling adequacy that can be used to inform the modelling of a minimally invasive autopsy strategy (thesis parts 3 & 5).
Chapter two: Methods used in the application of minimally invasive autopsy to a large cohort

2.2.1 Case selection

The study was approved by a national research ethics committee (REC 09/H0713/2) and all samples handled in accordance with the Human Tissue Act (2004). One hundred and ninety perinatal / paediatric autopsies referred to our centre as part of routine clinical care were prospectively recruited to LIA between June 2011 and October 2016 (Figure 14), following the first ten cases previously reported in a feasibility study. The cases were unselected, other than provision of parental consent for LIA or where specifically agreed between HM Coroner and the performing pathologist. Where examinations were consented procedures (rather than medico-legally required), parents were counselled regarding standard operating procedures of the role of and indications for standard autopsy, and offered standard autopsy initially, with LIA offered following refusal of standard autopsy. Parents could also restrict the MinImAL or standard autopsy to a specific body cavity or organ cavity, termed a “limited” autopsy, despite the lack of information regarding the added value of laparoscopic assisted sampling in this context. Some parents specified that a MinImAL could be performed initially, with conversion to a standard autopsy if required. Parents who declined full or MinImAL autopsy were offered Non-Invasive Autopsy (NIA) involving PMMRI, external examination and placental examination, with no tissue sampling. The study
Figure 14. Flowchart demonstrating recruitment for MinImAL procedure and less invasive autopsy. Adapted from Hutchinson et al.\textsuperscript{115} with permission.
2.2.2 MinImAL Procedure Protocol

Pre-autopsy 1.5 T PMMRI was performed in all cases. PMMRI results were reported by a specialist paediatric radiologist with expertise in post-mortem imaging and discussed with the author prior to the autopsy. Routine external examination of the body along with genetic and microbiological sampling were performed as usual for all cases. Placental examination was performed as part of the fetal autopsy, where available. The brain was only extracted when there was a clinical indication to do so, or if an abnormality was discovered on post-mortem MRI, with appropriate parental consent.

Sebire et al describe the creation of a paraumbilical port site (incision i, figure 15) as part of MinImAL examination. In practice, this is problematic, as peri-umbilical incisions require the operator to clear the bowel and liver prior to accessing the chest cavity at some distance from the port site. Due to the anatomical connections of the heart, it can be difficult to extract at MinImAL without damage to the lungs and great vessels. An incision in the left upper abdominal quadrant (incision ii, figure 15), made below the left costal margin, allows the operator to examine, extract or biopsy the lungs and heart by angling the incision rostrally from the initial perpendicular plane of the incision, or to examine the abdominal cavity by angling the initial incision caudally to avoid the diaphragm. Incision ii also reduces the distance from the port site to the target organs in the chest cavity, potentially making extraction easier when it may be important to preserve anatomy.
Figure 15. Schematic representing possible paraumbilical (i) and left subcostal (ii) incisions.
A straight laparoscope (2, 4, or 10mm diameter according to body size and availability) was passed into the abdominal cavity via a small incision (1-2cm), made either sub-xiphisternally (Figure 15) or in the left hypochondrium, and used to visualise organs for sampling within the limits of parental consent. If indicated, internal organs (such as heart and lungs) were removed en-bloc through the port-incision via grasping forceps through the port incision, examined externally, and subsequently returned to the body (Figure 16).

Initially, the first seven cases all had a MinImAL procedure followed by standard autopsy (with parental consent) of the abdomen and thorax by extending the MinImAL incision to a standard ‘T’ or ‘Y’ incision; however, in all cases, the major organs had been successfully laparoscopically eviscerated or sampled, and no further useful information was gained through the extensively invasive procedure. Conversion to standard autopsy was therefore subsequently only performed for inadequate visualisation. An attempt to sample major organs (pre-defined as: heart, lung, kidney, liver, spleen, adrenal, pancreas and thymus) was made in all cases that were not limited by consent.

Following autopsy examination, all organs were returned to the body, which was released to the families following reconstruction. An autopsy report was then generated, containing the post-mortem radiology, histology, microbiology and genetic results, as normal clinical practice.
Figure 16. Incision size (approximately 1 cm) required for MinImAL procedure in a term stillbirth (A), alongside the post-reconstruction photo (B). Adapted from Hutchinson et al. with permission.
Figure 17. Heart and lung block extracted intact following MinImAL procedure, prior to macroscopic dissection and examination. Even in small fetuses, organs can be extracted with relative ease using this technique. Adapted from Hutchinson et al.\textsuperscript{115} with permission.
2.2.3 Evaluating the MinImAL procedure: Sampling adequacy & timing

The primary outcome of this study was the rate of tissue sampling success following MinImAL procedure, which was pre-defined as sufficient material for the reporting pathologist to categorise into / make a diagnosis of normal or abnormal. Sampling failure was pre-defined as either insufficient material for comment, cases where the tissue sample was too small to survive histological processing or where the target organ was not sampled. Autopsy and histological findings were compiled contemporaneously using the Great Ormond Street Hospital autopsy database and analysed retrospectively according to specific organ pathology/normality and analysis of sampling adequacy using Microsoft Access and Microsoft Excel (Microsoft, Seattle, USA).

The secondary outcomes were evaluation of MinImAL procedure duration in a subgroup of cases, cause of death analysis of the cohort, and comparison of the proportion of intrauterine fetal deaths (IUFDs) and stillbirths that remained unexplained following MinImAL procedure with that of a previously reported, unselected cohort of >1,000 IUFDs examined at the same centre during a similar period.\textsuperscript{54-57}

As part of overall evaluation of the MinImAL procedure as a method of autopsy, the author was trained and subsequently timed for an unselected series of complete MinImAL procedures, following three familiarisation cases (Figure I8). Although this study was not a formal diagnostic accuracy trial of
the MinImAL procedure, a preliminary evaluation of the rate of ‘unexplained’ cases was necessary in order to establish whether use of the MinImAL procedure resulted in a statistically significant increase in the number of ‘unexplained’ deaths as compared to standard autopsy. In order to do this, the ‘unexplained’ rate across the stillbirth and IUFD cases within the MinImAL cohort was compared to the published rate from a large case series of >1,000 IUFDs that underwent standard autopsy at the same centre using Chi-Squared analysis.
Chapter three: Results

2.3.1 MinImAL cohort: Demographics

Of 1,900 referrals to our institution for autopsy examination between June 2011 and October 2016, 190 cases underwent LIA (Figure 14). Of these, 20 early gestation fetuses were specifically referred for micro-CT examination and have been excluded from this analysis. In 67 cases, the parents consented only for Non-Invasive Autopsy involving PMMRI, external examination and placental examination, with no tissue sampling. The remaining 103 cases underwent MinImAL procedure with both PMMRI and laparoscopically-assisted organ examination and sampling. Of the 103 MinImAL cases, 99 were consented cases, with another four undertaken on the authority of HM Coroner but at parental request (demographics of all cases are presented in Table 6). Ninety-three cases underwent complete MinImAL procedure, without restriction to a body system or cavity, and without sampling restriction. Ninety-one of these cases (97.8%) were successfully completed as minimally invasive procedures, as per the aforementioned protocol. As mentioned previously, seven cases were initially converted to standard autopsy at the beginning of the study as part of technical optimisation (in each of these cases, a successful MinImAL examination had been completed). In two further cases, one due to poor visualisation due to small fetal size, the other due to poor visualisation in Prune-Belly Syndrome, conversion to standard autopsy was required (with appropriate parental consent; unplanned conversion rate: 2/93, 2.2%). In no
case was conversion to standard autopsy preferred by the author where parental consent was not present to do so.

<table>
<thead>
<tr>
<th>Type of MinImAL (n = 103)</th>
<th>Coronial</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Consented</td>
<td>99</td>
</tr>
<tr>
<td>Indication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Termination of pregnancy</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>Intrapartum stillbirth</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>IUFD</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Neonatal death</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Infant (of which, SUDI)</td>
<td>3 (1)</td>
<td></td>
</tr>
<tr>
<td>Child/Adolescent</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

| Gestational age of fetal cases (n = 93) | Mean       | 25.5 gw |
|                                         | Median     | 23 gw   |
|                                         | Range      | 15-41 gw|

Table 6. Demographics of the 103 cases accepted for any form of MinImAL examination. Adapted from Hutchinson et al.\textsuperscript{115} with permission.

2.3.2 Evaluating the MinImAL procedure: duration

Examination duration time were collected in a sub-series of 21 initial cases. This series indicates a considerable learning effect, with the mean time of the first 10 cases (28 minutes, 6 seconds) being considerably higher than the mean of the last 10 cases timed (18 minutes, 12 seconds), as shown in Figure I8.
Figure 18. Time taken to perform MinImAL procedure by a single operator over 21 consecutive cases, with trend line. Adapted from Hutchinson et al. with permission.
2.3.3 Evaluating the MinImAL procedure: sampling adequacy

In each complete MinImAL case (n = 93), an attempt was made to sample pre-defined major organs (Table 7). Heart, lung and kidney were successfully sampled in every case (100%). Liver was successfully sampled in 96.7%, spleen in 94.5% and adrenal gland, pancreas and thymus in 89.0%, 82.4% and 56.0% of cases respectively (Table 7). Of the 91 cases in the cohort that underwent successful complete MinImAL examination, significant histological abnormalities were demonstrated in 16 organs (excluding CNS) in nine cases (Tables 7 & 8). Of the organs with a histological abnormality present, in all but two (both involving the heart), a clinical, radiological or macroscopic abnormality was present (Figure 19). Both cases with unsuspected cardiac abnormalities were neonatal deaths (one at day 11, one at four months). In no case of fetal death did histological sampling without a clinical, radiological or pathological indication reveal additional useful information. A Chi-squared comparison between the success rate of MinImAL and needle core sampling of organs (using back-calculated numerical data from previously published needle-core studies)\textsuperscript{75,76,94,95,99} revealed a significant difference between the two techniques, with MinImAL sampling more likely to lead to successful sampling of any organ on a per-attempt basis (p<0.0001, Table 9).
Table 7. Histological sampling success rates and normality/abnormality rates across major organs in the 91 complete, unconverted MinImAL cases. Adapted from Hutchinson et al.\textsuperscript{115} with permission.

<table>
<thead>
<tr>
<th></th>
<th>Heart (n=91)</th>
<th>Lung (n=91)</th>
<th>Kidney (n=91)</th>
<th>Liver (n=88)</th>
<th>Adrenal gland (n=81)</th>
<th>Pancreas (n=75)</th>
<th>Spleen (n=86)</th>
<th>Thymus (n=51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampled successfully, histology normal or non-contributory to death</td>
<td>89 (97.8%)</td>
<td>88 (96.7%)</td>
<td>84 (92.3%)</td>
<td>85 (93.4%)</td>
<td>81 (89.0%)</td>
<td>75 (93.4%)</td>
<td>85 (93.4%)</td>
<td>51 (56.0%)</td>
</tr>
<tr>
<td>Sampled successfully, histology abnormal and contributed to death</td>
<td>2 (2.2%)</td>
<td>3 (3.3%)</td>
<td>7 (7.7%)</td>
<td>3 (3.3%)</td>
<td>0</td>
<td>0 (1.1%)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total sampling success</td>
<td>91 (100%)</td>
<td>91 (100%)</td>
<td>91 (100%)</td>
<td>88 (96.7%)</td>
<td>81 (89.0%)</td>
<td>75 (94.5%)</td>
<td>86 (56.0%)</td>
<td>51 (56.0%)</td>
</tr>
<tr>
<td>Sampling failure</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 (3.3%)</td>
<td>10 (11.0%)</td>
<td>16 (17.6%)</td>
<td>5 (5.5%)</td>
<td>40 (44.0%)</td>
</tr>
</tbody>
</table>
Table 8. Evaluation of the significant abnormalities found on non-neurological histological examination within the cohort of complete MinImAL cases, and the presence of clinically, radiologically or macroscopically suspected abnormalities in these cases. Sixteen organs from nine complete MinImAL cases showed significant abnormalities that contributed to death. ALI = acute lung injury, DAD = diffuse alveolar damage, ARPKD = autosomal recessive polycystic kidney disease, ATN = acute tubular necrosis. Adapted from Hutchinson et al.115 with permission.
Figure 19. Illustration of a 24gw fetus with hepatosplenomegaly seen on T2-weighted PMMRI (coronal section of the thorax and abdomen) (A), with spleen histology obtained via laparoscopic sampling as part of a MinImAL procedure (B) [Haematoxylin and Eosin] & (C) [Diastase-PAS]). Numerous storage cells filled with DPAS positive material are present (arrowheads). Adapted from Hutchinson et al.\cite{115} with permission.
Uncorrected $\chi^2 = 255.957588$  $P < 0.0001$

Computed using StatsDirect (Cambridge, UK)

Table 9. Chi-squared comparison between the overall sampling success rates for aggregated previously published needle core autopsy studies and the MinImAL examination. MinImAL examination was more likely to yield a successful sample on a per-attempt basis ($p<0.0001$).

<table>
<thead>
<tr>
<th></th>
<th>Successful attempt</th>
<th>Unsuccessful attempt</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Needle-core sampling</td>
<td>631</td>
<td>525</td>
<td>1,156</td>
</tr>
<tr>
<td>MinImAL sampling</td>
<td>654</td>
<td>74</td>
<td>728</td>
</tr>
<tr>
<td>Total</td>
<td>1,285</td>
<td>599</td>
<td>1,884</td>
</tr>
</tbody>
</table>
2.3.4 Evaluating the MinImAL procedure: unexplained rate

Whilst this study was not designed as trial to evaluate the accuracy of the MinImAL procedure, Chi-squared analysis revealed no significant difference in the ‘unexplained’ rate between this cohort and over 1,000 IUFDs previously published (Table 10).\textsuperscript{54-57}

<table>
<thead>
<tr>
<th></th>
<th>Explained</th>
<th>Unexplained</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Man et al. 2016\textsuperscript{55}</td>
<td>412</td>
<td>652</td>
<td>1064</td>
</tr>
<tr>
<td>This study</td>
<td>18</td>
<td>16</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>430</td>
<td>668</td>
<td>1098</td>
</tr>
</tbody>
</table>

Uncorrected $\chi^2 = 2.79601$  $p = 0.0945$

Computed using StatsDirect (Cambridge, UK)

Table 10. Chi-squared comparison between the overall unexplained rate for stillbirths previously reported at Great Ormond Street Hospital and those undergoing MinImAL procedure. No statistically significant difference ($p=0.09$) was found.
2.3.5 Evaluating the MinImAL procedure: Overall cause of death

A summary of the overall causes of death obtained across the cohort is shown in Figure 20, with a further breakdown of the IUFD and stillbirth cases in Figure 21. The unexplained rate across the cohort (defined as a non-diagnostic autopsy in ToP cases) was 47% in IUFD and stillbirth cases, 25% in infant / childhood deaths, 17% in neonatal cases and 5% in ToP cases. Placental causes of death were identified in 29% of IUFD / stillbirth cases. The presence of genetic disorders and congenital anomalies also contributed significantly to death within the cohort, with 50% of neonatal, infant and childhood deaths causally affected by genetic disorders and 90% of ToP cases due to either a congenital anomaly or genetic disorder that was confirmed at MinImAL examination.
Figure 20. Causes of death within the cohort as percentages of the total number of terminations of pregnancy (ToP, $n = 59$), intrauterine fetal deaths and stillbirths (IUFD / SB, $n = 34$), neonatal deaths (Neonatal, $n = 6$) and infant and childhood deaths ($n = 4$). Adapted from Hutchinson et al. with permission.
Figure 21. Analysis showing the causes of death within the intrauterine fetal death and stillbirth groups from the cohort. Adapted from Hutchinson et al.115 with permission.
Chapter four: Discussion

2.4.1 Implications of this work

This study presents our initial experience with a large, unselected cohort of perinatal and paediatric autopsies performed using Minimally Invasive Autopsy with Laparoscopy (MinImAL procedure), along with analysis of sampling adequacy and histological abnormality analysis. This is the largest study to date utilising a minimally invasive approach to autopsy within the perinatal and paediatric setting. Through this research we have demonstrated that such approaches can be learnt and performed in a reasonable time frame, with a high accuracy and low failure rate (2/93 (2.2%)). Our findings suggest that MinImAL procedure has a similar performance to standard autopsy in terms of the proportion of IUFD / stillbirth cases in which a cause of death is determined. Given that recent data indicate that LIA techniques have a high degree of acceptance for parents and religious communities, these findings have implications for perinatal autopsy practice globally, as the acceptability of the standard autopsy continues to decline.

Our study had a low rate of conversion to standard autopsy due to technical difficulty or poor visualisation and good sampling accuracy (adequate sampling >80% in all organs other than thymus). This compares favourably with previously published studies (Table II). Potential reasons for sampling inadequacy include operator error (e.g. failure to sample the organ), identification error (e.g. sampling fat instead of adrenal gland), failure to...
locate the target organ, and failure of a sample to survive histological processing (e.g. due to size / amount of tissue). Furthermore, there was no significant difference in the unexplained rate between the MinImAL cohort and a previously published cohort of >1,000 IUFD cases from the same centre, suggesting that overall performance is likely to be similar to standard autopsy in this population. Examination of the placenta is clearly important within the IUFD / stillbirth sub-group as it accounted for 14/34 (41%) of these deaths.
Table II. MinImAL histological sampling performance compared to other published studies, along with methodology used and cohort type. The best performing figures compared with other studies are underlined gw = gestational weeks; USS = ultrasound scan. Adapted from Hutchinson et al.\textsuperscript{115} with permission.
While perceived to be traditionally important as part of the autopsy process by pathologists, the wider role of tissue sampling in autopsy is yet to be interrogated on a large scale using objective criteria. In this study, non-neurological histology provided a significant contribution to the cause of death in nine cases (in six terminations of pregnancy, two neonatal deaths and a non-sudden infant death). In only two cases (both neonatal deaths, both involving the heart) did histology of a macroscopically, clinically and radiologically normal organ provide the cause of death. In all other cases within the cohort, histological abnormalities were discovered within the context of clinical, radiological or morphological indications to sample the organ. The autopsy database at our institution facilitates large-scale comparisons of such data, whereby the contribution of histology to the cause of death can be objectively analysed across different death categories (e.g. stillbirth, neonatal death), and this is further evaluated in part three of this thesis. A previous study performed examining the contribution of routine histology in the context of IUFD found that only around 1% of cases demonstrated histological abnormalities which provided the cause of death when the internal organs appears normal macroscopically,\textsuperscript{110} which is in keeping with the findings of this study.

2.4.2 Time, cost and expertise

The rationale for the development of the MinImAL procedure derives from multiple studies demonstrating that needle biopsy of organs within the post-mortem setting yields high rates of ‘inadequate’ tissue samples,\textsuperscript{75,76,99} thus
potentially hindering investigation after death. This work has demonstrated that the MinImAL procedure can be learned by an autopsy practitioner, and applied within clinical practice, with good sampling rates of most organs, and a reasonable performance duration. Although the time required for examination of the thoracic and abdominal cavities may be prolonged by the use of MinImAL procedure, the use of pre-autopsy PMMRI in this study meant that in many cases, further extraction and examination of the brain was not necessary, balancing this out. Moreover, in another study, PMMRI has been reported to increase diagnostic accuracy in cases with marked maceration. The MinImAL approach therefore provides a potential solution to the problem of sampling adequacy for tissue-based investigations, including histology, microbiology, toxicology, and proteomics. The equipment used for the laparoscope-assisted portion of this study (Karl-Storz Tele Pack X LED) is a relatively low cost, portable option that avoids the need for an endoscopy stack and can be used with a wide range of scopes, providing visualisation in fetuses as small as 15 weeks. Laparoscopy data can be saved directly to a secure local USB drive if required, avoiding the need for expensive data storage systems. Although an incision is required through which to insert the laparoscope, this is much smaller than a standard autopsy incision, and could theoretically protect the operator from aerosolisation of pathogens when sampling tissue in potentially infectious cases (insufflation is not required for a MinImAL procedure). Parents already have a preconception about incisional approaches from the use of laparoscopic compared to open surgical approaches in the adult surgical setting. The MinImAL approach could therefore be applied
within low and middle-income settings, following suitable training of local operators. This has significant implications for investigations after death in high income countries, where acceptability of standard autopsy remains low, and low or middle-income countries, where community acceptance of autopsy and resources can be patchy, even in major cities.

2.4.3 Limitations

This study has some limitations. Some selection bias is possible within the study recruitment, as parents were required to specifically consent to minimally invasive autopsy. Potential sources of bias include the influence of the clinician taking consent, the clinical context of the loss, and the pre-existing attitudes of the parents to autopsy. It is possible that this contributed to the relatively high proportion of ToP cases within the cohort, although the methodology was largely the same across all cases, as is true for standard autopsy. It is not currently possible to extract the brain for a detailed neuropathological examination or extract an entire long bone using the MinImAL approach. Clinical suspicion of pathology in these areas may therefore necessitate an additional incision in some cases.

It is difficult to compare the findings of the MinImAL procedure to a reference standard (i.e. standard autopsy), as following a complete MinImAL procedure, few internal organs remained to be examined within the body cavity following conversion to full autopsy. There was no statistically significant difference between the ‘unexplained’ rate of the IUFD deaths within this cohort compared to a previously published cohort; due to the rarity of some of the
conditions encountered in clinical practice, a full diagnostic accuracy study of
the MinImAL procedure would potentially require thousands of cases
performed in a double-blinded manner and compared with a historical cohort
of standard autopsy cases (as no direct comparison between MinImAL and full
autopsy can be made).

Further evaluation of the MinImAL procedure compared with standard
autopsy is required for introduction into routine clinical practice. Economic
cost-benefit analysis addressing, for example, the cost of training pathologists
and a potential increase in case numbers with improved acceptability should
also be performed. With adequate training, the MinImAL approach could also
potentially be applied to low and middle-income settings, where community
acceptance of autopsy and resources are limited, although organisational
implications for introduction of such a service remain undetermined. Finally,
optimisation of algorithms for selection of cases most appropriate for
MinImAL across a wide range of clinical scenarios is required.

Part two summary

This study shows that the MinImAL procedure can be applied to current
clinical practice. Tissue sampling rates of the MinImAL autopsy are generally
better than those by needle sampling reported in the literature, with successful
sampling of heart, lung and kidney in every case.
Part three: Retrospective review of Great Ormond Street Hospital autopsy database

Pre-existing evidence

Consent rates to standard autopsies are falling, with minimally invasive approaches preferred by some parents, who may otherwise reject autopsy examination entirely. Perinatal autopsy guidelines established by the Royal College of Pathologists advise histological sampling of every organ in the investigation of intrauterine fetal death, termination of pregnancy, and sudden unexpected deaths in infancy/childhood, however, these are based on expert opinion and few studies have examined the yield of macroscopic and histological examination as part of autopsy. In order to better inform the rationale for organ sampling in autopsy examinations, this chapter will examine the yield of macroscopic and histological examination across perinatal and paediatric deaths.

Added value of this work

Retrospective analysis of the yield of both macroscopic and histological examination of the brain, heart, lungs, liver, kidneys, adrenal glands, pancreas, and thymus was performed in 5,311 cases (1,957 IUFD/stillbirth, 1739 SUDI, 824 SUDC, 791 ToP). Macroscopic examination of organs revealed a definite cause of death in around 21.6% of SUDC, 12.2% of SUDI, 1.7% of IUFD/stillbirth and 25.8% of ToP cases. Histological examination of
macroscopically normal organs revealed a definite cause of death in around 9.4% of SUDC, 5.2% of SUDI, 1.4% of IUFD and 0.3% of ToP cases.

Implications of all available evidence

The yield from macroscopic and histological examination of viscera is highly dependent on the clinical context of the death being investigated. Modelling of these data suggest that minimally invasive approaches to autopsy are likely to provide a similar yield to standard autopsy in the context of IUFD/stillbirth or ToP. Thorough sampling of all major organs for histological examination remains important in the context of SUDC or SUDI, where microscopically detectable pathologies within macroscopically normal organs are more frequently encountered.

Data entry to the Great Ormond Street Hospital database has been performed by consecutive research fellows and database architects, including:

Dr. Martin Weber, Dr. Jeremy Pryce, Dr. Andrew Bamber, Dr. Julie Man, Dr. Victoria Bryant, Ms. Xhanan Gholesi, Ms. Aimee Avery, Mr. John Booth and myself. All data mining and calculations presented within were performed by the author for the purposes of this thesis, unless otherwise stated.
Chapter one: The Great Ormond Street Hospital

Autopsy Database

3.1.1 Introduction

As discussed in the introductory chapter, standard perinatal autopsy is thought to provide useful additional clinical information in the management of future pregnancies in up to 22-76% of cases depending on the type of referral. This information may be used to manage future pregnancies differently, aid counselling regarding recurrence risk or be of medical use to either the parents or living siblings. However, these statistics do not allow determination of the diagnostic yield of each component of the standard autopsy (e.g. independent review of the clinical history, external examination, internal examination, organ histology, placental examination, placental histology radiology, microbiology and genetic analysis), and assume a standard autopsy to be a single process. In fact, a standard autopsy report is formed of a number of different components (Figure 22).
Figure 22. Components of a standard autopsy examination.
Until recently, there has been little need to examine the overall yield of each component of the standard autopsy. As consent rates have declined over recent years, the reasons for this decline have been examined. It has become increasingly evident from a number of qualitative studies that a major source of this decline is increasing parental awareness, sensitivity and dissatisfaction regarding the most invasive components of the standard autopsy (internal examination and organ histology), with parents associating internal examination with perceptions of disfigurement.\textsuperscript{9,14,47} Despite parental objections, at most centres performing perinatal and paediatric autopsy, options available to parents regarding the extent of examination are restricted to full autopsy with all investigations, cavity-limited autopsy (with a similar size of incision to full autopsy), placental examination (with or without external examination of the body), or to decline consent to any investigation. In effect, because the size of incisions made in traditional and limited autopsies is similar, the choice available is reduced to a binary one, which relates to whether parents accept major incisions or not.

Alternatives are beginning to emerge, and alongside the growth of modern radiological techniques, the field of post-mortem imaging has grown. Post mortem MRI, CT, and ultrasound have been explored as potential alternatives to internal examination, with varying degrees of success. PMCT is now well established in adult autopsy practice, while in paediatric and perinatal practice, PMMRI demonstrates excellent concordance (approximately 95%) with standard fetal autopsy, when genetics, microbiology and placental examination are included.\textsuperscript{107} In contrast, concordance rates of imaging alone
with the cause of death in older children are less good (76%) MARIAS, as infection may be difficult to determine without histological examination of a tissue. Some degree of invasive internal examination or exploration remains necessary in order to obtain visceral histology, as percutaneous needle-puncture autopsy procedures show poor reliability for the acquisition of a diagnostic quantity of tissue.\textsuperscript{75,76,94,95,99} Moreover, visceral histology has been shown to be very important in the investigation of a number of specific scenarios, including evaluation of SUDI\textsuperscript{50,90} and SUDC,\textsuperscript{111} where full histology is currently recommended in the evaluation of intrauterine fetal death, termination of pregnancy and neonatal death by the Royal College of Pathologists.\textsuperscript{88,89} Additionally, internal tissue may also be necessary for adjunctive tests performed as part of the autopsy process, such as microbiology, virology, metabolic, toxicological and chemical tests (depending on the clinical circumstances), though in general, less tissue is required for these tests than for histological analysis, and in fetal cases, many of these tests can be performed from placental tissue. Although there is good evidence that histology is valuable in some clinical circumstances, published data in a large cohort of intrauterine fetal deaths demonstrates that the yield of combined internal examination and histological sampling in this context is very low (approximately 1% of additional findings representing the cause of death which would not have been detected by a less invasive sample (e.g. placental histology).\textsuperscript{54-56}
In order to inform the rationale for routine organ sampling as part of a minimally invasive approach to autopsy it is necessary to establish a base of evidence regarding the likely yield of routine organ histology by clinical indication, which can be used to inform parents, clinicians and pathologists of the circumstances when invasive histology is essential and indicate scenarios when it is likely to add little to the overall investigation.
To explore this, the retrospective autopsy database used for recording data from >5000 perinatal autopsies performed at Great Ormond Street Hospital between 2005 and 2016 was interrogated.
Chapter two: Methods used in analysis of Retrospective data analysis

3.2.1: Case identification and classification

The Great Ormond Street Hospital autopsy database was established to record case data from SUDI cases\textsuperscript{34,50-52,90,91} and was subsequently expanded to cover IUFDs\textsuperscript{54-57,92,93}, SUDC\textsuperscript{111} and ToP. Additional data fields were created over time to capture information regarding antenatal history, clinical information provided in the referral, and pre-existing risk factors.

As part of this analysis, cases were entered into the database retrospectively by several different operators (identified on the chapter overview page), using the final post-mortem reports and the clinical information made available to the pathologist at the time of reporting. As part of data entry, interpretations made by the reporting pathologist regarding the abnormalities present at internal examination, and on histological examination were recorded according to pre-defined categories and definitions (causality categories, CCs), as laid out below (Figures 24 & 25). CCs were able to be allocated to system findings independently of other findings (e.g., ‘abnormal and definite cause of death’ could be allocated to both placental findings and organs showing evidence of abnormality in a case of intrauterine fetal death). CCs were allocated for both macroscopic and microscopic examination for all major organ systems (brain, heart, lungs, liver, kidneys, adrenal glands, pancreas, thyroid, placenta). All cases entered into the database were reviewed by a
senior pathology trainee prior to analysis. Additionally, all findings allocated as “abnormal and definitive cause of death”, or where a query arose regarding the importance of a finding, were further reviewed by consultant pathologist and a consensus opinion reached. If necessary, subtleties were explored further within free text boxes within the database adjacent to each overall finding. It was anticipated in advance of performing the analysis that an attrition rate would be present within the cohort, whereby a pathology report may indicate that an organ had been sampled, but there may not be enough information within the report to judge whether the tissue was normal or abnormal (e.g. due to autolysis of the tissue preventing pathologist comment). This was accounted for within the scoring system using an ‘insufficient comment’ category. Following completion of data entry, data was extracted for all completed cases between 2005 and 2016, according to referral category (SUDI, SUDC, IUFD, or ToP). Non-sudden deaths, referred organs (e.g. isolated hearts and brains referred for specialist opinion) and forensic cases were excluded from the analysis. Sudden and unexpected neonatal deaths were interrogated within the SUDI category. Following the application of inclusion and exclusion criteria, the cases were anonymised for the purposes of this study, and subsequently analysed using Microsoft Access, Microsoft Excel and Stats Direct.
<table>
<thead>
<tr>
<th>SUDC</th>
<th>SUDI</th>
<th>IUFD</th>
<th>ToP</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Sudden and</td>
<td>• Sudden and</td>
<td>• All intrauterine</td>
<td>• All terminations</td>
</tr>
<tr>
<td>unexpected</td>
<td>unexpected</td>
<td>fetal deaths regardless of</td>
<td>of pregnancy for</td>
</tr>
<tr>
<td>deaths</td>
<td>deaths</td>
<td>gestation</td>
<td>fetal abnormality or</td>
</tr>
<tr>
<td>• &gt;365 days of age</td>
<td>• 0 -365 days</td>
<td>• All stillbirths (including</td>
<td>placental</td>
</tr>
<tr>
<td></td>
<td>• Sudden</td>
<td>(including intrapartum), as</td>
<td>indications.</td>
</tr>
<tr>
<td></td>
<td>unexpected</td>
<td>long as no signs of life</td>
<td>• No ToP cases for</td>
</tr>
<tr>
<td></td>
<td>neonatal deaths</td>
<td>demonstrated</td>
<td>non-medical reasons were</td>
</tr>
<tr>
<td></td>
<td>included</td>
<td></td>
<td>included.</td>
</tr>
</tbody>
</table>

Figure 24. Criteria for the classification of deaths within the GOSH Access database.
<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Pathologist satisfied that histology or organ morphology lies within physiological limits.</td>
</tr>
<tr>
<td>Abnormal but not contributed to death</td>
<td>An abnormality is present, however, this is incidental to the death, with no causal relationship to the mechanism or cause of death. e.g. Mild vascular congestion, peripheral airway collapse</td>
</tr>
<tr>
<td>Abnormal and possibly contributed to death</td>
<td>An abnormality is present that may or may not have contributed to death. e.g. Presence of a small retroplacental clot</td>
</tr>
<tr>
<td>Abnormal and definitive cause of death</td>
<td>An abnormality is present that is highly likely to have caused death. Causal relationship with the cause of death. e.g. Florid necrotising chorioamnionitis on placental histology</td>
</tr>
</tbody>
</table>

Figure 25. Criteria for Causality Categories (CCs); these were applied to any macroscopic or microscopic abnormalities present in an autopsy case.
3.2.2 Methods: Attrition rate

The attrition rate (representing analysis of organs in cases where the presence of normality or abnormality could not be definitively determined) is documented for each organ by indication, within each section (Tables 12 - 15). Reasons that an organ may have been unsuitable for analysis included omission of sufficient detail within the autopsy report, autolysis of the organ preventing interpretation, failure to sample the organ in question, or failure of the target tissue to survive histological processing. In some cases, organ specific histology was taken but was not specifically commented on in the report; these were recorded as ‘normal’ value if a general comment was provided stating that in effect ‘histology from all organs was within normal limits’, otherwise excluded.

As part of evaluating the yield of macroscopic and microscopic evaluation, the following analyses were performed:

1. The presence of macroscopic abnormalities, categorised by CC, for each visceral organ by case type.

2. The presence of microscopic abnormalities, categorised by CC, for each visceral organ by case type.

3. The yield of macroscopic examination for each visceral organ (yield defined as the percentage of cases where a definitive cause of death is identified by visual inspection of an organ).
4. The yield of routine microscopic examination for each visceral organ (Yield defined as the percentage of cases where a definitive cause of death was identified by routine histological examination, where macroscopic examination of the organ was normal).

To maximise the objectivity of the dataset, analysis of cases where abnormalities were present classified as either “non-contributory” or “possibly contributory” were not further examined. The overall numbers were calculated for these CCs, but further analysis of these cases would have required application of a subjective interpretation to the pathologist’s reported comments.
Chapter three: Results of retrospective analysis of autopsy cases.

3.3.1 Case breakdown

5,311 eligible cases were identified within the period 2005-2016. The overall distribution of cases is shown in Figure 26. The main results are presented below; detailed organ-by-organ findings are presented in Appendix 2a-2d.

Figure 26. Overall distribution of cases within the database by classification of the clinical presentation.
3.3.2 Sudden Unexpected Death in Childhood

There were 824 total cases of SUDC recorded in the database with enough information to code the organs appropriately for both macroscopic and histological examination in the majority (range 56.6 - 95.4%, mean 80.3%, Table 12).

<table>
<thead>
<tr>
<th>Organ</th>
<th>Number of SUDC cases with adequate macroscopic and histological comment (total n = 824)</th>
<th>Number of indices excluded due to insufficient comment for classification (attrition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>739 (89.7%)</td>
<td>85</td>
</tr>
<tr>
<td>Lungs</td>
<td>786 (95.4%)</td>
<td>38</td>
</tr>
<tr>
<td>Brain</td>
<td>661 (80.2%)</td>
<td>163</td>
</tr>
<tr>
<td>Adrenals</td>
<td>654 (79.4%)</td>
<td>170</td>
</tr>
<tr>
<td>Thyroid</td>
<td>466 (56.6%)</td>
<td>358</td>
</tr>
<tr>
<td>Liver</td>
<td>735 (89.2%)</td>
<td>89</td>
</tr>
<tr>
<td>Kidney</td>
<td>715 (86.8%)</td>
<td>109</td>
</tr>
<tr>
<td>Pancreas</td>
<td>539 (65.4%)</td>
<td>285</td>
</tr>
</tbody>
</table>

Table 12. Analysis of SUDC reports, showing the proportion of reports where an index was considered sufficient for classification and the number lost to attrition (due to insufficient information in the report to allocate a causation category).

Macroscopic examination of organs at autopsy in the context of SUDC revealed a cause of death in 21.6% of cases overall, with most major anomalies occurring in the brain (7.8%), heart (6.6%) and lungs (5.1%), Figure 27.
The yield of microscopic examination of macroscopically normal organs in SUDC was 9.4% overall, with abnormalities in the lungs (3.7%), heart (3.4%) and brain (1.1%) representing areas of particularly high yield; the remaining organs combined demonstrated a yield of 2.3%, Figure 28.
Figure 28. Yield of microscopic examination of macroscopically normal organs in SUDC by organ.
3.3.3 SUDC summary

Macroscopic examination of the organs revealed a high proportion of the causes of death in this sub-group, with around 20% of cases receiving a 'definite' cause of death at this stage. The yield from histological examination of macroscopically normal organs in SUDC is also good, with around 10% of cases receiving a cause of death in this manner.

Some form of examination of the internal organs and histological investigation is therefore essential as part of investigation of SUDC deaths. Macroscopic examination of the brain (7.8%), heart (6.6%) and lungs (5.1%) were particularly important, with these organs representing areas of particularly high yield. Histology of macroscopically normal organs was also an important facet of the examination, particularly heart and lungs, and is therefore indicated in SUDC.

Recommendation

In SUDC cases, the yield from organ visualisation and routine histopathology is relatively high. There is, therefore, an evidence base to support routine examination of at least some internal organs along with routine histology in SUDC presentations.
3.3.4 Sudden Unexpected Death in Infancy

There were 1,739 SUDI cases with enough information to code both the macroscopic and microscopic appearances of the organs in the majority (range 69.6 – 96.6%, mean 88.7%, Table 13).

<table>
<thead>
<tr>
<th>Organ</th>
<th>Number of SUDI cases with adequate macroscopic and histological comment (total n = 1,739)</th>
<th>Number of indices excluded due to insufficient comment for classification (attrition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>1,638 (94.2%)</td>
<td>101</td>
</tr>
<tr>
<td>Lungs</td>
<td>1,680 (96.6%)</td>
<td>59</td>
</tr>
<tr>
<td>Brain</td>
<td>1,503 (86.4%)</td>
<td>236</td>
</tr>
<tr>
<td>Adrenals</td>
<td>1,586 (91.2%)</td>
<td>153</td>
</tr>
<tr>
<td>Thyroid</td>
<td>1,210 (69.6%)</td>
<td>529</td>
</tr>
<tr>
<td>Liver</td>
<td>1,647 (94.7%)</td>
<td>92</td>
</tr>
<tr>
<td>Kidney</td>
<td>1,631 (93.8%)</td>
<td>108</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1,447 (83.2%)</td>
<td>292</td>
</tr>
</tbody>
</table>

Table 13. Analysis of SUDI reports, showing the proportion of reports where an index was considered sufficient for classification and the number lost to attrition (due to insufficient information in the report to allocate a causation category).

Macroscopic examination of organs at autopsy in the context of SUDI revealed a cause of death in 12.2% of cases overall, with most major anomalies occurring in the heart (8.0%), brain (2.1%) and lungs (1.5%), Figure 29.
Figure 29. Yield of macroscopic examination in SUDI by organ.

The yield of microscopic examination of macroscopically normal organs in SUDI was 5.2% overall, with abnormalities in the lungs (2.7%), heart (0.8%) and brain (0.9%) representing areas of higher yield; the remaining organs demonstrated a combined yield of 0.8% (Figure 30).
Figure 30. Yield of microscopic examination of macroscopically normal organs in SUDI by organ.
3.3.5 SUDI summary

Macroscopic examination of the organs revealed a relatively high proportion of the causes of death in this sub-group, with around 10% of cases receiving a ‘definite’ cause of death via this process. The yield from histological examination of macroscopically normal organs in SUDI also reasonable, with around 5% of cases receiving a cause of death in this manner.

Some form of examination of the internal organs and histological investigation is therefore appropriate as routine part of investigation of SUDI deaths. Histological sampling of the lungs (2.7%), heart (0.8%) and brain (0.9%) showed the highest yield, although kidney (0.2%) and liver (0.4%) showed the cause of death in a number of cases, with a further 0.2% contributed by the adrenals and pancreas combined.

Recommendation

In SUDI cases, the yield from organ visualisation and routine histopathology is relatively high. There is adequate evidence to support routine examination of at least some internal organs, especially heart and lungs, along with histology of these organs in presentations of SUDI or neonatal death.
3.3.6 IUFD & stillbirth

There were 1,957 IUFD & stillbirth cases, with enough information to code both the macroscopic and microscopic appearances of the organs in most cases (range 56.7 – 85.3%, mean 75.8%, Table 14).

<table>
<thead>
<tr>
<th>Organ</th>
<th>Number of cases with adequate macroscopic and histological comment (total n = 1,957)</th>
<th>Number of indices excluded due to insufficient comment for classification (attrition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>1,619 (82.7%)</td>
<td>338</td>
</tr>
<tr>
<td>Lungs</td>
<td>1,642 (83.9%)</td>
<td>315</td>
</tr>
<tr>
<td>Brain</td>
<td>1,364 (69.7%)</td>
<td>593</td>
</tr>
<tr>
<td>Adrenals</td>
<td>1,563 (79.9%)</td>
<td>394</td>
</tr>
<tr>
<td>Thyroid</td>
<td>1,110 (56.7%)</td>
<td>847</td>
</tr>
<tr>
<td>Liver</td>
<td>1,670 (85.3%)</td>
<td>287</td>
</tr>
<tr>
<td>Kidney</td>
<td>1,604 (82.0%)</td>
<td>353</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1,300 (66.4%)</td>
<td>657</td>
</tr>
</tbody>
</table>

Table 14. Analysis of IUFD reports, showing the proportion of reports where an index was considered sufficient for classification and the number lost to attrition (due to insufficient information in the report to allocate a causation category).

Macroscopic examination of organs at autopsy in the context of IUFD or stillbirth revealed a cause of death in only 1.7% of cases overall, with most major anomalies occurring in the heart (0.5%), brain (0.6%) and lungs (0.3%). Macroscopic examination of the remaining organs yielded a cause of death in 0.3% of cases, Figure 3l.
Figure 31. Yield of macroscopic examination in IUFD by organ.
The yield of microscopic examination of macroscopically normal organs in IUFD / stillbirth was 1.4% overall, with abnormalities only identified in the lungs (1.3%) and kidney (0.1%), Figure 32. Of the cases where abnormalities representing the cause of death were identified in the lung, all of the cases were in fact fetal pneumonia in the context of ascending maternal genital tract infection, and were allocated to the database category of 'lung findings' because no placental findings were submitted with the fetus (had placental analysis shown severe ascending maternal genital tract infection, these cases would have been allocated under 'placental findings', irrespective of the findings in the lung and hence lung sampling would be unnecessary). Therefore, given this information, in our study, where a placenta was submitted with an IUFD/stillborn fetus and failed to show a cause of death, the routine histological sampling of macroscopically normal organs yielded a cause of death in only a tiny proportion (0.1%) of cases.

This finding is entirely consistent with the findings of Man et al.,\textsuperscript{55} whose data were generated from an earlier version of the same GOSH database, with implications for the practical protocol involved with the performance of minimally invasive autopsies (see Chapter 5).
Figure 32. Yield of microscopic examination of macroscopically normal organs in IUFD by organ.
3.3.7 IUFD & stillbirth summary

Macroscopic examination of the organs revealed a low frequency of cause of death in this sub-group, with only around 2% of cases receiving a ‘definite’ cause of death via this process. The yield from histological examination of macroscopically normal organs in IUFD was low, with almost no cases (only 0.1%) cases receiving a cause of death in this manner (excluding cases where placental examination would have revealed the cause; 1.4% without this exclusion applied). In the absence of a suspected anomaly from either antenatal investigations, clinical history, post-mortem imaging and placental examination, there is little evidence-based justification from these data for either organ visualisation or routine sampling of normal organs in the context of stillbirth or IUFD. This raises broader questions about resource allocation and novel investigative strategies, which will be explored further in the general discussion chapter (Part 5). However, mechanisms of death in IUFD and stillbirth remain poorly understood and it seems unlikely that breakthroughs will follow without a broader shift in investigative strategy.

Recommendation

In stillbirth and IUFD cases, the yield from organ visualisation and routine histopathology is low. The placenta is important in this scenario, revealing a cause of death in approximately one-third of cases, and should always be thoroughly examined by a specialist pathologist along with thorough clinical review.\textsuperscript{56}
3.3.8 Termination of pregnancy

There were 791 cases of ToP recorded in the database with enough information to code the organs appropriately for both macroscopic and histological examination in most cases (range 65.1% - 87.5%, mean 81.5%, Table 15).

<table>
<thead>
<tr>
<th>Organ</th>
<th>Number of cases with adequate macroscopic and histological comment (total n=791)</th>
<th>Number of indices excluded due to insufficient comment for classification (attrition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>675 (85.3%)</td>
<td>116</td>
</tr>
<tr>
<td>Lungs</td>
<td>692 (87.5%)</td>
<td>99</td>
</tr>
<tr>
<td>Brain</td>
<td>635 (80.3%)</td>
<td>156</td>
</tr>
<tr>
<td>Adrenals</td>
<td>662 (83.7%)</td>
<td>129</td>
</tr>
<tr>
<td>Thyroid</td>
<td>515 (65.1%)</td>
<td>276</td>
</tr>
<tr>
<td>Liver</td>
<td>686 (86.7%)</td>
<td>105</td>
</tr>
<tr>
<td>Kidney</td>
<td>669 (84.6%)</td>
<td>122</td>
</tr>
<tr>
<td>Pancreas</td>
<td>624 (78.9%)</td>
<td>167</td>
</tr>
</tbody>
</table>

Table 15. Analysis of ToP reports, showing the proportion of reports where an index was considered sufficient for classification and the number lost to attrition (due to insufficient information in the report to allocate a causation category).

Macroscopic examination of organs at autopsy in the context of ToP revealed a cause of death / major diagnosis in 25.8% of cases overall, with most major anomalies occurring in the brain (10.6%), heart (7.7%), kidneys (4.6%), lungs (2.2%) and liver (0.7%), Figure 33. Macroscopic examination of the remaining organs (adrenals, thyroid and pancreas) did not yield a cause of death in any further cases.
Figure 33. Yield of macroscopic examination in ToP by organ.
The yield of microscopic examination of macroscopically normal organs in ToP was only 0.3%, with abnormalities only identified in the brain (0.2%) and kidneys (0.1%), Figure 34. Review of these cases (one case in both categories) revealed that one case ventriculomegaly-associated changes in the presence of other visceral anomalies, while the abnormality present within the kidneys was autosomal recessive polycystic kidney disease in a case where the diagnosis had been confirmed antenatally using genetic analysis (array CGH) and the termination performed at 12gw, in which the kidneys still appeared macroscopically normal. Therefore, in only one case of 791 were there significant additional unsuspected findings from routine histology of macroscopically normal organs. This has significant implications for the implementation of a minimally invasive autopsy service when considering cases of ToP.
Yield (% of cases) of histological examination in ToP (microscopic abnormality representing cause of death, where macroscopy is normal)

<table>
<thead>
<tr>
<th></th>
<th>Heart</th>
<th>Lungs</th>
<th>Brain</th>
<th>Adrenals</th>
<th>Thyroid</th>
<th>Liver</th>
<th>Kidney</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>100.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>90.0</td>
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<td></td>
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<td></td>
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<tr>
<td>80.0</td>
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<tr>
<td>70.0</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30.0</td>
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<td></td>
</tr>
<tr>
<td>20.0</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>10.0</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Figure 34. Yield of microscopic examination of macroscopically normal organs in ToP by organ.
3.3.9 Termination of pregnancy summary

Macroscopic examination of the organs revealed a high proportion of the causes of death / major diagnoses in this sub-group, with around 25% receiving a ‘definite’ cause /diagnosis via this process. This is unsurprising, since visceral structural anomalies represent a common indication for ToP. Additional yield would have been provided by genetic analysis and placental examination, which were not considered as part of the current analysis. In contrast, the yield from histological examination of macroscopically normal organs in ToP is almost zero, with only 0.3% of cases receiving a cause of death / major diagnosis in this manner.

Recommendation

In ToPs, adequate examination for structural abnormalities of major organs is an essential as part of the autopsy process. Macroscopic examination of both clinically abnormal and clinically normal organs remains important, as there is the potential to change or refine the antenatal diagnosis. However, there is little to no value in sampling phenotypically normal organs for routine histology in this group.
Chapter four: Modelling of these data within a minimally invasive autopsy paradigm

3.4.1 Introduction

To better understand the challenges involved with implementing a stepwise, evidence-based minimally invasive autopsy service, the data was re-analysed for the ‘definite’ cause of death category to identify the proportion of causes likely to be picked up by a minimally invasive autopsy approach in future, and if any shortcomings of such an approach were evident.

As part of this analysis, cases with definite causes of death from each referral category (SUDC, SUDI, IUFD/SB, ToP) were re-analysed from the perspective of concordance with standard autopsy according to both 1) a non-invasive (Figure 35) and 2) a minimally invasive approach (Figure 36). For both, results are presented using concordance data from MIA/PM imaging studies. Due to the substantial learning curve present in reporting PM imaging studies, the actual diagnostic accuracy / concordance rates are likely to be better than the modelled rates presented below.
Figure 35. Steps involved in NIA-type investigation after death. Note the absence of internal examination and visceral histology.
Figure 36. Steps involved in investigation after death with MinImAL examination.
3.4.2 Methods used for modelling of data

3.4.2 Assumptions made as part of modelling data

The assumed paradigms for non-invasive and minimally invasive autopsy are shown above (Figures 35 and 36). The main difference between them is, by definition, the lack of internal examination and visceral histology within the non-invasive paradigm.

Many published studies present the concordance of imaging investigations with standard autopsy as follows:

1. Fetuses <24gw
2. Fetuses >24gw
3. Neonates and Children

For ToP cases, concordance rates were modelled using data from MRI studies reported as ‘Fetuses <24gw’ where available, as many terminations of pregnancy take place prior to 24gw. For IUFD cases, concordance rates were modelled using data from MRI studies reported as ‘Fetuses >24gw’ where available. For SUDI and SUDC cases, concordance rates were modelled using data from MRI studies reported as ‘Neonates and Children’. Although ideally any fetus from an IUFD or ToP cases of less than 18gw would be reported using micro-CT imaging (see part four of this thesis), the literature regarding concordance with standard autopsy is still evolving, and as such, MRI data has been chosen as the basis for the model presented in the following section.
3.4.3 Modelling discovery and non-discovery of autopsy findings

Published concordance rates for PMMRI investigation of fetal, neonatal and childhood death were applied to the cohorts identified using the GOSH autopsy database. The 95% limits of confidence were used to calculate the number of potential ‘non-discovered’ (missed) macroscopic abnormalities, according to three models:

1. NIA (imaging only)

For this model, it is assumed that PM imaging alone would detect a range of macroscopic abnormalities responsible for death based on published 95% confidence intervals concordance of radiology with autopsy by system, but that any histological abnormalities would be missed entirely.

2. Incremental approach (PMMRI imaging, followed by targeted MinImAL sampling of macroscopically abnormal organs only

For this model, it is assumed that PM imaging would detect a range of macroscopic abnormalities deemed responsible for death based on published 95% confidence intervals concordance of radiology with autopsy by system. Further, it is assumed that organs with a histological abnormality present deemed to be responsible for death would show an arbitrary secondary effect (e.g. change of signal on MRI imaging / haemorrhage / oedema / calcification) that would flag them for targeted sampling (with sampling success rates from
MinImAL data applied to this task to calculate the number of histological abnormalities detected). In cases where there was a histological abnormality responsible for death but no concurrent macroscopic abnormality, it is assumed that these causes of death would be missed entirely.

3. Routine MinImAL sampling post PMMRI

For this model, it is assumed that PM imaging would detect a range of macroscopic abnormalities deemed responsible for death based on published 95% confidence intervals concordance of radiology with autopsy by system. Further, it is assumed that routine MinImAL sampling of thoracic and abdominal organs will then be undertaken in every case, with sampling success rates from MinImAL data applied to this task to calculate the number of histological abnormalities detected. Further, in cases where there was a histological abnormality responsible for death but no concurrent macroscopic abnormality, it is assumed that these causes of death would be detected at the MinImAL sampling success rate.

For each model, the maximum potential number of missed causes of death is presented per 1,000 cases examined.

The advantages of modelling in this manner are as follows:
1. Data are presented on a system-by-system basis, and are largely independent of concurrent non-invasive ancillary placental, genetic and microbiological investigations which would be carried out in all cases.

2. The models presented represent clinically relevant data which may impact choice to parents that could be employed in the future.

Limitations of modelling in this manner are as follows:

1. Using “maximum potential non-discovery rate” is a pessimistic presentation of the data and assumes the worst-case scenario.

2. Similar data for placental and genetic abnormalities are not available.

3. Exclusion of all abnormalities deemed “possibly contributory to death” may exclude some actual causes of death.

4. Failure to consider improvement in interpretation of PM imaging and improvement in MinImAL sampling, following the publication of concordance rates.
Chapter five: Results & Discussion

3.5.1 Modelling of NIA and MIA effectiveness

The concordance rates of PM imaging (PMMRI) are presented in the context of new-borns and children below (Table 16).

<table>
<thead>
<tr>
<th>Organ (Neonates and children)</th>
<th>Criteria</th>
<th>PMMRI Sens</th>
<th>PMMRI Spec</th>
<th>PMMRI % concordance with autopsy</th>
<th>MinImAL sampling success rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain 32</td>
<td>Overall brain pathology</td>
<td>98.0 % [89.5 – 99.6]</td>
<td>80.9 % [70.0 – 88.5]</td>
<td>88.1 % [81.1 – 92.8]</td>
<td>N/A</td>
</tr>
<tr>
<td>Cardiac pathology 113</td>
<td>Structural or non-structural disease</td>
<td>61.9 % [40.9–79.3]</td>
<td>98.0 % [93.1–99.5]</td>
<td>93.3 %</td>
<td>Heart 100%</td>
</tr>
<tr>
<td>Thoracic pathology 112</td>
<td>Overall abnormalities (non-cardiac)</td>
<td>45.2 % [33.4, 57.5]</td>
<td>60.7 % [48.1, 71.9]</td>
<td>52.8 % [44.1, 61.4]</td>
<td>Lung 100%</td>
</tr>
<tr>
<td>Abdominal pathology 114</td>
<td>Abdominal pathological lesions</td>
<td>70.6 % [46.9–86.7]</td>
<td>86.8 % [79.0, 92.0]</td>
<td>84.6 % [77.1, 89.9]</td>
<td>Kidney 100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Liver 97%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Adrenal 89%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pancreas 82%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Spleen 93%</td>
</tr>
</tbody>
</table>

Table 16. Sensitivity, specificity and concordance of PM MRI in neonates and children from published studies,32, 112–114 along with MinImAL sampling success rates of those respective organs.

These data inform the modelling of SUDC and SUDI cases as follows in Table 17.
<table>
<thead>
<tr>
<th>Children (SUDC presentation) Retrospective cohort total: 824</th>
<th>Number of macroscopic abnormalities definitively representing the cause of death</th>
<th>Predicted range of macroscopic CODs modelled to be detected by PM imaging (PMMRI) using 95% CI (where available)</th>
<th>Number of histological abnormalities definitively representing the cause of death</th>
<th>Predicted number of histological CODs detected using MinImAL</th>
<th>Number of definitive histological causes of death in retrospective cohort database where macroscopic examination was normal</th>
<th>Maximum potential rate of COD non-discovery using NIA (PMMRI only, no histological sampling)</th>
<th>Maximum potential rate of COD non-discovery by adopting incremental approach (PMMRI, then MinImAL if abnormal)</th>
<th>Maximum potential rate of COD non-discovery by adopting routine MinImAL post PMMRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>55</td>
<td>45 – 51</td>
<td>70</td>
<td>N/A</td>
<td>7</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Heart</td>
<td>52</td>
<td>Approx. 49</td>
<td>96</td>
<td>96</td>
<td>25</td>
<td>120.1 per 1,000 cases</td>
<td>34.0 per 1,000 cases</td>
<td>3.6 per 1,000 cases</td>
</tr>
<tr>
<td>Lung</td>
<td>40</td>
<td>18 - 25</td>
<td>237</td>
<td>237</td>
<td>29</td>
<td>314.3 per 1,000 cases</td>
<td>61.9 per 1,000 cases</td>
<td>26.7 per 1,000 cases</td>
</tr>
<tr>
<td>Kidney</td>
<td>6</td>
<td>5 - 5</td>
<td>14</td>
<td>14</td>
<td>3</td>
<td>18.2 per 1,000 cases</td>
<td>4.9 per 1,000 cases</td>
<td>1.2 per 1,000 cases</td>
</tr>
<tr>
<td>Liver</td>
<td>5</td>
<td>4 - 4</td>
<td>14</td>
<td>14</td>
<td>3</td>
<td>18.2 per 1,000 cases</td>
<td>4.9 per 1,000 cases</td>
<td>1.2 per 1,000 cases</td>
</tr>
<tr>
<td>Adrenal</td>
<td>2</td>
<td>2 - 2</td>
<td>7</td>
<td>6</td>
<td>1</td>
<td>8.5 per 1,000 cases</td>
<td>2.4 per 1,000 cases</td>
<td>1.2 per 1,000 cases</td>
</tr>
<tr>
<td>Pancreas</td>
<td>3</td>
<td>2 - 3</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>6.1 per 1,000 cases</td>
<td>2.4 per 1,000 cases</td>
<td>2.4 per 1,000 cases</td>
</tr>
</tbody>
</table>

Table 17. Modelling of the rate of missed causes of death in SUDC by organ, informed by PMMRI concordance rates and MinImAL sampling success from Table 16.
In the context of SUDC, the retrospective analysis of histological findings and the modelling data presented demonstrate additional yield from routine sampling of most organs, even where macroscopically / radiologically normal. This is particularly the case for the heart, lung, kidney and liver, but also applies to the adrenal glands and pancreas. The modelling data for SUDI cases are presented on the following page (Table 18).

Based on this data, some causes of death originating in the thoraco-abdominal cavities are likely to be missed by adopting the use of routine MinImAL autopsy following PMMRI when compared with standard autopsy in the context of SUDC (36.3 non-discoveries per 1,000 cases performed). Of those causes of death modelled to be missed, the clear majority (26.7 per 1,000 cases) would occur in the lung. However, several factors limit the applicability of MinImAL autopsy in the context of SUDC. Firstly, the sampling accuracy of MinImAL is based predominantly on fetal cases, with only one SUDC case within the cohort, which was a limited examination. Additionally, gross evaluation of the brain is not covered by MinImAL examination; this is known to be important in older children.\textsuperscript{51,52,111,116} Moreover, the large variation of body size in SUDC is likely to preclude adequate sampling of organs through one incision site in many cases. As a result, it is not possible to recommend the routine use of PM imaging with MinImAL examination in the context of SUDC, though it may be useful to answer a targeted clinical question around the death. PM imaging is likely to be a useful adjunct to standard autopsy in SUDC cases.
Table 18. Modelling of the rate of missed causes of death in SUDI by organ, informed by PMMRI concordance rates and MinImAL sampling success from Table 16. ¶ = unable to calculate due as zero definitive causes of death present in the autopsy cohort.
The findings from the modelling of SUDI data demonstrate similarities to those of the SUDC cohort. Both the retrospective analysis of histological findings and the modelling data presented demonstrate a degree of additional yield from routine sampling of most organs, even where macroscopically/radiologically normal. This is particularly the case for the heart, lung, kidney and liver.

Based on this data, some causes of death originating in the thoraco-abdominal cavities are likely to be missed by adopting the use of routine MinImAL autopsy following PMMRI when compared with standard autopsy in the context of SUDI (approximately 14.9 cases per 1,000 cases). Of those causes of death modelled to be missed, the clear majority would occur in the heart (5.2 per 1,000 cases) or lung (8.6 per 1,000 cases). However, the sampling accuracy of MinImAL is based predominantly on fetal cases, with relatively little data from SUDI cases within the cohort. Additionally, gross evaluation of the brain is not covered by MinImAL examination; this is known to be important in SUDI cases. As a result, it is not possible to recommend the routine use of PM imaging with MinImAL examination in the context of SUDI, though it may be useful to answer a targeted clinical question around the death. PM imaging is likely to be a useful adjunct to standard autopsy in SUDI cases.

The concordance rates for post-mortem fetal PMMRI are shown in the following table. The data from fetuses of greater than 24 weeks was used to model data from the IUFD and stillbirth cohort. The data from fetuses of less than 24 weeks was used to model data from the ToP cohort (Tables 21 – 22).
<table>
<thead>
<tr>
<th>Organ (Fetal &gt;24 gw)</th>
<th>Criteria</th>
<th>PMMRI Sens</th>
<th>PMMRI Spec</th>
<th>PMMRI % concordance with autopsy</th>
<th>MinImAL sampling success rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain 32</td>
<td>Overall brain pathology</td>
<td>71.0 % [53.4 – 83.9]</td>
<td>76.9 % [63.9 – 86.3]</td>
<td>74.7 % [64.4 – 82.8]</td>
<td>N/A</td>
</tr>
<tr>
<td>Cardiac pathology 113</td>
<td>Structural or non-structural disease</td>
<td>83.3 % [43.7–97.0]</td>
<td>94.1 % [86.8–97.4]</td>
<td>93.3%</td>
<td>Heart 100%</td>
</tr>
<tr>
<td>Thoracic pathology 112</td>
<td>Overall abnormalities (non-cardiac)</td>
<td>37.5 % [18.5, 61.4]</td>
<td>88.2 % [79.0, 93.6]</td>
<td>79.3 % [70.0, 86.4]</td>
<td>Lung 100%</td>
</tr>
<tr>
<td>Abdominal pathology 114</td>
<td>Abdominal pathological lesions</td>
<td>64.7 % [41.3 – 82.7]</td>
<td>89.3 % [80.3, 94.5]</td>
<td>84.8 % [76.1, 90.7]</td>
<td>Kidney 100%</td>
</tr>
</tbody>
</table>

Kidney 100%
Liver 97%
Adrenal 89%
Pancreas 82%
Spleen 93%

Table I9. Sensitivity, specificity and concordance of PM MRI in fetuses of >24gw from published studies,32,112-114 along with MinImAL sampling success rates of those respective organs.
Table 20. Modelling of the rate of missed causes of death in IUFD by organ, informed by PMMRI concordance rates and MinImAL sampling success. ¶ = unable to calculate due as zero definitive causes of death present in the autopsy cohort. * = deaths including congenital pneumonia where no placenta was submitted for examination. ** = amended rate, assuming placenta had been available.

<table>
<thead>
<tr>
<th>IUFD (all)</th>
<th>Retrospective cohort total: 1957</th>
<th>IUFD (all)</th>
<th>Data modelled using &gt;24gw charts</th>
<th>Number of macroscopic abnormalities definitively representing the cause of death</th>
<th>Predicted number of macroscopic CODs modelled to be detected by PM imaging (PMMRI) using 95% CI (where available)</th>
<th>Number of histological abnormalities definitively representing the cause of death</th>
<th>Predicted number of histological CODs detected using MinImAL</th>
<th>Number of definitive histological causes of death in retrospective cohort database where macroscopic examination was normal</th>
<th>Maximum potential rate of COD non-discovery using NIA (PM MRI only, no histological sampling)</th>
<th>Maximum potential rate of COD non-discovery by adopting incremental approach (PMMRI, then MinImAL if abnormal)</th>
<th>Maximum potential rate of COD non-discovery by adopting routine MinImAL post PMMRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>9</td>
<td>6 - 7</td>
<td>70</td>
<td>N/A</td>
<td>7</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Heart</td>
<td>8</td>
<td>7</td>
<td>0</td>
<td>a</td>
<td>a</td>
<td>1.0 per 1,000 cases</td>
<td>1.0 per 1,000 cases</td>
<td>1.0 per 1,000 cases</td>
<td>1.0 per 1,000 cases</td>
<td>1.0 per 1,000 cases</td>
<td>1.0 per 1,000 cases</td>
</tr>
<tr>
<td>Lung</td>
<td>6</td>
<td>4 - 5</td>
<td>28</td>
<td>28</td>
<td>21*</td>
<td>15.3 per 1,000 cases*</td>
<td>11.8 per 1,000 cases*</td>
<td>1.0 per 1,000 cases</td>
<td>1.0 per 1,000 cases</td>
<td>1.0 per 1,000 cases</td>
<td>1.0 per 1,000 cases</td>
</tr>
<tr>
<td>Kidney</td>
<td>3</td>
<td>2 - 3</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>2.6 per 1,000 cases</td>
<td>1.5 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
</tr>
<tr>
<td>Liver</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>&lt;1.0 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
</tr>
<tr>
<td>Adrenal</td>
<td>0</td>
<td>¶</td>
<td>0</td>
<td>¶</td>
<td>¶</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0</td>
<td>¶</td>
<td>0</td>
<td>¶</td>
<td>¶</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>
In the context of stillbirth or IUFD, almost no causes of death are likely to be missed by adopting either an incremental or MIA approach (PMMRI followed by targeted sampling of abnormal organs) when compared with standard autopsy, provided that the placenta is available for examination. If the placenta is not available for examination, targeted sampling of the fetal lungs may be useful to evaluate the presence or absence of an inflammatory, which is known to occur in association with established cases of ascending maternal genital tract infection. NIA in this group is also likely to miss few causes of death compared with standard autopsy if targeted sampling of clinically suspected or radiologically identified anomalies is factored into the sampling strategy, and the placenta is available for examination. If there are clinical questions regarding the mechanism or cause of death in a peri-partum or intrapartum stillbirth, standard autopsy may still be necessary, as definitive proof regarding the presence or absence of certain findings may be medicolegally important (e.g. following obstructed labour or traumatic delivery).
<table>
<thead>
<tr>
<th>Organ (Fetal &lt;24 wks)</th>
<th>Criteria</th>
<th>PMMRI Sens</th>
<th>PMMRI Spec</th>
<th>PMMRI % concordance with autopsy</th>
<th>MinImAL sampling success rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain 32</td>
<td>Overall brain pathology</td>
<td>87.1 % [71.1 – 94.9]</td>
<td>69.2 % [59.9 – 77.1]</td>
<td>73.2 % [65.2 – 79.9]</td>
<td>N/A</td>
</tr>
<tr>
<td>Cardiac pathology 113</td>
<td>Structural or non-structural disease</td>
<td>82.4 % [59.0 – 93.8]</td>
<td>96.2 % [91.3 – 98.4]</td>
<td>94.5%</td>
<td>Heart</td>
</tr>
<tr>
<td>Thoracic pathology 112</td>
<td>Overall abnormalities (non-cardiac)</td>
<td>30.3 % [17.4 – 47.3]</td>
<td>96.0 % [91.0 – 98.3]</td>
<td>82.3 % [75.6, 87.4]</td>
<td>Lung</td>
</tr>
<tr>
<td>Abdominal pathology 114</td>
<td>Abdominal pathological lesions</td>
<td>77.1 % [61.0 – 87.9]</td>
<td>95.1 % [89.8, 97.7]</td>
<td>91.1 % [85.7, 94.6]</td>
<td>Kidney</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Adrenal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pancreas</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Spleen</td>
</tr>
</tbody>
</table>

Table 21. Sensitivity, specificity and concordance of PM MRI in fetuses of <24gw from published studies,32,112-114 along with MinImAL sampling success rates of those respective organs.
Table 22. Modelling of the rate of missed causes of death in ToP by organ, informed by PMMRI concordance rates and MinImAL sampling success.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Number of macroscopic abnormalities definitively representing the cause of death</th>
<th>Predicted range of macroscopic CODs modelled to be detected by PM imaging (PMMRI) using published concordance data and 95% CI (where available)</th>
<th>Number of histological abnormalities definitively representing the cause of death</th>
<th>Approximate number of histological CODs modelled to be detected using MinImAL</th>
<th>Number of definitive histological causes of death in retrospective cohort database where macroscopic examination was normal</th>
<th>Maximum potential rate of COD non-discovery using NIA (PM MRI only, no histological sampling)</th>
<th>Maximum potential rate of COD non-discovery by adopting incremental approach (PMMRI, then MinImAL if abnormal)</th>
<th>Maximum potential rate of COD non-discovery by adopting routine MinImAL post PMMRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>74</td>
<td>48 - 59</td>
<td>40</td>
<td>N/A</td>
<td>7</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Heart</td>
<td>56</td>
<td>53</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>5.1 per 1,000 cases</td>
<td>3.8 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
</tr>
<tr>
<td>Lung</td>
<td>16</td>
<td>12 - 14</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>17.7 per 1,000 cases</td>
<td>5.1 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
</tr>
<tr>
<td>Kidney</td>
<td>33</td>
<td>28 - 31</td>
<td>23</td>
<td>23</td>
<td>1</td>
<td>35.4 per 1,000 cases</td>
<td>7.6 per 1,000 cases</td>
<td>1.3 per 1,000 cases</td>
</tr>
<tr>
<td>Liver</td>
<td>5</td>
<td>4 - 5</td>
<td>0</td>
<td>¶</td>
<td>¶</td>
<td>1.3 per 1,000 cases</td>
<td>1.3 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
</tr>
<tr>
<td>Adrenal</td>
<td>0</td>
<td>¶</td>
<td>0</td>
<td>¶</td>
<td>¶</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0</td>
<td>¶</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1.3 per 1,000 cases</td>
<td>&lt;1 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
</tr>
</tbody>
</table>

¶ = unable to calculate due as zero definitive causes of death present in the autopsy cohort.
In the context of termination of pregnancy, few additional causes of death are likely to be missed by adopting routine MinImAL examination of the major internal organs following PMMRI when compared with standard autopsy. An incremental approach to autopsy (PMMRI followed by targeted sampling of abnormal organs) will also miss few causes of death compared with standard autopsy if targeted sampling of clinically suspected anomalies is factored into the sampling strategy (this is likely to negate most of the additional ‘misses’ within the modelling data, as the model does not take into account pathologist autonomy with the presence of an antenatally known abnormality). Furthermore, an incremental strategy informed by antenatal findings and specific clinical questions makes sense within the remit of the pathologist and the need to communicate results with the team in charge of patient care.
These data from a large series of unselected cases demonstrate that the contribution of the invasive components of perinatal and paediatric autopsy (macroscopic examination and routine organ histology) to the overall cause of death is dependent upon patient group. Specific types of case are associated with high yields.

Macroscopic examination of organs contributed the final cause of death in 25.8% of ToP cases, 21.6% of SUDC cases, 12.2% of SUDI cases and 1.7% of stillbirth/IUFD cases. Evaluation of internal organs therefore remains a recommended component of the autopsy process in ToP, SUDC and SUDI. The methodology used to evaluate the organs in this chapter of the thesis relied upon the use of a major incision to extract and examine the organs, however, alternative methodologies such as laparoscopically assisted MIA (discussed at length in part 2) may provide more acceptable surrogate to open examination of the organs with similar concordance with traditional autopsy examination. The published concordance for PM imaging as compared to macroscopic examination varies by methodology and case type, but is generally high, with MRI showing up to approximately 93% concordance with autopsy for assessment of pathology vs normality in fetuses and children and micro-CT showing up to 97.5% concordance for assessment of pathology vs. normality across any body system of small fetuses.32,59,67,112,113

In 5-10% of SUDC/SUDI cases, the final cause of death is determined by routine histological sampling of macroscopically normal organs, predominantly heart and lungs, with a few cases contributed by brain, liver
and kidney examination. Therefore, routine histological sampling of major organs remains an important aspect of investigation after death in SUDC and SUDI case, even if PM imaging appears normal.

Conversely, in ToP and IUFD cases, routine histological sampling of macroscopically normal organs contributes the cause of death in 0.3% and 1.4% of cases respectively (although the true figure for IUFD may be as low as 0.1%, as the vast majority of these cases were accounted for by fetal pneumonia where a placenta was unavailable).

When coupled with the knowledge that PMMRI has a very high concordance with traditional autopsy, it is logical that if PMMRI shows normal appearances of the internal organs in the context of stillbirth, IUFD or ToP, there is little clinical indication to aggressively sample normal organs. Targeted sampling of abnormal organs and lung may be sufficient to identify abnormal/contributory cases. The importance of placental examination cannot be understated in the context of IUFD/stillbirth, with approximately one third of causes of death provided through placental examination in this context.
Part three summary

Macroscopic evaluation of organs contributes the cause of death in 12-26% of SUDC, SUDI, and ToP cases. This process, or an appropriate and accurate surrogate with high concordance (e.g. post-mortem imaging) remains essential for investigation after death in most circumstances. The value of macroscopic examination in stillbirth/IUFD is less certain, with a contribution of only 1.7% of causes of death. Routine histological sampling of macroscopically normal organs provides significant contribution to the cause of death in 5-10% of SUDI/SUDC cases and is recommended in these contexts. Histological sampling of macroscopically normal organs in fetal cases, including ToP, SB and IUFD provides almost no (<1%) useful information for determining the cause of death or main diagnosis and for many organs the yield is zero. Therefore routine sampling in such cases is of limited value yet consumes resource and hence should not be routinely performed. In cases with contributory histological findings, almost all cases relate to heart, lungs, liver and kidney.
Part four: Development of post mortem microfocus computed tomography (Micro-CT) for early fetal loss.

Pre-existing evidence

Post mortem 1.5 T and 3 T MRI may be an alternative to conventional fetal autopsy but do not provide adequate diagnostic imaging of early gestation fetuses, particularly below 400 g body weight or 18 weeks gestation. Micro-CT could be developed and used for diagnostic purposes in the assessment of human pathology. This was assessed in extracted organs prior to assessing the diagnostic accuracy of post mortem micro-CT imaging against conventional autopsy in whole human fetuses.

Added value of this work

In addition to providing high resolution, three dimensional morphological assessments of congenital abnormalities and neoplastic pathology, micro-CT shows high levels of agreement with conventional autopsy across multiple organ systems in 20 cases of fetal loss or termination of pregnancy (700/718 indices, agreement = 97.5%, 95% CI, 96.6 – 98.4). In autopsy cases where the gestational age is under 14 weeks, micro-CT analysis yielded significantly fewer non-diagnostic indices than autopsy examination (22 / 440 vs 48 / 348 respectively; p<0.001).
Implications of all available evidence

Post mortem fetal micro-CT may offer an acceptable, non-invasive method of post mortem examination after early gestation fetal loss or early termination of pregnancy. High resolution fetal post mortem imaging facilitates discussion between all medical practitioners involved in the counselling of parents for future pregnancies.
Chapter one: Introduction to micro-CT

4.1.1: Rationale for the use of micro-CT

The introduction of clinical CT in the 1970s, revolutionised the diagnosis and treatment pathways of many medical conditions, offering clinicians new insights into pathological processes without the need for invasive surgery. Current CT scanners can image structures down to a voxel size of approximately 1 mm³ whilst minimising exposure to ionising radiation. In parallel to the development of clinical CT, the expansion of mass precision engineering (e.g. engine blocks / turbine blades) helped to provide a demand for higher resolution imaging of non-biological specimens, in order to improve the quality of manufacturing, to non-destructively analyse materials and to reduce faults. This led to the first microfocus CT (micro-CT) scanners being developed in the 1980s. Over the subsequent decades, micro-CT imaging technology has advanced considerably, with current scanners able to achieve sub-micron voxel-level resolution. The increasing availability and usage of this technique in modern science is reflected by the rising number of publications on the topic. This has been predominantly due to work in small animal studies which include phenotyping, bone morphology analysis and plant biology. Micro-CT has also found an important role in archaeology, where historical artefacts can be analysed and “virtually dissected” to investigate their contents, thereby minimising disruption and degradation due to handling.
Micro-CT may therefore appeal as part of a minimally invasive autopsy strategy, where the combination of high-resolution imaging whilst preserving tissue integrity could offer a means of investigation after death to parents who decline standard autopsy. Additionally, perinatal autopsies can be technically difficult procedures in smaller fetuses, for example, brain extraction in early gestations. Where complex anomalies require documentation, photography or demonstration, a non-destructive dataset offers the opportunity of a permanent record of clinical abnormalities (or of normal anatomy, in the process of exclusion of disease). This can be preserved as part of the mother’s medical notes, correlated with antenatal imaging, communicated to colleagues and used to base management decisions regarding future pregnancies. In particular, autopsy examination of early miscarriages or terminations can be technically challenging, with the possibility that congenital abnormalities (such as delicate intracranial cystic structures) may be missed or misinterpreted.

From a clinical perspective, there is a drive towards earlier diagnosis of fetal abnormalities, with numerous groups reporting the accuracy of first trimester ultrasound for fetal abnormalities. In combination with increasing uptake of non-invasive prenatal testing (NIPT), these circumstances could potentially lead to increased numbers of early terminations, with subsequent challenges for pathologists (and therefore clinicians) in confirming the diagnosis. Imaging is increasingly used to guide the autopsy process in both adults and children. Post-mortem 1.5 T MRI shows excellent correlation with autopsy findings over 18gw / 400g bodyweight, however, its diagnostic accuracy is reduced below
these thresholds.\textsuperscript{28} High field MRI (7 or 9.4 T) can provide the necessary resolution to meaningfully examine small fetuses and embryos,\textsuperscript{32,107} but it is expensive and available only in specialist centres and at a prohibitive cost. Initial experience with 3 T PMMRI shows improvement in image quality but no improvement in diagnostic accuracy when compared to 1.5 T MRI.\textsuperscript{68} Alternative high-resolution imaging modalities must be sought may therefore be more successful than MRI-based imaging in early pregnancy loss or terminations of pregnancy. Although proof of principle of the use of micro-CT has been demonstrated in a limited number of human fetuses,\textsuperscript{87} technical aspects regarding tissue preparation and assessment of diagnostic accuracy by body site compared to autopsy remain to be demonstrated, both in extracted organs and in whole fetuses.

This section aims to evaluate the potential role of micro-CT in perinatal autopsy. Firstly, an outline of the most important facets of micro-CT function is presented. This will aid understanding of the design of the following experiments. Following on from the work presented within this thesis on the feasibility of micro-CT examination of extracted organs samples, the scope of post-mortem micro-CT examination was expanded to include whole body fetal micro-CT including an assessment of the diagnostic accuracy of this technique and areas for future optimisation.
4.1.2 Part four aims

1. Evaluate technical issues around the use of micro-CT for
   a. Choice of contrast medium
   b. Effect of contrast on subsequent sampling and histology

2. Demonstrate proof of principle for imaging of extracted organs using micro-CT.

3. Demonstrate proof of principle for whole body fetal imaging using micro-CT.
4.1.3 On the theory and practicalities of Micro-CT

Like clinical CT scanners, micro-CT scanners produce X-rays by using a hot metal cathode (often made of tungsten or tungsten-rhenium) to produce an electron beam within a vacuum. The beam is focussed by a series of magnetic lenses onto a metal target (anode); as a result, X-rays are produced from the energy imparted by the electron beam to the electrons in the target material (characteristic radiation) and the deceleration of the beam (Bremsstrahlung (‘braking’) X-rays, Figure 37). The size of the focal spot on the metal anode (the site responsible for the production of the majority of X-rays) is an order of magnitude smaller than clinical CT scanners, thus improving image resolution by producing a pinhole camera-like effect\textsuperscript{122} but necessitating longer scans due to a relative reduction in the number of X-ray photons produced (minutes rather than seconds). It is this characteristic which gives rise to the nomenclature ‘microfocus computed tomography’; the size of the focal spot represents a major difference between micro-CT and clinical CT. This mechanism produces a polychromatic X-ray spectrum, where photons are emitted with wide range of energy values (from minimal energies (<1kV, may be stopped by paper) up to the maximum accelerating voltage applied between the cathode and anode (set by the user)). This is unlike a synchrotron, which utilises a coherent source (free electrons travelling at nearly the speed of light) to produce monochromatic X-rays (e.g. 140kV X-rays only), but similar to clinical CT.
Figure 37. X-ray spectrum for a Tungsten anode. Characteristic peaks in X-ray photon production are seen at 58-59kV, 67kV and 69kV. Generated using SpekCalc from NIST data.⁸⁵

Through precise control of the imaging z-axis, the range of magnification achievable using micro-CT is much greater than in clinical CT scanners. As a result, the resolution further improves proportionally to increasing magnification (albeit with decreasing field of view on the detector). This demonstrates a second important difference with clinical CT; in micro-CT, resolution generally improves with decreasing field of view (or sample size) as the object can be brought closer to the X-ray source, unlike in CT. Rotation of the sample within the beam (Figure 38) allows computer algorithms (e.g. using modified filtered back projection), to reconstruct detailed 3D internal
structures using the intensity values of the projected images. This process is similar to clinical CT, but may be calculated using different techniques (iterative reconstruction methods may provide a superior result in clinical CT where the number of X-rays taken (projections) may be limited to reduce radiation exposure, whereas non-iterative filtered back projection methods excel where large numbers of projections are available, such as in micro-CT).

Figure 38. Schematic of a micro-CT machine, demonstrating geometric magnification of a star-shaped object within a cone of X-rays. Adapted from Hutchinson et al.\textsuperscript{86} with permission.
4.1.4 Choice of Exogenous Contrast

As previously discussed, soft tissues have little inherent variation in X-ray absorption. As such, investigations utilising a standard incoherent X-ray source (such as those used in medical radiography, clinical CT and micro-CT) require either exogenous contrast or extremely long scan times to visualise differential levels of tissue contrast within soft tissues. Studies examining non-contrasted paraffin embedded human tissue taking the latter approach have routinely used scan times in the region of 12 hours.\textsuperscript{123-125} This may be acceptable for a mechanically stable object, such as a tissue block mounted in a secure holder, within a temperature and humidity-controlled environment. However, it is unlikely to be successful when scanning wet tissue such as an extracted tissue biopsy, human organ or fetal body, as these may be prone to slumping during the scan, or changes in dimension due to evaporation. The margins for movement error in micro-CT are small, as movement of as little as 0.01 mm may render a scan uninterpretable due to the amplification of movement artefact (due to geometric magnification of all movement, alongside with the specimen). Additionally, were the service to be implemented clinically, scan times of 12 hours with a high rate of unsuccessful scans may thwart its utility to clinicians and acceptability to parents.

Thus, exogenous contrast is required in order to reduce the scan times required to a manageable level. Though several exogenous contrast agents have been published within the literature regarding contrast-enhanced micro-CT\textsuperscript{80,81,126-128}, not all are relevant to this project if the ultimate goal is to
provide a safe, fast imaging of whole fetuses with minimal tissue distortion, whilst minimising cost (Table 23). For the purposes of minimally invasive autopsy, the ideal micro-CT contrast agent should:

1. Diffuse freely, without the need for injections or incisions

2. Diffuse quickly, within 72 hours (assuming a further 48 hours for scan, reporting and follow-on investigations if necessary)

3. Be safe to store and easy to dispose of

4. Be cheap and easy to acquire

5. Be scalable in volume / concentration

6. Be reversible

7. Not prevent histology and immunohistochemistry
<table>
<thead>
<tr>
<th>Reagent costs (approximation)</th>
<th>Hazards</th>
<th>Preparation</th>
<th>Storage</th>
<th>Disposal</th>
<th>Diffusion rate</th>
<th>Effect on histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>I$_2$KI (Kl$_3$) £61.70 / 100g</td>
<td>Irritant at high concentrations</td>
<td>Dilute to 1-10% w/v prior to use.</td>
<td>Store away from ammonium compounds.</td>
<td>Standard laboratory disposal.</td>
<td>Fast</td>
<td>Does not prevent histology</td>
</tr>
<tr>
<td>I$_2$E / I$_2$M £48.00 / 100g</td>
<td>As above, also flammable and toxic</td>
<td>Requires progressive dehydration of specimens prior to use.</td>
<td>Store away from ammonium compounds.</td>
<td>Standard laboratory disposal.</td>
<td>Fast</td>
<td>Does not prevent histology</td>
</tr>
<tr>
<td>PTA / PMA £73-78.50 / 100g</td>
<td>Requires use of respirator. Both are powerful oxidising agents, risk of severe burns.</td>
<td>Mix with 100% ethanol to 0.3% concentration.</td>
<td>Store in hazardous chemicals hood.</td>
<td>Special disposal required.</td>
<td>Slow</td>
<td>Use prevents histology</td>
</tr>
<tr>
<td>OsO$_4$ £28,600 / 100g</td>
<td>Carcinogenic, highly toxic</td>
<td>Create 2-4% w/v solution in water.</td>
<td>Immerse tissue in solution</td>
<td>Special disposal required.</td>
<td>Fast</td>
<td>May affect H&amp;E production.</td>
</tr>
</tbody>
</table>

Table 23. Comparison of contrast agents typically used when scanning micro-CT specimens.
From the above characteristics, I$_2$KI was chosen as the first line contrast agent for micro-CT studies throughout this project. I$_2$KI is safe, relatively inexpensive, easy to manufacture, and with relatively few effects on tissue (e.g. effect on subsequent histology production). Although iodine acts as a contrast agent, it does not provide histological fixation of tissues; ongoing biochemical reactions within biological specimens immersed in iodine may therefore lead to putrefaction, loss of mechanical stability and impede pathology investigations (e.g. autopsy or histology) unless a fixative is included in the scanning process. To expedite the iodination process, it would be ideal to combine the iodination and fixation steps; this should also help to prevent tissue degradation during the iodination period. As part of the optimisation process, extracted organs were imaged prior to attempting ‘virtual autopsies’. Initially, optimisation was performed in human fetal kidneys$^{129}$ and subsequently replicated the results in hearts$^{130,131}$ and brain biopsies.$^{132}$ This broad range of tissue types has been partly chosen to inform the challenges of iodinating different tissue types in whole fetuses, and partly chosen because research consent is available for the tissue in question.
4.1.5 Production of $\text{I}_2\text{KI}$ for micro-CT experiments

Lugol's iodine refers specifically to an aqueous solution of organic iodine ($\text{I}_2$) and potassium iodide (KI). Organic iodine (as a non-polar, covalent molecule) is insoluble in water, but will react with soluble potassium iodide (a polar, ionic compound) to form soluble tri-iodide as follows:

$$\text{I}_2(s) + \text{KI}(aq) \rightleftharpoons \text{I}_2\text{KI}(aq)$$

The tri-iodine ion is responsible for the brown-red colour of the aqueous solution. A ratio of approximately 2:1 KI:$\text{I}_2$ is used to ensure that the organic iodine is entirely dissolved at room temperature, however, not every published protocol requires the use of a 2:1 ratio, and as such, the total iodine content may differ between protocols using the same initial amount of elemental iodine.

Due to the different properties and proportions of the compounds involved in the formation of triiodide, there is considerable confusion (and possibly deliberate ambiguity) within some of the published literature regarding the concentration of $\text{I}_2\text{KI}$ used for experiments. Reported concentrations may refer to the weight/volume of $\text{I}_2$ (including or excluding KI, which may or may not be present in a 2:1 ratio), total iodine content (mg/L) or refer to a further dilution of the resulting $\text{I}_2\text{KI}$ cocktail (i.e. “20%” may either be used to indicate that one part of the mixed solution was subsequently made up to volume with four additional parts of water, or could refer to a combined iodine
concentration of 20%). Other studies report molecular iodine mass (mol/ml) (Table 24).

<table>
<thead>
<tr>
<th>Overall concentration</th>
<th>Organic iodine (I₂) (wt/v)</th>
<th>Potassium iodide (KI) [1:2 ratio] (wt/v)</th>
<th>Total iodine content (mg/ml)</th>
<th>Iodine mass (mol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30%</td>
<td>10%</td>
<td>20%</td>
<td>253.0</td>
<td>1.18x10⁻³</td>
</tr>
<tr>
<td>22.5%</td>
<td>7.5%</td>
<td>15%</td>
<td>189.6</td>
<td>8.82x10⁻⁴</td>
</tr>
<tr>
<td>15%</td>
<td>5%</td>
<td>10%</td>
<td>126.5</td>
<td>5.88x10⁻⁴</td>
</tr>
<tr>
<td>7.5%</td>
<td>2.5%</td>
<td>5%</td>
<td>63.3</td>
<td>2.94x10⁻⁴</td>
</tr>
<tr>
<td>3.75%</td>
<td>1.25%</td>
<td>2.5%</td>
<td>31.6</td>
<td>1.47x10⁻⁴</td>
</tr>
</tbody>
</table>

Table 24. Comparison of iodine solutions described by the concentration of various components within.

To avoid confusion within this thesis, all I₂KI solutions used in experiments will be reported according to total molecular concentration of iodine (mol/ml), which accounts for both the concentration of I₂ and KI present in the solution.
4.1.6: Theoretical Optimisation of X-ray settings

The accelerating voltage of a Nikon XT H 225 ST Micro-CT scanner can be set from 30-225kV. The effect of this setting is that it determines the distribution of X-rays being produced (spectrum) with energies varying from <1kV up to the maximum energy set by the user. Spectra differ between metal anodes; two of the most commonly used spectra being those of Molybdenum and Tungsten. Generally speaking, using lower kV values will result in greater amounts of contrast between substances in the final images following reconstruction. This is because ‘softer’ X-rays are more likely to be differentially attenuated by different tissue types. However, there are several important factors that exert an effect on this principle.

The first is that unlike a synchrotron where X-rays are produced within a very narrow energy range by a coherent source that can be controlled by the user, Micro-CT produces polychromatic X-ray spectra from an incoherent source. A broad spectrum of x-rays is produced, spread over a range of energies. There are however a large number of x-rays at about 60kV.

This increase in photons occurs when the energy imparted to the metal anode by the electron beam is equal to the binding energy of an electron shell (K, L, M etc). This results in a spike of photons being produced at the energy in question. The energy required to produce a spike in photons is the least at which a vacancy can be created in the particular shell and is referred to as the ‘edge’. Characteristic X-ray emissions are created when an initial vacancy in an inner shell is filled by transfer of an electron from another shell, thus leaving a
final vacancy in that shell. The energy of the emission equals the difference in binding energies between the initial and final vacancy. These peaks are therefore characteristic for any given element and are important in the final spectra produced by a target made of that element.

Secondly, as with X-ray production described above, in the sample being imaged there is a sudden increase in the attenuation coefficient of photons occurring at the photon energy just above the binding energy of the K shell electron of the atoms interacting with the photons. In theory contrast can be optimised by producing a high number of photons at the K edge of the material being imaged. Using an on-line database it is possible to obtain the photon cross section data for a compound such as I$_2$KI (XCOM from the National Institute of Standards and Technology,\textsuperscript{85} available at: http://physics.nist.gov/PhysRefData/Xcom/html/xcom1.html) and plot this against soft tissue photon cross-section attenuation data (Figures 39 and 40)). From these graphs, it is possible to see that I$_2$KI enhanced soft tissue will attenuate markedly in the low energy area of the spectrum, with edges around 5kV, 15kV and 33kV. With regard to the XT H 225 ST system available for use, this data indicates that a silver (K-lines at 22 and 24kV) or molybdenum (K-lines at 17 and 19kV) target may be preferable to a tungsten target (K-lines at 57-59kV and 67kV) for imaging I$_2$KI enhanced soft tissue, as a large number of photons can be generated in the vicinity of I$_2$KI's K-edge values by these metals. A copper target (K-lines at 8kV and 9 kV) may also be useful, though achieving enough penetration of larger specimens may be challenging in this case. Ideally, a rare earth metal target (lanthanum, cerium, praseodymium or
neodymium) would provide K-lines at almost the exact K-edge of I$_2$KI, though such a target is unlikely to survive prolonged exposure to a high energy electron beam given that these metals are relatively soft and oxidise readily.

Figure 39. Attenuation coefficients of soft tissue (red) and I$_2$KI (blue) for X-ray energies from 0 kV – 150kV. The green line represents the manufacturer’s stated lower limit of detection for the detector in a standard Nikon micro CT machine.
Figure 40. Attenuation coefficients of soft tissue (red) and I$_2$KI (blue) for X-ray energies from 20 kV – 150kV. The green line represents the manufacturer’s stated lower limit of detection for the detector in a standard Nikon micro CT machine.
Thirdly, the impact of noise and artifacts on the final images must be considered. Although theoretically the best contrast between soft tissue types should result from low X-ray energies (approximately 35kV according to the above calculations), care must be taken to ensure that sufficient photons penetrate the specimen and reach the detector in order to avoid degeneration of the image due to noise levels. Steps to minimise noise can include increasing the filament current (increasing the intensity of the electron beam and therefore also the number of photons produced), though this can also result in loss of resolution due to increased focal spot size. Similarly, the photon count at the detector can be improved by increasing the exposure time (and therefore the overall rotation time) for each X-ray projection, though doing so risks introducing artifact due to specimen movement. Also, using higher accelerating voltages results in a larger number of photons being produced and reaching the detector (due to the enlarged area under the spectral energy curve, improved penetration and K edge effects), though this negatively impacts on contrast.
4.1.7 Sample preparation for micro-CT experiments

Due to the prolonged duration of micro-CT scans compared with clinical CT (even when utilising a contrast agent), it is necessary to carefully prepare and mount the specimen in order to prevent specimen movement (including dehydration) and minimise the distance of the X-ray beam path through the tissue in order to reduce noise by improving the number of X-ray photons successfully reaching the detector. Although containers help to reduce movement and dehydration by creating a sealed micro-environment around the specimen, the material and shape of the container should be considered carefully, as glass containers will create a large amount of X-ray artefact by causing scatter and refraction. Additional artefacts will be created by non-cylindrical containers as the X-rays interact with the edges of the material surrounding the specimen. Parafilm M (Bemis, Oshkosh, USA) is a paraffin-based stretchable plastic film that can act as a mounting agent (by using wraps of Parafilm to secure the specimen to a mount or wedge) and as a physical barrier to prevent dehydration and movement. It has been used extensively throughout the experiments described below. Hydrogels or agar may also be used to mount specimens, but require a greater degree of preparation (including the use of a fume cupboard and respirator or some hydrogels) and excess material increases the attenuation of X-rays by increasing the beam path through the specimen (Figure 4I), potentially reducing signal and increasing noise as fewer photons reach the detector as a result.
Figure 4. Schematic of a micro-CT machine, demonstrating the effect of orientating a specimen such that the long axis of a specimen parallel to the X-ray path (A) and perpendicular to the X-ray path (B). In figure A, the increased amount of tissue within the beam path results in a decrease in the number of photons reaching the detector, increasing noise within the projection images.
Chapter two: Micro-CT imaging of extracted organs

Experiments: 4.2.1 – 4.2.6, Methods & Results

Feasibility (4.2.1 - 4.2.5)

Diagnostic accuracy (4.2.6)
4.2.1 Feasibility of extracted organ imaging using micro-CT

Experiment 4.2.1, Methods: Effects of iodination on tissue properties

To evaluate the potential use of micro-CT for the examination of biological tissue and obtain pilot data from extracted organs micro-CT imaging was performed in extracted human organs with abnormalities, in order to explore the extent to which micro-CT may visualise abnormalities within tissue and evaluate potential effects on production of histology from iodinated samples.

Experiment 4.2.1: Tissue distortion following I₂KI soak

To investigate optimal I₂KI preparation protocols including concentration and time of immersion for ex-vivo organs, and to obtain data on how CT artefacts that could affect diagnosis, an experiment was designed to demonstrate potential adverse effects of I₂KI immersion. Porcine lung specimens were contrasted using increasing concentrations of I₂KI; examined using a micro-CT scanner and then the resulting datasets and tissue was then examined to evaluate any adverse effects.

Experiment 4.2.1: Tissue preparation

Porcine lungs were used as a model in this experiment, as relatively porous tissue was felt likely to easily demonstrate tissue distortion following
iodination. Tissue was divided into approximately 3cm³ pieces and fixed in formalin for 48 hours prior to immersion in various concentrations of I₂KI for a further 48-hour period prior to scanning as follows:

A: 1.47x10⁻⁴ mol/ml (approximately 3.75% overall concentration)

B: 2.94x10⁻⁴ mol/ml (approximately 7.5% overall concentration)

C: 5.88x10⁻⁴ mol/ml (approximately 15% overall concentration)

D: 8.82x10⁻⁴ mol/ml (approximately 22.5% overall concentration)

E: 1.18x10⁻³ mol/ml (approximately 30% overall concentration)

Experiment 4.2.1: Scan parameters

Scans were performed using a Nikon XT H 225 ST micro-CT scanner (Nikon Metrology, Tring, UK). Target material was set to tungsten, with an accelerating voltage of 100 kV and a current of 100 μA (equivalent to 10W power). Detector gain was set to 30dB, with one frame per projection and 3141 projections per scan. Scans were reconstructed using Feldkamp filtered back projection algorithms with proprietary software (CTPro3D; Nikon Metrology) and post-processed using VG Studio MAX (Volume Graphics, Heidelberg, Germany).
Experiment 4.2.1, Results: Effects of iodination on tissue properties;

Effects of iodination on tissue properties

Micro-CT examination demonstrated that all tissue had been fully penetrated by iodine after 48 hours of immersion (Figure 42). There was an increasing degree of beam hardening artefact seen with increasing concentrations of iodine, and the tissue architecture of lung pieces immerse in 8.82x10^{-4} mol/ml I₂KI or greater was severely collapsed.

Figure 42. Micro-CT of porcine lung immersed in increasingly concentrated solutions of I₂KI (A-E). All specimens showed good internal contrast but beam hardening artefact and distortion worsened with increasing concentration. Scale bar = 2cm.
As expected, tissue distortion and discolouration increased as the concentration of iodine increased (Figure 18). Lung A (immersed in the weakest $1.47 \times 10^{-4}$ mol/ml iodine) could still macroscopically be identified as lung tissue and was easy to cut with a standard microtome blade following scanning. However, tissue immersed in concentrations of $8.82 \times 10^{-4}$ mol/ml or over was impossible to meaningfully examine visually, having totally collapsed, appearing featureless and uniform in colour (Figure 43). Specimens C, D and E required increasing amounts of effort to slice, even with a fresh microtome blade, as iodine concentration increased, and laboratory staff found specimens D & E difficult to orientate for histology and slide production. A good balance of tissue integrity and micro-CT contrast was found specimens A & B (Figures 42-43).
Figure 43. Macroscopic appearance of the porcine lung segments immersed in increasingly concentrated solutions of I$_2$KI. Macroscopic features were retained in the specimens immersed in the least concentrated solution (A) but lost in the remaining specimens.
These results show that the concentration of iodine should be kept as low as possible if the tissue is to be examined by a pathologist following the completion of micro-CT studies (or removed prior to macroscopic examination), with the caveat that the iodine concentration should be high enough to ensure that the tissue is fully contrasted. Application of Fick’s first law of diffusion suggests that iodination of unstained tissues should occur more quickly when higher concentrations of I₂KI are used, as the diffusion gradient will be steeper. The minimum concentration of iodine needed to fully contrast a whole fetus is unknown, but a pragmatic approach to iodination by selecting a concentrated enough solution to stain the tissue without deforming it represents a reasonable first step. A solution of approximately $2.94 \times 10^{-4}$ mol/ml I₂KI therefore appears to be a good initial contrast medium from extrapolation of this data, as this would lie approximately midway between the solutions used for pieces A & B.
Experiment 4.2.2, Methods: Use of iodination with fixation to prevent tissue degradation

To expedite the iodination process, it would be ideal to combine the iodination and fixation steps. Excess human lung from an explant for pulmonary hypertension were used as part of the experiment.

Experiment 4.2.2: Tissue preparation

Four histology block sized pieces of fresh human lung tissue (approx. 1.5cm x 1.5cm x 0.5cm) were selected at random following diagnostic sampling for surgical pathology analysis and allocated to either iodination alone (in a solution of $2.94 \times 10^{-4}$ mol/ml), or iodination in a solution doped with an equal proportion of 10% formalin. In order to match the iodine concentration across the specimens, solutions of 10% formalin were mixed with an equal volume of $\text{I}_2\text{KI}$ containing $4.98 \times 10^{-4}$ mol/ml total iodine content (based on favourable results from section 4.2.1); tissue for examination was immersed in this solution for 48 hours prior to imaging.

Experiment 4.2.2: Scan parameters

Scans were performed using a Nikon XT H 225 ST micro-CT scanner (Nikon Metrology, Tring, UK). Target material was set to tungsten, with an accelerating voltage of 100 kV and a current of 100 micro amps. Detector gain was set to 30dB, with one frame per projection and 3141 projections per scan.
Scans were reconstructed using Feldkamp filtered back projection algorithms with proprietary software (CTPro3D; Nikon Metrology) and post-processed using VG Studio MAX (Volume Graphics, Heidelberg, Germany).
Experiment 4.2.2, Results: Use of iodination with fixation to prevent tissue degradation

All samples that underwent iodination showed good contrast resolution on micro-CT examination. Furthermore, iodination did not interfere with tissue processing, embedding or H&E staining of slides. However, sections from the unfixed iodinated samples showed nuclear smudging and variations in the intensity of Haematoxylin and Eosin staining, artefacts which are associated with tissue degradation (Figure 44). The tissue contrasted with formalin/I$_2$KI mix showed good preservation of cellular detail (Figure 45). Histological examination of the lung samples demonstrated changes of severe pulmonary arterial hypertension, including plexiform lesions and thickening of the muscular arteries, pulmonary veins and bronchioles. Micro-CT volumes demonstrated adequate tissue contrast with diagnostically thickened vessels within the volume renderings and stack images (Figure 46).
Figure 44. Haematoxylin and Eosin stained slide (x40 objective) showing a plexiform lesion affecting a pulmonary arteriole in experiment 4.2.2, characteristic of changes secondary to pulmonary hypertension. The nuclei (dark purple) appear smudged and indistinct, reflecting tissue degradation, likely due to lack of fixation. Scale bar = 1mm
Figure 45. Micro-CT (A) and Haematoxylin and Eosin slide (B) from a similar area of the specimen, showing vascular tortuosity, in keeping with pulmonary hypertension.
These findings show the potential for micro-CT to produce data from ex-vivo samples but highlight the need for adequate tissue fixation prior to or during iodination.
Experiments 4.2.3 – 4.2.5: Feasibility of extracted organ imaging using micro-CT: visualisation and modelling of disease processes
Experiment 4.2.3, Methods: Examination of extracted human fetal kidneys;

Congenital renal anomalies are a frequent indication for termination of pregnancy and are a considerable source of perinatal morbidity and mortality. Although PMMRI and antenatal imaging may identify gross changes in kidney morphology that can indicate an underlying anomaly, often, tissue biopsies are required to confirm the underlying diagnosis and inform counselling for future pregnancies. The aim of the experiment was to evaluate whether micro-CT would permit visualisation of the sub-structures of normal and abnormal human kidney.

Experiment 4.2.3: Case selection

Micro-CT was performed on three human kidneys that were extracted at autopsy. One was a structurally and macroscopically normal kidney (termination undertaken for other reasons), one was suspected autosomal recessive polycystic kidney disease, and one case with suspected dysplastic kidneys secondary to bladder outlet obstruction. All parents provided full written consent to research as part of this study.

Experiment 4.2.3: Tissue preparation

Extracted specimens were immersed in equal parts of 10% formalin and I$_2$KI containing 2.94x10$^{-4}$mol/ml total iodine content for 48 hours prior to imaging.
Specimens were secured using Parafilm M (Bemis, Oshkosh, USA) within light plastic containers.

Experiment 4.2.3: Scan parameters

Scans were performed using a Nikon XT H 320 micro-CT scanner (Nikon Metrology, Tring, UK). Target material was set to molybdenum, with an accelerating voltage of 100 kV and a current of 100 micro amps. Detector gain was set to 30dB, with one frame per projection and 3141 projections per scan. Scans were reconstructed using Feldkamp filtered back projection algorithms with proprietary software (CTPro3D; Nikon Metrology) and post-processed using VG Studio MAX (Volume Graphics, Heidelberg, Germany).
Experiment 4.2.3, Results: Examination of extracted human fetal kidneys;

Micro-CT was performed on three human fetal kidneys: one structurally normal kidney, one with autosomal recessive polycystic kidney disease and one with multicystic dysplastic kidney disease. All kidney specimens showed excellent internal contrast on micro-CT examination (Figures 47 – 49), with no apparent detriment from the combination of formalin and iodine on subsequent H&E examination. There was no overt delay in the diffusion of iodine contrast of the solid renal parenchyma used in this experiment when compared with the relatively porous lung tissue used in previous experiments. All histology produced from the tissue showed excellent cellular preservation, with no artefacts such as nuclear smudging or variability in staining, as was shown in the unfixed tissue in a previous experiment (4.2.2). Comparison of structures visualised within the kidney by different methodologies is presented in Table 25.
Figure 47. (a) Coronal three-dimensional image using ex-vivo microcomputed tomography (micro-CT) at 15.5 microns through a normal fetal kidney from an unexplained stillbirth at 37 weeks’ gestation, showing normal renal cortex, medulla and collecting system. (b) Higher resolution (6.0 microns) micro-CT of the highlighted area in (a) showing the arcuate circulation (*). Adapted from Hutchinson et al. with permission.
Figure 48. (a) Postmortem whole-body ultrasound in fetus with autosomal recessive polycystic kidney disease after termination of pregnancy at 23 weeks' gestation following antenatal diagnosis of large, bright kidneys. Ex-vivo microcomputed tomography (b) of kidney showing radially aligned cysts, which were confirmed at histological examination (1.5× lens (c); 4× lens (d)). Adapted from Hutchinson et al.\textsuperscript{129} with permission.
Figure 49. Coronal three-dimensional image using ex-vivo microcomputed tomography (a) of a multicystic dysplastic kidney from a 20-week fetus after termination of pregnancy, with comparative histological examination (b). Potassium tri-iodide staining of kidney appears to bind avidly to blood, facilitating visualization of the vascular system, which may be segmented on the basis of grayscale values (c) or overlaid onto maximum-intensity projection images (d). Adapted from Hutchinson et al.\textsuperscript{129} with permission.
Table 25. Comparison of radiological and pathological investigation modalities and their ability to analyse different structures of the kidney. Adapted from Hutchinson et al.\textsuperscript{129} with permission.

<table>
<thead>
<tr>
<th>Renal Structure</th>
<th>CT (μm)</th>
<th>MRI (μm)</th>
<th>Ultrasound (μm)</th>
<th>Micro-CT (μm)</th>
<th>Macroscopic examination (μm)</th>
<th>Histological Examination (μm)</th>
<th>Electron Microscopy (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approximate Resolution Limit</td>
<td>600</td>
<td>300</td>
<td>120-400</td>
<td>0.5</td>
<td>10-100</td>
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<td>0.2</td>
</tr>
<tr>
<td>Whole Kidney</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Corticomedullary</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
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<tr>
<td>Corticomedullary differentiation</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>-</td>
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<tr>
<td>Collecting system</td>
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<tr>
<td>Overall vasculature</td>
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<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
</tr>
<tr>
<td>Vascular architecture</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
</tr>
<tr>
<td>Collecting tubules</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Individual cells</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Cellular substructures</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yes (some)</td>
<td>Yes (ultrastructure)</td>
</tr>
</tbody>
</table>

Adapted from Hutchinson et al.\textsuperscript{129} with permission.
Stack images and volume data generated by micro-CT demonstrated normal kidney anatomy, with a resolution comparable to that of low-power histology, in the macroscopically normal fetal kidney from an unexplained stillbirth at approximately 37 weeks’ gestation. In a case of antenatally known polycystic kidney disease, radially arranged, elongated cysts could be easily identified more easily from the micro-CT data than the histology from the case; these findings were diagnostic for Autosomal Recessive Polycystic Kidney Disease. Imaging of the multicystic dysplastic kidney revealed multiple cysts of varying sizes that corresponded to the subsequent histology. These initial results also show that combining fixation and iodination solutions is feasible, with no practical effect on the ability to obtain a satisfactory micro-CT image when compared with the use of formalin and iodine in sequential staining protocols.
Experiment 4.2.4, Methods: Disease detection and modelling utilising micro-CT

In addition to diagnosis of congenital anomalies, perinatal and paediatric pathologists are also concerned with the diagnosis of neoplastic processes. Although these are rare in neonates (and extremely rare within the fetal age range), childhood cancers (diagnosed between the ages of 1 and 14) cause considerable morbidity and mortality, with approximately 20% of childhood deaths related to neoplastic processes.² It would therefore be advantageous to examine whether micro-CT can identify the presence of such tumours, and to evaluate preliminary data with regards to what extent they can be characterised. Micro-CT was used to evaluate possible visualisation of tumour pathology in a suspected case of Tuberous Sclerosis, as part of autopsy examination of the heart.

Experiment 4.2.4: Case selection & tissue preparation

One case was opportunistically recruited following referral to Great Ormond Street Hospital for autopsy confirmation of antenatal imaging findings. Antenatal sonography at 34+5 weeks' gestation demonstrated the presence of multiple cardiac nodules, alongside fetal bradycardia. Post-mortem whole-body CT and MRI demonstrated cortical tubers and subependymal nodules, but no rhabdomyomas were identified. Following extraction of the heart at autopsy, it was immersed in a solution of equal parts 10% formalin and I₂KI
containing $2.49 \times 10^{-4}$ mol/ml total iodine content for 48 hours prior to micro-CT examination.

Experiment 4.2.4: Scan parameters

Scans were performed using a Nikon XT H 225 ST micro-CT scanner (Nikon Metrology, Tring, UK). Target material was set to molybdenum, with an accelerating voltage of 120 kV and a current of 83 micro amps. Detector gain was set to 30dB, with one frame per projection and 3141 projections per scan. Scans were reconstructed using Feldkamp filtered back projection algorithms with proprietary software (CTPro3D; Nikon Metrology) and post-processed using VG Studio MAX (Volume Graphics, Heidelberg, Germany).
Experiment 4.2.4, Results: Disease detection and modelling utilising micro-CT

Macroscopic examination of the heart revealed some subtle changes in thickness of the interventricular septum and ventricular free walls, but no obvious rhabdomyomas. However, micro-CT examination demonstrated multiple individual cardiac tumours in keeping with rhabdomyomas, widely dispersed throughout the heart, consistent with a diagnosis of tuberous sclerosis (Figure 50-51). These were confirmed on histology of the heart, with haematoxylin & eosin, Masson’s trichrome and diastase periodic acid-Schiff stains unaffected by iodination of the heart. These highlighted the clear cells with glycogen vacuoles separated by stands of cytoplasm within the tumour areas. The results from this case demonstrate that neoplastic pathologies can be detected by micro-CT in addition to congenital anatomical malformations.
Figure 50. The fetal heart following iodination (a–c), with the corresponding volume-rendered micro-CT images (d–f). Following removal at autopsy, the heart was soaked in iodine to improve soft tissue X-ray contrast. Rhabdomyomas were seen on micro-CT examination (arrows). Iodination was not felt to hinder dissection, at which no definite rhabdomyomas were seen by the pathologist. Adapted from Hutchinson et al. with permission.
Figure 51. Comparison of histology from the case [Masson’s trichrome (a-c), PAS with diastase (d-f), haematoxylin and eosin (g-i)] with slice images from micro-CT imaging (j-l).

Multiple well-circumscribed lesions can be identified on histology and micro-CT examination within the myocardium and papillary muscles (arrows). High-power examination (a,d,g) reveals disorganised, vacuolated cells with strands of cytoplasm extending between cell membrane and nucleus (‘spider’ cells) compressing normal myocardium (dashed line indicates the border of a rhabdomyoma). Scale bars A, B, C = 200 μm. Scale bars b, c, e, f, h, i, l = 2 mm. Scale bar j = 0.65mm. Scale bar k = 3 mm. Adapted from Hutchinson et al. with permission.
Experiments 4.2.5, Methods: Disease detection and modelling utilising micro-CT

Following success with a monocellular tumour type, it was decided to attempt to replicate the results utilising a more complex tumour type. Adamantinomatous craniopharyngiomas (ACP) contain several different cellular compartments of different cellular density (including palisading epithelium, stellate reticulum, epithelial whorls/clusters and “wet keratin”) and a complex pattern of invasion, such as finger like protrusions of tumour within an often-florid glial tissue reaction. As Micro-CT imaging of tissues relies on differential X-ray absorption between tissue components, it was hypothesised that micro-CT could be used to delineate ACPs and their intrinsic components.

Experiment 4.2.5 & 4.2.5a: Case selection

Three anonymised archival primary frozen ACP samples were fixed in 10% formalin and then placed in potassium tri-iodide for at least 72 hours to improve CT contrast. Images were acquired using a Nikon XT H 225 ST micro-CT scanner. After imaging samples were embedded in paraffin and processed by standard protocols, including staining with Haematoxylin and Eosin and immunohistochemistry for glial fibrillary acidic protein (GFAP) and beta-catenin.
Experiments 4.2.5: Scan parameters

Scans were performed using a Nikon XT H 225 ST micro-CT scanner (Nikon Metrology, Tring, UK). Target material was set to molybdenum, with accelerating voltages of between 70 and 100 kV and a current of between 100 and 142 uA. Detector gain was set to 24dB, with one frame per projection and 3141 projections per scan. Scans were reconstructed using Feldkamp filtered back projection algorithms with proprietary software (CTPro3D; Nikon Metrology) and post-processed using VG Studio MAX (Volume Graphics, Heidelberg, Germany). Modelling of human tumour tissue growth was undertaken jointly by John Apps and the author using Imaris 8.0 (Bitplane AG).
Experiments 4.2.5, Results: Disease detection and modelling utilising micro-CT

Three anonymised archival frozen ACP samples were fixed in 10% formalin and then placed in potassium tri-iodide for at least 72 hours to improve CT contrast. Images were acquired using a Nikon XT H 225 ST micro-CT scanner. After imaging samples were embedded in paraffin and processed by standard protocols, including staining with Haematoxylin and Eosin and immunohistochemistry for glial fibrillary acidic protein (GFAP) and beta-catenin. The results presented in the following figures demonstrate that micro-CT imaging can non-destructively give detailed 3D structural information of tumours in volumes with isotropic voxel sizes of 4-6\(\mu\)m (equivalent to a resolution of 5-7\(\mu\)m when taking account of the focal spot size of 3\(\mu\)m) with excellent internal contrast, equivalent to that of low power histological examination (Figure 52). Such information complements classical histology by facilitating virtual slicing of the tissue in any plane and providing unique detail of the three-dimensional relationships of tissue compartments, which would support clinical assessment and scientific understanding of the morphology of tumour invasion. The spatial relationship of these clusters to tumour infiltration was further explored in 3D by utilising advanced semi-automated image processing software (Imaris (Bitplane AG) and VG Studio MAX (Volume Graphics GmbH)) to extract contour lines for both tumour and epithelial whorls from the micro-CT image stacks. Differential grey values allowed tumour boundaries and clusters to be segmented from reactive glial
tissue within manually determined regions. Segmentation tools merged the largest connected areas bounded by the maximum intensity of voxels within a user-defined range, creating a three-dimensional model (Figure 53). This highlighted the complex relationships of tumour and reactive tissue with nodules and islands interspersed across a region of the sample. An area of apparent “finger like protrusions” was further analysed and found to be part of a relatively larger complex area of tumour tissue.
Figure 52. Micro-CT imaging of adamantinomatous craniopharyngioma: a virtual and matched histological tissue section of ACP case 1 showing areas of tumour interspersed by reactive glial tissue. Scale bar indicates 1 mm. b 20x images of specific tumour compartments from boxed regions of A. The left panel shows epithelial whorls (“clusters”) within an area of tumour and the right panel shows “wet keratin” which has a higher grey value on CT imaging. Scale bars indicate 100 μm. EW = Epithelial Whorls, SR = Stellate Reticulum, PE = Palisading Epithelium, G = Reactive Glial Tissue, WK = Wet Keratin. Adapted from Apps/Hutchinson et al.\textsuperscript{132} with permission.
Figure 53. A. Three-dimensional annotation of an area of case 1. Green indicates the border of tumour demonstrating nodules and islands with some interconnections. Connections of less than 5 μm will not be well visualised at this resolution, possibly explaining discontinuities. Purple indicates epithelial whorls/clusters. B. An area of finger-like protrusions. The upper panel shows the micro-CT image; the lower panel shows 3D annotation revealing a complex 3D structure in this region. C. Immuno-histochemical staining of the post micro-CT samples in (A) demonstrating appropriate antigenic reactivity following iodination. Upper panel beta-catenin showing a cluster with nucleo-cytoplasmic accumulation (case 3), lower panel glial fibrillary acidic protein (GFAP) (case 1). Scale bars indicate 100 μm. Adapted from Apps/Hutchinson et al.132 with permission.
These results confirm that micro-CT does not preclude subsequent histological processing or staining. All diagnostic features were preserved and immuno-staining successful following potassium tri-iodine staining. This experiment presents the first 3D assessment of the cellular relationships involved in tumour infiltration from human samples using micro-computed tomography (Micro-CT) imaging.
Diagnostic accuracy of extracted organ imaging using micro-CT

Experiment 4.2.6, Methods: Diagnostic accuracy of micro-CT for extracted fetal hearts

To better inform the approach to whole body fetal micro-CT and obtain preliminary data regarding the diagnostic utility of micro-CT examination when applied to autopsy practice, it was necessary to investigate micro-CT examination of extracted organs.

4.2.6 Case selection

Great Ormond Street Hospital pathology department acts as a national referral centre for cardiac pathologies. Accordingly, many terminations of pregnancy for complex congenital heart disease are referred to the pathology department for autopsy. These cases can be diagnostically challenging due to small fetal size, the complexity and variability of the pathologies involved, the presence of maceration, and improving sonographic techniques leading to earlier gestation terminations of pregnancy.

These pathologies therefore offered an opportunity to acquire clinically meaningful data and a robust assessment of the strengths and weaknesses of micro-CT imaging as applied to pathology specimens.
Five cases referred to Great Ormond Street Hospital for perinatal autopsy with consent to research were obtained and the heart-lung blocks or individual hearts extracted intact at autopsy examination, according to the preference of the performing pathologist. One normal heart from a termination of pregnancy for another indication (with research consent) was also be scanned. The specimens were then fixed (in 10% formalin) and iodinated prior to micro-CT, which was reported blinded to the antenatal information. As these examinations were performed as part of clinical care, the scan findings were discussed with an experienced specialist paediatric cardiac pathologist prior to dissection (single blind design).

4.2.6 Micro-CT examination

Five cases with congenital heart disease were prospectively selected from referrals to Great Ormond Street Hospital for formal perinatal autopsy examination. In every case, specific morphological information on the cardiovascular system was requested by the clinical team as part of standard care. At formal autopsy examination, the heart or heart-lung block was extracted and fixed in 10% neutral buffered formalin prior to iodination. Iodination involved the addition of I$_2$KI in a 1:1 ratio with the formalin solution to minimise sample preparation time. I$_2$KI with a total iodine content of 63.25 mg/mL (iodine mass of 2.49 × 10$^{-4}$ mol/mL) was chosen based on previous animal studies and contrast optimisation experiments (experiments
4.2.1 – 4.2.5). Specimens were left to soak in the iodination solution for at least 48 hours prior to scanning.

Immediately prior to micro-CT examination, the specimens were removed from iodination solution and rinsed in distilled water to remove excess surface iodine, in order to minimise artefacts generated during scanning, either through X-ray attenuation or fluid movement during the scanning process. The specimens were then padded dry using gauze and wrapped in Parafilm M or a similar low-density plastic film, prior to being secured in a low-density plastic cylinder in an attempt to ensure stability during the scan. Isotropic voxel sizes varied according to the geometric magnification achieved (inversely correlated with specimen size) and ranged between 19 and 31 µm. Following micro-CT examination (duration of between 35–70 minutes), the specimens were fixed in 10% formalin to prevent tissue degradation and aid with the removal of iodine prior to macroscopic examination.

X-ray images were acquired using an XT H 320 microfocus-CT scanner with a multi-metal target (Nikon Metrology, Tring, UK). Parameters including target material (tungsten or molybdenum), X-ray energies, and current were optimised on a case-by-case basis with the aim of maximising detector saturation without requiring filtration of the X-Ray spectrum (expected energy range, 85–125 kilovolts; current range, 50–135 micro amps). Although filtration of an X-ray spectrum reduces noise, contrast to noise ratios in soft tissue specimens have also been observed, therefore no filtration was be used. Scans were reconstructed using Feldkamp filtered back projection algorithms with
proprietary software (CTPro3D; Nikon Metrology) and post-processed using VG Studio MAX (Volume Graphics, Heidelberg, Germany).

The resulting stack images were assessed by an experienced paediatric radiologist with expertise in fetal post-mortem imaging. Twenty-one indices normally assessed at autopsy were evaluated in the datasets generated for each case, including: atrial situs, atrioventricular connection, ventriculoarterial connection, superior and inferior caval veins, right atrium, coronary sinus, right ventricle, pulmonary trunk, pulmonary veins, left atrium, left ventricle, aortic arch, coronary arteries, the cardiac valves (tricuspid, pulmonary, mitral, aortic), arterial duct, interatrial septum and interventricular septum.

4.2.6 Macroscopic examination

Cardiac dissection was performed by Dr. Michael Ashworth, a consultant paediatric pathologist with a specialist interest in cardiac pathology, within 24 hours of the micro-CT examination. Using a dissecting microscope, the same 21 indices were assessed. Features identified by dissection and micro-CT images were then compared to evaluate both modalities before the final autopsy report was issued. Potential discrepancies were reviewed and agreed by consensus, with pathological examination used as the gold standard. Concordance was defined as the combination of true positives and true negatives, i.e. complete agreement between micro-CT and autopsy.
4.2.6 Statistical analysis

‘Apparent advantages of micro-CT’ were defined as cases in which micro-CT is diagnostic but autopsy is non-diagnostic, or the heart is macerated. ‘Apparent misses on micro-CT’ were defined as a case in which autopsy is diagnostic, but micro-CT is non-diagnostic for these indices. Apparent discrepancies included all false positives, false negatives, apparent advantages and apparent misses.\textsuperscript{130}
Experiment 4.2.6, Results: Diagnostic accuracy of micro-CT for extracted fetal hearts

The aforementioned iodination protocol provided excellent internal contrast; with no areas of shadowing typical of under-iodination and only minor beam hardening corrections required (these were anticipated given the lack of filtration used). All micro-CT scans acquired provided the necessary level of detail to make an accurate morphological diagnosis without the need for further dissection. In all cases, the correct overall diagnosis could be made on blinded micro-CT examination prior to examination by an expert pathologist and there was complete agreement between micro-CT and pathological examination for overall diagnosis in all cases. Following iodination, there was brown discoloration of the hearts, which did not significantly hinder gross dissection. There was agreement for 95.8% (114/126) indices assessed on micro-CT and autopsy dissection, with a median concordance of 19/21 per case and a range of 18–20/21 per case. This consisted of 23 true positives and 91 true negatives, giving an overall concordance of 95.8% (95% CI, 90.5–98.2%) following removal of seven indices that were non-diagnostic at either micro-CT or autopsy. The sensitivity and specificity of micro-CT are given in Table 26. There were 12/126 (9.5%) discrepancies between techniques.
Table 26. Overall sensitivity and specificity data of post-mortem examination by microcomputed tomography compared with traditional autopsy dissection as assessed by identification of 21 indices. Adapted from Hutchinson et al.\textsuperscript{130} with permission.
There were four apparent false negatives on micro-CT assessment (Table 27); in one case of hypoplastic right heart syndrome (Case 3), both a Chiari network and a pulmonary venous aberration were missed on micro-CT examination but seen on autopsy, with three pulmonary veins branching from the left lung and only one from the right; in one case of hypoplastic left heart syndrome (Case 2), the right atrium was interpreted as normal on micro-CT but was thick walled at autopsy; and the fourth discrepancy related to the under-calling on micro-CT of a dilated pulmonary trunk in one case of tetralogy of Fallot (Case 4). There was one apparent false positive (overcall) on micro-CT assessment; a pulmonary trunk appeared wide on micro-CT examination owing to the presence of a blood clot but was normal when later dissected (Case 2).

There were two indices for which micro-CT was non-diagnostic, both of which were analysis of the coronary sinus opening and were normal at autopsy. For five indices, micro-CT provided additional information (apparent advantages) when dissection was non-diagnostic, or the heart was macerated. In all cases, these indices related to micro-CT demonstrating features in the ventricles in which the myocardium was found subsequently to be heavily autolysed at macroscopic examination. Micro-CT identified thickened abnormal myocardium in two cases of tetralogy of Fallot (Cases 1 and 4), and, in one other case, the myocardium was normal at micro-CT but macerated at dissection (Case 5, Figure 54).
<table>
<thead>
<tr>
<th>Case</th>
<th>Gestational age</th>
<th>Micro-CT findings</th>
<th>Macroscopic autopsy findings</th>
<th>Discrepancies</th>
<th>Agreement</th>
<th>Overall diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22gw</td>
<td>VSD, over-riding aorta with right sided arch, atretic pulmonary valve, hypertrophic right ventricle.</td>
<td>VSD, over-riding aorta with right sided arch, atretic pulmonary valve, macerated ventricular myocardium.</td>
<td>Myocardium too macerated to comment at autopsy but good detail on micro-CT.</td>
<td>19/21</td>
<td>Tetralogy of Fallot</td>
</tr>
<tr>
<td>2</td>
<td>17gw</td>
<td>Hypoplastic left ventricle with atretic aorta, wide pulmonary trunk, and normal right atrium.</td>
<td>Hypoplastic left ventricle with atretic aorta, normal pulmonary trunk, and thickened right atrium.</td>
<td>Thickened right atrium appeared normal on micro-CT. Normal pulmonary trunk appeared wide on micro-CT.</td>
<td>19/21</td>
<td>Hypoplastic left heart syndrome</td>
</tr>
<tr>
<td>4</td>
<td>23gw</td>
<td>Double outlet right ventricle, atretic pulmonary valve, VSD, overriding aorta, normal pulmonary trunk, right ventricular hypertrophy.</td>
<td>Double outlet right ventricle, atretic pulmonary valve, VSD, overriding aorta, dilated pulmonary trunk, macerated myocardium.</td>
<td>Dilated pulmonary trunk appeared normal on micro-CT. Myocardium too macerated to comment at autopsy but good detail on micro-CT. Coronary sinus not visualised on micro-CT.</td>
<td>18/21</td>
<td>Tetralogy of Fallot</td>
</tr>
<tr>
<td>5</td>
<td>23gw</td>
<td>Normal anatomy, normal ventricular myocardium.</td>
<td>Normal anatomy. Macerated ventricular myocardium.</td>
<td>Myocardium too macerated to comment at autopsy but good detail on micro-CT.</td>
<td>19/21</td>
<td>Normal heart</td>
</tr>
<tr>
<td>6</td>
<td>23gw</td>
<td>Double outlet right ventricle, atretic pulmonary valve, VSD, overriding aorta, narrow pulmonary trunk, right ventricular hypertrophy.</td>
<td>Double outlet right ventricle, atretic pulmonary valve, VSD, overriding aorta, narrow pulmonary trunk, right ventricular hypertrophy.</td>
<td>Coronary sinus not visualised on micro-CT.</td>
<td>20/21</td>
<td>Tetralogy of Fallot</td>
</tr>
</tbody>
</table>

Table 27. Detailed case breakdown for the experiment, alongside findings and discrepancies at micro-CT and autopsy. Adapted from Hutchinson et al. with permission.
Figure 54. Case 5: Virtual dissection of a normal fetal heart (23gw). Micro-CT examination facilitates examination of structures without the need for dissection. Adapted from Hutchinson et al.\textsuperscript{130} with permission.
Figure 55. Case 4: A 23gw heart and lung block from a case of tetralogy of Fallot. Blood clot expands the pulmonary trunk, which was called normal on micro-CT, but interpreted as widened at macroscopic examination (apparent false negative at micro-CT). In the same case, the RV appeared hypertrophic at micro-CT but was described as macerated at macroscopic examination. All diagnostic features were identified on micro-CT. Adapted from Hutchinson et al. with permission.
In all cases routine histological examination as part of the standard autopsy protocol was adequate, with no adverse effect of iodine immersion, and in no case did histological examination provide additional useful diagnostic information. These findings demonstrate that micro-CT has the potential to provide diagnostic quality morphological information on complex pathologies in organs from small fetuses.
Chapter 3: Whole body post-mortem fetal micro-CT

Experiments: 4.3.1 – 4.3.4, Methods & Results

Feasibility (4.3.1 - 4.3.3)

Diagnostic accuracy (4.3.4)
Feasibility of whole-body post-mortem fetal micro-CT

Experiment 4.3.1, Methods: Feasibility of whole-body imaging using micro-CT in an adult rat model

To establish whether micro-CT can provide imaging of whole fetuses, and whether there were any severe adverse effects of I₂KI immersion or micro-CT examination, an experiment was designed based around the examination of pre-deceased adult (approximately 90 days old) Sprague-Dawley rats (Royal Veterinary College, Potters Bar, UK) that had been euthanised following completion of an unrelated study at the Royal Veterinary College.

4.3.1 Case selection and specimen preparation

Following euthanasia of the rats, the carcasses were transported to Great Ormond Street Hospital for subsequent preparation examination. Euthanasia of the animals was not solicited as part of this research project. The rodents were shaved to removed excess fur, marked to aid identification, and immersed whole into I₂KI solutions of $1.47 \times 10^{-4}$ mol/ml or $5.88 \times 10^{-4}$ mol/ml for 72 hours. One rat was immersed in 10% formalin, for use as a negative control. A total of three rats were used initially, to establish whether there were possible differences in diffusion of I₂KI through the carcasses over a period of up to 72 hours. The time limit of 72 hours was chosen as it represents a clinically reasonable time for the preparation of a fetus prior to full autopsy (allowing for booking in, allocation to a consultant, and
preliminary imaging investigations as appropriate). Prior to scanning, the rats were removed from solution and rinsed to remove excess I₂KI, prior to being dried and vacuum sealed inside a polyamide plastic bag using a commercially available vacuum food sealer (Andrew James Ltd, Seaham, UK).

4.3.1 Micro-CT examination

X-ray images were acquired using an XT H 320 microfocus-CT scanner with a multi-metal target (Nikon Metrology, Tring, UK). Target material was set to tungsten, with an accelerating voltage of 100 kV and a current of 100 microamps. Detector gain was set to 30dB, with one frame per projection and 3141 projections per scan. Scans were reconstructed using Feldkamp filtered back projection algorithms with proprietary software (CTPro3D; Nikon Metrology) and post-processed using VG Studio MAX (Volume Graphics, Heidelberg, Germany).

4.3.1 Image analysis

Diffusion distances through the skin were measured to give a measure of overall I₂KI ingress using VG Studio MAX.
Experiment 4.3.1, Results: Feasibility of whole-body imaging using micro-CT in an adult rat model

No visible exogenous contrast was visible in the mouse immersed in 10% formalin, as expected for the negative control mouse. The rat immersed in 1.47x10^{-4} mol/ml I_2KI showed contrast within the skin and subcutis only, with a measurable diffusion distance of 3.48mm. The rat immersed in 5.88 x10^{-4} mol/ml I_2KI also showed minimal internal contrast, with some contrast in the subcutaneous tissues (diffusion distance 5.80mm), as shown in Figure 56. Despite immersion in exogenous contrast for 72 hours (longer than many of the extracted organ studies previously presented), the specimens in this experiment failed to adequately contrast in a clinically relevant period. Possible explanations for contrast failure include an inadequate volume of contrast solution, an inadequate amount of time for diffusion of the contrast agent, and the effect of the mechanical keratin barrier of the skin (or a combination of the above). Given the results obtained from the extracted organs, it is likely that the keratin layer of the skin is acting as a major barrier to diffusion. This is unlikely to be a major constraint in perinatal autopsy cases, as human skin remains non-keratinised until relatively late in gestation (approximately 185 days). Further micro-CT experiments will use mouse embryos to examine further feasibility for human autopsy studies.
Figure 56. Coronal sections through micro-CT scans of nude adult mice following immersion for 72 hours in formalin (a), I$_2$KI \( [1.47 \times 10^{-4} \text{ mol/ml total iodine content}] \) (b), and I$_2$KI \( [5.88 \times 10^{-4} \text{ mol/ml total iodine content}] \) (c). Contrast penetration was poor in both iodinated specimens, with no meaningful increase seen with increased concentration of I$_2$KI.
Experiment 4.3.2, Methods: Feasibility of whole-body imaging using micro-CT: Embryonic mice

Following failure of experiment 4.3.1, it was decided to attempt micro-CT examination of embryonic mice. One possible explanation for the failure of iodination in experiment 4.3.1 is the presence of extensive keratinisation of the skin of the adult rats. As embryonic mice possess non-keratinised skin prior to E14 and given previous success from multiple other groups in phenotyping a mouse embryo using I2KI based micro-CT, it was thought that a successful result would identify the probable cause of failure of 4.3.1 to be keratinisation of the skin.

4.3.2 Case selection and specimen preparation

Surplus E13 mouse embryos were acquired from a collaborative group seeking confirmation of the potential utility in phenotyping genetic defects in mouse embryos. Three mice were received frozen in 100% methanol. They were soaked in equal volumes (25ml each) of 10% formalin and I2KI for 8 hours to impart tissue contrast, prior to being rinsed, dried and sealed within Parafilm M (Bemis, Oshkosh, USA). The specimens were then mechanically stabilised using 3% w/v aqueous agar (Sigma-Aldrich, Irvine, UK).
4.3.2 Micro-CT examination

X-ray images were acquired using an XT H 225 ST microfocus-CT scanner with a multi-metal target (Nikon Metrology, Tring, UK). Target material was set to tungsten, with an accelerating voltage of 90 kV and a current of 111 microamps. Detector gain was set to 30dB, with one frame per projection and 3141 projections per scan. Scans were reconstructed using Feldkamp filtered back projection algorithms with proprietary software (CTPro3D; Nikon Metrology) and post-processed using VG Studio MAX (Volume Graphics, Heidelberg, Germany).
Experiment 4.3.2, Results: Feasibility of whole-body imaging using micro-CT: Embryonic mice

Three mouse embryos were iodinated and scanned as part of this experiment. Excellent internal contrast was demonstrated, with good views of all organ systems obtained. Specific abnormalities identified include a VSD (0.24mm), exencephaly and foreface disruption. Excellent views of normal central nervous system, respiratory system, cardiovascular system, genitourinary and digestive tract systems were also obtained (Figure 57 – 60).
Figure 57. Saggital section through a volume rendering of an E13.5 mouse embryo. A pericardial effusion is identified (star), along with a small ventricular septal defect (white arrow). Approximate scale: one background block = 1mm²
Figure 58. Micro-CT volume rendering of an E13.5 mouse embryo showing exencephaly and foreface disruption.
Figure 59. Micro-CT data showing maximum intensity projections of an E13.5 mouse embryo at low (a) and high (b) magnification, with windowing applied to highlight vasculature (high contrast). Key: Yellow star – carotid artery. Red star – descending thoracic aorta. White star – distal descending aorta. Blue star – ascending thoracic aorta. Pink star – Ductus arteriosus.
Figure 60. Micro-CT examination of an unaffected embryo utilising Lugol’s Iodine (I2KI) staining. A: Sagittal view, soft tissue windowing B: Sagittal view, maximum intensity projection. Micro-CT images of iodinated tissue can be windowed to view soft tissues (A), or vasculature (B). No pathologies were identified within this embryo.
Micro-CT technology can non-destructively phenotype embryos at high resolution, which can then be virtually dissected; 3D printed or indefinitely stored and could provide a solution to current issues affecting the use of human embryonic tissue for diagnosis, teaching and research.
Experiment 4.3.3, Methods: Feasibility of whole-body imaging using micro-CT: human embryos

Any novel imaging modality requires an accurate knowledge of normal findings, and in this setting, accurate assessment of the gestational age and thus expected developmental anatomy are of utmost importance to prevent disease misdiagnosis. This case study presents post-mortem micro-CT imaging of a very early pregnancy loss where accurate dating of embryonal age was possible, and to a similar level as histopathology. The study not only represents the earliest human embryo assessed by micro-CT in the medical literature but also offers an understanding for the possibilities of this technology in future pregnancy losses.\textsuperscript{134}

4.3.3 Case selection and specimen preparation

Fully informed, written parental consent for conventional autopsy, imaging, and the use of tissue for research was obtained. The embryo was referred to our institution as fresh tissue following a termination of pregnancy for social reasons, without any history of underlying congenital anomalies. The estimated gestational age was approximately 11 to 12 weeks (by date of maternal last menstrual period). The specimen was first immersed at room temperature in a solution of 10% formalin (to prevent tissue degradation) and potassium triiodide (I\textsubscript{2}KI / Lugol's iodine, to impart tissue contrast), with a total iodine content of 63.25 mg/mL (iodine mass of $2.94 \times 10^{-4}$ mol/ml), in a
1:1 ratio for 48 hours prior to imaging. Immediately prior to imaging, the fetus was removed from the iodine solution, rinsed in water, and dried with gauze.

4.3.3 Micro-CT examination

Images were acquired using an XT H 225 ST micro-CT scanner with a multi-metal target (Nikon Metrology, Tring, UK). Scanning parameters included X-ray energies and beam current at 80 kV and 87 μA, respectively. Exposure time was 354 milliseconds, with the number of projections optimized at 3080 with 1 X-ray frame per projection. The resultant images were reconstructed using modified Feldkamp filtered back-projection algorithms with proprietary software (CTPro3D; Nikon Metrology, UK) and post-processed using VG Studio MAX 3.0 (Volume Graphics GmbH, Heidelberg, Germany). The isotropic voxel size was 9.7 μm. Following imaging, the embryo was de-iodinated using sodium thiosulphate pentahydrate dissolved in water (4% w/v) for 12 hours. The specimen was then embedded within a paraffin block and sectioned. A traditional autopsy was not possible due to the very small fetal size.

4.3.3 Image analysis

To date the embryo on imaging, a similar method utilised by pathology was adopted based on the identification of various morphological features as early development occurs. These can be divided into the “embryonic” (i.e., first 8 weeks post-fertilisation) and “fetal” period, with the former subdivided into 23
“Carnegie stages,” as described and developed by O'Rahilly and Muller\textsuperscript{135,136} (Table 28). Whilst the presence of different structures do not provide a specific age \textit{per se}, only a developmental stage, rough approximations of gestational age can be inferred. Interpretation of the imaging findings were made using a computer graphics program (VG Studio MAX 3.0 [Volume Graphics GmbH]) by consensus reading between a consultant pathologist and a radiologist. Figure 61 demonstrates the external and internal morphological appearances, respectively, on micro-CT, with the latter compared with the histological examination.
Experiment 4.3.3, Results: Feasibility of whole-body imaging using micro-CT: human embryo

Prior to imaging, the embryo had an estimated gestational age of approximately 11-12 weeks, however detailed analysis showed this to be in fact several weeks younger around 49-52 days post ovulation (Carnegie stage 21) corresponding to approximately 7 weeks (Figure 61 & Table 28). It is known that estimation of the gestational age from the first day of the LMP of the mother can pose inherent inaccuracies given inconsistencies in dates of ovulation during a menstrual cycle and inaccurate maternal recollection, which may explain the discrepancy in this case.
Figure 61. Volume rendered three-dimensional image (A) reconstructed from micro-CT imaging data from a 7 week human embryo. A sagittal section (B) with accompanying haematoxylin & eosin stained slide (C) are also demonstrated. Adapted from Shelmerdine/Hutchinson et al.\textsuperscript{134} with permission.
<table>
<thead>
<tr>
<th>Embryonic (Carnegie) Stage</th>
<th>Approx. day³ (post ovulation)</th>
<th>External morphological features</th>
<th>Case Study</th>
</tr>
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<tbody>
<tr>
<td><strong>Weeks (post ovulation)</strong></td>
<td><strong>Week 6-7 (Day 42-49)</strong></td>
<td><strong>Week 7-8 (Day 50-57)</strong></td>
<td><strong>Week 8-9 (Day 58+)</strong></td>
</tr>
<tr>
<td>Embryonic (Carnegie) Stage</td>
<td>18</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td><strong>Embryonic (Carnegie) Stage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Approx. day³ (post ovulation)</strong></td>
<td><strong>42-45</strong></td>
<td><strong>45-47</strong></td>
<td><strong>47-50</strong></td>
</tr>
<tr>
<td><strong>External morphological features</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Head</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head relatively larger (from week 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cartilaginous skull develops</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membranous part of the skull develops</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular plexus appears in superficial tissues of head</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular plexus extends ¾ of the way above eye/ear level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular plexus approaches vertex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular plexus bends towards erect position.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head bends towards erect position.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head more rounded</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head relatively larger (from week 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Face</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eyelid folds begin, tip of nose distinct</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lens cavity obliterated, lens suture forms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eyelids rapidly encroach upon the eyes. External ear more developed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tip of nose distinct. Lens present. Eyelids and external ear beginning to develop, eyelids not yet encroaching or fusing.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estimated stage 21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estimated stage 21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trunk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body more</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elongating and</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trunk appears</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trunk elongating, not mature in shape yet.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal morphological features: Skeletal</td>
<td>Estimated stage &lt;22</td>
<td>Estimated stage &lt;23</td>
<td>Estimated stage 20-21</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>---------------------</td>
<td>----------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Thorax</td>
<td>First generation of sub-segmental bronchi complete</td>
<td>Further division of bronchi (primary bronchi at week 4-5)</td>
<td>No bony collar. No bone marrow present.</td>
</tr>
<tr>
<td>Humerus</td>
<td>Middle of shaft becomes</td>
<td>Cartilaginous phases 1-</td>
<td>Bony collar appears and all 5 cartilaginous phases now present</td>
</tr>
<tr>
<td>Skull and vertebrae</td>
<td>Cartilaginous skull develops</td>
<td>Membranous part of the skull develops</td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>Morphology</td>
<td>Feature Description</td>
<td>Stage</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>Radial/Ulna</td>
<td>Bony collar appears</td>
<td></td>
<td>No bony collar (Stage &lt;22)</td>
</tr>
<tr>
<td>Femur</td>
<td>Bony collar appears</td>
<td></td>
<td>No bony collar (Stage &lt;22)</td>
</tr>
<tr>
<td>Tibia/Fibula</td>
<td>Bony collar appears</td>
<td></td>
<td>No bony collar (Stage &lt;22)</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>Hemispheres cover 2/3 of the diencephalon (stage 20), ¾ by stage 21</td>
<td>Cortical plate begins to appear Whole lateral surface of diencephalon covered</td>
<td>Estimated stage &lt;22</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>Outer swelling appears = future flocculus</td>
<td>Cerebellar commissures appear</td>
<td>Present, but not well formed Estimated stage &lt;23</td>
</tr>
<tr>
<td>Eyes/Ears</td>
<td>Semi-circular ducts form:</td>
<td>Some optic fibres reach the optic chiasma Optic tract reaches lateral geniculate body</td>
<td>Difficult to identify optic tract/ optic nerve. Eyelids not well formed Estimated stage &lt;22</td>
</tr>
<tr>
<td></td>
<td>1: anterior, 2: posterior, 3: lateral</td>
<td>Eyelids now rapidly encroach upon the eyes. Optic nerve sheath forms. Formation of auricle</td>
<td></td>
</tr>
</tbody>
</table>
Table 28 Internal and external morphological appearances expected at differing stages of embryonal and fetal development with estimation of corresponding gestational age. The features present in our case study are provided in the far-right column with estimated developmental stage in bold text. Overall assessment of the specimen was for an embryo of Carnegie stage 21, roughly corresponding to gestational age of 49 to 52 days (i.e., 7 weeks). Adapted from Shelmerdine/Hutchinson et al.\textsuperscript{134} with permission.
During life in utero, antenatal ultrasound assessment of the greatest length of the embryo (i.e. crown rump length, CRL) is well regarded as the most accurate measure for estimating early gestational age (usually measurable by 6-7 weeks). Nevertheless, in this evaluation, there was no information on CRL from the referring hospital and the measured CRL on micro-CT suggested an embryo of an even earlier gestational age (at approximately 6 weeks gestation, 42-45 days post ovulation; Carnegie stage 18). Whilst several pathological reasons may exist for this, the discrepancy is likely to have arisen from potential tissue shrinkage after the iodination and fixation process prior to scanning, which has previously been described\textsuperscript{87,137} in isolated tissue samples, although this has yet to be formally assessed in human embryos and fetuses. Alternative and less likely explanations (given lack of significant antenatal history and that the tissue was obtained ‘fresh’) may be from stunted embryonal growth secondary to factors such as poor maternal nutrition, an underlying skeletal dysplasia or maceration. In fact, on initial radiological review a skeletal dysplasia had been an area of some debate, given the long bones of both upper and lower limbs appeared shortened in proportion to the hands and feet. Nevertheless, this was later discounted as during normal developmental stages it is usual for long bones to lengthen over several few days following Carnegie stage 21. Although maceration is known to be detrimental to micro-CT analysis of early gestational embryos, in this case our tissue was obtained fresh and there was no maceration on imaging or visual examination.
This case has demonstrated that even at very early gestational pregnancy losses, micro-CT imaging can demonstrate internal and external morphological structures in an embryo similar to histopathology. This may provide a future viable alternative to autopsy where parental consent is refused.
Experiment 4.3.4, Methods: Diagnostic accuracy of whole-body post-mortem fetal micro-CT

To date, there has been no formal evaluation of diagnostic accuracy for the use of micro-CT in the context of perinatal autopsy. Establishing preliminary diagnostic accuracy would be an important step in the development of a clinical service that could be offered to parents who reject full autopsy.

4.3.4 Case selection and preparation

Cases were prospectively recruited for a double-blinded diagnostic accuracy study from two centres that regularly perform post mortem perinatal imaging, Great Ormond Street Hospital for Children, London or University Hospital Brugmann, Brussels for formal perinatal autopsy examination.

Following sampling for cytogenetic investigations (where necessary) fetuses were immersed at room temperature in a solution of 10% formalin (to prevent tissue degradation) and potassium triiodide (I₂KI / Lugol's iodine, to impart tissue contrast), with a total iodine content of 63.25 mg / mL (iodine mass of $2.49 \times 10^{-4} \text{ mol / mL}$), in a 1:1 ratio for 72 hours prior to imaging. Prior to imaging, the fetuses were removed from the I₂KI, rinsed in water to remove excess surface iodine and dried using gauze. Specimens were secured using foam supports, Parafilm M (Bemis, Oshkosh, USA) and carbon fibre rods to ensure mechanical stability during micro-CT examination. Following micro-CT examination, fetuses were de-iodinated using sodium thiosulphate
pentahydrate dissolved in water (4% w / v) for at least 12 hours prior to autopsy, and then transferred to a solution of 10% formalin to prevent tissue degradation prior to autopsy examination.

4.3.4 Scan parameters

Micro-CT images of the specimens were acquired using an XT H 225 ST microfocus-CT scanner with a multi-metal target (Nikon Metrology, Tring, UK). X-ray energies and beam current values ranged between 80 – 110 kV and 87-180 µA respectively. Exposure times ranged from 250 ms to 354 ms, with the number of projections optimized for the size of the specimen (number of pixels covered within area of interest x 1.5) and one X-ray frame per projection. Where possible, each fetus was scanned three times (approximately 19 minutes each, total scan time approximately 57 minutes), to provide one overview whole body dataset at lower magnification followed by two higher-magnification scans of the brain, and thorax & abdomen. Projection images were reconstructed using modified Feldkamp filtered back-projection algorithms with proprietary software (CTPro3D; Nikon Metrology, UK) and post processed using VG Studio MAX 3.0 (Volume Graphics GmbH, Heidelberg, Germany). Isotropic voxel sizes varied according to specimen size.
4.3.4 Image analysis and reporting

Micro-CT images were independently evaluated by two pediatric radiologists with experience of fetal post mortem imaging and a final diagnosis based on the consensus read. The radiologists were provided with the same clinical information as is available to the pathologist but blinded to any autopsy results. 40 individual indices were assessed for each case, including seven neurological (cortex, cerebellum, midbrain, brainstem, spine, CSF spaces, and eyes), ten thoracic (mouth, neck, larynx, trachea, bronchi, thymus, thyroid, lungs, chest wall, and diaphragm), nine cardiac (right inflow tract, right outflow tract, left inflow tract, left outflow tract, pericardium, interatrial septum and interventricular septum, coronary arteries, and ductus arteriosus), thirteen abdominal (esophagus, stomach, small bowel, large bowel, pancreas, liver, adrenals, spleen, abdominal wall, kidneys, ureters, bladder, and gonads), and one musculoskeletal indices. Twenty fetuses (11–21 weeks gestational age, median 14 weeks) were prepared for micro-CT investigation. The cohort included seven intrauterine fetal deaths and 13 terminations of pregnancy (ten of which were performed for fetal anomalies; Table 29). 40 indices were assessed in each fetus at both micro-CT and autopsy and compared. Of the 800 potential indices for analysis, 12 neurological indices were not assessed at autopsy (due to parental preference that the head not be opened unless an abnormality was detected on imaging) and 70 non-diagnostic indices (non-diagnostic either by micro-CT, autopsy or both modalities) were also removed from further analysis, leaving 718 indices for analysis.
4.3.4 Autopsy examination

Autopsies were performed blinded to the micro-CT findings by specialist perinatal pathologists, in the centre that recruited the case, according to standard clinical procedures, recording the same diagnostic indices as reported by the radiologists. Histology was taken at the pathologist’s discretion as part of routine clinical investigation. Features identified by dissection, histological examination and micro-CT imaging were compared. Potential discrepancies were reviewed, with pathological examination used as the reference standard for the purposes of this analysis.

4.3.4 Statistical analysis

The primary outcome was concordance between micro-CT and conventional autopsy for overall diagnosis. Concordance was defined as the sum of true positives and true negatives divided by all diagnostic cases. Secondary outcomes were sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), expressed as the proportion of undetected pathological lesions (false negatives) and apparent overcalls (false positives), with subgroup analysis of five different body indices / categories with 95% confidence intervals (CI). Exact methods were used to calculate confidence intervals and SPSS (Version 19 for Macintosh, SPSS Inc., IBM, New York, USA) was used for data analysis by (initials redacted for reviewer blinding). p<0.05 was taken as the threshold for statistical significance, where appropriate.
Experiment 4.3.4, Results: Diagnostic accuracy of whole-body post-mortem fetal micro-CT

All specimens demonstrated excellent internal contrast on micro-CT examination with the iodination protocol (Figure 62). Tissue processing for micro-CT (iodination in a mix of formalin and iodine, reversal of iodination with sodium thiosulphate pentahydrate) did not cause significant tissue degradation or prevent adequate autopsy dissection in any case. Mean image resolution was $27 \pm 13.4 \, \mu m$ (range $7.4 - 51 \, \mu m$).
<table>
<thead>
<tr>
<th>Case</th>
<th>Age (weeks)</th>
<th>Mode of death</th>
<th>( \mu )CT resolution (( \mu )m)</th>
<th>Brain µCT</th>
<th>Brain Autopsy</th>
<th>( \mu )CT Body</th>
<th>Body Autopsy</th>
<th>Agreement</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>ToP</td>
<td>7.4</td>
<td>Complex NTD</td>
<td>Complex NTD</td>
<td>Y</td>
<td>Abdominal wall defect</td>
<td>Y</td>
<td>Complex NTD</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>ToP</td>
<td>13.7</td>
<td>NTD</td>
<td>NTD</td>
<td>Y</td>
<td>Acardia</td>
<td>Y</td>
<td>TRAP sequence</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>IUFD</td>
<td>20.2</td>
<td>Non-diagnostic, macerated</td>
<td>Non-diagnostic, macerated</td>
<td>Y</td>
<td>Normal</td>
<td>Normal</td>
<td>TRAP sequence</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>ToP</td>
<td>15.8</td>
<td>Alobar holoprosencephaly</td>
<td>Alobar holoprosencephaly</td>
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<td>Cystic kidneys, omphalocoele</td>
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<td>Holoprosencephaly</td>
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<td>5</td>
<td>16</td>
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<td>42.3</td>
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<td>AVSD, facial dysmorphism</td>
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<td>Abnormal karyotype, cardiac anomaly</td>
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<td>6</td>
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<td>Y</td>
<td>Coarctation, webbed neck</td>
<td>Y</td>
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<td>7</td>
<td>15</td>
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<td>Non-diagnostic, macerated</td>
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<td>Cleft palate, VSD, chest &amp; abdominal wall defects</td>
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<td>Limb body wall complex / ADAM</td>
<td>Limb body wall complex / ADAM</td>
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<td>Normal</td>
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<td>Normal</td>
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<td>19</td>
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<td>50.9</td>
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<td>Cleft, absent radius</td>
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Table 29. Comparison of diagnoses from micro-CT and autopsy. Adapted from Hutchinson et al.\textsuperscript{109} with permission.
Figure 62. Micro CT of a phenotypically normal fetus (case 12) at 12 gestational weeks (A). Axial (B) and Coronal (C) analysis of the heart reveals the aorta (star) and pulmonary trunk (hexagon). Adapted from Hutchinson et al.\textsuperscript{109} with permission.
4.3.4 Overall results

Autopsy demonstrated 13 fetuses with structural abnormalities overall, 12 of which were also identified by micro-CT (Figure 63). Overall, micro-CT agreed with overall autopsy findings in 35 / 38 diagnoses across the 20 fetuses; sensitivity 93.8% (95% CI: 71.7, 98.9%), specificity 100% (95% CI: 82.4, 100%). Agreement for body imaging diagnoses was 100%, in two cases there was no consent to remove and examine the brain at autopsy. In one case at 15 weeks gestation, micro-CT was non-diagnostic due to degradation of brain tissue, and an autopsy diagnosis was reached following specialist neuropathological examination following brain extraction. In two further cases, micro-CT reported the brain to be normal, but autopsy was non-diagnostic.

4.3.4 Agreement by body organ system

Overall, there was full agreement for 700/718 indices assessed on both micro-CT and autopsy dissection (agreement 97.5%; 95% CI 96.6, 98.4%; Table 30). This consisted of 104 true positives and 596 true negatives, giving overall sensitivity of 89.7% (82.8, 94.0%) and specificity of 99.0% (97.8, 99.5%). Overall, sensitivity was 87% or greater, and specificity was 98% or greater for each organ system and overall.

Analysis of the 70 non-diagnostic indices within the cohort revealed that Micro-CT was non-diagnostic (when autopsy was diagnostic) in 10/788 indices (1.27%), with autopsy non-diagnostic (when micro-CT was diagnostic) in 41/788 indices (5.20%; p<0.001). Both modalities were non-diagnostic in 19/788 indices (2.41%).
Figure 63. Micro-CT of a 13 week fetus with alobar holoprosencephaly (case 4, A & B). Autopsy (C & D) confirmed the abnormal finding shown on micro-CT. Scale bar C & D = 1cm. Adapted from Hutchinson et al.\textsuperscript{109} with permission.
<table>
<thead>
<tr>
<th>Body System</th>
<th>NE</th>
<th>ND</th>
<th>TP / FP</th>
<th>FN / TN</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuro</td>
<td>12</td>
<td>27</td>
<td>17 / 1</td>
<td>1 / 82</td>
<td>94.4% [74.2, 99.0]</td>
<td>98.8% [93.5, 99.8]</td>
<td>94.4% [75.2, 99.0]</td>
<td>98.8% [93.5, 99.8]</td>
<td>98.0% [93.1, 99.5]</td>
</tr>
<tr>
<td>Chest</td>
<td>0</td>
<td>6</td>
<td>20 / 2</td>
<td>3 / 169</td>
<td>87.0% [67.9, 95.5]</td>
<td>98.8% [95.8, 99.7]</td>
<td>90.9% [72.2, 97.5]</td>
<td>98.3% [95.0, 99.4]</td>
<td>97.4% [94.1, 98.9]</td>
</tr>
<tr>
<td>Cardiac</td>
<td>0</td>
<td>21</td>
<td>19 / 0</td>
<td>2 / 138</td>
<td>90.5% [71.1, 97.3]</td>
<td>100% [97.3, 100]</td>
<td>100% [83.2, 100]</td>
<td>98.6% [94.9, 99.6]</td>
<td>98.7% [95.5, 99.7]</td>
</tr>
<tr>
<td>Abdomen</td>
<td>0</td>
<td>16</td>
<td>41 / 3</td>
<td>5 / 195</td>
<td>89.1% [77.0, 95.3]</td>
<td>98.5% [95.6, 99.5]</td>
<td>93.2% [81.8, 97.7]</td>
<td>97.5% [94.3, 98.9]</td>
<td>96.7% [93.7, 98.3]</td>
</tr>
<tr>
<td>MSK</td>
<td>0</td>
<td>0</td>
<td>7 / 0</td>
<td>1 / 12</td>
<td>87.5% [52.9, 97.8]</td>
<td>100% [75.8, 100]</td>
<td>100% [64.6, 100]</td>
<td>92.3% [66.7, 98.6]</td>
<td>95.0% [76.4, 99.1]</td>
</tr>
<tr>
<td>Overall</td>
<td>12</td>
<td>70</td>
<td>104 / 6</td>
<td>12 / 596</td>
<td>89.7% [82.8, 94.0]</td>
<td>99.0% [97.8, 99.5]</td>
<td>94.5% [88.6, 97.5]</td>
<td>98.0% [96.6, 98.9]</td>
<td>97.5% [96.1, 98.4]</td>
</tr>
</tbody>
</table>

Table 30. Diagnostic performance of micro-CT by body system. All confidence intervals are 95%. Adapted from Hutchinson et al.\textsuperscript{109} with permission.
4.3.4 Discrepant findings

There were 18/718 (2.5%) apparent discrepancies between micro-CT and autopsy findings (Table 30). Three false negative indices (apparent ‘misses’ on micro-CT) were however easily detected on external examination of the fetus (1x polydactyly, 1x sacral neural tube defect (Figure 64), 1x ambiguous genitalia), leaving nine apparent false negatives of internal abnormalities; one VSD, malrotation of the bowel (x3), abnormalities of lung lobation (x2), laryngeal atresia, hypoplastic bladder, right inflow tract anomaly (Table 31).

There were six apparent false positives (‘overcalls’) on micro-CT assessment; an apparently hypoplastic thymus reported as normal at autopsy, caudal regression sacral change not identified at autopsy, an incidental cystic neck lesion not identified at autopsy (Figure 65), a utero-vesical connection not identified at autopsy, one overcall of histologically normal kidneys and a megarectum that was mistaken for megacystis (Table 31).
Figure 64. An apparent miss on micro-CT examination was a sacral neural tube defect (case 4), which was easily detected at external examination (star), but overlooked on the micro-CT scan (B). Adapted from Hutchinson et al.\textsuperscript{109} with permission.
<table>
<thead>
<tr>
<th>Region</th>
<th>True positives</th>
<th>Correct Diagnoses</th>
<th>FP</th>
<th>Overcalls</th>
<th>FN</th>
<th>Misses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuro</td>
<td>17</td>
<td>1 x ventricular malformation, 1x cortical malformation, 1x hypotelorism, 1x hypertelorism, 1x vertebral anomalies, 2x cranio-rachischisis, 10x structural abnormality</td>
<td>1</td>
<td>Caudal regression</td>
<td>1</td>
<td>1 x neural tube defect</td>
</tr>
<tr>
<td>Chest</td>
<td>20</td>
<td>1x narrow chest, 1x neck webbing, 2x cleft palate, 2x neck disruption, 3x chest wall disruption, 5x diaphragmatic disruption, 6x structural abnormality</td>
<td>2</td>
<td>Thymic hypoplasia, incidental neck lesion</td>
<td>3</td>
<td>2 x lung lobation anomalies 1 x laryngeal atresia</td>
</tr>
<tr>
<td>Cardiac</td>
<td>19</td>
<td>1x AVSD (6 indices), 1 x Truncus arteriosus (4 indices) 9x structural abnormality</td>
<td>0</td>
<td>None</td>
<td>2</td>
<td>1 x VSD 1 x right heart anomaly</td>
</tr>
<tr>
<td>Abdo</td>
<td>41</td>
<td>1x omphalocele, 1x cystic kidneys, 1x absent kidney, 1x absent ureter, 1x bladder anomaly, 23x visceral disruption/displacement, 13x structural abnormality</td>
<td>3</td>
<td>Normal kidneys megarectum interpreted as megacystis, uterovesical connection</td>
<td>5</td>
<td>3 x Bowel malrotation 1 x ambiguous genitalia 1 x hypoplastic bladder</td>
</tr>
<tr>
<td>MSK</td>
<td>7</td>
<td>1x complex NTD, 1x rib defects, 1x symmetrical limb foreshortening</td>
<td>0</td>
<td>None</td>
<td>1</td>
<td>1 x polydactyly</td>
</tr>
</tbody>
</table>
Table 31. Breakdown of true positives (correct diagnoses), false positives (overcalls) and false negatives (misses) of micro-CT examination within the cohort by body system. (AVSD – atrioventricular septal defect, FN – false negative, FP – false positive, NTD – neural tube defect, TD – Thanatophoric Dysplasia, VSD – ventricular septal defect). Adapted from Hutchinson et al.\textsuperscript{109} with permission.

<table>
<thead>
<tr>
<th></th>
<th>1x radial absence, 1x femoral absence, 1x sternal anomaly, 1x skeletal dysplasia (TD)</th>
<th>6</th>
<th>-</th>
<th>12</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL</td>
<td>104</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 65. A cystic neck lesion (star) was identified in case 5 on micro-CT examination. This was overlooked at autopsy, as this region is not routinely dissected, and appears within the data as an apparent overcall. Adapted from Hutchinson et al.\textsuperscript{109} with permission.
4.3.4 Agreement by gestation

We further divided cases into first (≤14 weeks gestation) and second (>14 weeks gestation) trimester (mean gestational age 12.4 weeks (n=11; SD 1.2 weeks) and 17 weeks respectively (n=9; SD 2.6 weeks)). The non-diagnostic rate was higher for micro-CT in first trimester (22 / 440; 12.04%) than second trimester fetuses (7 / 360; 4.7%; p<0.001; Table 32). However, within first trimester cases, micro-CT analysis yielded significantly fewer non-diagnostic indices than autopsy examination (22 / 440 vs 48 / 348 respectively; p<0.001). There was no statistical difference in non-diagnostic rates between micro-CT and autopsy in second trimester cases (p=0.35). There were no differences between diagnostic accuracy indices across individual organ systems (Table 32), but PPV was significantly higher in younger fetuses (<14 weeks) at 97.3% vs 85.7% (p<0.001).
<table>
<thead>
<tr>
<th></th>
<th>NE</th>
<th>ND</th>
<th>TP / FP</th>
<th>FN / TN</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuro &lt;14 wks</td>
<td>0</td>
<td>18</td>
<td>16 / 0</td>
<td>1 / 42</td>
<td>94.1%</td>
<td>100%</td>
<td>100% **</td>
<td>97.7%</td>
<td>98.3%</td>
</tr>
<tr>
<td>&gt;14 wks</td>
<td>12</td>
<td>9</td>
<td>1 / 1</td>
<td>0 / 40</td>
<td>100%</td>
<td>97.6%</td>
<td>50.0%</td>
<td>100%</td>
<td>97.6%</td>
</tr>
<tr>
<td>Chest &lt;14 wks</td>
<td>0</td>
<td>6</td>
<td>14 / 1</td>
<td>3 / 86</td>
<td>82.4%</td>
<td>98.9%</td>
<td>93.3%</td>
<td>96.6%</td>
<td>96.2%</td>
</tr>
<tr>
<td>&gt;14 wks</td>
<td>0</td>
<td>0</td>
<td>0 / 1</td>
<td>0 / 83</td>
<td>100%</td>
<td>98.8%</td>
<td>85.7%</td>
<td>100%</td>
<td>98.9%</td>
</tr>
<tr>
<td>Cardiac &lt;14 wks</td>
<td>0</td>
<td>16</td>
<td>9 / 0</td>
<td>1 / 73</td>
<td>90.0%</td>
<td>100%</td>
<td>100%</td>
<td>98.6%</td>
<td>98.8%</td>
</tr>
<tr>
<td>&gt;14 wks</td>
<td>0</td>
<td>5</td>
<td>0 / 0</td>
<td>1 / 65</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>98.5%</td>
<td>98.7%</td>
</tr>
<tr>
<td>Abdomen &lt;14 wks</td>
<td>0</td>
<td>13</td>
<td>36 / 1</td>
<td>3 / 90</td>
<td>92.3%</td>
<td>98.9%</td>
<td>97.3% **</td>
<td>96.8%</td>
<td>96.9%</td>
</tr>
<tr>
<td>&gt;14 wks</td>
<td>0</td>
<td>3</td>
<td>5 / 2</td>
<td>2 / 105</td>
<td>71.4%</td>
<td>98.1%</td>
<td>71.4%</td>
<td>98.1%</td>
<td>96.5%</td>
</tr>
<tr>
<td>MSK &lt;14 wks</td>
<td>0</td>
<td>0</td>
<td>5 / 0</td>
<td>1 / 5</td>
<td>83.3%</td>
<td>100%</td>
<td>100%</td>
<td>83.3%</td>
<td>90.9%</td>
</tr>
<tr>
<td>&gt;14 wks</td>
<td>0</td>
<td>0</td>
<td>2 / 0</td>
<td>0 / 7</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Overall &lt;14 (n=11)</td>
<td>0</td>
<td>53</td>
<td>80 / 2</td>
<td>9 / 296</td>
<td>89.7%</td>
<td>99.3%</td>
<td>97.6% **</td>
<td>97.0%</td>
<td>97.2%</td>
</tr>
<tr>
<td>&gt;14 wks (n=9)</td>
<td>12</td>
<td>17</td>
<td>24 / 4</td>
<td>3 / 300</td>
<td>88.9%</td>
<td>98.7%</td>
<td>85.7%</td>
<td>99.0%</td>
<td>97.9%</td>
</tr>
</tbody>
</table>
Table 32. Overall diagnostic performance of micro-CT vs. autopsy by gestation.

** indicates p < 0.001. Sensitivity was calculated as true positives (TP) divided by true positives and false negatives (FN); specificity calculated as true negatives (TN) divided by true negatives and false positives (FP); positive predictive value (PPV) calculated as true positives divided by true positives and false positives; negative predictive value (NPV) calculated as true negatives divided by true negatives and false negatives; and agreement calculated as sum of true positives and true negatives divided by all cases. (ABDO – abdominal pathology, CARDIAC – cardiac pathology, CHEST – non-cardiac thoracic pathology, MSK – musculoskeletal pathology, ND – non-diagnostic, NE – not examined, NEURO – Neurological pathology) Adapted from Hutchinson et al.109 with permission.
Experiment 4.3.4, Discussion

Micro-CT provided post mortem perinatal imaging findings with a high concordance with conventional autopsy in early gestation fetuses. Apart from one case of non-diagnostic brain imaging due to tissue degradation, non-invasive micro-CT examination reached the correct overall diagnosis in all cases. In first trimester cases, micro-CT examination performed better than standard autopsy examination, which may reflect improved reporter confidence when analysing a 3D volume, compared to an extremely challenging autopsy procedure.

Fetal autopsy has several important considerations that constrain its use. Information obtained from fetal autopsy may be limited due to autolysis and maceration or due to technical reasons (e.g. small fetal size). Many early miscarriages are not reported to medical practitioners, possibly due to a perceived inability to offer adequate investigation after death, and some institutions may still treat the remains of an early miscarriage as clinical waste. Consequently, there is a substantial unmet clinical need with regards to investigation of first and early second trimester fetal loss for parents for whom standard autopsy is either not available or not acceptable. Furthermore, post-mortem confirmation of fetal anomalies following first trimester prenatal diagnosis and early termination of pregnancy has an important quality and governance role, in addition to parental reassurance. This experiment shows that micro CT can provide imaging volumes of fetal anatomy, which would become part of the medical record and could be demonstrated to parents or
clinicians in complex cases. In smaller cases, the relatively improved resolution obtained by micro CT (due to greater geometric magnification of the sample), has the potential to yield greater anatomical detail. Imaging of early gestation fetuses below 16 – 18 weeks is particularly challenging. The non-diagnostic rate (for micro CT) of 1 / 20 cases (5%) and 29 / 800 indices (3.63%) is significantly better than the reported literature. Although diagnostic accuracy of PMMR is related to both field strength and fetal size, non-diagnostic imaging rates <20 weeks were 50% at 1.5 T and 30% at 3 T and 23.5% at 9.4 T. Several issues remain to be addressed with regards to the optimisation of micro CT for widespread clinical practice, including methods to reduce staining time, the use of alternative contrast agents and acceptability of discolouration of the skin caused by fixation (as iodination is reversible). Additionally, the fetal brain is relatively high in water content, and therefore may be vulnerable to the relatively high osmolality of 12KI. Since the micro CT was non-diagnostic in this case of brain abnormality, in clinical practice, extraction of the brain for formal neuropathological examination should be performed when micro CT is non-diagnostic, although this also remains challenging at perinatal autopsy when autolysis is present.

In the UK, most fetal autopsies are performed at the request of the parents; as such, the investigation is tailored to the parents’ expectations, rather than those of the medical professional requesting or performing the examination. However, consent rates remain low and many parents find the idea of an invasive autopsy distressing, with some reporting that their baby has ‘suffered
enough’ or will fearing unsatisfactory cosmetic effects from a standard autopsy procedure. Furthermore, perinatal pathology is highly specialised, usually limited to tertiary referral centres. Thus, there may be delays due to logistical difficulties in transferring the body for autopsy or obtaining an autopsy in a timely manner, which can add to parental distress and may be an issue for Muslim or Jewish parents in whom delay in burial may be particularly problematic. High resolution imaging also facilitates discussions between medical practitioners involved in fetal diagnosis, including radiologists, pathologists, geneticists, paediatricians and fetal medicine specialists who are involved in counselling of parents for future pregnancies. The overall clinical utility in an unselected population remains to be assessed in a larger cohort of fetuses. This study was limited by patient recruitment, as only the fetuses of parents who agreed to participate in the micro CT study were enrolled, and the potential skin discolouration from the use of fixative (formalin) and iodine may have reduced patient participation. Further studies recruiting across a range of congenital malformations are required in order to provide personalised counselling regarding likely yield of micro CT examination in a range of clinical scenarios.
Part four summary

These data from a range of extracted organs and numerous unselected fetal cases demonstrate that iodinated post-mortem micro-CT is an accurate and feasible method of documenting perinatal anatomy. This is true for confirmation of normality, as seen in the normal extracted fetal heart and in the whole-body validation study, and in the context of congenital abnormalities (experiments 4.2.6 and 4.3.4) or neoplasia (experiment 4.2.5).

Micro-CT may be particularly useful for phenotyping tissue when the gestation is early, and autopsy is therefore technically difficult, or where there are concerns about maceration (although this was not formally tested within these experiments). Areas for future development include contrasting of the brain, which is particularly vulnerable to structural degradation in highly concentrated solutions of I$_2$KI, and methods to speed up contrast ingress into post-mortem fetuses. Additionally, there may be a role for expanding micro-CT autopsies to cover surgical terminations of pregnancy, as it would theoretically be possible to digitally examine disrupted fetuses for anatomical abnormalities.
Part five: Discussion

The discussion will summarise the main conclusions of the thesis, including general limitations, present evidence-based guidelines for the investigation of SUDC, SUDI, IUFD and ToP and areas for further research.
5.1 Conclusions

The research within this thesis has primarily sought to advance the development of the minimally invasive perinatal autopsy within the context of falling parental consent rates and the emerging field of post-mortem radiology.

The primary conclusions, outlined against the aims of this thesis, are stated below:

**Aim 1: Test the feasibility of laparoscopic autopsy by performing this procedure in a cohort of cases (estimated cohort size = 100), including an assessment of sampling adequacy.**

The MinImAL procedure was shown to be feasible in 103 cases, with sampling adequacy >80% for most body organs (100% for heart, lung and kidney), with low failure rate (2/93, 2.2%).

**Aim 2: Evaluate technical issues around the use of micro-CT, including choice of contrast medium and the effect of contrast on subsequent sampling and histology.**

I2KI offers cheap, reversible micro-CT contrast of biological tissues with no subsequent effect on haematoxylin and eosin slide production when used in low to moderate concentrations. Although more testing is required, no adverse effects were seen on human fetal organ histological evaluation when a limited
range of immunohistochemistry was tested, either post-iodination or following I$_2$KI removal with sodium thiosulphate.

_Aim 3:_ Demonstrate proof of principle for whole body fetal imaging using micro-CT and test diagnostic accuracy.

Micro-CT autopsy in fetal 20 cases showed high levels of agreement with conventional autopsy across multiple organ systems (97.5% agreement, 95% CI, 96.6-98.4) and may offer an acceptable, non-invasive method of post-mortem examination for early gestation fetal loss or termination of pregnancy.

_Aim 4:_ Interrogate the evidence base for macroscopic examination of organs and histological sampling in perinatal and paediatric deaths using the GOSH autopsy database.

The yield of invasive examination (macroscopic examination and visceral histology) to the overall cause of death was examined across 5,311 total cases, subcategorised by indication (ToP, IUFD, SUDI, SUDC).

Macroscopic examination of organs provided a cause of death in 25.8% of ToP cases, 21.6% of SUDC cases 12.2% of SUDI cases, and 1.7% of IUFDs. It is reasonable to expect that of the majority of such abnormalities would be detected by appropriate post-mortem imaging.

The value of routine histological sampling was relatively high for SUDC and SUDI, with 9.4% and 5.2% of cases respectively receiving a cause of death from
Histology of macroscopically normal organs. In contrast, the yields of routine sampling in IUFD and ToP are minimal with 0.1% and 0.3% of cases respectively receiving main diagnosis or cause of death from histology of macroscopically normal organs.

Aim 5: Establish clinical guidelines for less invasive autopsy given the above findings.

Proposed autopsy guidelines are presented later in this chapter.
5.2 General discussion

The data presented in parts 2-4 builds upon recent minimally invasive autopsy literature and introduces new methodologies and evidence to better inform the performance of minimally invasive autopsy in the perinatal and paediatric setting. As a result, there is now additional evidence to support several non-invasive or minimally invasive investigations that can be offered to parents who experience a fetal, perinatal or paediatric death. Moreover, the yield of invasive autopsy can be better articulated in different contexts, which may help clinicians and parents to make difficult decisions regarding the performance (or not) of an autopsy. Consequently, there is a strong case to be made in for a shift in practice away from the currently limited choice currently available to families (Figure 66), towards a spectrum-based approach (Figure 67) that considers the type of case (childhood death, infant death, IUFD, or ToP) along with the specific clinical questions to be answered.
Figure 66. Illustration of the current paradigm of autopsy presented to parents. The choice is effectively trinary, as even a limited autopsy requires a major incision to access a body cavity.
Figure 67. Illustration of a revised paradigm of perinatal autopsy proposed by this thesis, of which, the primary driver is the degree of consent provided by parents under a shared decision-making model. Guided by clinicians, parents may choose to consent either to imaging only, to imaging plus targeted sampling, or to standard autopsy. A variety of techniques may be used by the investigating clinician to answer the clinical question, whilst remaining within the bounds of parental consent.
The argument for a shift in practice towards a spectrum of investigations can be made from a pragmatic, practice-based perspective with the knowledge that the consensus of recent studies examining autopsy acceptability rates in high income countries reflects that most parents are not prepared to accept full autopsy. Moreover, acceptability rates are particularly poor for later gestation intrauterine deaths and neonatal deaths. Ultimately, regardless of how “good” a traditional invasive autopsy is, the poor acceptability of invasive techniques significantly undermines its status as the sole option for medical investigation after death, particularly given the poor yield of invasive examination in IUFD (demonstrated in part three of this thesis).

A further compelling argument for a shift in autopsy practice can be made from an ethical standpoint, based on improving patient autonomy. The paediatric and perinatal autopsy is one of the only medical procedures in existence where the extent and expectations of the procedure are largely dictated by those receiving care (in this case, the family of the deceased) by the scope of the consent granted for examination, rather than the clinician requesting the test. Compare, for example, an MRI scan, a blood test, or a minor operation, where the clinician explains the test to be performed, along with risks and benefits, and receives a yes/no authorisation to proceed. Presenting relatives with a spectrum of choices, with evidence-based information regarding the likely diagnostic yield of different options, facilitated by an expert clinician (e.g. a fetal medicine doctor, specialist bereavement midwife, pathologist, or similar) fits the shared decision-making model of healthcare championed by the NHS as part of the NHS Five Year
Forward View\textsuperscript{138} and represents a manifestation of moves towards “individualised healthcare”. This approach is recognised to empower patients and, if accompanied by information tailored to the specific circumstances, could provide the basis for a service built around parental involvement and research co-design, rather than the current approach, which seems rooted in medical paternalism. This is reinforced by numerous studies showing that acceptance rates of minimally invasive perinatal autopsy are $>90$.\textsuperscript{9,14,47} A minimally invasive approach also shows improved acceptability within Muslim and Jewish communities,\textsuperscript{65,71} although significant concerns remain regarding turnaround time to ensure timely burial of the body, independent of autopsy choice.

In addition to the general points raised above, the results presented to this point demonstrate numerous specific (data-based) implications for the field of perinatal and paediatric autopsy, the implications of which will be considered later in the ‘proposed autopsy guidelines’ (5.4 – 5.7). These findings include several areas where existing clinical practice could be altered significantly. In other contexts, the importance of tissue-based investigations has been reinforced.
5.3 Limitations and criticisms

This section presents the overall general limitations and criticisms of this work, in addition to the specific methodological limitations regarding each part of this thesis (previously presented in parts 2-4, respectively).

5.3.1 Acceptability

Numerous studies indicate that the acceptability rates of minimally invasive autopsy techniques are greater than 90%. Although this level of acceptability is a considerable improvement on traditional autopsy, it is incumbent on practitioners to examine the remaining reasons for non-consent.

Review of the literature indicated that the reasons for withholding consent are diverse. Within deeply religious communities, (particularly ultra-Orthodox Jewish communities and some Muslim populations), objections arise based on the potential delay to funeral arrangements, perceived insult to the body (and perceived shame that arises as a result), the need to be buried ‘whole’ and the futility of investigation, given that the death in question could be perceived to have been the will of God. Even if a minimally invasive service could provide a turnaround time of less than 12 hours from receipt of a body to release for burial or cremation, it is highly unlikely that the service would be perceived as ‘acceptable’ to those with deeply held religious or cultural objections to the procedure. Therefore, it is similarly unlikely that addressing the service turnaround time will lead to an increase in consented cases from deeply
religious communities, or those with a cultural opposition to the autopsy. Instead, a rapid turnaround service is likely to be of more benefit to Coroners, who come under pressure from communities to either avoid an autopsy altogether or to conclude investigations based on imaging alone. This has been seen in adult autopsy pathology, with Muslim and Jewish communities active in lobbying Coroners and parliamentarians for legally acceptable non-invasive alternatives to adult Coronial autopsies. A secondary effect of this community pressure may include the rise of private ‘digital autopsy’ companies offering ‘no cause of death, no fee’ type services to relatives (Figure 68).

From a medical perspective, by introducing potential major reporting bias in the form of financial incentives for positive results, this business model undermines the medical raison d’être of a traditional autopsy procedure, which is to exhaustively search for a specific cause of death (or to document the absence of one). However, from a medicolegal standpoint, this may prove acceptable, given the poor quality of Coronial services provided by pathologists in England & Wales (particularly in adult cases), the increasing caseload handled by Coroners, ongoing delays to the introduction of a medical examiner system, and the balance of probably (greater than or equal to 51% certainty) required by Coroners to establish a cause of death.
Figure 68. Fee structure model available on the website of a private company offering a ‘no win, no fee’ style of service to parents.

For some parents and medical practitioners, minimally invasive autopsy lacks the perceived thoroughness of a traditional autopsy, thus leaving the potential for diagnostic doubt to arise, or for a cause of death to be missed. Following appropriate counselling of the likely yield of an invasive autopsy (considering the clinical history, type of loss and parental expectations of the procedure), if parental preference for full autopsy remains, this should be taken as the option most likely to meet parental expectations. In this context, minimally invasive autopsy is probably inappropriate, but post-mortem imaging may still be useful in guiding potential areas for detailed examination during the autopsy or acting as a ‘second view’ to confirm normality. Consent rates suggests that these parents will be in the minority (<20%), however, and most of the parental feedback received during the course of this thesis has been to perform
“as minimally invasive an autopsy as is possible”, i.e. stopping with the least invasive autopsy which provides an adequate answer.

Overall, health and legal professionals are supportive of minimally invasive perinatal autopsy, with several procedural and psychological benefits identified in a recent qualitative study. These include preference for smaller incisions, improved autonomy for parents, potential improvement in turnaround times (both of the body and the report) and improved relationships between coroners and the communities they represent. There are also legitimate concerns raised by peers, which should be considered carefully as part of any future service implementation. Potential problems include inappropriate uptake of the procedure in situations where a traditional autopsy remains more appropriate, loss of certainty in the diagnosis (due to omission of some aspects of the procedure) and de-skilling of the pathology workforce.

Regarding acceptability within a legal setting, the scientific literature on MIA is now relatively well developed. Post-mortem imaging may therefore be entirely appropriate in helping to provide sanitised demonstration of complex injuries or abnormalities to lay members of juries and as a correlation with macroscopic findings. However, it would be unwise to advocate for isolated use of MIA autopsy techniques in a forensic setting, where the standard of proof requires findings to be established so that a jury is “so as to be sure” (beyond reasonable doubt). While MIA may be entirely appropriate
for a Coronal Court, where the standard of proof required is lower (on the balance of probabilities).

Extensive work on the parental acceptability has been undertaken by Dr. Celine Lewis, to which, the thesis author has contributed, in parallel to this thesis. The citations of this work are presented in Appendix 5.
5.3.2 Complexity

A spectrum of choices for investigation after death provides improved choice for parents may improve autonomy, however, the added complexity of consenting parents for autopsy has potential drawbacks for clinicians and consent takers, as the process may take longer, and require additional explanation and discussion during the consent process. In addition, if there are relatively few reasonable options available (e.g. a complex, post-operative childhood death is unlikely to be suitable for MinImAL examination), then this process may have the opposite effect of making parents feel like autonomy is being removed from them. To some extent these issues can be mitigated through comprehensive consent training of staff and patient education. Additional complexity regarding consent taking is likely to represent an unavoidable consequence of expanding the range of investigations available and promoting a shared decision-making model of consent.

5.3.3 Cost

The issue of cost from a strategic and public health perspective remains to be fully addressed in future work, however, some of the implications will be briefly discussed in this section. Currently, all perinatal post-mortem examinations in the UK are funded via central government at a standard tariff of approximately £900. This covers an examination, regardless of the specific components of the autopsy (whether external only, or full autopsy, the fee remains the same). Performance of PMMRI or micro-CT adds an additional
cost of approximately £250 to a standard autopsy examination, with negligible additional cost for a MinImAL procedure. To some extent, the costs of PM imaging may be negated through savings on the number of blocks taken, either through targeted sampling used to confirm abnormalities identified on imaging, or by leaving the skull intact (thus saving operator time at autopsy and approximately 5-10 histology blocks), due to the high negative predictive value of PMMRI for significant brain abnormalities.32

A potential issue closely related to cost is the availability of cross-sectional imaging. Many live patients wait months for routine MRI investigations; it may therefore be difficult to persuade local hospitals to open scan slots for post mortem investigations. However, this problem could potentially be overcome by facilitating an out-of-hours scanning service, as many diagnostic scanners do not yet run 24 hours / day and lie dormant outside of sociable working hours. Given that public demand has effectively driven the development of MIA services over the past decade, it is likely that there is a business case to be made for post-mortem scanning in out-of-hours slots, provided that local Anatomical Pathology Technicians (APTs) and radiographers can be trained and adequately reimbursed for their time. Scans can be acquired locally and reported centrally, provided that there are an adequate number of trained radiologists to meet the likely demand.

A full economic analysis, considering training of operators (reporting pathologists and radiologists), as well as logistical challenges involved with rolling out a minimally invasive approach on a larger scale should be
conducted, which must also include the additional ‘value’ of cause of death information gained from cases who currently do not undergo any form of autopsy but who would accept MIA.

5.3.4 Value of a negative autopsy

It is much harder to value or analyse the importance of a ‘negative’ autopsy result when compared one that generates a definitive cause of death, as the impact of a negative result is highly prone to context and subjectivity (as opposed to a definitive result, which usually has a specific implication for management). Following the death of a fetus or neonate, for some parents, there may be a desire to be certain that there was nothing more that could have been done to help their baby, or as part of ensuring that the chances of it happening in future are as small as possible. These parents may have genuine concerns about the possibility of an MIA approach generating a false negative result (failing to detect pathology), although the false negative rate of most imaging and laboratory tests are widely accepted. Parents may also have to weigh these perceived drawbacks against the cosmetic and psychological benefits of MIA, although there may be little difference between psychological advantages of different types of autopsy, with the most significant differences being between receiving and declining the autopsy of choice. Formal exploration of the benefits of negative autopsy and the impact of a negative MIA procedure is beyond the remit of this thesis. However, if a consent taker feels that there is a chance of significant parental distress after a negative or
unclear result following appropriate counselling of the risks and benefits, then MIA is unlikely to be a suitable procedure in this context.

5.3.5 Assessment of Neuropathology

The assessment of neuropathology in this study was generally undertaken by paediatric pathologists, with referral to neuropathologists undertaken in several cases, where judged necessary by the consultant in charge of the case. Post-mortem fetal brains are not routinely examined by neuropathologists at Great Ormond Street Hospital, with referral undertaken for the most challenging cases. Similarly, all PMMRI cases were reported by paediatric radiologists with experience of post-mortem imaging, rather than specialist neuro-radiologists. Although PMMRI has a high negative predictive value for the assessment of neuropathology, it is possible that subtle neuropathological abnormalities could have been missed throughout the studies described above, however, it is unlikely that these would have led to a change in overall diagnosis. Assessment of neuropathology by micro-CT remains an area for further development; this is further explored below.
5.4 Proposed Autopsy Guidelines for Investigation of SUDC

5.4.1 Imaging concordance and autopsy yield in SUDC

Based on data from the MARIAS study, which examined the concordance of autopsy and PM imaging in a double-blind manner, the concordance of PMMRI alone in the context of infant and childhood death (n = 123 cases) is as follows (Table 33):

<table>
<thead>
<tr>
<th>Concordance (95% CI)</th>
<th>69.1%, 60.5-76.6 (85/123)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discordance</td>
<td>27% (34/123)</td>
</tr>
<tr>
<td>Non-diagnostic</td>
<td>3% (4/123)</td>
</tr>
</tbody>
</table>

Table 33. Concordance of PMMRI alone with standard autopsy in 123 cases from the MARIAS study.

The most commonly missed cause of death by PMMRI alone without sampling of organs or blood is occult infection/sepsis (24/123, 19.5% of cases), for which, no reliable marker currently exists on PM imaging. From the retrospective database review (3.3.2 – 3.3.9), macroscopic examination of organs provides a cause of death in approximately 21.6% of cases (overall n = 824), with causes of death attributable as follows in Table 34.

<table>
<thead>
<tr>
<th>Heart</th>
<th>Lung</th>
<th>Brain</th>
<th>Adrenals</th>
<th>Thyroid</th>
<th>Liver</th>
<th>Kidney</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.6%</td>
<td>5.1%</td>
<td>7.8%</td>
<td>0.3%</td>
<td>Nil</td>
<td>0.6%</td>
<td>0.8%</td>
<td>0.4%</td>
</tr>
<tr>
<td>(52/789)</td>
<td>(40/792)</td>
<td>(55/704)</td>
<td>(2/772)</td>
<td>(0/732)</td>
<td>(5/780)</td>
<td>(14/779)</td>
<td>(5/774)</td>
</tr>
</tbody>
</table>

Table 34. Yield of macroscopic examination to the cause of death in 824 SUDC cases, split by organ system. Where the organ was not commented on in the report, an exclusion was made for that body system, leading to variation in the denominator.
From the retrospective database review, microscopic examination of macroscopically normal organs in SUDC provides a cause of death in approximately 9.4% of cases, distributed as follows in Table 35.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Heart</th>
<th>Lung</th>
<th>Brain</th>
<th>Adrenals</th>
<th>Thyroid</th>
<th>Liver</th>
<th>Kidney</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.4%</td>
<td>3.7%</td>
<td>1.1%</td>
<td>0.2%</td>
<td>Nil</td>
<td>0.4%</td>
<td>0.4%</td>
<td>0.2%</td>
</tr>
<tr>
<td></td>
<td>(25/739)</td>
<td>(29/786)</td>
<td>(7/661)</td>
<td>(1/654)</td>
<td>(0/466)</td>
<td>(3/735)</td>
<td>(3/715)</td>
<td>(1/539)</td>
</tr>
</tbody>
</table>

Table 35. Yield of microscopic examination to the cause of death in 824 SUDC cases, split by organ system. Where the organ was not commented on in the report, an exclusion was made for that body system, leading to variation in the denominator.

Although these rates appear small for each individual organ, cumulatively, they represent a considerable yield of the total causes of death in a cohort where investigation after death is mostly undertaken at the behest of the Coroner because the cause of death cannot be given clinically. Extrapolating from the MARIAS study, it is reasonable to consider that in the context of childhood death, the majority of macroscopic causes of death will be detected by PM imaging. The choice of PM imaging in SUDC should primarily be PMMRI, unless there is a concern regarding trauma or inflicted injury.63,74,116
5.4.2 Potential for non-discovery of cause of death in SUDC

There is a relatively high risk (estimated around 9% using current data) that a histologically detectable cause of death within a clinically, radiologically and macroscopically normal organ will be missed if an imaging only approach is used in the context of SUDC. (Table 36). Moreover, MinImAL examination in SUDC is likely to pose mechanical sampling problems (due to patient size / instrument length) and is unlikely to be successfully applied to whole body sampling in children. Only one case within the MinImAL cohort was a child, and organ sampling was restricted to specific organs as part of the Coronal Authority in that case. As such, MinImAL cannot be recommended as routine practice in SUDC (Figure 69).

<table>
<thead>
<tr>
<th>Children (SUDC presentation)</th>
<th>Maximum potential rate of COD non-discovery using NIA (PM MRI only, no histological sampling)</th>
<th>Maximum potential rate of COD non-discovery by adopting incremental approach (PMMRI, then MinImAL if abnormal)</th>
<th>Maximum potential rate of COD non-discovery by adopting routine MinImAL post PMMRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrospective cohort total: 824</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>120.1 per 1,000 cases</td>
<td>34.0 per 1,000 cases</td>
<td>3.6 per 1,000 cases</td>
</tr>
<tr>
<td>Lung</td>
<td>314.3 per 1,000 cases</td>
<td>61.9 per 1,000 cases</td>
<td>26.7 per 1,000 cases</td>
</tr>
<tr>
<td>Kidney</td>
<td>18.2 per 1,000 cases</td>
<td>4.9 per 1,000 cases</td>
<td>1.2 per 1,000 cases</td>
</tr>
<tr>
<td>Liver</td>
<td>18.2 per 1,000 cases</td>
<td>4.9 per 1,000 cases</td>
<td>1.2 per 1,000 cases</td>
</tr>
<tr>
<td>Adrenal</td>
<td>8.5 per 1,000 cases</td>
<td>2.4 per 1,000 cases</td>
<td>1.2 per 1,000 cases</td>
</tr>
<tr>
<td>Pancreas</td>
<td>6.1 per 1,000 cases</td>
<td>2.4 per 1,000 cases</td>
<td>2.4 per 1,000 cases</td>
</tr>
</tbody>
</table>

Table 36. Summarised data of potential rates of non-discovery of COD in SUDC (see part 3 of this thesis for full table) utilising different investigative strategies.
5.4.3 Proposed SUDC investigation strategy

Clinical review of notes

Imaging (cross-sectional & plain radiography)

External examination

Standard internal examination

Sample all organs thoroughly

Genetic analysis

Microbiology

Virology

Metabolic analysis

Toxicology

Specialist imaging

Figure 69. Evidence-based flowchart for optimal investigation of SUDC
The SUDC autopsy pathway should therefore remain relatively unchanged from standard autopsy practice currently in place. PMMRI may be a useful adjunct to help document macroscopic findings, or to guide internal examination. PMCT may also be useful for some indications, such as traumatic deaths or suspected inflicted injury. Even when PMCT is available, skeletal surveys should be performed in cases of suspected inflicted injury, as evidence regarding the utility of CT in this context is still emerging, although CT may prove superior to plain radiography in the fullness of time. The brain should be extracted and examined using standard techniques following sampling of cerebrospinal fluid for microbiology and virology.
5.5 Proposed Autopsy Guidelines for Investigation of SUDI

5.5.1 Imaging concordance and autopsy yield in SUDI

The MARIAS data for MRI concordance with standard autopsy includes both infants and children within the same diagnostic category (see Table 1 above). As such, occult infection / sepsis remains the most important missed cause of death in this cohort (in early neonatal deaths, prematurity and placental pathologies are also important causes). Macroscopic examination of organs at autopsy in the context of SUDI revealed a cause of death in approximately 12.2% of cases overall (overall n = 1,739), distributed as follows in Table 37.

<table>
<thead>
<tr>
<th>Heart</th>
<th>Lung</th>
<th>Brain</th>
<th>Adrenals</th>
<th>Thyroid</th>
<th>Liver</th>
<th>Kidney</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.0% (134/1686)</td>
<td>1.5% (26/1680)</td>
<td>2.1% (32/1543)</td>
<td>0.2% (3/1667)</td>
<td>Nil (0/1551)</td>
<td>0.5% (8/1674)</td>
<td>0.1% (2/1667)</td>
<td>0.1% (0/1651)</td>
</tr>
</tbody>
</table>

Table 37. Contribution of macroscopic examination to the cause of death in 1,739 SUDI cases, split by organ system. Where the organ was not commented on in the report, an exclusion was made for that body system, leading to variation in the denominator.
Microscopic examination of macroscopically normal organs yielded a cause of death in approximately 5.2% of SUDI cases, distributed as follows in table 38.

In SUDI cases, the cumulative total of causes of death returned by histological examination of macroscopically normal organs is clinically significant (Table 39). Cross-sectional imaging choices in this cohort should be PMMRI as first line, with PMCT used as an additional investigation in traumatic deaths or where inflicted injury is suspected.

<table>
<thead>
<tr>
<th>Heart</th>
<th>Lung</th>
<th>Brain</th>
<th>Adrenals</th>
<th>Thyroid</th>
<th>Liver</th>
<th>Kidney</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8%</td>
<td>2.7%</td>
<td>0.9%</td>
<td>0.1%</td>
<td>Nil</td>
<td>0.4%</td>
<td>0.2%</td>
<td>0.1%</td>
</tr>
<tr>
<td>(13/1638)</td>
<td>(45/1680)</td>
<td>(13/1503)</td>
<td>(1/1586)</td>
<td>0/1210</td>
<td>0/1647</td>
<td>0/3/1631</td>
<td>0/1/1447</td>
</tr>
</tbody>
</table>

Table 38. Contribution of microscopic examination to the cause of death in 1,739 SUDI cases, split by organ system. Where the organ was not commented on in the report, an exclusion was made for that body system, leading to variation in the denominator.
<table>
<thead>
<tr>
<th>Infant presentation</th>
<th>Maximum potential rate of COD non-discovery using NIA (PM MRI only, no histological sampling)</th>
<th>Maximum potential rate of COD non-discovery by adopting incremental approach (PMMRI, then MinImAL if abnormal)</th>
<th>Maximum potential rate of COD non-discovery by adopting routine MinImAL post PMMRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrospective cohort total: 1,739</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>84.0 per 1,000 cases</td>
<td>12.6 per 1,000 cases</td>
<td>5.2 per 1,000 cases</td>
</tr>
<tr>
<td>Lung</td>
<td>155.3 per 1,000 cases</td>
<td>34.5 per 1,000 cases</td>
<td>8.6 per 1,000 cases</td>
</tr>
<tr>
<td>Kidney</td>
<td>6.3 per 1,000 cases</td>
<td>2.3 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
</tr>
<tr>
<td>Liver</td>
<td>18.4 per 1,000 cases</td>
<td>5.2 per 1,000 cases</td>
<td>1.1 per 1,000 cases</td>
</tr>
<tr>
<td>Adrenal</td>
<td>3.45 per 1,000 cases</td>
<td>1.7 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
</tr>
<tr>
<td>Pancreas</td>
<td>&lt;1.0 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
</tr>
</tbody>
</table>

Table 39. Summarised data of potential COD non-discovery rates in SUDI (see part 3 of this thesis for full table) utilising different investigative strategies.
5.5.2 Potential for non-discovery of cause of death in SUDI

There is a small but significant (estimated around 5% based on current data) risk that a histologically detectable cause of death within a clinically, radiologically and macroscopically normal organ will remain undetected if an imaging only approach is used in the context of SUDI. However, unlike in SUDC, MinImAL examination is possible in the context of neonatal or infant death, with six neonates and three infants included in the MinImAL cohort of cases, none of which required conversion to full autopsy. MinImAL can therefore be recommended as a possible means of internal examination and organ sampling in infant or neonatal death (Figure 70). In terms of histological sampling, all organs should be sampled thoroughly in accordance with FRCPath guidelines within the setting of infant or neonatal death. If a MinImAL approach is used and there are strong objections to opening the head (which requires an additional large incision), the non-discovery rate of causes of death from the un-examined brain will be approximately 1% in the context of a clinically and radiologically normal brain (Appendix 2b, Figure 84).
5.5.3 Proposed SUDI investigation strategy

Figure 70. Evidence-based flowchart for optimal investigation of SUDI. Differences from standard practice are highlighted in red.
The infant death / neonatal death / SUDI autopsy pathway in non-forensic settings could therefore be amended to include the option of MinImAL examination, which may be particularly useful if consent to full autopsy is withheld. PMMRI may be a useful adjunct to help document macroscopic findings, or to guide internal examination. PMCT may also be useful for some indications, such as traumatic deaths or suspected inflicted injury. Even when PMCT is available, skeletal surveys should be performed in cases of suspected inflicted injury, as evidence regarding the utility of CT is still emerging, although CT may prove superior to plain radiography in the fullness of time.
5.6 Proposed Autopsy Guidelines for Investigation of IUFD

5.6.1 Imaging concordance and autopsy yield in IUFD

The MARIAS study showed overall concordance between MRI alone and standard autopsy of 63.0% (n=58) (95% CI, 52.8 – 72.2) in fetuses >24 weeks. In fetuses ≤24 weeks, the concordance of MRI alone with standard autopsy was 42.7% (n=185) (95% CI, 35.8 – 49.9). The concordance for both groups increased to approximately 95% once genetics, microbiology and placental investigations were considered in conjunction with PMMRI (i.e. excluding macroscopic examination of internal organs and organ histology). In fetuses of <400g bodyweight / 18gw, micro-CT imaging provides excellent concordance with standard autopsy.109 PM radiology can therefore provide an excellent surrogate for invasive autopsy across all gestational ages.

Retrospective analysis of 1,957 IUFD cases showed that macroscopic examination of organs at autopsy in the context of IUFD (all gestations) revealed a definite cause of death in approximately 1.5% of IUFD cases, distributed as follows in Table 40.

<table>
<thead>
<tr>
<th>Heart</th>
<th>Lung</th>
<th>Brain</th>
<th>Adrenals</th>
<th>Thyroid</th>
<th>Liver</th>
<th>Kidney</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5%</td>
<td>0.3%</td>
<td>0.6%</td>
<td>Nil</td>
<td>0.1%</td>
<td>0.1%</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>(8/1754)</td>
<td>(6/1739)</td>
<td>(9/1506)</td>
<td>(0/1723)</td>
<td>(1/1702)</td>
<td>(0/1544)</td>
<td>(0/1739)</td>
<td>(0/1651)</td>
</tr>
</tbody>
</table>

Table 40. Contribution of macroscopic examination to the cause of death in 1,957 IUFD cases, split by organ system. Where the organ was not commented on in the report, an exclusion was made for that body system, leading to variation in the denominator.
Histological examination of macroscopically normal organs revealed a definite cause of death in approximately 1.4% of all IUFD cases, distributed as shown below (Table 41).

<table>
<thead>
<tr>
<th>Organ System</th>
<th>Heart</th>
<th>Lung</th>
<th>Brain</th>
<th>Adrenal</th>
<th>Thyroid</th>
<th>Liver</th>
<th>Kidney</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nil (0/1619)</td>
<td>1.3% (21/1642)</td>
<td>Nil (0/1364)</td>
<td>Nil (0/1563)</td>
<td>Nil (0/1110)</td>
<td>Nil (0/1670)</td>
<td>0.1% (2/1604)</td>
<td>Nil (0/1300)</td>
</tr>
</tbody>
</table>

Table 41. Contribution of microscopic examination to the cause of death in 1,957 IUFD cases, split by organ system. Where the organ was not commented on in the report, an exclusion was made for that body system, leading to variation in the denominator.

The 1.3% of cases in which examination of the lungs revealed a cause of death were exclusively cases with congenital pneumonia, in which no placenta was available for examination. Had the placenta been available for examination, no additional yield would have been obtained from this body system. Both cases of renal ‘causes of death’ in the context of IUFD were unsuspected renal vein thrombosis, however, the authors of this report are sceptical as to whether this represented cause of death in the context of intrauterine demise or was an epiphenomenon. As such, routine histological sampling of visceral organs in the context of normal antenatal imaging and normal macroscopic examination (and by extension, PM radiology, given the high degree of concordance between the two techniques) yields almost no findings relevant to the cause of death.
5.6.2 Potential for non-discovery of cause of death in IUFD

Given the above findings, the risk that a histologically detectable cause of death within a clinically, radiologically and macroscopically normal organ will be missed if an imaging only approach is used in the context of IUFD is very low (Table 42). With regards to histological sampling, all organs clinically or radiologically suspected of being abnormal should be sampled thoroughly. Routine histological sampling of normal organs (clinically and radiologically normal) provides almost no yield relevant to the cause of death when the placenta is available for examination but may provide psychological reassurances in the form of a “negative autopsy”.

<table>
<thead>
<tr>
<th>IUFD (all)</th>
<th>Maximum potential rate of COD non-discovery using NIA (PM MRI only, no histological sampling)</th>
<th>Maximum potential rate of COD non-discovery by adopting incremental approach (PMMRI, then MinImAL if abnormal)</th>
<th>Maximum potential rate of COD non-discovery by adopting routine MinImAL post PMMRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>1.0 per 1,000 cases</td>
<td>1.0 per 1,000 cases</td>
<td>1.0 per 1,000 cases</td>
</tr>
<tr>
<td>Lung</td>
<td>1.0 per 1,000 cases</td>
<td>1.0 per 1,000 cases</td>
<td>1.0 per 1,000 cases</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.6 per 1,000 cases</td>
<td>1.5 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
</tr>
<tr>
<td>Liver</td>
<td>&lt;1.0 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
</tr>
<tr>
<td>Adrenal</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Table 42. Summarised data of potential COD non-discovery rates in IUFD (see part 3 of this thesis for full table) utilising different investigative strategies.
MinImAL examination is entirely feasible within this context, with 93 fetal deaths (including 28 IUFD cases and six intrapartum stillbirths alongside 59 ToPs) in the MinImAL cohort of cases, two of which required conversion to full autopsy (2/93, 2.2% conversion rate). MinImAL can therefore be recommended as a possible means of internal examination and organ sampling in IUFD or intrapartum stillbirth (Figure 71). If a MinImAL approach is used and there are strong objections to opening the head (which requires an additional large incision), the non-discovery rate of causes of death from the un-examined brain will be approximately 1% in the context of a clinically and radiologically normal brain (Appendix 2c, Figure 92).
5.6.3 Proposed IUFD investigation strategy

![Flowchart with steps]

- Clinical review of notes
- Imaging (cross-sectional: PMMRI or µCT)
- External examination & placental examination*
- Standard or MinImAL internal examination to be undertaken if clinical or radiological suspicion of abnormality
- Sample all organs relevant to potential abnormality

**Figure 71.** Evidence-based flowchart for optimal investigation of IUFD

Differences from standard practice are highlighted in red

*As an additional modification to this protocol, placental triage could be used to ascertain whether a significant abnormality is present prior to performance of an invasive procedure
The IUFD autopsy pathway should therefore be amended to place more emphasis on the importance of antenatal investigations and post-mortem radiology, with subsequent investigation of suspected internal anomalies by MinImAL examination (if consent to standard autopsy is refused) or standard autopsy. With regards to PM imaging, routine ‘babygram’ X-rays have been shown to provide low yield in cases where a skeletal disorder is not suspected and should therefore not be undertaken routinely. PMMRI should be the modality of choice in fetuses >18gw / 400g bodyweight, with iodinated micro-CT used in fetuses <18gw / 400g bodyweight where acceptable to the family. Micro-CT may also provide a corollary method of further examining internal anatomy if discrepancies arise between antenatal imaging and PMMRI, especially where parents refuse consent to MinImAL or standard autopsy. PMCT is unlikely to be of benefit in the context of fetal demise outside of the very specific context of complex skeletal dysplasia, where additional information to that available from plain radiography may be useful.
5.7 Proposed Autopsy Guidelines for Investigation of ToP

5.6.1 Imaging concordance and autopsy yield in IUFD

The concordance of PMMRI for fetal cases, discussed in the IUFD section above, similarly applies to ToP cases. Although terminations may be carried out at any gestational age, the majority occur before 24gw because combined screening, non-invasive prenatal testing and routine ultrasound scans in the first and second trimester are likely to detect chromosomal or structural abnormalities early in the pregnancy. Additionally, terminations after 24gw can be more difficult to obtain due to practicalities of the law. In fetuses of <500g bodyweight / l8gw, micro-CT imaging provides excellent concordance with standard autopsy and seems to produce fewer non-diagnostic indices when compared with standard autopsy in fetuses of ≤14gw.109

Retrospective analysis of 791 ToP cases showed that macroscopic examination of organs at autopsy in the context of ToP (all gestations) revealed a definite cause of death in approximately 25.8% cases, distributed as follows in Table 43.

<table>
<thead>
<tr>
<th>Heart</th>
<th>Lung</th>
<th>Brain</th>
<th>Adrenals</th>
<th>Thyroid</th>
<th>Liver</th>
<th>Kidney</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.7%</td>
<td>2.2%</td>
<td>10.6%</td>
<td>Nil</td>
<td>Nil</td>
<td>0.7%</td>
<td>4.6%</td>
<td>Nil</td>
</tr>
<tr>
<td>(56/727)</td>
<td>(16/724)</td>
<td>(74/695)</td>
<td>(0/723)</td>
<td>(0/672)</td>
<td>(5/707)</td>
<td>(33/724)</td>
<td>(0/700)</td>
</tr>
</tbody>
</table>

Table 43. Contribution of microscopic examination to the cause of death in 791 ToP cases, split by organ system. Where the organ was not commented on in the report, an exclusion was made for that body system, leading to variation in the denominator.
Histological examination of macroscopically normal organs revealed a definite cause of death in approximately 0.3% of all ToP cases, distributed as shown below (Table 44).

<table>
<thead>
<tr>
<th>Heart</th>
<th>Lung</th>
<th>Brain</th>
<th>Adrenals</th>
<th>Thyroid</th>
<th>Liver</th>
<th>Kidney</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil (0/675)</td>
<td>Nil (0/692)</td>
<td>0.2% (1/635)</td>
<td>Nil (0/662)</td>
<td>Nil (0/515)</td>
<td>Nil (0/686)</td>
<td>0.1% (1/669)</td>
<td>Nil (0/624)</td>
</tr>
</tbody>
</table>

Table 44. Contribution of microscopic examination to the cause of death in 791 ToP cases, split by organ system. Where the organ was not commented on in the report, an exclusion was made for that body system, leading to variation in the denominator.

These data show that in the context of a termination of pregnancy, there is almost no value in routine histological sampling of an organ that is macroscopically normal.

By extension, given the high rate of concordance between PMMRI or micro-CT and invasive autopsy, there is likely to be no value in the histological examination of a radiologically normal organ, on the proviso that the PM radiology findings are concordant with antenatal imaging. Conversely, there is likely to be value in the utilisation of autopsy techniques to resolve a discordance between antenatal and post-mortem imaging.
5.6.2 Potential for non-discovery of cause of death in ToP

Given the above findings, the risk that a histologically detectable cause of death within a clinically, radiologically and macroscopically normal organ will be missed if an imaging only approach is used in the context of ToP is very low (Table 45). With regards to histological sampling, all organs clinically or radiologically suspected of being abnormal should be sampled thoroughly. Routine histological sampling of normal organs (clinically and radiologically normal) provides almost no yield relevant to the cause of death.

<table>
<thead>
<tr>
<th>ToP</th>
<th>Maximum potential rate of COD non-discovery using NIA (PM MRI only, no histological sampling)</th>
<th>Maximum potential rate of COD non-discovery by adopting incremental approach (PMMRI, then MinImAL if abnormal)</th>
<th>Maximum potential rate of COD non-discovery by adopting routine MinImAL post PMMRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>5.1 per 1,000 cases</td>
<td>3.8 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
</tr>
<tr>
<td>Lung</td>
<td>17.7 per 1,000 cases</td>
<td>5.1 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
</tr>
<tr>
<td>Kidney</td>
<td>35.4 per 1,000 cases</td>
<td>7.6 per 1,000 cases</td>
<td>1.3 per 1,000 cases</td>
</tr>
<tr>
<td>Liver</td>
<td>1.3 per 1,000 cases</td>
<td>1.3 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
</tr>
<tr>
<td>Adrenal</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1.3 per 1,000 cases</td>
<td>&lt;1 per 1,000 cases</td>
<td>&lt;1.0 per 1,000 cases</td>
</tr>
</tbody>
</table>

Table 45. Summarised data of potential COD non-discovery rates in ToP (see part 3 of this thesis for full table) utilising different investigative strategies.
MinImAL examination is entirely feasible within this context, with 93 fetal deaths (including 28 IUFD cases and six intrapartum stillbirths alongside 59 ToPs) in the MinImAL cohort of cases, two of which required conversion to full autopsy (2/93, 2.2% conversion rate). MinImAL can therefore be recommended as a possible means of internal examination and organ sampling in ToP (Figure 72). If a MinImAL approach is used and there are strong objections to opening the head (which requires an additional large incision), the non-discovery rate of causes of death from the un-examined brain will be <1% in the context of a clinically and radiologically normal brain (Appendix 2d, Figure 100).
5.7.3 Proposed ToP investigation strategy

Clinical review of notes

Imaging (cross-sectional: PMMRI or uCT)

External examination & placental examination*

Standard or MinImAL internal examination to resolve discrepancy, obtain tissue or provide unifying diagnosis

Sample all organs relevant to potential abnormality

Genetic analysis

Microbiology

Virology

Metabolic analysis

X-ray only in skeletal anomalies

Figure 72. Evidence-based flowchart for optimal investigation of ToP. Differences from standard practice are highlighted in red.

*Placental examination only if relevant to clinical reason for ToP
The protocol for ToP cases can therefore be streamlined to include MinImAL examination or standard autopsy only where tissue-based investigations are necessary to discern underlying pathology in a common presentation (e.g. bright kidneys on antenatal USS), to resolve antenatal/PM imaging discrepancies, to obtain tissue for genetic investigations, or to confirm normality/abnormality when investigating a pathological association or syndrome as a possible unifying diagnosis. Plain radiography is likely to be useful in cases of skeletal dysplasia, with non-iodinated micro-CT also showing potential utility in this area.
5.8 Areas for further research

There are major challenges that remain to be addressed within the field of paediatric and perinatal pathology. However, continued performance of standard autopsy (as has now been done for decades) is unlikely to yield new insights that will improve the understanding of non-specific, umbrella entities such as SUDI, SUDC or placental dysfunction. However, the tissue obtained from standard autopsy could be vital for new, non-hypothesis-based approaches, such as metabolomics, proteomics, advanced imaging, or massively parallel sequencing. The sheer amount of information generated by these techniques, along with the complex and variable nature of phenotype expression makes it extremely difficult to delineate signal (pathology) from noise. Multicentre collaboration between different pathology groups and a unified approach to data analysis would be logical initial steps to improve the chances of delineating the complex and overlapping pathology-phenotype interactions.

Specific steps to further the research presented in this thesis include a full economic analysis examining potential service implications within pathology, radiology and fetal medicine for a nationwide rollout of MIA services. Additional further imaging research regarding the utility of post-mortem ultrasound (potentially with USS guided needle sampling of organs for tissue-based investigations) should also be considered a high priority. USS has several logistical advantages over cross-sectional imaging, including cost, availability, ease of use, ease of training, portability and the ability to perform real-time
image guided biopsies. The MIA techniques outlined within this thesis are heavily dependent on the availability of 1.5 T MRI and are largely suited to high-income countries with good availability of scan time. Validation of PM imaging using USS could improve the applicability of the MIA techniques presented within this thesis to low and middle-income countries. Moreover, use of USS has the potential to improve service availability of MIA where there are long waits for cross-sectional imaging services, where living patients are prioritised.

Several areas within developmental neuropathology are diagnostically challenging for pathologists and fetal medicine specialists. Abnormalities that are clearly visible at antenatal ultrasound may be very difficult to confirm at standard autopsy, in part due to the fragility of the fetal brain and the difficulty in extracting the area in question, without disrupting the anomaly in question. Some abnormalities, such as ventriculomegaly, are also known to spontaneously resolve following fetal death prior to PMMRI (possibly due to lack of CSF flow after death and fluid shifts). An additional area of diagnostic difficulty concerns the assessment of midline structures, including the development of the corpus callosum, at around 20gw. The corpus callosum is usually fully formed by 18-20gw, however, this corresponds with a gestational age range at which 1.5 T MRI may not be powerful enough to generate the required resolution required to confirm normality or abnormality of the relevant neuroanatomy. Clinical decisions around the management of agenesis of the corpus callosum are further complicated by the variation in clinical outcome for individuals affected by agenesis of the corpus callosum.
In this circumstance, micro-CT offers a method of assessing neuroanatomy in the post-mortem setting at an exponentially improved resolution; this could lead to an improvement in the quality of autopsy reports in this challenging area of clinical practice and thereby improve the quality of clinical advice and antenatal correlation provided to clinicians and parents regarding the management of subsequent pregnancies.

This thesis has demonstrated that micro-CT can provide information regarding the assessment of tumour growth within extracted organs (Experiments 4.2.4 and 4.2.5). This has potentially significant implications for macroscopic surgical pathology, where decisions regarding patient management may be made based on the presence or absence of tumour at a surgical margin, or the percentage of necrotic tumour or a cellular constituent within a resection (e.g. the proportion of blastema and necrosis in nephroblastoma, small cell undifferentiated components within a hepatoblastoma, yolk sac tumour within a teratoma). It may be possible to develop either a rapid-scan (to guide intra-operative management) or long-scan protocol (to guide macroscopic assessment of specimens) using micro-CT to better inform pathology practice.

Lastly, micro-CT offers an opportunity to better understand the structure and morphology of the normal and abnormal placenta. Initial work by Pratt et al. focused on the imaging of the fetal vascular supply, however, this represents only one half of placental function, and sheds little light on villous morphology, parenchymal function (or dysfunction), or maternal blood
supply. Investigation of different contrast agents, or the use of phase contrast-CT, used alongside the aforementioned techniques developed by Pratt et al. could enable quantification of factors such as vascularity, vessel tortuosity and fibrin deposition in normal and abnormal placentas, at both low-magnification across the whole placental disc and at high magnification for regions of interest (e.g. cord insertion, or within macroscopically abnormal areas).
References


Appendix 1: List of terminology

The following definitions define the terminology within the field of minimally invasive autopsy, in increasing order of invasiveness.

Non-invasive autopsy (NIA): Imaging based autopsy without incisions or punctures of the body. This may include detailed review of the clinical notes and investigations, external examination together with placental examination, medical photography, and radiological investigations. Radiological investigations performed may include conventional radiographs, ultrasound imaging and cross-sectional imaging. A non-invasive autopsy does not contain a standard autopsy examination of internal organs and the body is neither punctured nor incised.

Less invasive autopsy (LIA): An umbrella term referring to any autopsy approach other than that requiring a major incision. This includes NIA and minimally invasive autopsy (MIA), both with and without laparoscopic examination.

Minimally invasive autopsy (MIA): NIA plus needle puncture of the body to obtain blood, cerebrospinal fluid, and other microbiological swabs/cultures.

Minimally invasive autopsy + laparoscopy (MinImAL procedure): Modification of MIA to include a laparoscopic approach to internal examination and histological sampling of organs, without employing a major incision, with prior radiological investigation, at any gestation or age. This approach requires an incision between 1 and 5cm in length, which is proportional with the size of the body (1cm in fetuses, up to 5cm in older children).

Major incision: A traditional autopsy incision, used to facilitate access to the cranium or thoraco-abdominal cavities.

Limited autopsy: Organ or body cavity specific approach, such as examination of the heart, the brain, the chest, the abdomen, performed in a similar manner to that of a full autopsy (above). A limited autopsy is usually performed to
answer a very specific clinical question and may still requires a major incision with evisceration of the relevant organ(s).

Standard autopsy: Standard diagnostic autopsy including a combination of review of clinical notes and investigations, external examination, radiological investigations, major incisions with evisceration, dissection and subsequent internal organ examination with weights, macroscopic evaluation and histology. Forensic techniques (skin flaying, extraction of the cervical spine) may be included within some standard autopsies. This is generally regarded as the current standard of care against which, other approaches are compared.
Appendix 2a: Organ specific findings in SUDC cohort
Figure 73. Findings from examination of the heart in SUDC.
Figure 74. Findings from examination of the lungs in SUDC.
Brain findings in SUDC cohort

Figure 75. Findings from examination of the brain in SUDC.
Any macroscopic abnormality (%) | 8.3
Macroscopic abnormality, COD (%) | 0.3
Any microscopic abnormality (%) | 14.4
Microscopic abnormality, COD (%) | 1.1
Microscopic abnormality which is COD & macroscopic examination is normal (%) | 0.2

Figure 76. Findings from examination of the adrenals in SUDC.
Liver findings in SUDC cohort

Figure 77. Findings from examination of the liver in SUDC.
Kidney findings in SUDC cohort

Figure 78. Findings from examination of the kidneys in SUDC.
Figure 79. Findings from examination of the pancreas in SUDC.
Figure 80. Findings from examination of the thyroid in SUDC.
Appendix 2b: Organ specific findings in SUDI cohort

![Heart findings in SUDI cohort](chart)

**Figure 8i.** Findings from examination of the heart in SUDI.
Figure 82. Findings from examination of the lungs in SUDI.
Figure 83. Findings from examination of the brain in SUDI.
Figure 84. Findings from examination of the adrenals in SUDI.
Liver findings in SUDI cohort

Figure 85. Findings from examination of the liver in SUDI.
Any macroscopic abnormality (%)  
Macroscopic abnormality, COD (%)  
Any microscopic abnormality (%)  
Microscopic abnormality, COD (%)  
Microscopic abnormality which is COD & macroscopic examination is normal (%)

Figure 86. Findings from examination of the kidneys in SUDI.
Figure 87. Findings from examination of the pancreas in SUDI.
Figure 88. Findings from examination of the thyroid in SUDI.
Appendix 2c: Organ specific findings in IUFD & stillbirth cohort

Figure 89. Findings from examination of the heart in IUFD / stillbirth.
Figure 90. Findings from examination of the lungs in IUFD / stillbirth.
Brain findings in IUFD cohort

Figure 91. Findings from examination of the brain in IUFD / stillbirth.
### Liver findings in IUFD cohort

<table>
<thead>
<tr>
<th></th>
<th>Any macroscopic abnormality (%)</th>
<th>Macroscopic abnormality, COD (%)</th>
<th>Any microscopic abnormality (%)</th>
<th>Microscopic abnormality, COD (%)</th>
<th>Microscopic abnormality which is COD &amp; macroscopic examination is normal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.1</td>
<td>0.1</td>
<td>7.7</td>
<td>0.1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

**Figure 92.** Findings from examination of the liver in IUFD / stillbirth.
Adrenal findings in IUFD cohort

Figure 93. Findings from examination of the adrenals in IUFD / stillbirth.
Figure 94. Findings from examination of the kidneys in IUFD / stillbirth.
Figure 95. Findings from examination of the pancreas in IUFD / stillbirth.
Figure 96. Findings from examination of the thyroid in IUFD / stillbirth.
Appendix 2d: Organ specific findings in ToP cohort

Heart findings in ToP cohort

Figure 97. Findings from examination of the heart in ToP.
Figure 98. Findings from examination of the lungs in ToP.
Brain findings in ToP cohort

Figure 99. Findings from examination of the brain in ToP.
### Liver findings in ToP cohort

<table>
<thead>
<tr>
<th>Category</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any macroscopic abnormality (%), COD</td>
<td>3.3</td>
</tr>
<tr>
<td>Macroscopic abnormality, COD (%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Any microscopic abnormality (%), COD</td>
<td>1.7</td>
</tr>
<tr>
<td>Microscopic abnormality, COD (%)</td>
<td>0.0</td>
</tr>
<tr>
<td>Microscopic abnormality which is COD &amp; macroscopic examination is normal (%)</td>
<td>0.0</td>
</tr>
</tbody>
</table>

**Figure 100.** Findings from examination of the liver in ToP.
Adrenal findings in ToP cohort

Any macroscopic abnormality (%) 2.8
Macroscopic abnormality, COD (%) 0.0
Any microscopic abnormality (%) 3.8
Microscopic abnormality, COD (%) 0.0
Microscopic abnormality which is COD & macroscopic examination is normal (%) 0.0

Figure I01. Findings from examination of the adrenals in ToP.
Figure 102. Findings from examination of the kidneys in ToP.
Figure I03. Findings from examination of the pancreas in ToP.
Thyroid findings in ToP cohort

Figure I04. Findings from examination of the thyroid in ToP.
Appendix 3: Publications arising directly from this thesis


Appendix 4: Further publications arising from work undertaken during this thesis


Appendix 5: Acceptability of less invasive autopsy

This appendix presents citations of work on the parental acceptability of less invasive autopsy undertaken in parallel to this thesis. These works were primarily undertaken by Dr. Celine Lewis, with contribution from the author of this thesis.


Appendix 6: Prizes and presentations arising directly from this thesis

1. Royal College of Pathologists Silver Research Medal (Presented February 2019) for the following paper and implications for clinical service:

2. Articles featured on the cover of three issues:

Ultrasound in Obstetrics and Gynecology, January 2016
“Micro-CT for fetal autopsy”

4. Best Scientific Presentation, European Society of Paediatric Radiology, Davos, June 2017
“Micro-CT for fetal autopsy”

“Fostering successful collaborations with industry”

6. European Society of Paediatric Radiology travel bursary (500 Euros) to present at International Paediatric Radiology 2016, Chicago
“Micro-CT of congenital heart disease”

7. Highly Commended in Best Newcomer category, UCL Institute of Child Health Open Day, 18th November 2015
“Micro-CT of congenital heart disease”

8. Best Poster Presentation, National Academic Pathology Trainees Network Meeting, 7th-8th October 2015
“Micro-CT of congenital heart disease”