Study Documentation Background

The work presented here is partly reproduced from Hutchinson et al.[1] *Feasibility and experience of the MinImAL procedure: Minimally Invasive perinatal and paediatric Autopsies with Laparoscopically assisted tissue sampling*. Ultrasound Obstet Gynecol. doi: 10.1002/uog.20211. [Epub ahead of print]

Initial experience with laparoscopic assisted MIA

At GOSH, laparoscopic MIA has been available to selected referring trusts as part of a REC approved research study (Development of the Minimally Invasive Autopsy) being performed in parallel with the HTA study, for parents undergoing standard autopsy who consent to MIA in addition, and also for parents who refuse standard autopsy.

This study was not part of the HTA study and was not funded by the HTA, but the application stated that in the final report we would relate findings to those of the initial experience performed MIA in clinical practice, hence the write up below. This work will be written up and submitted as a separate study but is included here for context as stated in the original HTA application.

Methods

190 cases of perinatal / paediatric autopsies referred to our centre as part of routine clinical care were prospectively recruited to minimally invasive autopsy with laparoscopy between June 2011 and October 2016. The study does not include cases previously reported in a feasibility study.[2] The total number of non-forensic Coronial and consented autopsies performed at our institution over this period was 1,900 (MIA in 10% of cases).

Cases were unselected, and where examinations were consented procedures (rather than medico-legally required), parents were counselled regarding standard operating procedures regarding the role and indications for autopsy and offered full autopsy initially, with less invasive options offered following refusal of full autopsy. The study group included cases who only consented to limited autopsy, despite the lack of information regarding the added value of laparoscopic assisted sampling, and those who consented to standard autopsy but additionally agreed to a MinImAL procedure being carried out initially as part of the research study. The study was approved by a local research ethics committee and all parents provided informed written consent.

MinImAL Procedure Protocol

Pre-autopsy 1.5T PMMRI was performed as previously described in all cases.[3] The PMMRI results were reported by a specialist paediatric radiologist with expertise in postmortem imaging and discussed with the pathologist prior to the autopsy (NJS or

JCH), at which, routine external examination of the body along with genetic and microbiological sampling were performed as usual for a full autopsy. If the PMMRI of the brain was normal, with no additional specific neurological indications, the brain was not subsequently extracted at autopsy due to low yield of additional anomalies in these circumstances.[4] If suspicions of an underlying neurological pathology were noted from joint pathologist/radiologist review of the clinical history, referral form or pre-autopsy PMMRI, the parents were informed that an additional incision would be required to extract and examine the brain, as per standard autopsy, in order to confirm the diagnosis.

A 2, 4, or 10mm diameter straight laparoscope (according to gestational age) was then passed into the abdominal cavity via a small incision (1-2cm), made either subxiphisternally or in the left hypochondrium, and used to visualise organs for sampling within the limits of parental consent. If necessary, internal organs (e.g. heart and lungs) were either sampled in-situ or could be eviscerated and removed through the incision, examined externally, and subsequently returned to the body.

With parental consent for both procedures at the beginning of the study, the first seven cases had a MinImAL procedure followed by standard autopsy examination of abdomen and thorax by extending the MinImAL incision to a standard 'T' or 'Y' incision; however, in all cases, the organs had either been successfully laparoscopically eviscerated or sampled, and no further useful information was gained through the invasive procedure or examination of the brain. Conversion to standard autopsy was subsequently therefore only performed for specific indications or inadequate sampling, if parental consent allowed.

An attempt to sample major organs (defined as: heart, lung, kidney, liver, with spleen, adrenal, pancreas and thymus) was made in all cases which were not limited by consent. Placental examination was performed as part of the fetal autopsy, where available, as per usual protocol.

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 postmortem radiology, histology, microbiology and genetic results, as normal.

<u>Evaluating the MinImAL Procedure: Timing</u>

To demonstrate whether the MinImAL procedure could be applied in day-to-day autopsy practice, it was decided to record the timing of organ inspection, evisceration (if deemed necessary by the operator), dissection and sampling for an unselected cohort of the cases. Cases undergoing limited MinImAL procedure or requiring conversion to full autopsy due to poor visualisation were excluded from this analysis.

Evaluating the MinImAL Procedure: Sampling adequacy

Autopsy and histological findings were prospectively compiled using the GOSH Access Autopsy Database and analysed retrospectively according to body cavity for comparisons with radiology data (CNS, Cardiac, Thoracic, Abdominal/Pelvic and Musculoskeletal) and according to specific organ pathology/normality for autopsy and analysis of sampling adequacy (thymus, heart, lungs, liver, adrenals, kidneys, spleen pancreas) using Microsoft Access and Microsoft Excel (Microsoft, Seattle, USA).

The primary outcome was MinImAL sampling success, which was pre-defined as sufficient material to provide a pathological comment on normality, abnormality or degree of autolysis. 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.

The secondary outcomes were evaluation of timing of the MinImAL procedure in a subgroup of cases, cause of death analysis of the cohort, and comparison of the proportion of IUFDs and stillbirths that remained unexplained following MinImAL procedure with that of a previously reported, unselected cohort of >1,000 intrauterine fetal deaths that had undergone standard autopsy at the same centre.

Evaluating the MinImAL Procedure: Overall unexplained rate

Although this study has not been designed as a diagnostic accuracy trial of the MinImAL procedure, since no randomisation or mandatory consent to standard autopsy was possible, an evaluation of the overall outcomes was performed by comparing the proportion of 'unexplained' cases in order to establish whether use of the MinImAL procedure resulted in a statistically significant increase in 'unexplained' verdicts arising following completion of the procedure as compared to standard autopsy. In order to do this, the 'unexplained' rate across the stillbirth and IUFD cases within the MinImAL cohort will be compared to the published rate from a large case series of >1,000 IUFDs that had undergone standard autopsy at the same centre (Ref Man) using Chi-Square analysis.

Results

Of 1,900 referrals to our institution for autopsy examination between June 2011 and October 2016, 190 cases underwent some form of LIA according to parental consent requirements. Of these, 20 were early gestation fetuses specifically referred for microCT examination[1] and were excluded from further analysis. 67 parents specifically only consented for NIA, involving PMMRI, external examination and placental examination. The remaining 103 cases underwent MinImAL procedure with both PMMRI and laparoscopic-assisted organ examination and sampling.

MinImAL cohort:
Of the 103 MinImAL cases, 99 were consented cases, with another four undertaken on the authority of HM Coroner following specific parental request for a less invasive approach.

93/103 cases were fetal deaths (IUFD, stillbirths or terminations of pregnancy; age range 15gw – 41gw, median 23gw, mean 25gw), six were neonatal deaths (four early neonatal deaths (<7 days following birth), one late neonatal death (7-28 days following birth), one duration unknown), three were infant deaths (two non-sudden, one sudden and unexpected death in infancy (SUDI)) and one childhood death at 13 years. 11 cases were limited by consent to a specific body cavity/organ.

92 cases underwent complete MinImAL procedure, without restriction to a body system or cavity, and without sampling restriction. 90 of these cases (97.7%) were successfully completed as minimally invasive procedures, as per the aforementioned protocol. As noted above, the seven initial cases were converted to full autopsy at the beginning of the study as part of technical optimisation. In two further cases, one due to fetal size, the other due to poor visualisation in Prune-Belly Syndrome, conversion to standard autopsy was performed with consent of the parents, (unplanned conversion rate: 2/85, 2.4%). In no cases was conversion to standard autopsy suggested by the performing pathologist in any cases in this series in which parental consent for conversion was not present.

Table 1: Demographics of the 103 cases accepted for any form of MinImAL examination

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

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

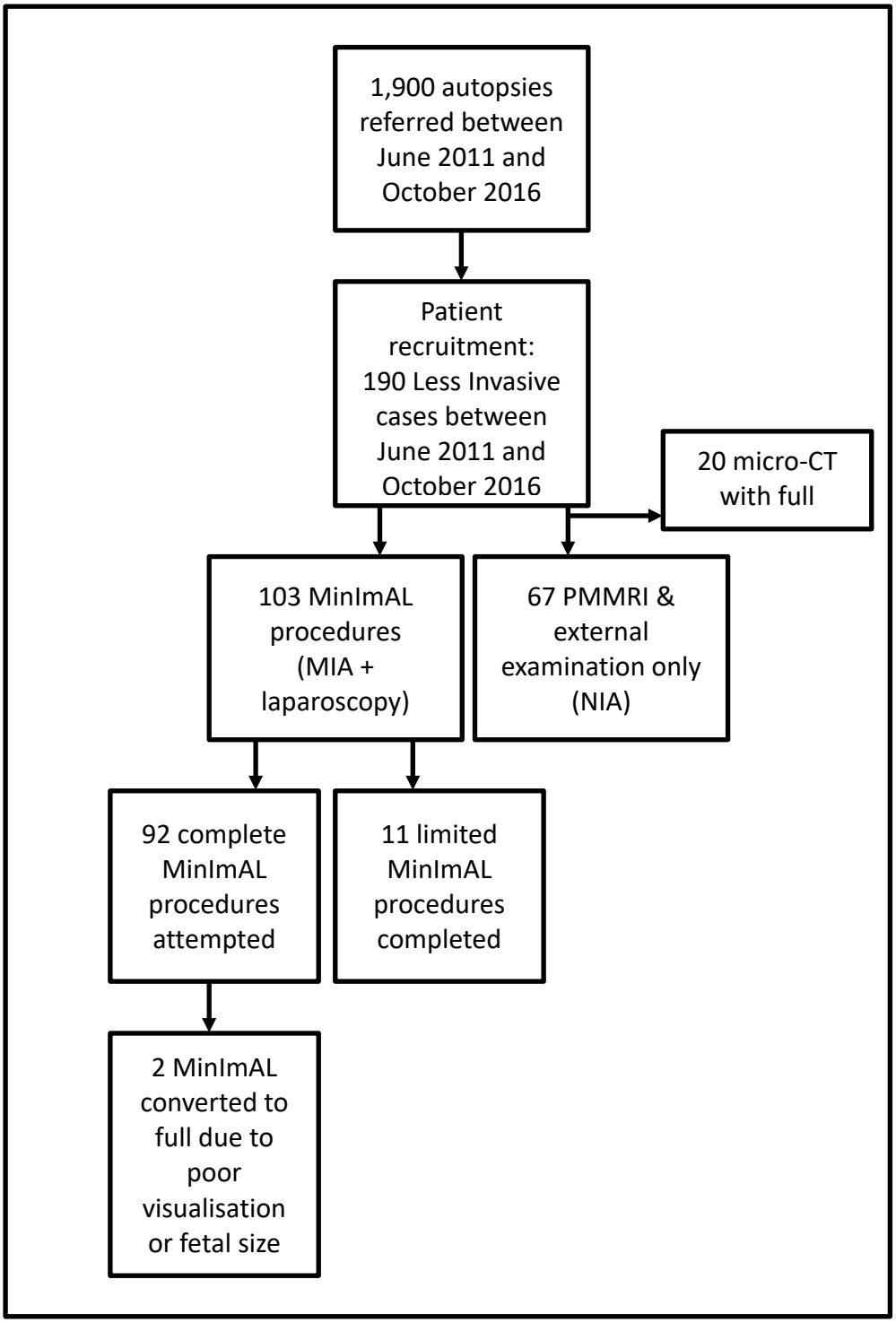


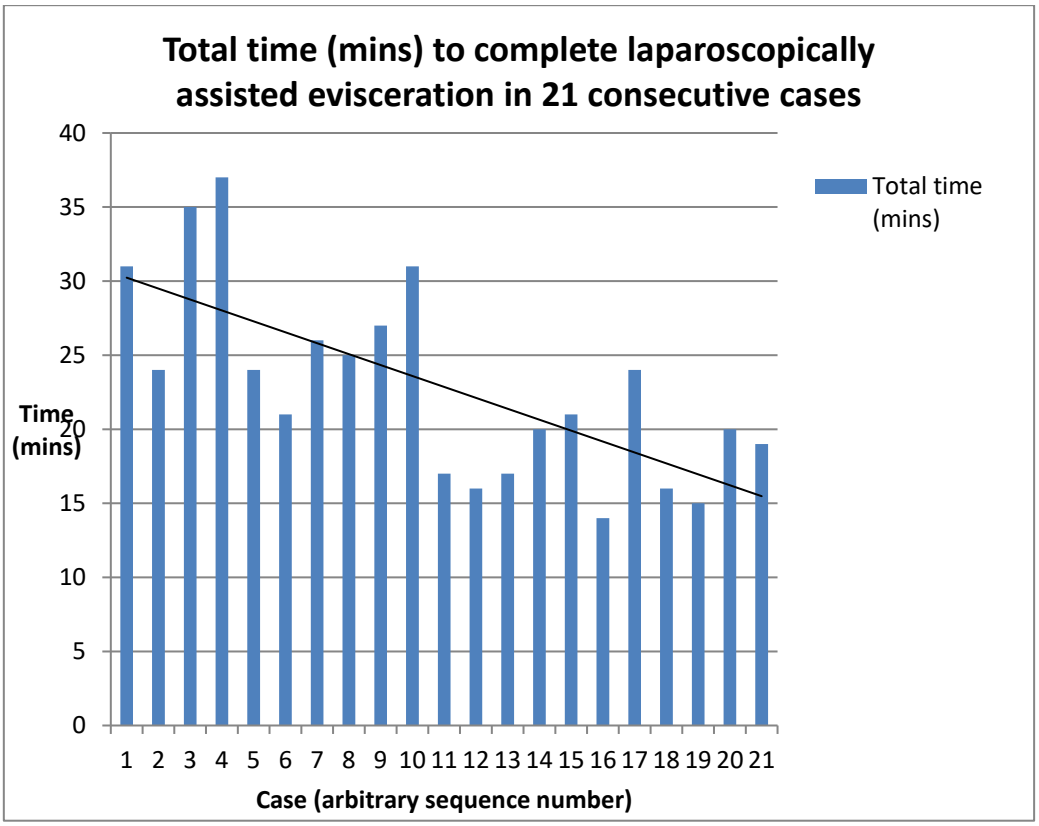
Figure 1: Flow chart of MIA inclusion case

Evaluating the MinImAL procedure: timing

As part of overall evaluation of the MinImAL procedure as a method of autopsy, a skilled operator familiar with standard autopsy but inexperienced with MinImAL (JCH) was trained and subsequently timed for an unselected series of complete MinImAL procedures, following three familiarisation cases.

Limited MinImAL procedures and those which were converted due to poor visualisation were excluded from this series, leaving a sub-group of 21 cases in which timings were collected. 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 subsequent 10 cases timed (18 minutes, 12 seconds).

Figure 2: Time for MIA examination by experience.



Evaluating the MinImAL procedure: sampling adequacy

In each MinImAL case, an attempt was made to sample major organs. Heart, lung and kidney were successfully sampled in every case. Liver was successfully sampled in 98%, spleen in 94% (with implications for genetic analysis following MinImAL), and adrenal gland, pancreas and thymus in 89%, 82% and 55% of cases respectively.

Potential reasons for sampling inadequacy include operator error (e.g. failure to specifically 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 or amount of tissue).

Of the 90 cases in the cohort that underwent successful complete MinImAL examination, histological abnormalities were demonstrated in 16 organs across 10 cases. Of the organs with a histological abnormality present, in all but two (both involving the heart), a clinical, radiological or autopsy abnormality was present. Both of the cases with unsuspected cardiac abnormalities were neonatal deaths (one at day 11, one at 4 months).

In no case of fetal death did histological sampling without a clinical, radiological or pathological indication reveal additional useful information.

Table 2: Histological sampling success rates and normality/abnormality rates across major organs in the 90 complete MinImAL cases

	Heart	Lung	Kidney	Liver	Adrenal gland	Pancreas	Spleen	Thymus
Sampling failure	0	0	0	3 (%)	10 (%)	16 (%)	5 (%)	40 (%)
Sampled and histology normal	88 (%)	86 (%)	84 (%)	84 (%)	80 (%)	74 (%)	81 (%)	50 (%)
Sampled and histology abnormal	2 (%)	4 (%)	6 (%)	3 (%)	0	0	1 (%)	0
% Sampling success	100	100	100	98	89	82	94	55

Evaluating the MinImAL procedure: unexplained rate

Whilst this study was not designed as trial to evaluate the accuracy of the MinImAL procedure, Chi-Square analysis revealed no significant difference in the 'unexplained' rate between SB/IUFD in this cohort and over 1,000 IUFDs previously published)[5];

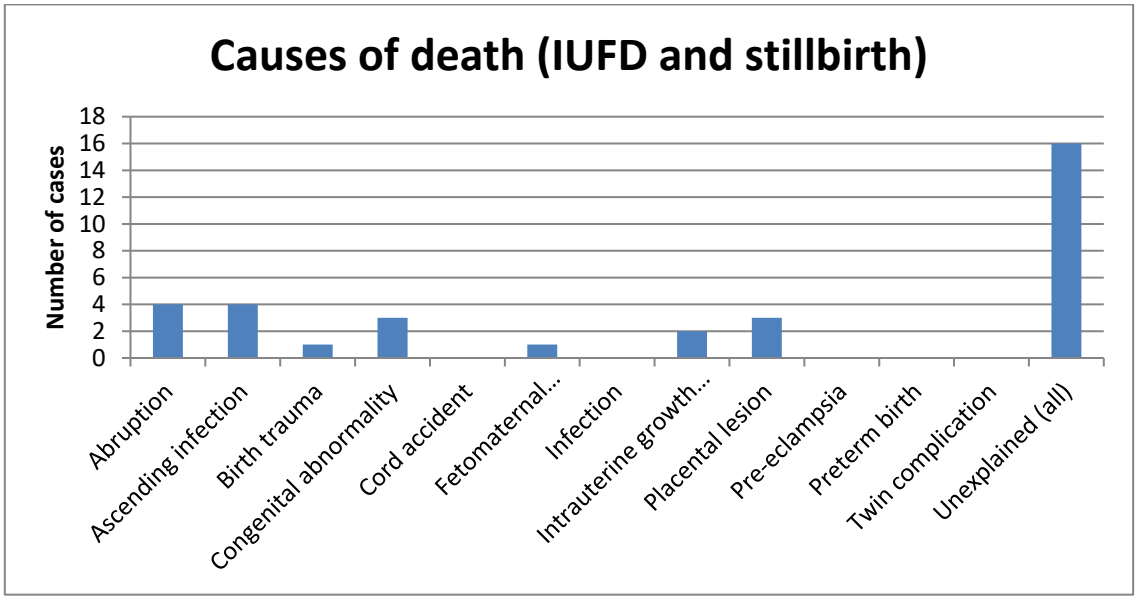
indeed, the unexplained rate was slightly lower in the current cohort. This suggests that MinImAL procedure has a similar performance to standard autopsy in terms of the proportion of cases in which a cause of death is determined.

Table 3:

	Explained	Unexplained	Total
Man et al 2016	412	652	**1064**
This study	18	16	**34**
Total	**430**	**668**	**1098**

Chi-square without Yates correction, Chi squared 2.8, two-tailed P=0.09

Figure 3: Causes of death in IUFD/Stillbirth cases



Limited MinImAL sub-group

In 11 cases, either by parental or Coronial request, a limited MinImAL procedure was performed (examination restricted to specific body cavity or organ cavity). In these cases, there was usually a specific clinical question to be answered based on the clinical presentation. This group contained eight fetal cases (five TOP, two IUFD, one stillbirth), one neonatal death, one infant death and one childhood death.

Discussion

This descriptive study presents first experience with a large, unselected cohort of perinatal and paediatric autopsies performed using less invasive techniques, including MinImAL autopsy, along with analysis of sampling adequacy and histological abnormality analysis.

The findings demonstrate that such approaches can be learnt and performed with a low failure rate (2/87 MinImAL cases converted to standard autopsy due to technical difficulty) and good sampling accuracy (Adequate sampling of heart, lung, kidney, liver and spleen >94%, sampling of pancreas and adrenal gland >80%). In all other cases within the cohort, histological abnormalities would have been discovered because of clinical, radiological or pathological indications to sample the organ.

This study was based on laparoscopic-assisted sampling since previous data on postmortem needle biopsies suggested poor performance. However, since the data from the database series indicates that limited sampling of major organs provides adequate diagnostic coverage and that more recent evidence using real-time ultrasound guidance and large bore biopsy needles suggests that needle biopsies of major organs are likely to provide adequate material for evaluation. This would mean that sampling can be performed more quickly and less invasively with no requirements for special equipment, which has significant implications for investigation after death in developing countries, in which the concept of LIA is becoming more acceptable.[6]

Summary of findings

The findings of this section have demonstrated that:

-MIA is a feasible alternative to standard PM, which can be learned and performed to a high degree of reliability in terms of adequacy of tissue sampling.
-Initial data suggests similar rates of determination of cause of death or main finding to standard autopsy.

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