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Post mortem microfocus computed tomography for early gestation fetuses: a validation study against conventional autopsy.

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Condensation: Micro CT is a highly accurate imaging tool for whole body fetal imaging following early gestation fetal loss, which parents may prefer to conventional invasive autopsy.

Implications and Contributions

A. To evaluate the diagnostic accuracy of micro CT as a high resolution imaging technique for non-invasive autopsy following early gestational fetal loss.

B. Micro CT shows high levels of agreement with conventional autopsy across multiple organ systems in fetal loss or termination of pregnancy (700/718 indices, agreement 97.5%; 95% CI 96.6, 98.4%)

C. The study shows that micro CT can provide non-invasive high-resolution imaging 3D volumes of fetal anatomy, which facilitate autopsy and subsequent discussions between medical professionals involved in patient care and counselling for future pregnancies

Short version of title: Micro CT for fetal autopsy

Abstract

Background: Perinatal autopsy provides useful clinical information in up to 40% of cases. However, there is a substantial unmet clinical need with regards to post mortem investigation of early gestation fetal loss for parents for whom standard autopsy is either not available or not acceptable. Parents dislike the invasive nature of autopsy, but current clinical imaging techniques do not provide high-enough imaging resolution in small fetuses. We hypothesized that microfocus computed tomography, a rapid, high resolution imaging technique, could give accurate diagnostic imaging following early gestation fetal loss.

Objectives: The objective of the study was to evaluate the diagnostic accuracy of microfocus computed tomography for non-invasive human fetal autopsy for early gestation fetuses, using conventional autopsy as the reference standard.

Study design: We compared iodinated whole body microfocus computed tomography in 20 prospectively recruited fetuses (11-21 weeks' gestation; from two centers), to conventional autopsy in a double-blinded manner for (a) main diagnosis, and (b) findings in specific body organs. Fetuses were prepared using 10% formalin / potassium tri-iodide. Images were acquired using XT H 225 ST microfocus CT scanner, using size-appropriate parameters. Images were independently evaluated by two pediatric radiologists across 40 individual indices to reach consensus, blinded to formal perinatal autopsy results. The primary outcome was agreement between micro CT and conventional autopsy for overall diagnosis.

Results: Post-mortem whole body fetal micro CT gave non-invasive autopsy in minutes, at a mean resolution of 27μm, with high diagnostic accuracy in fetuses below 22 weeks gestation. Autopsy demonstrated 13/20 fetuses with structural

abnormalities, 12 of which were also identified by micro CT (92.3%). Overall, micro CT agreed with overall autopsy findings in 35/38 diagnoses (15 true positive, 18 true negative; sensitivity 93.8% (95% CI: 71.7, 98.9%), specificity 100% (95% CI: 82.4, 100%)), with 100% agreement for body imaging diagnoses.

Furthermore, following removal of non-diagnostic indices, there was agreement for 700 / 718 individual body organ indices assessed on micro CT and autopsy (agreement 97.5%; 95% CI 96.1, 98.4%), with no overall differences between fetuses ≤14 or >14 weeks gestation (agreement 97.2%, 97.9% respectively). Within first trimester fetal loss cases (below 14 weeks gestation), micro CT analysis yielded significantly fewer non-diagnostic indices than autopsy examination (22/440 vs 48/348 respectively; $p<0.001$).

Conclusion: Post mortem whole-body fetal micro CT gives non-invasive, detailed anatomical examinations, achieved in minutes, at high resolution. Micro CT may be preferable to MRI in early gestation fetuses and may offer an acceptable method of examination after fetal loss for parents who decline invasive autopsy. This will facilitate autopsy and subsequent discussions between medical professionals involved in patient care and counselling for future pregnancies.

Keywords: anatomy, autopsy, computed tomography, microfocus computed tomography, micro-CT, post-mortem, termination of pregnancy, virtual autopsy.

Introduction

Fetal loss is a common event which impacts a million women each year in the US [1] and there is a growing Western trend to postponing pregnancy despite an increased risk of fetal death with advancing maternal age [2]. Perinatal autopsy provides diagnostic information regarding fetal anomalies, the etiology of intrauterine fetal death, and influences the management of future pregnancies and living relatives [3-5]. Although perinatal autopsy is an important source of epidemiological data regarding developmental abnormalities and complications of pregnancy and labour, the majority of parents decline standard invasive perinatal autopsy [6-8], mainly due to its invasive nature [7-12].

Post mortem MRI (PMMRI) can be offered as part of a less invasive approach [13, 14], but although PMMRI has high diagnostic accuracy in fetuses (approximately 94% concordance with autopsy) [14], it does not provide adequate high resolution imaging in early gestation loss (below 500 g bodyweight / 18 weeks' gestation) [15, 16], even at higher field strengths [17]. Furthermore, following the use of modern high-resolution ultrasound [18-24] and in the era of cell-free DNA testing [25-49], earlier antenatal diagnoses of congenital malformations are being made, subsequently leading to terminations of pregnancy at earlier gestations [50,51]. The combination of limited fetal size and tissue breakdown following *in-utero* death (termed maceration) makes conventional fetal autopsy challenging at lower gestations and fetal body weights, in addition to the issue of availability of specialist fetal post mortem examination [3, 52]. Availability of high-quality clinical imaging techniques for first and early second trimester perinatal post mortem use would realize the parents' need for non-invasive investigation with high diagnostic accuracy and may improve access to specialist opinion.

Microfocus computed tomography (Micro CT) is an attractive alternative technique in terms of resolution, cost, speed and accessibility. Micro CT has been used previously to phenotype animal models of disease [53-55], in maxillofacial research [56], in archeology [57], and for non-destructive testing of components within industry. Recently, three-dimensional imaging of human tissue at histological resolution has been shown to be possible [58, 59], and feasibility for post mortem imaging of ex-vivo organs and whole fetuses has been demonstrated [60-62].

In this study, we evaluated the diagnostic accuracy of micro CT for non-invasive human fetal autopsy for early gestation fetuses using conventional autopsy as the reference standard.

Materials and Methods

Case selection

This study was performed as part of an ethically approved larger study investigating minimally invasive autopsy techniques and novel methods of post mortem imaging (CE13/LO/1494 and CE2015/81). All samples 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, and all material was handled in accordance with parental instructions. Twenty cases (11-21 weeks' gestation; median 14 weeks' gestation) were recruited prospectively from two centers that regularly perform post mortem perinatal imaging for formal perinatal autopsy examination between June 2015 and September 2016. The trial conforms to the STARD statement [63].

Tissue preparation

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 (I_2KI / Lugol's iodine, to impart tissue contrast), with a total iodine content of 63.25 mg / mL (iodine mass of 2.49×10^{-4} mol / mL), in a 1:1 ratio for 72 hours prior to imaging. Before scanning, the specimens were removed from the iodine solution, 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. Fetuses were further fixed in 10% formalin to prevent tissue degradation prior to autopsy examination when needed.

Micro CT examination

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 were between 80 – 110 kV and 87-180 μA respectively. Exposure times were 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 and magnification achieved and ranged from 7.4 μm to 51.0 μm.

Image analysis

Micro CT images were independently evaluated by two pediatric radiologists (OJA, MMC) 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 was available to the pathologist, but blinded to any autopsy results. We assessed 40 individual indices in 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.

Autopsy examination

Autopsies were performed blinded to the micro CT findings by specialist perinatal pathologists (JCH, NJS, VS) according to standard clinical procedures recording the same diagnostic indices. Histology was taken where appropriate as part of routine clinical investigation. Features identified by dissection, histological examination and micro CT imaging were then compared. Potential discrepancies were reviewed, with pathological examination used as the reference standard for the purposes of this analysis.

Statistical evaluation

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 valve (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 [64] and SPSS (Version 19 for Macintosh, SPSS Inc.,

IBM, New York, USA) was used for data analysis. $P<0.05$ was taken as the threshold for statistical significance, where appropriate.

Results

20 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 1). 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. All specimens demonstrated excellent internal contrast on micro CT examination with the iodination protocol (Figure 1 & Video 1). 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 ± 13.4 μm (range 7.4 – 51 μm).

Overall results

Autopsy demonstrated 13 fetuses with structural abnormalities overall, 12 of which were also identified by micro CT (Figure 2 & Video 2). 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%; Table 1). Agreement for body imaging diagnoses was 100%, and 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.

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 2). 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%).

Discrepant findings

There were 18/718 (2.5%) apparent discrepancies between micro CT and autopsy findings (Table 2). 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 3), 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 3). 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 4), a uterovesical connection not

identified at autopsy, one overcall of histologically normal kidneys and a megarectum that was mistaken for megacystis (Table 3).

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 4). 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 4), but PPV was significantly higher in younger fetuses (<14 weeks) at 97.3% vs 85.7% ($p<0.001$).

Comment

Principal findings of the study

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.

Classical autopsy

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. We have shown 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. There is a sharp decline in diagnostic utility of conventional clinical 1.5T PMMR in fetuses < 500 g [15], hence the need to examine other imaging techniques and modalities. 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 [17, 65] and fetal size [15], non-diagnostic imaging rates <20 weeks were 50% at 1.5 T and 30% at 3 T [17] and 23.5% at 9.4 T [65].

Research perspectives

Several issues remain to be addressed with regards to the optimization 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 I_2KI. Since the micro CT was non-diagnostic in this case of brain abnormality, in clinical practice, extraction of the brain for formal neuropathological examination is advised when micro CT is non-diagnostic, although this also remains challenging at perinatal autopsy when autolysis is present.

Clinical implications of the study

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 [66,67] and many parents find the idea of an invasive autopsy

distressing. Some parents feel that their baby has 'suffered enough',or fear unsatisfactory cosmetic effects from a standard autopsy procedure [68]. Furthermore, perinatal pathology is highly specialized, usually limited to tertiary referral centers. 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 [69]. High resolution imaging also facilitates discussions between medical practitioners involved in fetal diagnosis, including radiologists, pathologists, geneticists, pediatricians and fetal medicine specialists who are involved in counselling of parents for future pregnancies.

Strengths and limitations of the study

Our study is the first to document diagnostic accuracy of micro CT for early gestation human fetuses, but represents proof of principle in this cohort. The overall clinical utility in an unselected population remains to be assessed in a larger cohort of fetuses. We were 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 personalized counselling regarding likely yield of micro CT examination in a range of clinical scenarios.

Conclusions

Post mortem whole body fetal micro CT allowed us to perform non-invasive autopsy in minutes, at 27 µm resolution (mean resolution) with 92% diagnostic accuracy in fetuses below 22 weeks gestation.

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Table 1: Comparison of diagnoses obtained for micro CT and autopsy.

Case	Age (weeks)	Mode of death	µCT resolution (µm)	Brain µCT	Brain Autopsy	Agree	Body µCT	Body Autopsy	Agree	Diagnosis
1	11	ToP	7.4	Complex NTD	Complex NTD	**Y**	Abdominal wall defect	Abdominal wall defect	**Y**	Complex NTD with gastroschisis
2	14	ToP	13.7	NTD	NTD	**Y**	Acardia	Acardia	**Y**	Multisystem disorder
3	14	IUFD	20.2	Non-diagnostic, macerated	Non-diagnostic, macerated	**Y**	Normal	Normal	**Y**	Normal
4	13	ToP	15.8	Alobar holoprosencephaly	Alobar holoprosencephaly	**Y**	Cystic kidneys, omphalocoele	Cystic kidneys omphalocoele	**Y**	Multisystem disorder
5	16	ToP	42.3	Normal	Normal	**Y**	AVSD, facial dysmorphism	AVSD, facial dysmorphism	**Y**	Abnormal karyotype, cardiac anomaly
6	16	IUFD	23.5	Normal	Normal	**Y**	Coarctation, webbed neck	Abnornal karyotype, webbed neck	**Y**	Turner syndrome
7	15	ToP	29.7	Non-diagnostic, macerated	Holoprosecephaly	**N**	Normal	Normal	**Y**	Holoprosecephaly
8	13	ToP	17.2	Non-diagnostic, macerated	Non-diagnostic, macerated	**Y**	Cleft palate, VSD, chest & abdominal wall defects	Cleft palate, VSD, chest & abdominal wall defects	**Y**	Ectopia cordis
9	21	ToP	46.0	Normal	Non-diagnostic, macerated	**N**	Skeletal dysplasia	Skeletal dysplasia	**Y**	Skeletal dysplasia - TD

10	11	ToP	15.2	Normal	Normal	**Y**	Normal	Normal	**Y**	Normal
11	11	ToP	15.3	Normal	Normal	**Y**	Normal	Normal	**Y**	Normal
12	11	ToP	16.6	Normal	Normal	**Y**	Normal	Normal	**Y**	Normal
13	12	IUFD	16.9	Normal	Non-diagnostic, macerated	**N**	Normal	Normal	**Y**	Missed miscarriage
14	15	IUFD	30.6	Normal	Normal	**Y**	Thoraco-abdominal schisis	Thoraco-abdominal schisis	**Y**	Unexplained IUFD
15	14	ToP	27.1	Normal	Normal	**Y**	Skeletal anomaly	Skeletal anomaly	**Y**	Skeletal anomaly - NOS
16	13	ToP	34.4	Normal	Normal	**Y**	Limb body wall complex / sacral teratoma	Limb body wall complex / sacral teratoma	**Y**	Limb body wall complex / sacral teratoma
17	21	IUFD	43.0	Normal	Normal	**Y**	Normal	Normal	**Y**	Unexplained IUFD
18	15	IUFD	22.8	Normal	Normal	**Y**	Truncus arteriosus, CRS / VACTERL	Truncus arteriosus, CRS / VACTERL	**Y**	Multisystem disorder
19	15	ToP	50.9	Normal	Not examined	**-**	Cleft, absent radius	Cleft, absent radius	**Y**	Cleft palate, limb anomaly
20	19	IUFD	51.0	Normal	Not examined	**-**	Normal	Normal	**Y**	Unexplained IUFD
			TOTAL			**15/18**			**20/20**	

Table 1. Comparison of diagnoses obtained for micro CT and autopsy in 20 cases. Age given in gestational weeks (gw). A = abnormal, N = normal, ND = non-diagnostic.

(µCT – micro CT, ADAM – amniotic deformity adhesions and mutilations, AVSD – atrioventricular septal defect, CNS – central nervous system, CRS – caudal regression syndrome, GW – gestational weeks, IUFD – intrauterine fetal death, NOS – skeletal dysplasia not otherwise specified (symmetrical limb foreshortening), NTD – neural tube defect, PPV – positive predictive value, ToP – termination of pregnancy, TD – thanatophoric dysplasia, TRAP – twin reversed arterial perfusion, VACTERL – vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, renal anomalies, and limb abnormalities)

Table 2: Diagnostic performance of Micro CT vs autopsy by body system

	NE	ND	TP / FP	FN / TN	Sensitivity %	Specificity %	PPV (%)	NPV (%)	Concordance
Neuro	12	27	17 / 1	1 / 82	94.4% [74.2 , 99.0]	98.8% [93.5 , 99.8]	94.4% [75.2 , 99.0]	98.8% [93.5 , 99.8]	98.0% [93.1, 99.5]
Chest	0	6	20 / 2	3 / 169	87.0% [67.9 , 95.5]	98.8% [95.8 , 99.7]	90.9% [72.2 , 97.5]	98.3% [95.0 , 99.4]	97.4% [94.1 , 98.9]
Cardiac	0	21	19 / 0	2 / 138	90.5% [71.1 , 97.3]	100% [97.3 , 100]	100% [83.2 , 100]	98.6% [94.9 , 99.6]	98.7% [95.5, 99.7]
Abdomen	0	16	41 / 3	5 / 195	89.1% [77.0 , 95.3]	98.5% [95.6 , 99.5]	93.2% [81.8 , 97.7]	97.5% [94.3 , 98.9]	96.7% [93.7, 98.3]
MSK	0	0	7 / 0	1 / 12	87.5% [52.9 , 97.8]	100% [75.8 , 100]	100% [64.6 , 100]	92.3% [66.7 , 98.6]	95.0% [76.4, 99.1]
Overall (n=800)	**12**	**70**	**104 / 6**	**12 / 596**	**89.7% [82.8, 94.0]**	**99.0% [97.8 , 99.5]**	**94.5% [88.6, 97.5]**	**98.0% [96.6 , 98.9]**	**97.5% [96.1, 98.4]**

Table 2. Diagnostic performance of Micro CT vs autopsy by body system.

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)

Table 3. Detailed true positives, false positives and false negatives on micro CT by body system

	True positives	Correct Diagnoses	FP	Overcalls	FN	Misses
Neuro	**17**	1 x ventricular malformation, 1x cortical malformation, 1x hypotelorism, 1x hypertelorism, 1x vertebral anomalies, 2x cranio-rachischisis, 10x structural abnormality	**1**	Caudal regression	**1**	1 x neural tube defect
Chest	**20**	1x narrow chest, 1x neck webbing, 2x cleft palate, 2x neck disruption 3x chest wall disruption, 5x diaphragmatic disruption 6x structural abnormality	**2**	Thymic hypoplasia, incidental neck lesion	**3**	2 x lung lobation anomalies 1 x laryngeal atresia
Cardiac	**19**	1x AVSD (6 indices), 1 x Truncus arteriosus (4 indices) 9x structural abnormality	**0**	None	**2**	1 x VSD 1 x right heart anomaly
Abdo	**41**	1x omphalocele, 1x cystic kidneys, 1x absent kidney, 1x absent ureter 1x bladder anomaly, 23x visceral disruption/displacement, 13x structural abnormality	**3**	Normal kidneys megarectum interpreted as megacystis, uterovesical connection	**5**	3 x Bowel malrotation 1 x ambiguous genitalia 1 x hypoplastic bladder
MSK	**7**	1x complex NTD, 1x rib defects, 1x symmetrical limb foreshortening, 1x radial absence, 1x femoral absence, 1x sternal anomaly,	**0**	None	**1**	1 x polydactyly

		1x skeletal dysplasia (TD)				
TOTAL	**104**	-	**6**	-	**12**	-

Table 3. Detailed true positives, false positives and false negatives on micro CT by body system.

(AVSD – atrioventricular septal defect, FN – false negative, FP – false positive, NTD – neural tube defect, TD – Thanatophoric Dysplasia, VSD – ventricular septal defect)

Table 4: Overall diagnostic performance of Micro CT vs autopsy by gestational group (≤14 or >14 weeks gestation)

	NE	ND	TP / FP	FN / TN	Sensitivity %	Specificity %	PPV (%)	NPV (%)	Concordance
Neuro ≤14 wks	**0**	**18**	**16 / 0**	**1 / 42**	94.1% [73.0, 99.0]	100% [91.6, 100]	100% ** [80.6, 100]	97.7% [87.9, 100]	98.3% [91.0, 99.7]
>14 wks	12	9	1 / 1	0 / 40	100% [20.7, 100]	97.6% [87.4, 99.6]	50.0% [9.5, 90.5]	100% [91.2, 100]	97.6% [87.7, 99.6]
Chest ≤14 wks	**0**	**6**	**14 / 1**	**3 / 86**	82.4% [59.0, 93.8]	98.9% [93.8, 99.8]	93.3% [70.2, 98.8]	96.6% [90.6, 98.8]	96.2% [90.5, 98.5]
>14 wks	0	0	6 / 1	0 / 83	100% [61.0, 100]	98.8% [93.6, 99.8]	85.7% [48.7, 97.4]	100% [95.6, 100]	98.9% [94.0, 99.8]
Cardiac ≤14 wks	**0**	**16**	**9 / 0**	**1 / 73**	90.0% [59.6, 98.2]	100% [95.0, 100]	100% [70.1, 100]	98.6% [92.7, 99.8]	98.8% [93.5, 99.8]
>14 wks	0	5	10 / 0	1 / 65	90.9% [62.3, 98.4]	100% [94.4, 100]	100% [72.2, 100]	98.5% [91.9, 99.7]	98.7% [92.9, 99.8]
Abdomen ≤14 wks	**0**	**13**	**36 / 1**	**3 / 90**	92.3% [79.7, 97.3]	98.9% [94.0, 99.8]	97.3% ** [86.2, 99.5]	96.8% [90.6, 98.9]	96.9% [92.4, 98.8]
>14 wks	0	3	5 / 2	2 / 105	71.4% [35.9, 91.8]	98.1% [93.4, 99.5]	71.4% [35.9, 91.8]	98.1% [93.4, 99.5]	96.5% [91.3, 98.6]
MSK ≤14 wks	**0**	**0**	**5 / 0**	**1 / 5**	83.3% [43.6, 97.0]	100% [56.6 100]	100% [56.6, 100]	83.3% [43.6, 97.0]	90.9% [62.3, 98.4]
>14 wks	0	0	2 / 0	0 / 7	100% [34.2, 100]	100% [64.6, 100]	100% [34.2, 100]	100% [64.6, 100]	100% [70.1, 100]
Overall ≤14 (n=11)	**0**	**53**	**80 / 2**	**9 / 296**	**89.7% [81.9, 94.6]**	**99.3% [97.6 , 99.8]**	**97.6% ** [91.5, 99.3]**	**97.0% [94.5 , 98.4]**	**97.2% [95.0, 98.4]**
>14 wks (n=9)	**12**	**17**	**24 / 4**	**3 / 300**	**88.9% [71.9, 96.1]**	**98.7% [96.7 , 99.5]**	**85.7% [68.5, 94.3]**	**99.0% [97.1 , 99.7]**	**97.9% [95.7, 99.0]**

Table 4. 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)

FIGURE LEGENDS

Figure 1. Micro CT of a phenotypically normal fetus (case 12) at 11 gestational weeks (A). Axial (B) and Coronal (C) analysis of the heart reveals the aorta (star) and pulmonary trunk (hexagon).

Figure 2. Micro CT of a 13 gestational week fetus with holoprosencephaly (case 4, A&B). Autopsy confirmed the abnormal finding identified on micro CT.

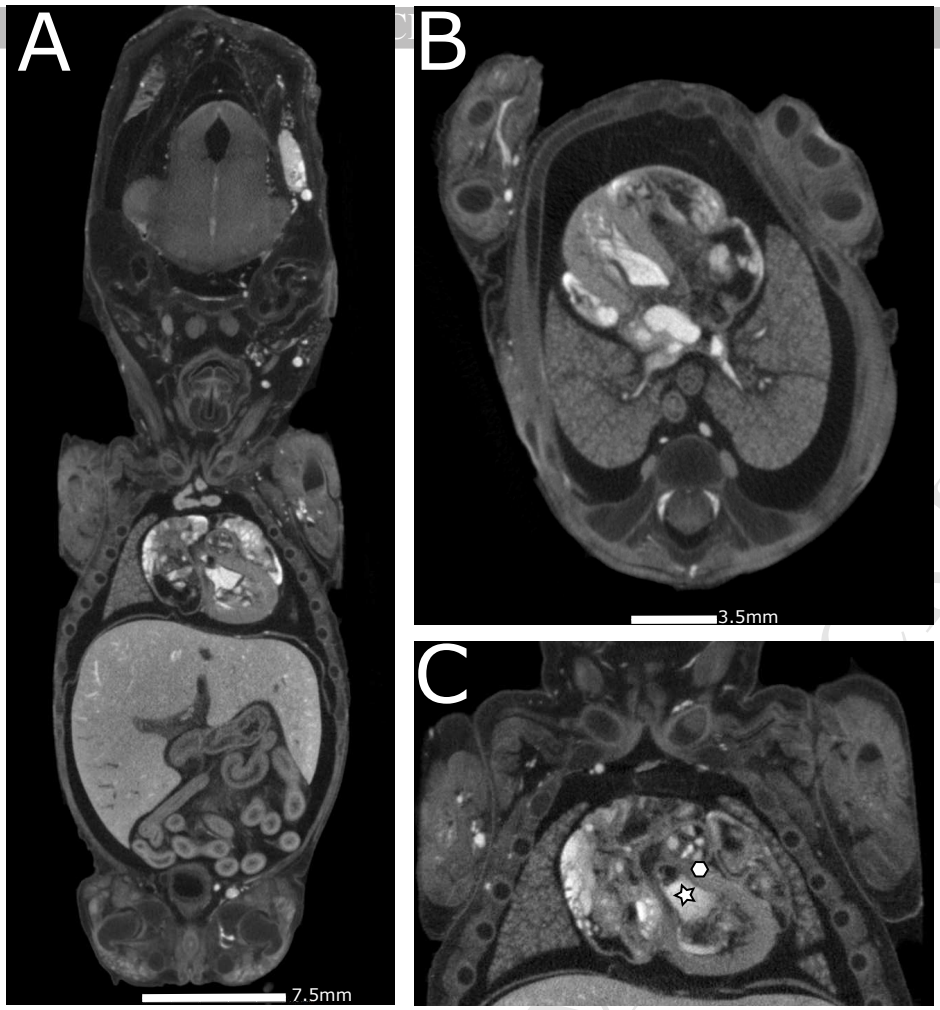
Figure 3. An apparent miss from case 4 at micro CT examination was a sacral NTD, which was easily detected at external examination (star), but overlooked on the micro CT data (B).

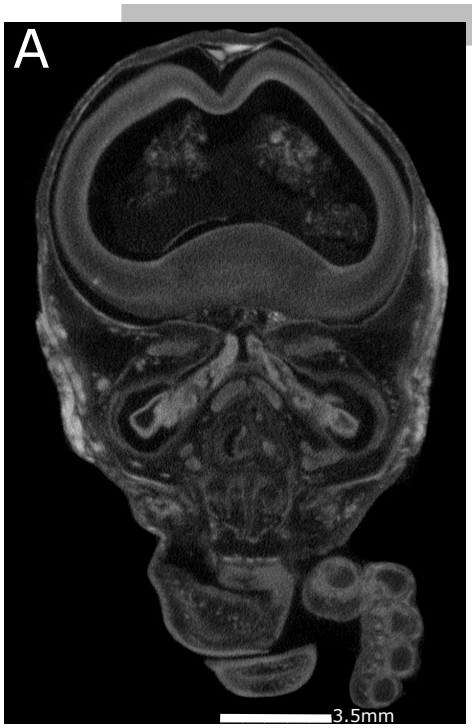
Figure 4. An apparent overcall from micro CT data in case 5 was a cystic neck lesion (star), which was overlooked at autopsy, as this region is not routinely dissected.

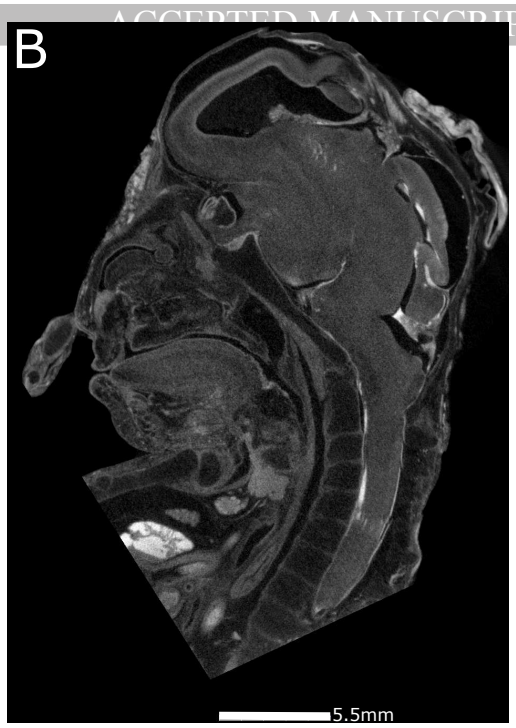
VIDEO LEGENDS

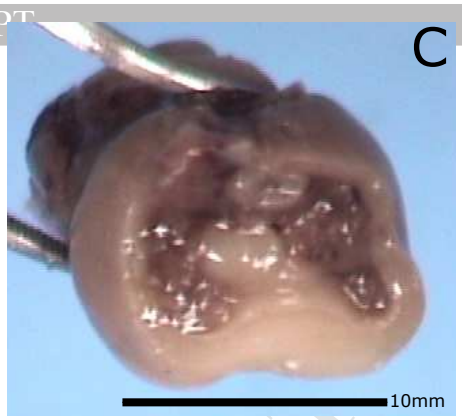
Video 1. Micro CT volume rendering demonstrating virtual autopsy of a fetus from the cohort (case 10, 11 weeks' gestation) with a normal phenotype. Scan resolution approximately 15.2µm.

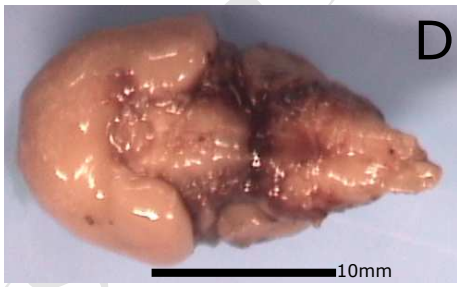
Video 2. Micro CT volume rendering demonstrating virtual autopsy of a fetus from the cohort (case 19, 15 weeks' gestation) with a cleft lip and palate, and limb abnormalities. Scan resolution approximately 50.9µm.

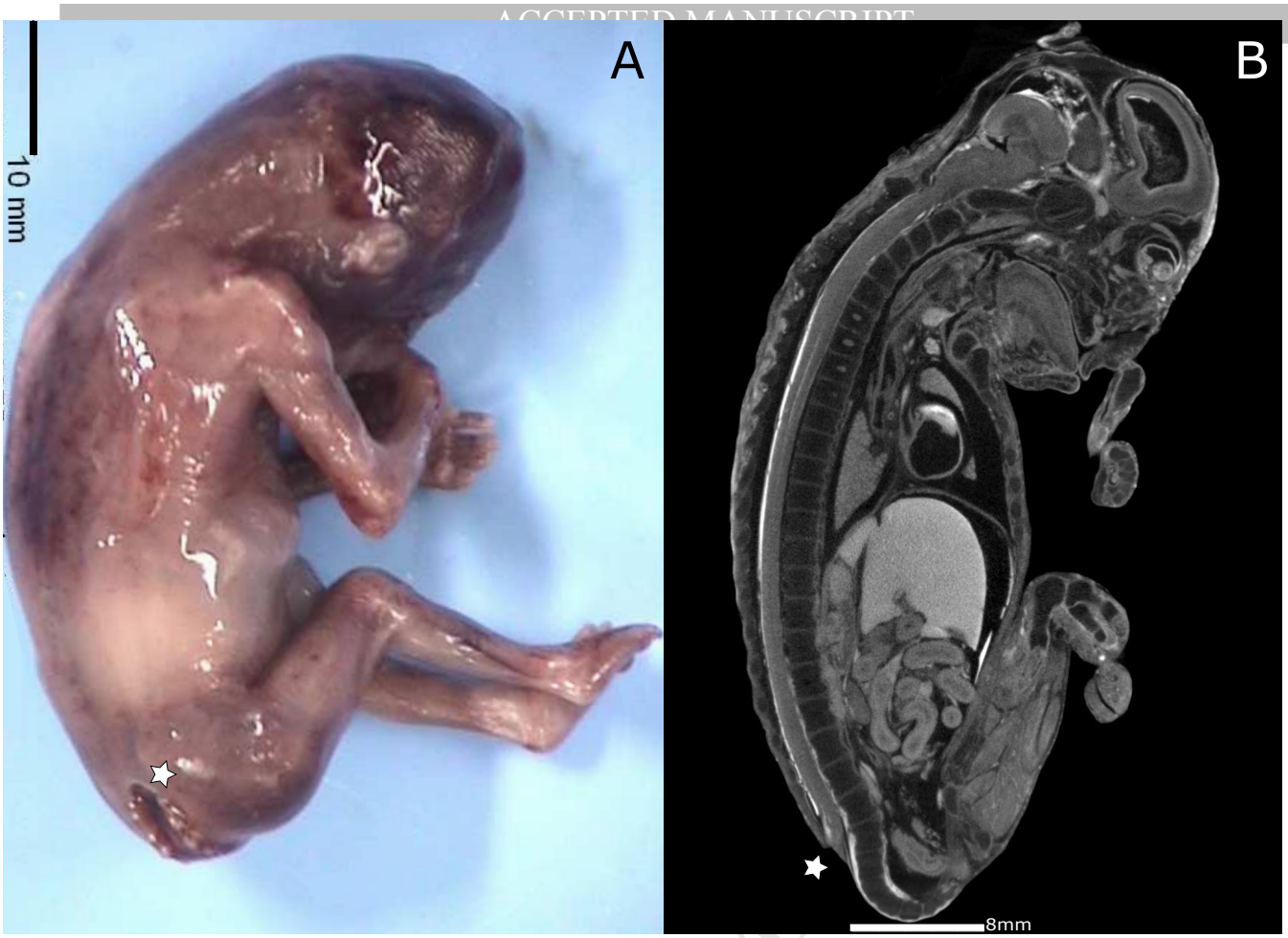


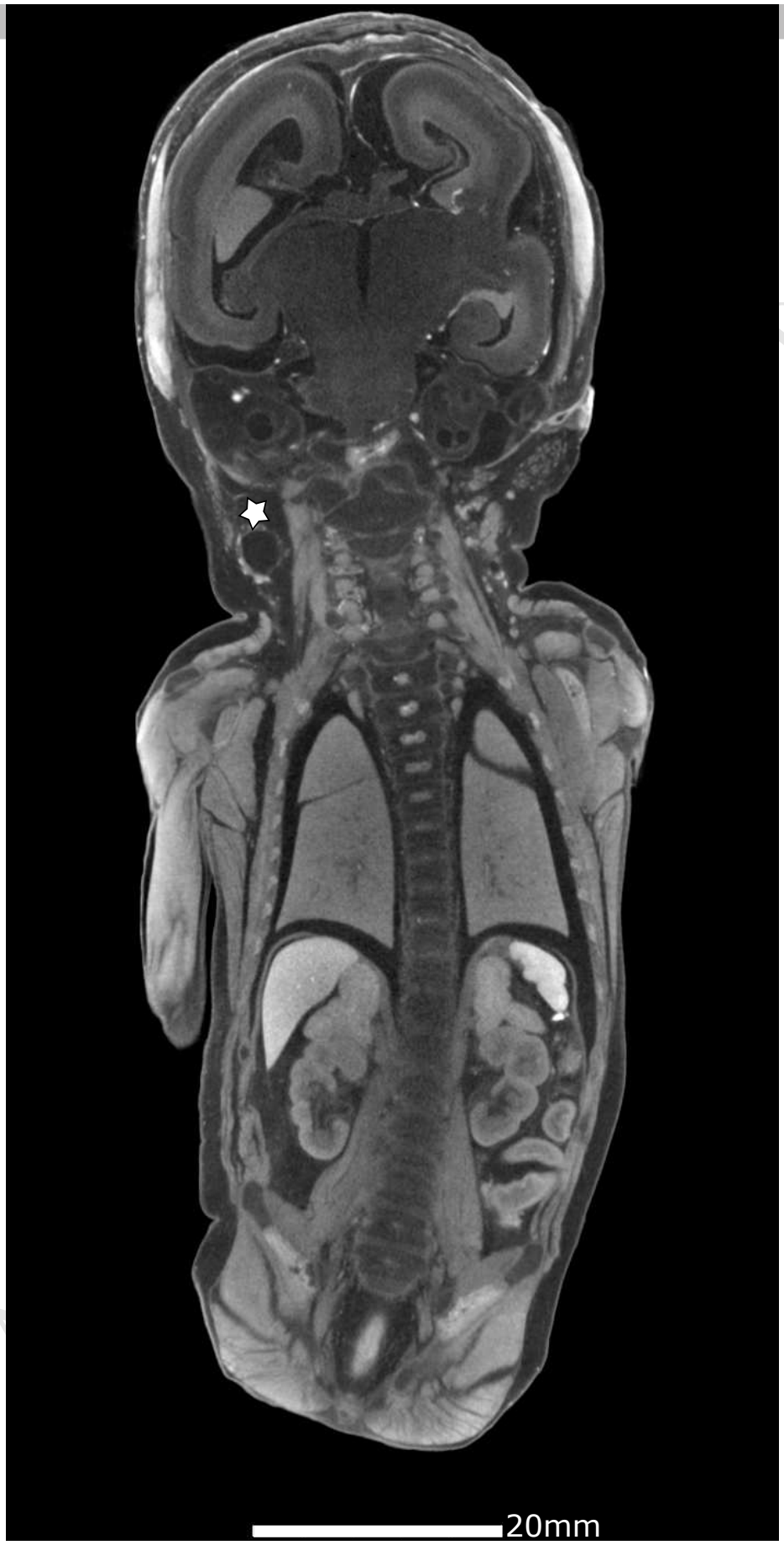








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UCL GREAT ORMOND STREET INSTITUTE OF CHILD HEALTH

Post mortem microfocus computed tomography for early gestation fetuses: a validation study against conventional autopsy.

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Introduction - background

- Perinatal autopsy can provide important information regarding management of future pregnancies but its invasive nature is poorly accepted by parents.

- Post-mortem imaging, including post-mortem MRI (PMMRI), has been shown to be acceptable to women who refuse full autopsy.*(Cannie et al., 2012)*

- However, many women who experience first trimester losses may not be eligible for PMMRI due to small fetal size, and autopsy is technically challenging at early gestations.

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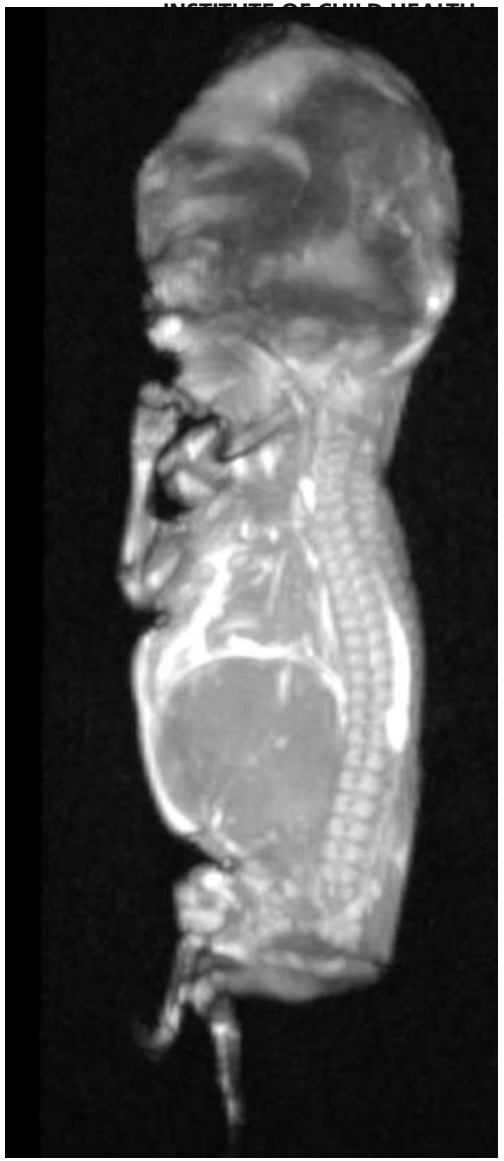
Introduction – clinical problem

- 1.5T PMMRI is non-diagnostic at low bodyweight *(Jawad et al. 2016)*
- 3T PMMRI shows only minor improvements at the lower limit of diagnosis *(Kang et al. Eur Radiol. 2017)*
- Microfocus computed tomography (micro CT) offers a potential imaging method for early gestation fetal loss and termination of pregnancy at high resolution.



Example of imaging resolution of 1.5T PMMRI at 14 weeks

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Objective

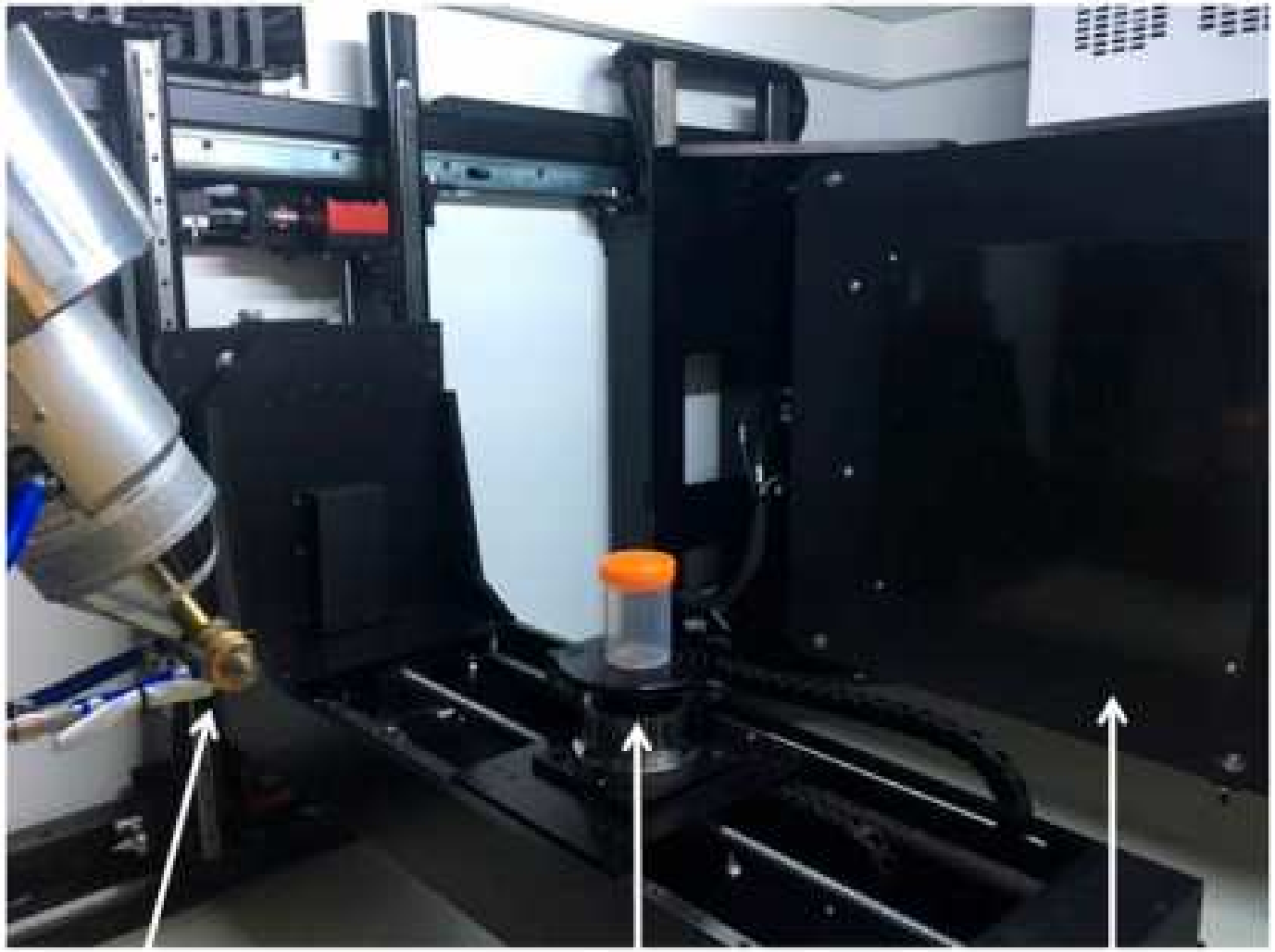
Examination of whole fetuses using micro-CT and comparison with autopsy as the gold standard

Micro CT: Principles

- Micro CT is used extensively in industry for non-destructive testing.

- Imaging uses a focal spot of several microns (compared with several millimetres in a clinical CT).

- Micro CT can provide high resolution volumes of biological tissue with excellent contrast, but requires either very prolonged scan times (8 hours +) or administration of contrast medium.



Hutchinson et al., BJR 2017

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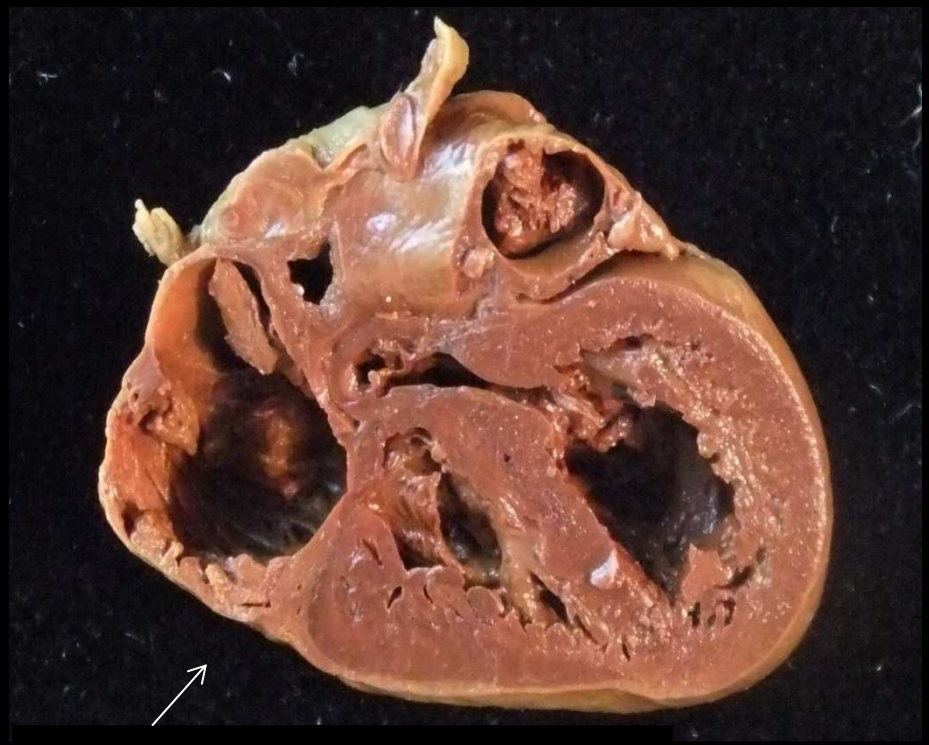
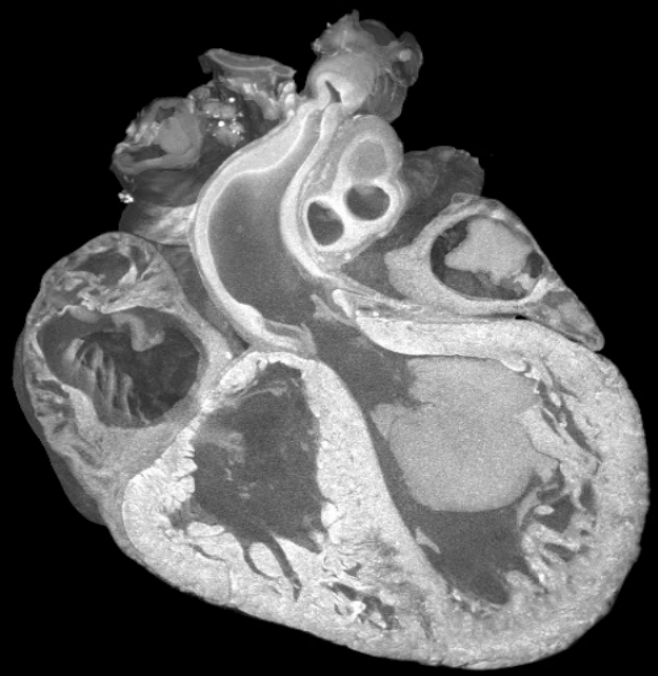
Micro CT in extracted organs



Max diameter 16mm



Example of excised fetal heart at 20 weeks gestation (left excised specimen, right Micro CT)

Study Design

- Twenty cases were prospectively recruited from two centres.
  - iodinated using Lugol's Iodine (I_2KI) following formalin fixation
- Micro CT examination was performed using a Nikon XT H 225 ST system (voxel size range: 7.4 - 51.0 µm).
- All fetuses received a full autopsy following de-iodination with Sodium Thiosulphate ($Na_2S_2O_3$).
- Double blinded reporting
- Forty pre-defined indices across the body were compared between micro-CT and autopsy.

Study Design

- Primary outcome
- Concordance between micro CT and conventional autopsy for overall diagnosis

- Secondary outcomes
- Diagnostic indices: sensitivity, specificity, positive predictive valve, negative predictive value (NPV)

Results: Overall

- Autopsy demonstrated 13 fetuses with structural abnormalities overall, 12 / 13 identified by micro CT.

- In one case at 15gw, micro CT was non-diagnostic due to degradation of brain tissue, and an autopsy diagnosis was reached following specialist neuropathological examination following brain extraction.

- 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%)

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Results

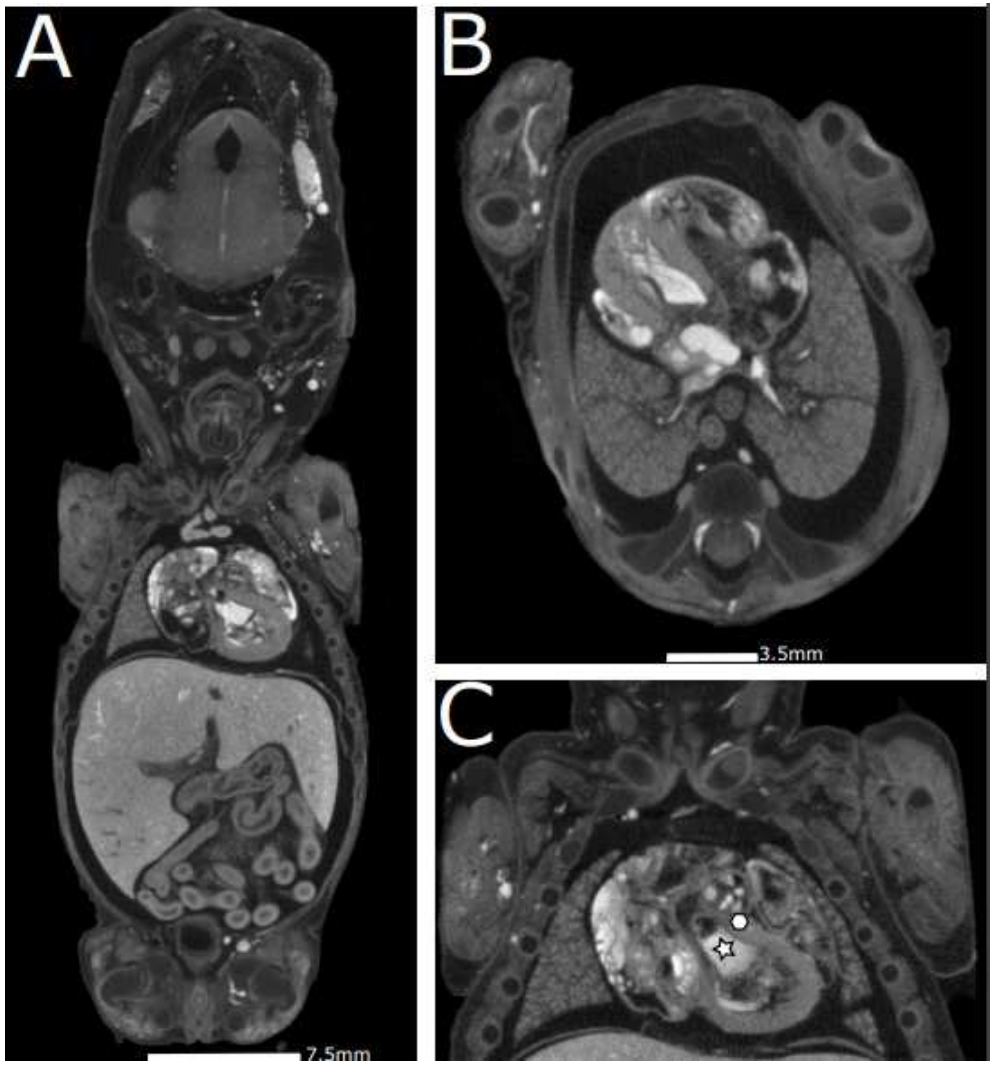

Figure 1. Micro CT of a phenotypically normal fetus (case 12) at 11 gestational weeks (A). Axial (B) and Coronal (C) analysis of the heart reveals the aorta (star) and pulmonary trunk (hexagon).

Results

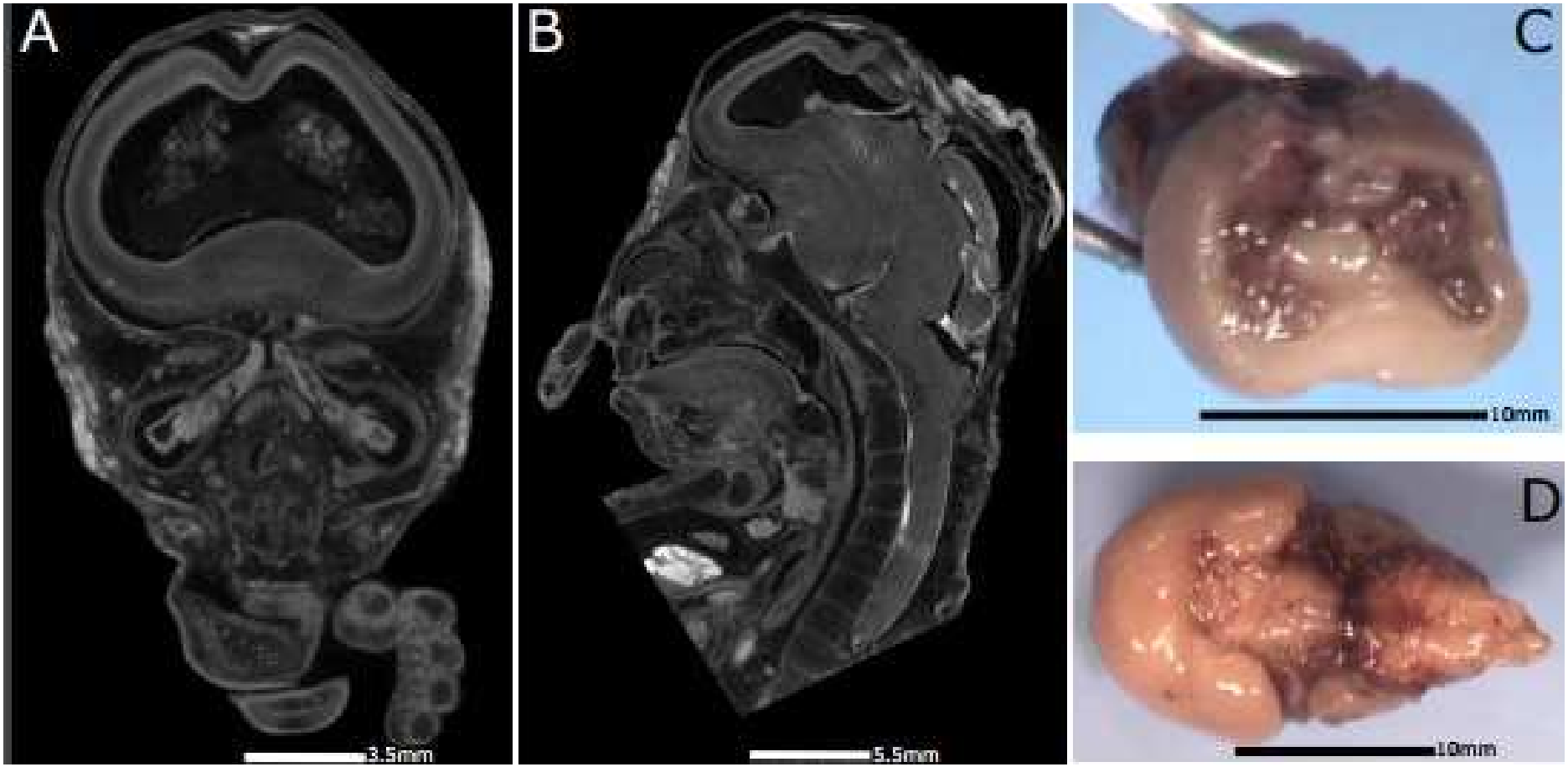

Figure 2. Micro CT of a 13 gestational week fetus with holoprosencephaly (case 4, A&B). Autopsy (C&D) confirmed the abnormal finding identified on micro CT.

Results: Body system / organ level

- There was full agreement for 700/718 indices between micro CT and autopsy across all body systems
  (82 indices removed that were non-diagnostic or not examined)

- Agreement 97.5% (95% CI 96.6, 98.4%; Table 2)

- Overall, sensitivity was >87%, and specificity was >98% for each body system and overall (Table 2).

	NE	ND	TP / FP	FN / TN	Sensitivity %	Specificity %	PPV (%)	NPV (%)	Concordance
Neuro	12	27	17 / 1	1 / 82	94.4% [74.2 , 99.0]	98.8% [93.5 , 99.8]	94.4% [75.2 , 99.0]	98.8% [93.5 , 99.8]	98.0% [93.1, 99.5]
Chest	0	6	20 / 2	3 / 169	87.0% [67.9 , 95.5]	98.8% [95.8 , 99.7]	90.9% [72.2 , 97.5]	98.3% [95.0 , 99.4]	97.4% [94.1 , 98.9]
Cardiac	0	21	19 / 0	2 / 138	90.5% [71.1 , 97.3]	100% [97.3 , 100]	100% [83.2 , 100]	98.6% [94.9 , 99.6]	98.7% [95.5, 99.7]
Abdomen	0	16	41 / 3	5 / 195	89.1% [77.0 , 95.3]	98.5% [95.6 , 99.5]	93.2% [81.8 , 97.7]	97.5% [94.3 , 98.9]	96.7% [93.7, 98.3]
MSK	0	0	7 / 0	1 / 12	87.5% [52.9 , 97.8]	100% [75.8 , 100]	100% [64.6 , 100]	92.3% [66.7 , 98.6]	95.0% [76.4, 99.1]
Overall (n=800)	**12**	**70**	**104 / 6**	**12 / 596**	**89.7% [82.8, 94.0]**	**99.0% [97.8 , 99.5]**	**94.5% [88.6, 97.5]**	**98.0% [96.6 , 98.9]**	**97.5% [96.1, 98.4]**

Table 2. Diagnostic performance of Micro CT vs autopsy by body system. (ABDO – abdominal pathology, CARDIAC – cardiac pathology, CHEST – non-cardiac thoracic pathology, MSK – musculoskeletal pathology, ND – non-diagnostic, NE – not examined, NEURO – Neurological pathology)

Results

- We further divided cases into
  - first trimester (≤14 weeks gestation, n=11), and
  - second trimester (>14 weeks gestation, n=9).

- The non-diagnostic rate was higher for micro CT in first (22 / 440; 12.04%) compared to second trimester fetuses (7 / 360; 4.7%; $p<0.001$).
- Within first trimester cases, micro CT analysis yielded significantly fewer non-diagnostic indices than autopsy examination (22 / 440 vs 48 / 348 respectively; $p<0.001$, Table 4).

- No statistical difference in non-diagnostic rates between micro CT and autopsy in second trimester cases ($p=0.35$).

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	NE	ND	TP / FP	FN / TN	Sensitivity %	Specificity %	PPV (%)	NPV (%)	Concordance
Neuro ≤14 wks	**0**	**18**	**16 / 0**	**1 / 42**	94.1% [73.0, 99.0]	100% [91.6, 100]	100% ** [80.6, 100]	97.7% [87.9, 100]	98.3% [91.0, 99.7]
>14 wks	12	9	1 / 1	0 / 40	100% [20.7, 100]	97.6% [87.4, 99.6]	50.0% [9.5, 90.5]	100% [91.2, 100]	97.6% [87.7, 99.6]
Chest ≤14 wks	**0**	**6**	**14 / 1**	**3 / 86**	82.4% [59.0, 93.8]	98.9% [93.8, 99.8]	93.3% [70.2, 98.8]	96.6% [90.6, 98.8]	96.2% [90.5, 98.5]
>14 wks	0	0	6 / 1	0 / 83	100% [61.0, 100]	98.8% [93.6, 99.8]	85.7% [48.7, 97.4]	100% [95.6, 100]	98.9% [94.0, 99.8]
Cardiac ≤14 wks	**0**	**16**	**9 / 0**	**1 / 73**	90.0% [59.6, 98.2]	100% [95.0, 100]	100% [70.1, 100]	98.6% [92.7, 99.8]	98.8% [93.5, 99.8]
>14 wks	0	5	10 / 0	1 / 65	90.9% [62.3, 98.4]	100% [94.4, 100]	100% [72.2, 100]	98.5% [91.9, 99.7]	98.7% [92.9, 99.8]
Abdomen ≤14 wks	**0**	**13**	**36 / 1**	**3 / 90**	92.3% [79.7, 97.3]	98.9% [94.0, 99.8]	97.3% ** [86.2, 99.5]	96.8% [90.6, 98.9]	96.9% [92.4, 98.8]
>14 wks	0	3	5 / 2	2 / 105	71.4% [35.9, 91.8]	98.1% [93.4, 99.5]	71.4% [35.9, 91.8]	98.1% [93.4, 99.5]	96.5% [91.3, 98.6]
MSK ≤14 wks	**0**	**0**	**5 / 0**	**1 / 5**	83.3% [43.6, 97.0]	100% [56.6 100]	100% [56.6, 100]	83.3% [43.6, 97.0]	90.9% [62.3, 98.4]
>14 wks	0	0	2 / 0	0 / 7	100% [34.2, 100]	100% [64.6, 100]	100% [34.2, 100]	100% [64.6, 100]	100% [70.1, 100]
Overall ≤14 (n=11)	**0**	**53**	**80 / 2**	**9 / 296**	**89.7% [81.9, 94.6]**	**99.3% [97.6 , 99.8]**	**97.6% ** [91.5, 99.3]**	**97.0% [94.5 , 98.4]**	**97.2% [95.0, 98.4]**
>14 wks (n=9)	**12**	**17**	**24 / 4**	**3 / 300**	**88.9% [71.9, 96.1]**	**98.7% [96.7 , 99.5]**	**85.7% [68.5, 94.3]**	**99.0% [97.1 , 99.7]**	**97.9% [95.7, 99.0]**

Table 4. Overall diagnostic performance of micro CT vs. autopsy by gestation.
** indicates $p < 0.001$. (ABDO – abdominal pathology, CARDIAC – cardiac pathology, CHEST – non-cardiac thoracic pathology, MSK – musculoskeletal pathology, ND – non-diagnostic, NE – not examined, NEURO – Neurological pathology)

Results

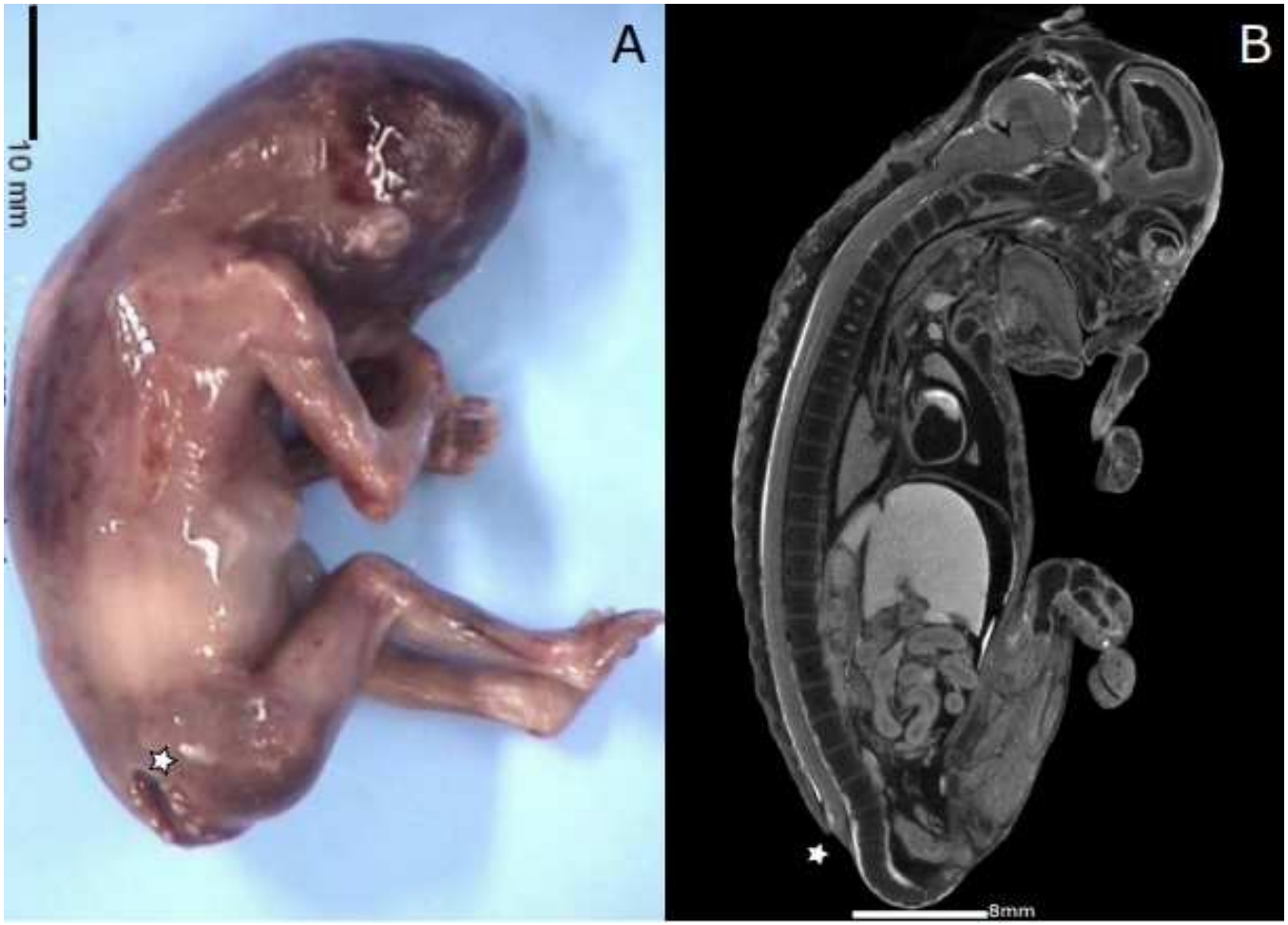

Figure 3. An apparent miss from case 4 at micro CT examination was a sacral NTD, which was easily detected at external examination (star), but overlooked on the micro CT data (B).

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Results

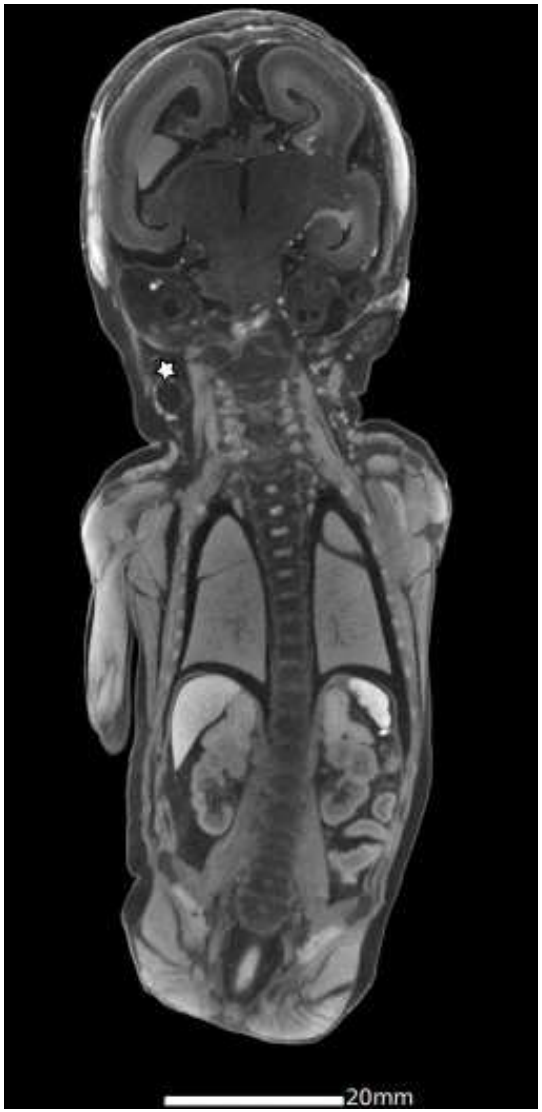

Figure 4. An apparent overcall from micro CT data in case 5 was a cystic neck lesion (star), which was overlooked at autopsy, as this region is not routinely dissected.

Strengths and limitations

- Proof of principle study
- First study to document diagnostic accuracy of micro CT for early gestation human fetuses
- Micro CT better than standard autopsy with respect to non-diagnostic indices (may reflect reporter confidence)

- Potential recruitment bias in consented cases
- Small population
- Clinical utility to be assessed in larger cohort

Conclusions

- Micro CT shows high levels of agreement with conventional autopsy across multiple organ systems in fetal loss or termination of pregnancy

- Micro CT may be useful in early gestational fetal loss when conventional autopsy is declined

- Micro CT can provide non-invasive high-resolution imaging 3D volumes of fetal anatomy, which facilitate autopsy and subsequent discussions between medical professionals involved in patient care and counselling for future pregnancies

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