Minimally invasive, imaging guided virtual autopsy compared to conventional autopsy in the foetus, infant and child: study protocol for the paediatric virtual autopsy trial

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Abstract

Background
In the light of declining autopsy rates around the world, post-mortem MR imaging appears to be a promising alternative for conventional autopsy in the investigation of children’s death. A major drawback of this non-invasive autopsy approach is the fact that histopathological and microbiological examination of the tissue is not possible. The objective of this prospective study is therefore to compare the performance of minimally invasive, virtual autopsy including CT-guided biopsy with conventional autopsy in a paediatric population.

Methods/Design
Foetuses, newborns and children referred for autopsy at three different institutions associated with the University of Zurich will be eligible for recruitment. All bodies will be examined with a commercial CT and a 3 Tesla MRI scanner, blinded to the results of conventional autopsy. After cross-sectional imaging, CT-guided tissue sampling will be performed by a multifunctional robotic system (Virtobot) allowing for automated post-mortem biopsies. Virtual autopsy results will be classified with regards to the likely final diagnosis and major pathological findings and compared to the results of conventional autopsy as the diagnostic gold standard.

Discussion
There is urgent need for the development of alternative post-mortem examinations, not only as a counselling tool for families and as quality control for clinical diagnosis and treatment, but also as an instrument to advance medical knowledge and clinical practice. This interdisciplinary study will determine whether virtual autopsy will narrow the gap of information between non-invasive and traditional autopsy.
**Trial Registration**
ClinicalTrials.gov: NCT01888380

**Keywords:**
Autopsy, post-mortem imaging, minimally invasive virtual autopsy, guided biopsy, virtopsy®, stillbirth, newborn.

**Background**
Despite the long clinical success of post-mortem examinations [1], there has been a sustained decline in autopsy rates around the world [2]. This development can not only be observed in adults, but even more frequently in neonates, infants, and children [3-5] and is in spite of the fact that perinatal and paediatric autopsies are still of particular value in several ways.

First, perinatal post-mortem examinations are able to identify genetic and obstetric factors of relevance to the management of future pregnancies and allow appropriate counselling of families. In approximately 30% of cases following termination of pregnancies, foetal autopsy changed the recurrence risk given to parents [6, 7].

Second, foetal and paediatric autopsies are important in confirming and refuting pre-morbid diagnoses and are capable of making further specific diagnoses. Stambouly et al. demonstrated that paediatric autopsies reveal additional findings in up to 50% of cases, and in around 10% of these, identify diagnoses which, had they been known earlier may have prevented death [8]. At last, autopsy has besides the benefits to individuals and families the potential to advance medical knowledge and improve clinical practice [9].

The reasons for the fall in autopsy rates are multifactorial and complex. Importantly, a large majority of parents deny consent to autopsy because of concerns about disfigurement of their dead child when opening the body. The wish for their infant to “be left in peace” and scepticism of any benefit are further causes for this denial [10,
Additionally, the controversial perception that improved technology renders the autopsy redundant might have changed interest and attitude of public and clinicians towards traditional post-mortem examinations.

Questions have therefore been raised if traditional autopsy can still maintain its role as a means of quality control for clinical diagnosis and treatment. For this reason, many clinicians have called for a minimally invasive autopsy technique, which may be better accepted by parents and healthcare professionals and could therefore provide an alternative for those parents for whom traditional autopsy is not an option. Previous research indicates a better acceptance of this autopsy approach among healthcare professionals [12] and parents [13].

Less invasive methods, such as post-mortem ultrasound- and laparoscopic examinations [14] were reported in the last two decades, but did not gain widespread acceptance due to lack of evidence from studies comparing such an approach in a rigorous and blinded way with conventional autopsy. Post-mortem magnetic resonance imaging (post-mortem MRI) and computed tomography (CT), generically referred to as post-mortem cross-sectional imaging [15] are other non-invasive autopsy techniques and in this context first reported in 1996 [16]. They have the advantage that the position of the different organs can be determined in situ and that the integrity of the human body is not touched. Numerous studies have now investigated the diagnostic accuracy of post-mortem magnetic resonance imaging as compared to conventional autopsy. Most of them showed good results in the detection of central nervous system abnormalities, but rather poor results for the detection of cardiac anomalies [17-21]. The latest and so far largest prospective validation study in foetuses and children demonstrated an overall concordance of 89.3% regarding the cause of death or major pathological lesions detected by
minimally invasive autopsy (post-mortem MRI and blood sampling) compared with conventional autopsy [22]. In children and adolescents, the concordance was generally lower than in foetuses (53.6% vs. 94.6%), mainly because of undetected sepsis affecting the lungs, the heart, the intestine, or because of disseminated sepsis. In such situations, analysing microstructure down to the electron microscopic level as well as specific staining would be helpful [23]. This, however, is not possible with an imaging based, non-invasive autopsy approach. To overcome this shortcoming, percutaneous organ biopsies with or without image guidance have been proposed [24, 25]. Biopsies leave only minor lesions and enable to process the specimen similar to that obtained by conventional autopsy. Major limitations are the relatively small amount of tissue that can be collected and the risk that representative parts are missed. Breeze et al. investigated the feasibility of percutaneous foetal organ biopsies using ultrasound guidance ± surface landmarks in the context of a minimally invasive autopsy and stated that this technique cannot yet be considered to provide useful clinical information because less than 50% of all biopsies were adequate for histological examination [26]. Percutaneous needle biopsies under CT guidance are therefore suggested to be a more reliable method for targeted sampling of tissue probes narrowing the information gap between non-invasive and traditional autopsy [27]. However, a large prospective study of such a minimally invasive autopsy approach by post-mortem cross-sectional imaging and CT-guided biopsies in foetuses, infants and children is missing. With this interdisciplinary study, we therefore aim to compare the performance of cross-sectional imaging combined with CT-guided biopsy with conventional autopsy. For this project, an approach combined of post-mortem imaging and imaging guided biopsies was defined as minimally invasive- or virtual autopsy.
Methods/Design

Trial design
This is a prospective, comparative, interdisciplinary, clinical trial with three study sites: Department of neonatology of the University Hospital Zurich, Children’s University Hospital Zurich and Institute of Forensic Medicine. All three study sites are associated with a single academic institution – University of Zurich, Switzerland. Consecutive foetuses, newborns and children referred for autopsy at these three institutions will be eligible for recruitment. All recruited cases will undergo post-mortem imaging (MRI and CT) with CT-guided biopsies as well as conventional autopsy. The study has been approved by the local ethics committee and is registered at clinicaltrials.gov under number NCT01888380. The study will be conducted in accordance with principles enunciated in the current version of the Declaration of Helsinki, the guidelines of Good Clinical Practice (GCP) issued by the International Conference on Harmonization, and Swiss regulatory authority’s requirements. Except for forensic cases, parental consent will be required for each subject.

Inclusion and exclusion criteria
Inclusion criteria for participation in the paediatric virtual autopsy study comprise still- and live born infants above 16 0/7 weeks of gestational age until adolescents up to 16 years of age no matter of the manner of death (trauma, homicide, suicide and intoxication). Deceased infants who are donors of organs will be excluded from the study.

Objectives
Our primary objective is to compare the accuracy of post-mortem imaging combined with guided biopsy for detecting the cause of death and/or major pathological lesions
with that of conventional autopsy in foetuses, newborns and children. Secondary objectives primarily aim at evaluating which clinical indications are best suited for which of the two approaches. Second, to optimize a protocol for MRI examinations in deceased neonates, infants and children, third, to report the number of cases where there is a change in the ante-mortem diagnosis following post-mortem cross-sectional imaging with CT-guided biopsy, and last, to investigate the reasons why parents consent to or decline autopsy.

**Recruitment**

As routinely practiced, post mortem examinations will be offered to still- and live born infants until adolescents up to 16 years of age no matter of the manner of death was natural or non-natural cause. In non-medico legal cases parents will be approached by appropriately trained staff (consultant, experienced midwife) whether they consent to an autopsy. If parents agree, a precedent cross sectional imaging with CT-guided biopsy will be proposed. They receive an information leaflet and at least one parent has to give written consent.

Regardless of whether parents agreed to post-mortem examinations, they will be asked about their reasons for or against one of the two autopsy approaches. The person obtaining consent will note these answers in a questionnaire specifically designed for this study, addressing issues previously reported as important in post-mortem decision-making.
Post-mortem imaging

All bodies will be examined with a commercial CT (Siemens Dual Source SOMATOM®) and a 3 Tesla MRI scanner (Philips Achieva 3.0T) at the Institute of Forensic Medicine, University of Zurich. The first 20 subjects will be used to improve the imaging sequences and to optimize the robotic system allowing for CT-guided tissue sampling. These subjects will not be included in the main study. In a first step, a specialized forensic radiologist will report the MR- and CT image on to a large internet-based secure database (SecuTrial®). All diagnoses will be specified according to the international classification of diseases (ICD-10).

CT-guided tissue sampling

After cross-sectional imaging CT-guided tissue sampling will be performed by a multifunctional robotic system at the Institute of Forensic Medicine, University of Zurich. This “Virtobot System” allows for automated post-mortem biopsies and is composed of a six-axis industrial robotic arm (Stäubli TX90L; Stäubli, Freienbach, Switzerland) that has been mounted to an external axis, aligned with the CT table [28]. For performing biopsies, the Virtobot has been combined with a surgical navigation system. In every case, the same predefined organs (heart, lung, brain, liver, kidney, bowels) will be biopsied under close monitoring by a forensic pathologist specialized in paediatric forensic medicine. Specimens will be fixated in formalin and examined by an independent pathologist, who is blinded to the results of post-mortem imaging- and conventional autopsy.

Virtual autopsy – final classification

Based on cross-sectional imaging and histology, the findings will then be reclassified with regards to the likely final diagnosis and major pathological findings. An
interdisciplinary team (forensic radiologist, forensic pathologist and pathologist) blinded to conventional autopsy will have to decide, whether one or several pathological findings will be present for each organ system. This final classification based on virtual autopsy will be reported on to the same database and again specified according to the ICD-10.

**Conventional autopsy**

All conventional autopsies, providing the gold standard against which the virtual autopsy is assessed, will be performed by experienced perinatal, paediatric, and forensic pathologists according to the guidelines of the Swiss Society of Pathology [29]. The same database with independent access portals will be used to maintain blinding from virtual autopsy. For each organ system, the same categorization (likely final diagnosis and major pathological findings) will be used and specified according to the ICD-10.

**Sample size calculation and statistical analysis**

A sample size of 100 cases allows computing a 95% confidence interval of width +/-8% for the primary outcome if the true percentage of cases for which virtual autopsy correctly identifies the diagnostic category is 80%. The width of the confidence interval will decrease to +/- 6% if the true percentage is 90%, and further to +/- 4% if the true percentage is 95%. This precision is considered as sufficient to determine the quality of virtual autopsy.

The diagnoses established in both ways by the two independent teams will be compared, the autopsy results being the gold standard. The primary outcome will be the percentage of cases for which virtual autopsy correctly identifies the diagnostic
category, which will be given together with a 95% confidence interval. Sensitivity, specificity and predictive values will be given for each diagnostic category, as defined by the gold standard, again with 95% confidence interval. Because of the small sample sizes, the confidence interval by Wilson will be used throughout, as recommended in Altman et al Chapter 6 and 10 [30]. Finally, the data of the virtual autopsy will be assessed for accuracy of associated lesions, clinical usefulness of information and determination of the cause of death.

**Discussion**
Based on previous research, post-mortem MRI appears to be a promising alternative for conventional autopsy in foetuses, newborns and children. The only disadvantage of the imaging based autopsy is the fact that tissue for histopathological and microbiological examination cannot be provided resulting in a poor accuracy for MRI due to mostly infective causes in older children and adolescents.

This study protocol therefore describes the design of a new paediatric virtual autopsy study, focusing on a minimally invasive autopsy including tissue sampling with the aid of a robotic system. To the best of our knowledge, this is the first prospective study to evaluate the accuracy of such a virtual autopsy approach compared to conventional autopsy in a paediatric population.

**Trial Status**
The paediatric virtual autopsy study is not yet recruiting subjects.

**List of abbreviations**
MRI: magnetic resonance imaging

CT: computed tomography

GCP: Good Clinical Practice

ICD: International Classification of Diseases
Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
CR is responsible for the conception, design and conduction of the study. CR developed a data collection system and drafted the study protocol. CB will be responsible for minimally invasive virtual autopsy. SR will interpret the cross-sectional imaging data and report together with CB, RM, and SAB the final classification based on virtual autopsy. LS and BF provided input into the clinical aspects of the study and will be responsible for recruitment of cases. BK will give administrative support. LH will be responsible for the statistical analyses. EB will process the tissue derived from virtual autopsy and will report the results of the histological examination. PKB and RC will perform conventional autopsies. HUB has overall responsibility for the study and advised on the conception, design and conduction of the study. He critically revised the article. All authors read and approved the final manuscript.


Additional files provided with this submission:

Additional file 1: Decision_institutional_ethical_committee_virtopsy.PDF, 3385K