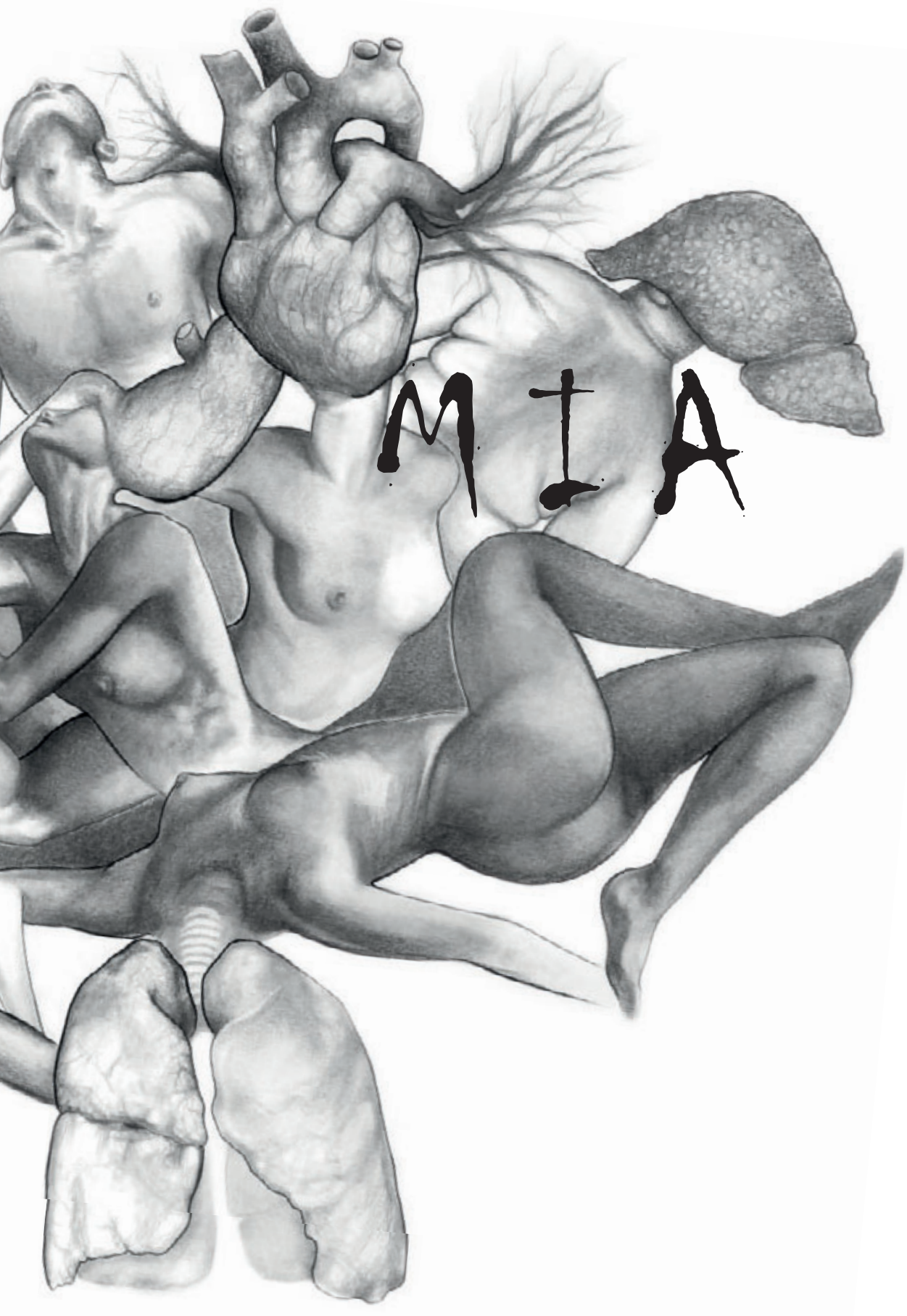
MIA

MIA

Britt M. Blokker

M I A

The Minimally Invasive Autopsy
De Minimaal Invasieve Autopsie

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. H.A.P. Pols

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 4 april 2018 om 11.30 uur

door
Brigitta Maria Blokker
geboren te Rijswijk

Erasmus University Rotterdam

Erasmus

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1

General introduction

AUTOPSY

Autopsy is also called dissection, necropsy, obduction or post-mortem examination. The word "autopsy" literally means "to see with one's own eyes", which is derived from the ancient Greek αὐτοψία (*autopsia*).[1] If physicians want to see for themselves what illnesses the deceased have suffered from, they perform this procedure that enables them to examine the corpse of the deceased both externally and internally.

In the Netherlands, consent from next-of-kin is required to perform a clinical autopsy.

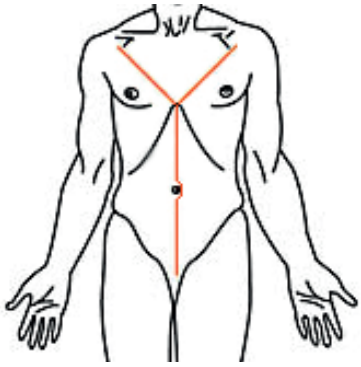
AUTOPSY TECHNIQUE

After an external examination of the deceased's body, a Y-shaped incision is made from the top of each shoulder to the centre of the chest, meeting at the sternum (breastbone) and running down to the pubic bone (figure 1).[2]

The anatomic relationships between organs and connected and surrounding structures (e.g. vessels, peritoneum) are examined in situ. Then, the organs are removed either one by one or 'en bloc' per body cavity, and further examined outside the body. After gross examination, small tissue samples from the main organs and pathologic processes are obtained for examination under the microscope, and, in some cases, for microbiological examination, toxicology or, if included in the consent by next-of-kin, for teaching and/or biomedical research.

To remove the brain an incision over the back of the head is made from ear to ear, and the scalp is folded forward, allowing removal of the skull (neurocranium) and the entire brain. For proper examination the brain has to be fixed in formalin for several weeks, which makes it impossible to put the brain back into the body after completion of the autopsy. In contrast, all organs of the torso are returned to the body cavities, unless otherwise agreed by the next-of-kin. After autopsy, the body is closed by firmly sewing the skin back together, and when (ritually) cleansed it is suitable for laying out.

Figure 1



HISTORY OF THE AUTOPSY

The autopsy marks the beginning of scientific medicine,[2-4] and, until today it supports the development of medicine by identifying and clarifying new diseases, and by providing feedback on new diagnostic and therapeutic interventions.

Back in time

The first knowledge about normal and abnormal anatomy was gained from descriptions made by prehistoric hunters, butchers and cooks, and from divination performed by animistic philosophers in the ancient Babylon (±3500 BC). The latter were believed to be able to communicate with divine powers and foretell the future using animal organs, mainly livers (figure 2).

Figure 2

In ancient Egypt, embalmers removed internal organs through small incisions from the deceased's body, in order to cleanse the body cavities. Instead of examining these organs, the Egyptians were interested in wounds and fractures and ascribed non-traumatic diseases to demons.

In the early Hellenic world, it was believed that the gods could cure patients in the Ἀσκληπιεῖον (*Asklepieion,* healing temple) from their diseases. The naturalistic philosophers of later years (±500 BC) did not believe in supernatural powers, but practiced physiology. Their study of nature included anatomy, however, it did not yet correlate anatomy with disease. At the time of Hippocrates (ca. 460-377 BC), the ἰατροί (*Iatri*, physicians) described external observations of disease (using all five senses), but autopsy on human corpses was probably not performed until the third century BC, when the Greek ruled over Egypt. In Alexandria, the so-called Μουσεῖον (*Mouseion*, museum) was introduced: a home for arts and sciences, including a library. Here, medical students were enabled to dissect bodies of criminals, and thereby learned how to distinguish normal structures from those changed by disease. It was Erasistratus (ca. 310-250 BC) who at that time discovered the association between diseases and changes in solid organs, and pointed out the relevance of autopsy.

For years to come the autopsy procedure was carried out without a clear protocol. According to the ancient documents, physicians often left many organs unexamined, because they finalized the autopsy as soon as they were convinced to have found the cause of death.

New developments started with Vesalius, who published the first book on human anatomy 'De Humani Corporis Fabrica Libri Septem' in 1543, based on his own observations taken directly from the many human dissections he performed, and founded a systematic approach for autopsy. In the eighteenth century, autopsy reports became more extensive and sophisticated. Physicians like Morgagni and Boerhaave started paying attention to the clinical history and it's correlation to autopsy findings (they realised that diseases develop over time); Bichat distinguished different kinds of human tissue through experiments and recognized how they got affected by disease. It wasn't until the nineteenth century, that the autopsy technique itself was subject to improvement: it was Prost who insisted on examining all the organs during a "complete autopsy" that required at least three hours. Later, Rokitansky pointed out how clinical practice could benefit from the knowledge gained from autopsy, and introduced the modern concepts of pathogenesis. In contrast to Rokitansky, who preferably disturbed the anatomical structures as little as possible and examined the organs in situ, it was Virchow who eviscerated all organs from the body one by one, to further dissect and examine them.

Microscopy

Van Leeuwenhoek discovered the microscope and used it to closely examine (σκοπέω/ σκοπώ, *skopeo/ skopo*) the small (μικρός, *mikros*) things, like microorganisms. Virchow was the first to apply state of the art microscopy for the examination of cells and tissues. He and other academic researchers comprehensively investigated disease processes, such as cancer, thromboembolism and inflammation, and made many contributions to the basic understanding of diseases. Over the years, the limitations of gross pathology were recognized and the added value of microscopy was generally acknowledged. Thus, with the further standardization of autopsy techniques, the protocols included macroscopy and microscopy.

CURRENT PRACTICE

In the twentieth century, medical progress and the understanding of disease, mainly depended on clinicians' observations and proving their diagnoses to be wrong or right through autopsy.

Until the present day, the conventional autopsy (CA) has proven to be a valuable tool in clinical medicine. On an individual level, by revealing the cause of death and other possibly relevant or missed diagnoses, the autopsy outcome may aid next-of-kin in their grieving process[5,6] and provide doctors with information for risk counselling and with feedback on clinical diagnoses and therapies. The importance of this feedback is borne out by the frequency of discrepancies between clinical diagnoses and post mortem findings that are still found, in spite of the advanced diagnostic techniques used in modern medicine.[7-14] These include major errors or class-I-discrepancies in up to one fourth of the cases, depending on the case mix. In the bigger picture, CA is relevant for healthcare quality control and policy making; for medical science and education; for accurate death certificates and epidemiologic databases; and for obtaining human tissue samples for laboratory research [15-27]

Attitudes toward autopsy in history

The Egyptians believed that major disfigurement of the deceased's body would prevent the deceased from entering the afterlife. Other (earlier) civilizations also had objections against autopsy, either based on religious grounds (the body must be treated with respect and buried promptly), on humanitarian and aesthetic grounds, or out of fear

that the person may still be alive after all. However, there was no formal ban on autopsy, and in 1231, Frederick II established the first law that authorized autopsy in his Holy Roman Empire.

After a low tide of autopsies in Europe during the (early) Middle Ages, objections to post-mortem investigation were more often put aside. It slowly became widely accepted that autopsies were performed, though mostly on victims of war or crimes, on criminals - to learn, show, and teach any interested parties present in the theatres (figure 3) - and, when there were hospitals, on deceased patients to compare symptoms and anatomical or pathological findings.[28,29]

Figure 3

DECLINING AUTOPSY RATE

After the 'glory days' of the autopsy, the clinical autopsy rates started to decline sometime after the Second World War, even in countries without financial restraints for autopsies and especially if consent from next-of-kin was required for autopsy.[22,23,30-33] In the Netherlands generally all non-forensic autopsies take place in hospitals, and almost all of them are performed on in-hospital deceased patients, which constitute about one third of all deceased. There has been a steady decline of autopsy rates in hospitals in the Netherlands between 1977 and 2011: from 31% to 11% in academic hospitals and from 24% to 9% in non-academic hospitals.[33]

There are many explanations for the declining autopsy rate, for example the increasing workload for pathologists, mainly due to extensive diagnostics for the living; the somewhat unclear budgeting of the autopsy, which is often hidden in the hospital budget; unwillingness among clinicians to be confronted with clinical 'failures', with the risk of lawsuits about alleged malpractice; lack of education about (the importance of) autopsy in the general medical curriculum; dissatisfaction with the quality of autopsy reports, and the long time it often takes to deliver them; few possibilities to discuss autopsy findings and gain from feedback (post-autopsy conference); poor or insufficient communication between clinicians and next-of-kin about autopsy; fear for disfigurement of the deceased's body; religious and cultural objections against autopsy; and overconfidence in the accuracy of clinically established diagnoses.[24,34-43] The latter argument is unchallenged because of the low autopsy rates[44] and readily accepted by next-of-kin from the clinician who treated their beloved one.[45]

REVIVING THE AUTOPSY

The decreasing number of post-mortems performed each year is a serious concern for healthcare quality control.[23,46] To change this trend, the communication and provided information about autopsy should be improved on many levels. In particular, the misconception should be addressed that with the advanced diagnostic techniques used in today's clinical practice, autopsy will hardly ever reveal new facts.

Rather, we should use these advanced diagnostic techniques to the advantage of the autopsy and develop less invasive, imaging-based methods,[47,48] which may help increase autopsy rates.

Post-mortem radiology

Imaging techniques have a long history in forensic pathology. Shortly after the discovery of x-rays by Roentgen, at the end of the nineteenth century, this technique was applied to locate bullets, detect fractures and identify bodies.[49] More recently, in the late seventies of the twentieth century computed tomography (CT) was used for similar purposes.[50] From the nineties on, the value of imaging techniques, including CT and magnetic resonance imaging (MRI), were increasingly recognised and applied.
Among the pioneers in the forensic field was the Virtopsy group in Bern. Their method included surface imaging methods to document injuries, CT-scans to detect bone injuries and gross pathologies and MRI-scans to identify soft tissue injuries. The Virtopsy was always followed by a conventional forensic autopsy.[51] In the Netherlands, modern cross-sectional imaging techniques, CT and MRI were increasingly used for forensic pathology from the end of the twentieth century, to complement the conventional forensic autopsy, among others to investigate child abuse.[52]
In the clinical setting, the radiological techniques were primarily used for perinatal autopsies.[53] X-rays commonly complement conventional autopsy on foetuses (babygram) or neonates, mainly for the detection of skeletal anomalies. CT-scanning also allows the examination of the body in 3D reconstructions. MRI, on the other hand, can be performed to identify pathologies of the internal organs and central nervous system. In addition, if invasive autopsy is not permitted, needle biopsies and aspiration of for example blood allow histological, microbiological and metabolic examination.[54,55]
Alongside these perinatal autopsies, alternative autopsy methods for naturally deceased adults, either non-invasive or minimally invasive, have been developed and compared to the CA. Many of the alternative methods use radiological imaging techniques, and the minimally invasive autopsy techniques often include tissue biopsies and/ or contrast-enhanced images (e.g. angiography).

In this thesis, we present what we learned from previous studies about alternative methods for the CA in non-forensic cases,[56] and how we aimed to improve our own version[57] of the Minimally Invasive Autopsy (MIA).

2

Autopsy rates in the Netherlands: 35 years of decline

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PLoS One. 2017 Jun 15;12(6):e0178200.

ABSTRACT

Objective: Although the autopsy still is a valuable tool in health statistics, healthcare quality control, medical education, and biomedical research, autopsy rates have been declining worldwide. The aim of this study was to examine trends of overall, clinical and forensic autopsy rates among adults in the Netherlands over the last four decades, and trends per sex, age (groups), and hospital type.

Methods: We performed a retrospective study covering 35 years of Dutch national death counts (1977 – 2011), the number of in-hospital deceased patients, the number of deaths due to external causes, and the proportion of autopsies performed in these populations. The effects of sex, age and hospital category were analysed by linear and logistic regression and differences were evaluated by chi-square tests.

Results: Overall autopsy rates declined by 0.3% per calendar year, clinical autopsy rates by 0.7% per calendar year (from 31.4% to 7.7%), and forensic autopsy rates did not decline. Per calendar year the fraction of in-hospital deceased patients decreased by 0.2%. Autopsy rates were highest among men and younger patients; clinical autopsy rates were highest for patients dying in academic hospitals.

Conclusions: In the Netherlands clinical autopsy rates have rapidly declined while at the same time the fraction of in-hospital deaths decreased, both contributing to the overall reduced absolute number of autopsies performed. It is important to improve awareness among both clinicians and general practitioners of the significance of the clinical autopsy.

INTRODUCTION

Background

The relevance of the clinical autopsy is well recognized; it provides bereaved relatives with information on the cause of death and clinicians with feedback on diagnosis and treatment, thus making it an important instrument for healthcare quality control.[21,22] In spite of the advanced diagnostic technologies used in modern medicine, there are still discrepancies found between clinical diagnoses and post-mortem findings[7,8,13] with a significant rate of class-I-discrepancies (major diagnoses).[10] By identifying these, the autopsy improves the accuracy of both death certificates[15] and epidemiologic databases.[19] Moreover, it contributes to medical knowledge[24] provides for evidence-based research, and is a resource for biomedical research, e.g. by procurement of normal and pathological tissues.[26] Despite these benefits, clinical autopsy rates have rapidly declined worldwide in the past decades, and alternative less invasive post-mortem methods are currently being developed to improve or replace the conventional autopsy.[56]

Several studies have shown local or national trends of autopsy rates.[22,23,32,58-61] Few studies have reported on Dutch autopsy rates[21,62] and only one study evaluated potential factors that might have influenced autopsy rates, based on a small population in the early sixties.[63]

Purpose

In this analysis of national statistics we describe the 35-year trends in the Netherlands of adult deaths, both in-hospital deceased patients and deaths due to external cause, and the clinical and forensic autopsy rates over the same period. We analysed the effects of age, sex and hospital type on the autopsy rates.

MATERIALS AND METHODS

Data collection

For each year in the period of 1977 to 2011, 35 years in all, we obtained the total number of registered adult deaths, the number of in-hospital deceased adult patients, the number of deaths due to external cause, the number of clinical and forensic autopsies performed, and if available, information on age, sex and hospital category. These variables were derived from logbooks of the Netherlands Forensic Institute (NFI),

and databases provided by Statistics Netherlands (SN, a.k.a. CBS Statline) and Dutch Hospital Data (DHD) in cooperation with Kiwa Carity's data services. The latter is a service organisation that aims to improve Dutch healthcare in various ways.

We analysed the SN databases for all registered adult deaths in the Netherlands per year, and we collected tables presenting the total number of deaths and the number of deaths due to external causes (S1 Table).
Kiwa Carity provided an anonymized set of aggregated data (S1 Table, S2 Table), including all cases of adult patients (≥18 years) deceased in Dutch hospitals, their age and gender, the type of hospital they died in (academic or non-academic), and whether or not an autopsy was performed. Compulsory forensic autopsies in the case of suspected unnatural death, as is the policy in the Netherlands, were excluded. Using the program Matlab®, we created files by year, consisting of one line per individual case. To ensure the privacy of individuals, the data were used according to the required protocol for data provided by DHD. Further ethical approval was not required for this part.
The information on performed forensic autopsies was collected from the logbooks kept by the forensic pathologists of NFI. For each case only gender and age were extracted and registered in an anonymized file. NFI has granted us ethical approval to use this file to create an overview of these forensic cases.

The emphasis of our analyses was on clinical autopsy rates. In the Netherlands, there are no extramural facilities for non-forensic autopsies. Therefore, if a person dies outside a hospital from a supposed natural cause of death, and next-of-kin ask for a post-mortem examination, the autopsy will be performed by clinical pathologists in the nearest hospital. Because this situation rarely occurs, we expect that only few cases of performed autopsies have been missed.

Data analysis

Excel® and SPSS® were used for data analysis. We calculated means, differences, ratios and percentages. Overall numbers were plotted with the exact autopsy percentages, and, to filter random noise within the subgroups and make trends visible, 4-year moving average plots were constructed. Linear regression was performed to show trends over time, logistic regression to analyse the effect of possible explanatory variables (year, sex, age and hospital type), and the chi-square test to analyse differences between academic and non-academic hospitals. To identify multiple trends within the collected

35 years, we stratified the years into three time periods of 12, 12 and 11 years (1977-1988, 1989-2000 and 2001-2011). For other analyses we created age subgroups (e.g. ±20-year groups).

RESULTS

General overview

From 1977 to 2011, 4,539,619 adults died in the Netherlands (mean: 129,703 per year, 95%CI: 110,093;142,355). The overall death counts have steadily increased with a mean of 805 per calendar year (95%CI: 640;971) and a total increase of 23.3%. The overall autopsy rates declined with 0.3% per year (Fig 1-A). Each year approximately one third of these overall deceased adults died in hospitals (mean: 44,075 per year, 95%: 36,601;48,341). The percentage of in-hospital deceased shows an overall decline of 0.2% per calendar year (95%CI: -0.003;-0.002).

On 249,178 of the in-hospital deceased patients autopsies were performed (mean: 7119 per year, 95%CI: 2,820;12,209). In 35 years, the absolute number of performed clinical autopsies decreased with an average of 4% per year, to less than a quarter of the former number; each year, 282 fewer autopsies were performed (95%CI: -295;-268). Per additional calendar year, the odds of performing an autopsy on an in-hospital deceased adult patient were reduced by 5% (95%CI: 0.950;0.950). The clinical autopsy rate decreased with a mean of 0.7% per calendar year, from 31.4% in 1977 to 7.7% in 2011 (Fig 1-B). When divided into the three time periods, we observed the steepest decline in the earliest period (1977-1988, Table 1).

Only a small number of deaths each year was due to external causes (mean: 5335, 95%CI: 4,783;6,104), over the years this number decreased with 13 per year (95%CI: -23;-3). Forensic autopsy was performed in 8.5% (95%CI: 6.4-10.6, Fig 1-C). The trend of forensic autopsy rates is not significant, but when divided into the three time periods we observed an increase followed by a decrease (Table 1).

Sex of the deceased

The mean increase of overall deaths per calendar year was 705 among women (95%CI: 600;811) and 100 among men (95%CI: 37;163). Regardless of this trend, the majority of in-hospital deceased patients (54.7%, 95%CI: 52.7-57.0) and deaths due to external causes (57.9%, 95%CI: 55.0;60.0) was always male.

Each year the majority of the clinical autopsies were performed on men. The number of performed clinical autopsies decreased with a mean of 167 per year (95%CI: -176;-157) among men and 115 (95%CI: -120;-111) among women. If an in-hospital deceased patient was male, the odds of performing an autopsy were higher by a factor of 15.4% (95%CI: 1.144;1.164). The difference between men and women was also present with respect to autopsy rates, both clinical and forensic. Clinical autopsy rates declined with 0.7% per year among men and 0.6% per year among women (Fig 2-A). When divided into the three time periods the decline was similar between the sexes (Table 2). The forensic autopsy rates, on the other hand, showed no trend among women; only the 35-year trend among men showed a small but significant increase (0.001, 95%CI: 0.000-0.001).

Age of the deceased

At least a quarter of young adults died in a hospital, this fraction of in-hospital deceased increases up to the age group of 69 years olds (44.5%) and then declines to less than 10%. A total of 249,178 clinical autopsies were performed, most at the age of 76. Until that age there is a mean increase of 152 autopsies per year of age (95%CI: 134;169), after that age the number of autopsies decreases by 467 per year of age (95%CI: -508;-426). Also, the autopsy rates were higher among patients who died at a younger age, with the highest peak at the age of 35. Until that age the autopsy rates increased with 0.2% per age year (95%CI: 0.001;0.003), and from the age 36 onwards the autopsy rates declined with 0.3% per age year (95%CI: -0.003;-0.003).

All four age groups showed a decline in performed autopsies. In absolute numbers most clinical autopsies were performed in the age group of 60 to 79. Autopsy rates, on the other hand, were highest in the younger age groups for both clinical autopsies (Fig 2-B) and forensic autopsies. Compared to the age group of 80 years and older, the odds of a clinical autopsy being performed were 2.276 (95%CI: 2.218;2.335) among the 18-39 year age group, 1.986 (95%CI: 1.959;2.014) among the 40-59 year age group, and 1.598 (95%CI: 1.582;1.614) among the 60-79 year age group. Each calendar year the clinical autopsy rates declined within the range of 0.8% (youngest group) and 0.6% (oldest group), see Table 2.

Hospital type

A minority of the in-hospital deceased patients died in an academic hospital, but the autopsy rates were always higher in academic hospitals than in non-academic hospitals (Fig 2-C and Table 3). Over the years, academic autopsy rates declined more

than non-academic autopsy rates, even when divided in the three time periods (Table 2). Compared to non-academic hospitals, the odds of an autopsy being performed in an academic hospital were 1.374 (95%CI 1.356,1.392).

DISCUSSION

Main findings

From 1977 to 2011 overall deaths increased, especially those among women and the age group of 80 years and older. The fraction of in-hospital deceased patients declined and there was a small decline of deaths due to external causes. Each year the majority of both the in-hospital deceased patients and deaths due to external causes were male. Also, more autopsies were performed on men. Both clinical and forensic autopsy rates were higher among men, and among patients who died at a young age (18 to 59 years). Over the 35-year time period there was a decline of performed clinical autopsies, and a decline in clinical autopsy rates for both sexes, all age groups, and for both hospital categories. Academic hospitals performed fewer autopsies, but had higher autopsy rates than non-academic hospitals.

Strengths and limitations of this study

We present primary results on 35 years of Dutch population-based data containing more than 4.5 million people overall, including over 1.5 million in-hospital deceased patients, and over 180 thousand deaths due to external causes. We assume that there are no missing cases, apart from those that were not officially registered with death certificates.

Of 264,450 of these cases we know that an autopsy was performed. However, it is unknown how many autopsies were performed on out-of-hospital deceased adults with a supposed natural cause of death. We assume that the number is negligible, based on publications in Dutch medical journals concerning the difference in autopsy rates between intramural and extramural diseased cases.[64] Also, from our experience we know that general practitioners or geriatricians rarely send in out-of-hospital deceased for clinical autopsy. To support this, we retrieved the numbers of autopsies performed in our own university medical centre from 2010 to 2015. We found that only 6.7% of all adult autopsies were performed on extramural cases, which correlates with the 6% reported in 1986.[65] Overall, the autopsy rates among all extramural deaths in the Netherlands are reported to be less than 1%.[65,66]

Comparison with the literature

According to the SN death counts in the Netherlands increased from 110,000 in 1977 to 136,000 in 2011, which could be explained by the overall population growth. There was also a relative increase from 7.9 per 1000 in 1977 to 8.1 per 1000 in 2011, which is possibly due to the substantially increased number of deceased women in the age group of 80 years and older. For years, the life expectancy at birth has been lower for Dutch men than for Dutch women, which must have led to an excess of women within the Dutch population. These women have eventually reached an older age, and passed away.
In 2003, the Dutch government eased budgetary constraints in the healthcare system, leading to increased healthcare delivery, including more active and life-prolonging treatments for the elderly.[67] As a result the life expectancy increased, and the increase of overall deaths ended.

A possible explanation for the overall decline of in-hospital deaths could be the shortening of in-hospital stays, that was initially due to budgetary constraints of the Dutch government[67] and is now continued by altered healthcare policy for the terminal phase of life. Ploemacher et al. suggested that patients are currently more often discharged from hospitals to receive palliative care from external facilities[68] and as a result more patients die at home or in nursing homes. The decline of in-hospital deceased could further be explained by an increase of deaths due to cancer, especially within the age groups of 60 years and older. According to Van der Wal et al. a substantial number of cancer patients (48%) died at home.[69] A factor possibly related to the excess of in-hospital deceased men (and performed autopsies), is that men more often have health problems that correlate with higher mortality rates, whereas women have health problems with a higher disease burden.[70]

As a direct result of decreasing in-hospital deaths, fewer autopsies were performed in the Netherlands. Also the autopsy rates declined, just as observed in other countries,[22,32,71] especially with increasing age of the deceased.[32,71] Among the age group of 60 to 79 years fewer autopsies were performed each year, which might be correlated with the increasing number of deaths due to cancer that is observed in that same age group. If a patient dies of cancer, the cause of death seems obvious to next-of-kin[45] and an autopsy superfluous.
At the same time, the clinician might be less eager to ask for an autopsy[43] especially if end-of-life decisions were made and euthanasia was performed. The requirements for requesting euthanasia in the Netherlands are extensive, for instance, it has to be shown that the disease is intolerable and that treatment options are lacking.[69] To support this

contention, the clinician must have documented all diagnoses and therapy options carefully, and may feel that the need for autopsy is less urgent. Hence, the decline in autopsy rates is multifactorial and cannot be explained only by fewer consents from next-of-kin. This conclusion is supported by Gaensbacher et al., who observed declining autopsy rates in Austria, where no consents are needed for clinical autopsies.[32]

Autopsy practices differ per country, for example policies on financing autopsy, the rate of forensic autopsies, sites where autopsies are performed, and the necessity of obtaining consent from next-of-kin.
Financing of the clinical autopsy is complicated.[72] Data on the exact costs per autopsy are not available; cost estimates per autopsy vary according to the number of autopsies being performed[73] and the extensiveness of the procedure.[74] At the same time gained benefits per autopsy are difficult to quantify, and, as a consequence, cost-benefits of autopsy cannot easily be determined. Due to competing business activities and scarce healthcare resources, autopsy financing appears not to be a priority of today's hospitals.[75] It is often not clear from which departmental or institutional budget the autopsy costs are, or should be, derived. The lack of a firm financial basis for autopsy services has very likely contributed to declining autopsy rates.[23] In Dutch hospitals, however, the costs for autopsy are paid off the general hospital budget. There are neither financial nor capacity constraints for clinicians or next-of-kin to have an autopsy performed, therefore, financial and capacity issues cannot explain the decline of the autopsy rate in the Netherlands.
There are also different policies for financing the medicolegal/ forensic autopsy. For example, in Denmark forensic autopsies are paid from the police budget and thus compete with other cost,[74] whereas in Finland the forensic autopsies are all payed for by the government. Even in recent years, the overall Finnish autopsy rates have been around 30%, which is explained by increasing medicolegal autopsy rates at the time when clinical autopsy rates started to decrease.

In the Netherlands, non-forensic autopsy cases with supposed natural death are carried out in general hospitals, whereas in the investigated period forensic cases were performed at NFI. In some countries, however, forensic autopsy may also be performed on cases that are not of interest to the police, such as deceased whose cause of death is classified as natural, but remains unclear.[74]
In many countries consent from next-of-kin is compulsory for a non-forensic autopsy, however in some countries, autopsy may be performed without consent (if there is a clear medical or scientific interest[32]). In some other countries, next-of-kin may object to autopsy even though consent for autopsy is not required; so-called opt out-system. In few countries autopsy has even been mandatory.[76]

Despite these and other policy differences, the general trend is declining autopsy rates. To illustrate this, we plotted national autopsy rates of Western European countries during the investigated time period, using overall autopsy rates collected from the WHO European Health Information Gateway, including deceased under 18 years of age (Fig 3, S3 Table).

Since we included adult cases only, our clinical autopsy rates are somewhat different from those reported in the literature. Fetuses and neonates are usually more often autopsied than adults.[77]
Autopsy rates were consistently higher for men than women. This phenomenon is also seen in other studies[58,78] and one could wonder why. Is it because men are usually younger than women, when they die? Do we try harder to explain the cause of death in men than that in women? Are bereaved wives more willing to give consent, than bereaved husbands?
That autopsy rates were higher in academic hospitals than in non-academic hospitals was expected.[61,73] Patients in academic hospitals generally have more complex pathologies than those in non-academic hospitals. If such patients die, it is more likely that the clinicians (and next-of-kin) feel the need for post-mortem investigation. In addition, academic doctors might have a more active approach to (further) investigation, than specialists in non-academic hospitals. Also, the teaching and research responsibilities in the academic hospital are probably in favour of autopsies.

Various other explanations for the (worldwide) declining autopsy rates have been mentioned, such as religious or cultural convictions of both doctors and next-of-kin, funeral delay, fear for mutilation of the deceased's body, absence of a defined minimum autopsy rate, cost reduction policies, pathologist's resistance to autopsy, adverse media attention[22,24,38,40] and improved pre-mortem diagnostic techniques. It is generally assumed that the decline of autopsy rates in the recent years was speeded up by the improved diagnostic value of the imaging techniques.
In our study, however, linear regression showed the largest decline of clinical autopsy rates in the first time period (1977-1988), when the two revolutionary new imaging techniques had not yet been implemented in Dutch hospitals. In the seventies ultrasound and endoscopic techniques were introduced in clinical practice, but due to restrictive governmental policies, computed tomography (CT) was introduced relatively late. Only since the late eighties all radiology departments in Dutch hospitals had a CT-scan, and at that same time magnetic resonance imaging (MRI) was introduced.[79] We hypothesize that the imaging techniques improved along with many other diagnostic techniques, and that together they may have led to the phenomenon of overconfident

clinicians[12] who underestimate the relevance of clinical autopsy. This was confirmed in a recent study, which showed that the main reason for clinicians not to request an autopsy was the assumption that the cause of death was known.[45]

To revive the interest of clinicians in the autopsy with its various significant applications is medicine, we may as well use these improved imaging techniques to our advantage. If, in the future, next-of-kin refuse conventional autopsy, clinicians could offer them alternatives, whereby state of the art imaging is the basis of a minimally invasive autopsy technique. Recently, the feasibility of both non-invasive and minimally invasive approaches, using CT and/ or MRI as alternatives for the autopsy, is being investigated.[57,80] With minimally invasive autopsy techniques tissue biopsies can be obtained for histologic examination and molecular analyses.[26]
Importantly, these alternatives may be more acceptable to populations that have fundamental problems with the conventional autopsy. Epidemiology might also benefit from introduction of imaging-based post-mortem investigation, because it makes a snapshot and a permanent record of the deceased that can be revisited as new questions arise.

CONCLUSIONS

Clinical autopsy rates have been declining rapidly, probably most of all because clinicians are convinced that the autopsy will not show anything other than what is already known through pre-mortem diagnostics. This is a major concern, because autopsies to this day disclose findings that might have changed the treatment of the patient, in addition to being an important tool for quality control, education and research in medicine. Efforts should be made to revive the interest in the clinical autopsy, in particular by introducing approaches whereby state of the art imaging is integrated with a minimally invasive autopsy technique.

TABLES

Table 1. Linear regression analyses of autopsy rates, per time period per variable

General autopsy rates		**Overall** Regression coefficient (95% CI)	**Clinical** Regression coefficient (95% CI)	**Forensic** Regression coefficient (95% CI)
Time period	**1977-1988**	-0.004 (-0.004; -0.003)	-0.012 (-0.012; -0.012)	0.001 (0.000; 0.002)
	1989-2000	-0.002 (-0.003; -0.002)	-0.006 (-0.006; -0.006)	0.003 (0.001; 0.004)
	2001-2011	-0.001 (-0.002; -0;001)	-0.003 (-0.003; -0.003)	-0.004 (-0.006; -0.003)
	1977-2011	-0.003 (-0.003; -0.002)	-0.007 (-0.007; -0.007)	0.000* (0.000; 0.001)

* not significant

Table 2. Linear regression analyses of clinical autopsy rates, per time period per variable

Clinical autopsy rates		**Male** Regression coefficient (95% CI)	**Female** Regression coefficient (95% CI)	**Academic** Regression coefficient (95% CI)	**Non-academic** Regression coefficient (95% CI)
Time period	**1977-1988**	-0.012 (-0.013; -0.012)	-0.012 (-0.012; -0.011)	-0.022 (-0.023; -0.020)	-0.011 (-0.012; -0.011)
	1989-2000	-0.006 (-0.006; -0.006)	-0.006 (-0.007; -0.006)	-0.009 (-0.010; -0.008)	-0.006 (-0.006; -0.005)
	2001-2011	-0.003 (-0.003; -0.003)	-0.003 (-0.003; -0.003)	-0.005 (-0.006; -0.004)	-0.003 (-0.003; -0.003)
	1977-2011	-0.007 (-0.007; -0.007)	-0.006 (-0.007; -0.006)	-0.009 (-0.009; -0.009)	-0.006 (-0.007; -0.006)
Clinical autopsy rates		**18-39 years** Regression coefficient (95% CI)	**40-59 years** Regression coefficient (95% CI)	**60-79 years** Regression coefficient (95% CI)	**80 years -older** Regression coefficient (95% CI)
Time period	**1977-1988**	-0.016 (-0.019; -0.014)	-0.014 (-0.015; -0.013)	-0.012 (-0.012; -0.011)	-0.009 (-0.010; -0.009)
	1989-2000	-0.005 (-0.007; -0.003)	-0.005 (-0.006; -0.005)	-0.006 (-0.006; -0.006)	-0.006 (-0.006; -0.006)
	2001-2011	-0.007 (-0.010; -0.005)	-0.003 (-0.004; -0.002)	-0.002 (-0.003; -0.002)	-0.003 (-0.003; -0.002)
	1977-2011	-0.008 (-0.008; -0.007)	-0.007 (-0.007; -0.007)	-0.007 (-0.007; -0.007)	-0.006 (-0.006; -0.006)

Table 3. In-hospital deceased patients, performed autopsies and clinical autopsy rates per hospital category per time period

Clinical autopsy rates		**Academic**			**Non-academic**		
		Deceased	Autopsies	Rate	Deceased	Autopsies	Rate
Time period	**1977-1988***	46357	14524	31.33%	472431	111184	23.53%
	1989-2000*	57816	11349	19.63%	504567	69790	13.83%
	2001-2011*	52474	6001	11.44%	408984	36330	8.88%
	1977-2011*	156647	31874	20.35%	1385982	217304	15.68%

* Difference per time period P<0.001 in Chi Square-test

FIGURES

Figure 1-A. Overall deaths and autopsy rates in the Netherlands per year.

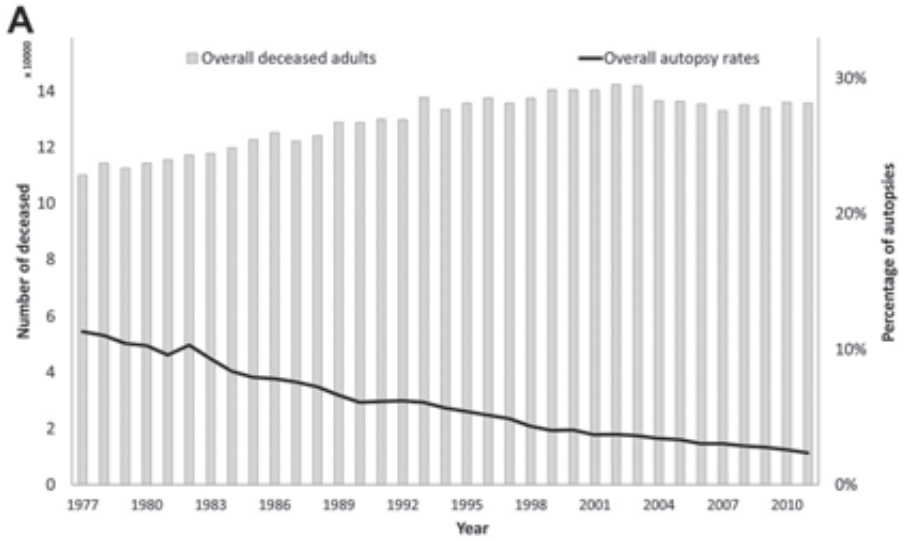


Figure 1-B. Clinical deaths and autopsy rates in the Netherlands per year.

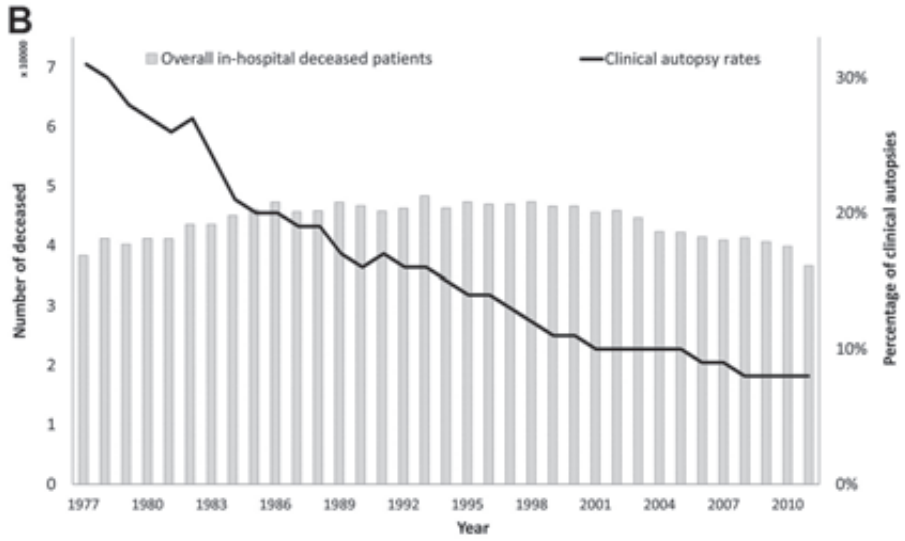


Figure 1-C. Forensic deaths and autopsy rates in the Netherlands per year.

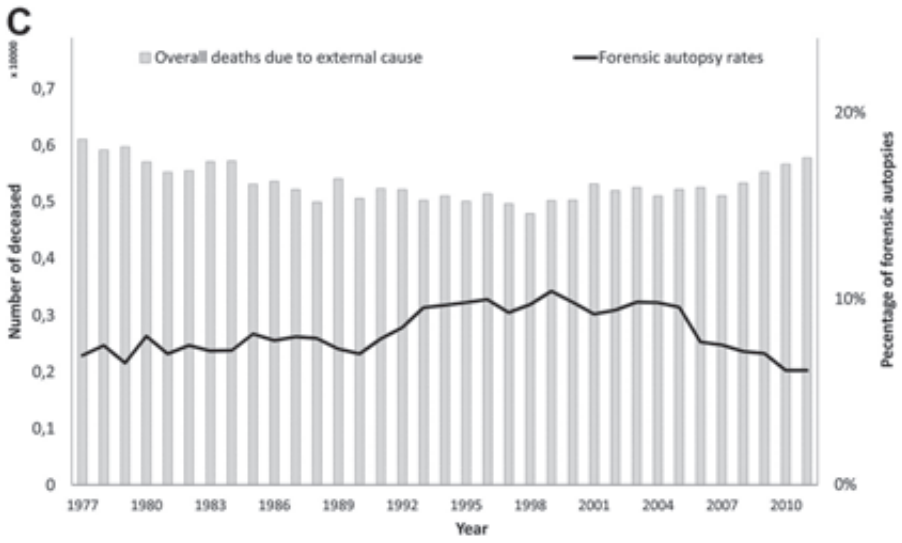


Figure 2-A. 4-year moving averages of clinical autopsy rates per sex.

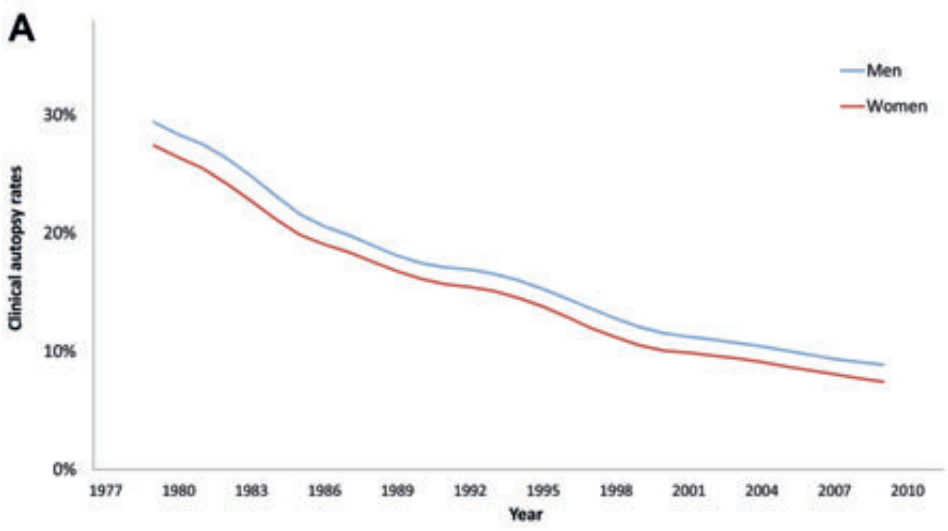


Figure 2-B. 4-year moving averages of clinical autopsy rates per age group.

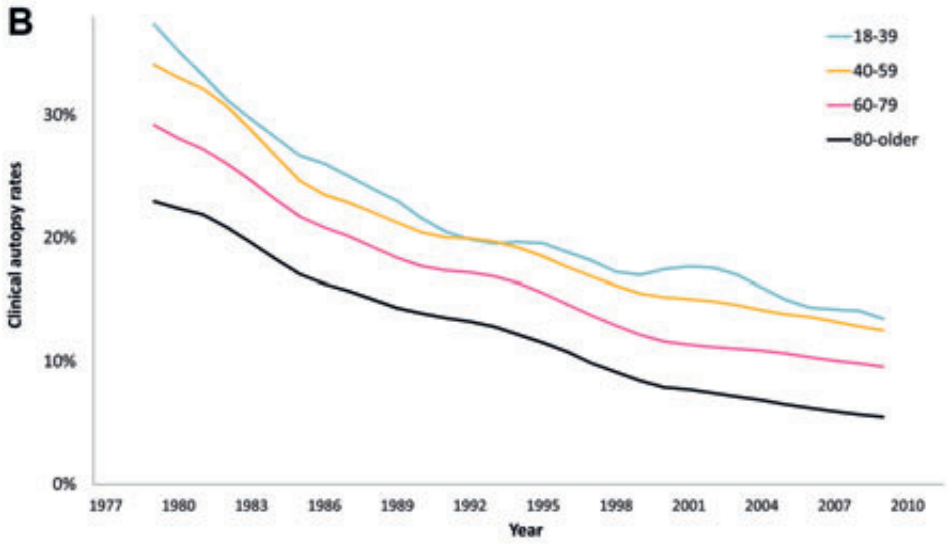


Figure 2-C. 4-year moving averages of clinical autopsy rates per hospital category.

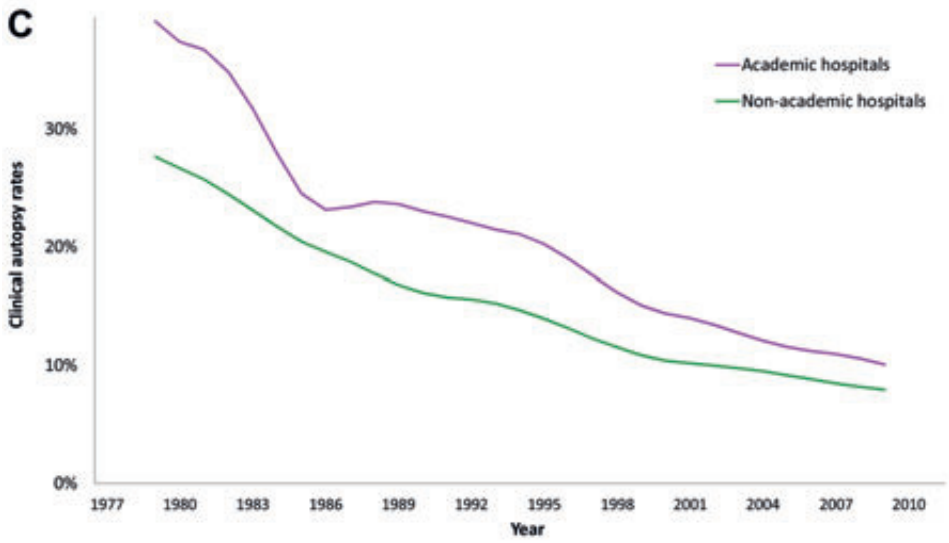
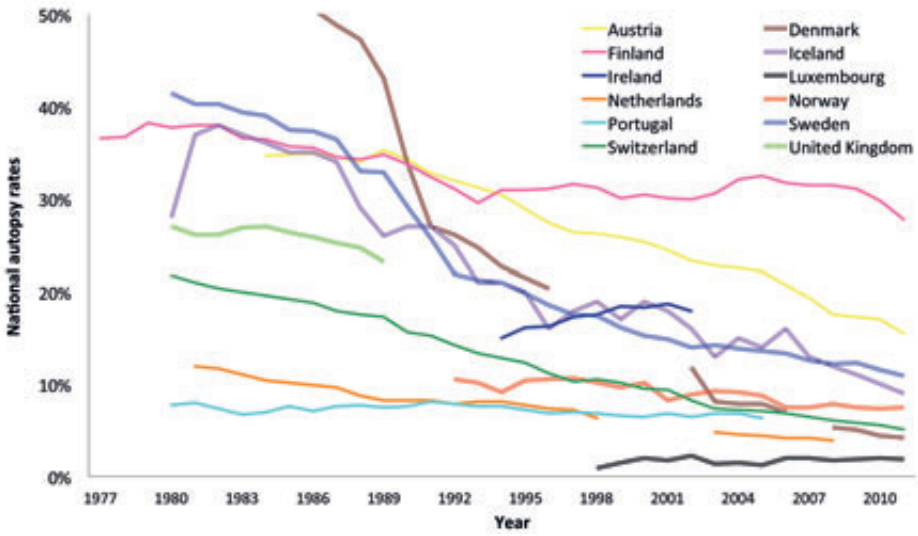


Figure 3. National autopsy rates of Western European countries according to the World Health Organization (European Health Information Gateway)



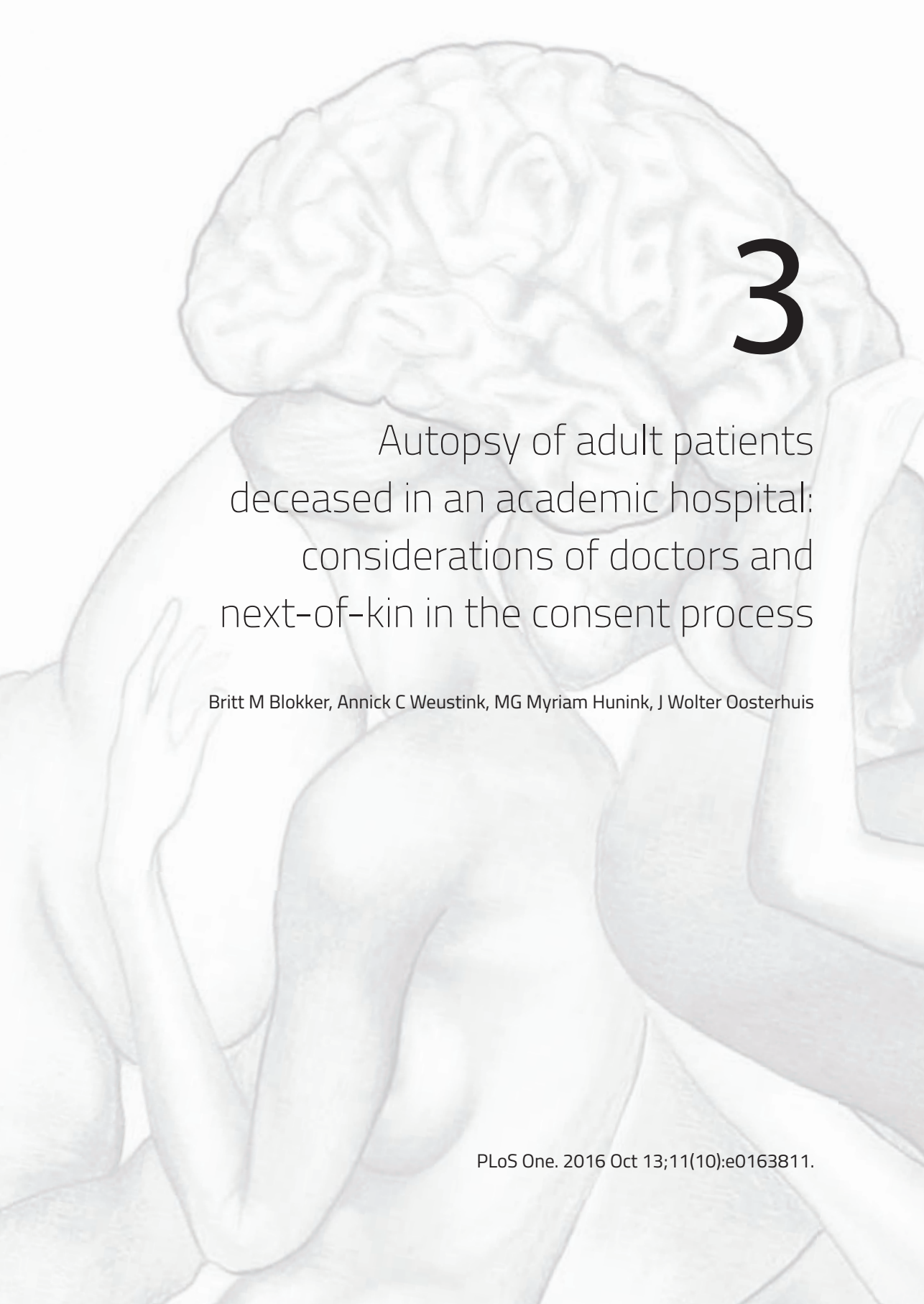
APPENDICES

Not included in this thesis:

S1 Table. Overview of cases in SN and DHD databases

S2 Table. SPSS database of all individual cases provided by Dutch Hospital Data (DHD) in cooperation with Kiwa Carity's data services

S3 Table. Overall autopsy rates collected from the WHO European Health Information Gateway (https://gateway.euro.who.int/en/indicators/hfa-indicators/hfa_545-6410-autopsy-rate-for-all-deaths/)



3

Autopsy of adult patients deceased in an academic hospital: considerations of doctors and next-of-kin in the consent process

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PLoS One. 2016 Oct 13;11(10):e0163811.

ABSTRACT

Introduction: Hospital autopsies, vanishing worldwide, need to be requested by clinicians and consented to by next-of-kin. The aim of this prospective observational study was to examine how often and why clinicians do not request an autopsy, and for what reasons next-of-kin allow, or refuse it.

Methods: Clinicians at the Erasmus University Medical Centre were asked to complete a questionnaire when an adult patient had died. Questionnaires on 1000 consecutive naturally deceased adults were collected. If possible, missing data in the questionnaires were retrieved from the electronic patient record.

Results: Data from 958 (96%) questionnaires was available for analysis. In 167/958 (17.4%) cases clinicians did not request an autopsy, and in 641/791 (81.0%) cases next-of-kin did not give consent. The most important reason for both clinicians (51.5%) and next-of-kin (51.0%) to not request or consent to an autopsy was an assumed known cause of death. Their second reason was that the deceased had gone through a long illness (9.6% and 29.5%). The third reason for next-of-kin was mutilation of the deceased's body by the autopsy procedure (16.1%). Autopsy rates were highest among patients aged 30-39 years, Europeans, suddenly and/or unexpectedly deceased patients, and tissue and/or organ donors. The intensive care and emergency units achieved the highest autopsy rates, and surgical wards the lowest.

Conclusion: The main reason for not requesting or allowing an autopsy is the assumption that the cause of death is known. This is a dangerous premise, because it is a self-fulfilling prophecy. Clinicians should be aware, and communicate with the next-of-kin, that autopsies not infrequently disclose unexpected findings, which might have changed patient management.

Mutilation of the deceased's body seems a minor consideration of next-of-kin, though how it really affects autopsy rates, should be studied by offering minimally or non-invasive autopsy methods.

INTRODUCTION

Background

Autopsies on in-hospital deceased patients are performed to confirm, revise or identify the cause of death and relevant pathology, in order to provide clinicians with appropriate feedback on diagnosis and treatment. Despite the use of advanced diagnostic technologies in modern medicine, autopsies still reveal major diagnostic errors.[10,13] Although the clinical autopsy thus remains an important healthcare quality control measure, the last 30 – 40 years have witnessed a worldwide decline of clinical autopsies.[22,23,32] Particularly in developed countries, with traditionally high autopsy rates, where financial and technical resources are available.

For a clinical autopsy, consent from next-of-kin is compulsory in most countries. The reluctance of next-of-kin to consent to autopsy, for example due to fear of mutilation of the body or concerns about organ retention of their loved ones,[24,38,40] may be one explanation for low autopsy rates. Moreover, there seems to be a declining interest in autopsies among both clinicians and pathologists.[43] Although many clinicians still recognize the importance and benefits of autopsies,[41,81] in practice they find it difficult to request consent for autopsy, and often do not ask for it.[30,81] In such circumstances, the next-of-kin will rarely consider the possibility of an autopsy.[22]

Purpose

The aim of this prospective observational study is to examine how often and why clinicians do not request an autopsy, and how often and for what reasons the next-of-kin allow or refuse it. We investigate the correlations between autopsy rate and certain patient characteristics and clinical aspects.

MATERIALS AND METHODS

Study population and study design

For this prospective observational study, a survey was carried out at the Erasmus University Medical Centre, the tertiary referral centre, with around 1200 beds, for 3 to 4 million people in the Southwestern part of the Netherlands. Clinicians were asked to fill in a questionnaire about the consent process in their conversation with the next-of-kin subsequent to the death of an adult patient. According to the policy at Erasmus MC they should always offer the next-of-kin the possibility of an autopsy. For the purpose of our study, we had them ask the next-of-kin about their reasons for either giving or refusing consent to an autopsy in the ensuing conversation. It was deemed unethical to confront

the bereaved with a separate questionnaire on this matter, which they had to fill out themselves immediately after the loss of a loved one. Thus the next-of-kin were not aware that their answers to questions of the doctor were collected to investigate their reasons for allowing or refusing an autopsy. This approach was considered acceptable because the questionnaire (S1 Fig), designed in consultation with the clinicians, was only meant for guidance and documentation of the conversation that clinicians at Erasmus MC normally have with next-of-kin upon demise of a patient.
As alluded to above, before starting the survey, we had some clinicians test the preliminary version of the questionnaire, which we adjusted according to their comments, before finalizing and implementing it. To boost clinicians' compliance, we presented the study at their research meetings, explaining them the purpose of the study, and how to they should use the questionnaire to document their conversation with the next-of-kin. During the survey we reminded them of the study, by giving a second round of presentations. Also, as a standing reminder for the clinicians, we had the questionnaires added to the compulsory forms to be completed upon demise of a patient.
From January 2012 to April 2013, questionnaires were collected from 1000 consecutive cases.
Only patients who had died in-hospital from a supposed natural cause of death were eligible. Excluded were all deceased under age 18, those who underwent euthanasia, and victims of traffic accidents and crime. Case inclusion was based on the mortuary logbook, in which all in-hospital deceased patients are registered. As mentioned consent from next-of-kin for this study was not obtained, therefore, all patient information was anonymized and de-identified prior to analysis. Approval of the Erasmus MC Institutional Review Board and Ethics Committee was obtained for this purely observational study.

Data collection

For each case we collected information on the consent process, the patients' characteristics and clinical factors. The information provided in the questionnaires was checked, and if possible, missing data was retrieved from the electronic patient record (EPR). If nonetheless the information was insufficient, or unclear, the clinicians were contacted as soon as possible. In general they appeared very co-operative in providing the missing data.

A number of potentially relevant variables, partially based on the literature,[32] was selected for registration in this study: patient characteristics (sex, age, ethnicity, religion, marital status) and clinical aspects, such as being a donor, the ward where the

patient died, the way of dying (an unexpected or sudden death/ death after being ill for a longer period of time, a so called "long illness"), and who decided on consent for autopsy (partner/relatives/non-relatives).
Outcome measures were defined as: consent for autopsy requested (yes/no); consent for autopsy given (yes/no); the motivation for either decision; autopsy performed (yes/no). An autopsy had at least to include examination of thorax and abdomen.
Multiple-choice questions, based on the literature,[30,38,40,41,81] were used to trace the considerations behind the decisions of clinicians and next-of-kin. Per multiple-choice question one or more motives could be given. A final, open question for "other motives" gave the option to enter any motives not yet addressed.
If a case record in the EPR mentioned "*autopsy not permitted*", this was interpreted as autopsy consent having been requested by clinicians and not given by next-of-kin. In such cases the motives of next-of-kin remained unknown. If the case record said "*no autopsy*", or if autopsy was not mentioned at all, it remained unknown whether or not consent was requested, and whether next-of-kin had been given the chance to consider autopsy.

Data analysis and presentation

All information obtained from the questionnaires and the EPR was entered in an SPSS database. Missing variables within a case were scored as 'unknown', and included in the analyses.
Autopsy rates were calculated for all cases combined, and for specific subgroups. Per clinical ward the total number of deceased, the number of autopsies requested, and the number of given consents were presented graphically.
Cases were not eligible for further analyses if all outcome variables on decisions and considerations concerning autopsy consent were missing. Per subgroup in the consent process the percentage of given motives was plotted.
Patient characteristics and clinical aspect outcomes were cross-tabulated. To this end they were sorted into three groups: the numbers of autopsies not requested, the number of autopsies requested but not performed (including two restricted autopsies, and one case in which family abroad could not finalize the consent by signing the consent form), and the number of autopsies requested and performed. For each variable the distribution of cases within these three groups was analysed by Chi Square tests. If necessary subgroups were combined to meet the criteria for a valid Chi Square test: 80% of the cells in the table should have expected frequencies greater than five, and all cells should have expected frequencies greater than one.

RESULTS

Overall

In 958 of the 1000 cases the information gained from the collected questionnaires and the EPR was eligible for our analyses. In 873 of the 958 (91.1%) eligible cases the clinicians had filled in the questionnaire, in the 85 (8.9%) remaining cases the information on the consent process could be retrieved from the EPR. In 167 cases (17.4%) of these the clinician reported not to have requested consent for autopsy, and in 147 (18.6%) of the remaining 791 cases the next-of-kin consented to an autopsy including at least thorax and abdomen (see Fig 1), resulting in an overall autopsy rate of 14.7%.

Autopsy percentages and distribution of cases

Among the 1000 cases, the highest overall autopsy rates measured per variable were 16.1% among women, 20.1% among deceased in the age group of 60-69 years old, 18.8% among deceased who had never been married, 16.7% among Europeans, 20.0% among Catholics, 15.2% among the sudden and/or unexpected deaths, and 40.0% among organ donors.

Considering only the 958 cases with information about the consent process, the measured autopsy rates were slightly different. Table 1 shows these cases and their distribution across the outcome measures concerning autopsy request and consent, per patient characteristic and clinical aspect.

The measured autopsy rates derived from this table are still highest among all the subgroups mentioned above, apart from the subgroup of ages. The autopsy rates in the subgroup of 30-39 years of age are the highest with 22.2%. The Chi Square tests showed that the outcomes of the consent process were unequally distributed over some of the variables.

The distribution of all 1000 cases per ward is shown in Fig 2. According to the Chi Square test, the outcome measures (autopsy not requested, autopsy not performed, autopsy performed) within the 958 cases were unequally distributed across the different wards ($P<0.001$, $df=18$).

Motives for decisions on autopsy consent

The main motive of clinicians to not request autopsy was a '*supposed known cause of death*' (Fig 3-A). This motive was mentioned in 86 of the 167 (51.5%) cases. Their second motive, '*a long illness after which an autopsy would be too much to request*' was mentioned in only 16 (9.6%) cases, in nine (5.4%) it was combined with the first motive. In 15 (9.0%) cases clinicians did not request an autopsy because of '*their expectation not to get consent from next-of-kin*'.

In 12 (7.2%) cases, which were not victims of traffic accidents, the conversation with next-of-kin was done by forensic doctors, who had been consulted for legal reasons. Being externally employed, they did not know about the autopsy policy in our institute and therefore did not ask permission for clinical autopsy. As motives for not requesting autopsy in these cases, we scored *'other'* and *'not the right person to ask permission'* for autopsy.

The two main motives of next-of-kin to not give consent for autopsy were similar to those of the clinicians (Fig 3-B). A '*supposed known cause of death*' was mentioned in 327 of the 641 (51.0%) cases, and '*a long illness whereby the deceased had suffered enough'* was mentioned in 189 (29.5%) cases. A combination of these two motives (with or without another motive) was reported in 70 cases (10.9%). Their third motive, '*fear of mutilation of the deceased's body'*, was mentioned in 103 cases (16.1%). The combination of '*fear of mutilation*' and a 's*upposed known cause of death*' was reported in 42 (6.6%) cases, and the combination of '*a long illness*' and '*fear of mutilation*' in 32 (5.0%) cases.
'*To find out about the cause of death*' was the most important motive for next-of-kin to give consent for autopsy (Fig 3-C). It was reported in 124 of the 150 cases (82.7%), followed by 42 (28.0%) cases in which next-of-kin wanted '*to find out if the deceased had any other disease'*. In 35 (23.3%) cases both of these motives were reported. In 40 (26.7%) cases the next-of-kin had decided to give consent for autopsy, because the clinician had requested it and/ or advised to have an autopsy performed.

DISCUSSION

Main findings

Autopsy rates were the highest among patients who had died suddenly and/or unexpectedly, were on an intensive care unit, 30 to 39 years, European, or a donor. The main motive for clinicians to not request an autopsy, and for next-of-kin to not consent to it, was the assumption that '*the cause of death was known'*. Their second motive was '*a long illness'*, whereby clinicians found an autopsy too much to request and next-of-kin found that the patient had suffered enough. A third concern for next-of-kin was '*fear of mutilation of the deceased's body'*.

Limitations of this study

The study was conducted in a single academic institution where autopsies are encouraged to the extent that physicians should always offer the next-of-kin the possibility of an autopsy, and where there are no financial restraints for clinicians to

request an autopsy. Therefore, the results may not directly apply to other hospitals with different autopsy policies. Nevertheless, the results are meaningful and in our view more generally applicable. Precisely because the study was carried out under conditions without financial and technical restraints it could trace considerations related to substance that may explain the present low autopsy rates. It is likely that where conditions and policies are less favourable towards autopsies, clinicians by similar considerations will feel even more justified to not pursue an autopsy.

In this survey the clinicians reported on the consent process in the final conversation they had with next-of-kin. The risk that this self-reporting method might introduce a bias towards desired answers was accepted, because it was for practical and ethical reasons, addressed under "Materials and methods" in the paragraph "Study population and study design", the only way to get the sought-after information. Assuming that the clinicians had always reported decisions in the consent process truthfully, we may conclude that they requested consent for autopsy in most cases (82.6%). In contrast, Burton and colleagues[82] found that consent for autopsy was requested in only 6.2% of eligible cases. In their study design, they did not investigate why clinicians did or did not request consent, because it might have introduced a bias. We believe that our results may indeed have been positively biased by our more extensive questioning and meticulous follow-up of the questionnaires, and also by the autopsy policy at our institute.

Among the patient characteristics, the Chi Square test did not show significant differences between religions, probably due to the high number of unknowns (63.4%). Probably the clinicians were reluctant to ask about religion, although the questionnaire included this item. Religion was more often reported in the EPR of patients who had suffered a long illness, than of those who had died suddenly and/or unexpectedly (respectively in 59% and 41%).

We were only able to evaluate univariable associations. Ideally, possible associations between variables and outcomes are evaluated with multivariable regression analyses, but to achieve a reasonable power for these analyses many more cases would have been necessary.

Theoretical explanations and comparison with the literature

The overall autopsy rates on surgical and neurological wards were under 10%, and those of the ICUs and the emergency room above 20%. In Sheffield, UK, autopsy rates were reported to be below 10% for many specialties, including neurology and neurosurgery, but 11.6% for general surgery.[82] In Belfast, UK, the worst decline in autopsy rates was

observed for surgical wards and ICUs, resulting in rates below 10%, whereas autopsy rates for neurosciences remained above 20%.[30] Apparently, attitudes and approaches of clinicians toward autopsy differ per specialty and hospital.
Several patient variables seem to influence the chance of an autopsy being requested and performed. Comparable to other studies[77,83] autopsy rates were higher in younger patients, lowest in the age group of 80-99, and similar between the sexes. In contrast to another study[32] autopsy rates appeared not to be different depending on marital status or religion. Religious objections and concerns about mutilation have been described in several studies.[38,40,41,81] Especially in Islam, removal of organs or disfigurement of the deceased's body is generally forbidden.[84] In our cohort, not a single autopsy was performed on a deceased patient who was known to be a Muslim. In 48.7% of these cases next-of-kin had religious motives for refusing autopsy, and in 2.6% they feared of mutilation, compared to 4.8% and 13.8%, respectively, among known Christians.
Some of the considerations to not request or consent to autopsy should be addressed in order to improve autopsy rates. In this study '*inadequate knowledge about the autopsy procedure keeping clinicians from requesting consent*'[81] was mentioned in only a single case, complex consent forms were not mentioned as discouraging, neither were a decreased quality of the autopsy procedure or delay of the final autopsy report.[24,30,75,81]

In several cases both next-of-kin and clinicians mentioned that '*the deceased had suffered enough*'[38,41,85] which correlates to the lower autopsy rate we found among patients who died after a long illness. Perhaps fear of the discovery of misdiagnoses or treatment errors[30,38,75,81] and the risk of malpractice suits[39] kept clinicians from requesting an autopsy in such cases. Or, more likely, both clinicians and next-of-kin had fewer unanswered questions than in cases of sudden death.
In general, clinicians tend to overestimate the reliability of advanced diagnostic technologies and therefore underestimate the value of autopsy.[22,30 ,32,44] They assume that '*the cause of death is known*', and may be unaware of the fact that there are still discrepancies found between premortem diagnoses and diagnoses found at autopsy.[7-10,13,86] If clinicians, when discussing the possibility of autopsy, tell the next-of-kin that the cause of death is already known and do not explain how or why an autopsy could still be of value, the next-of-kin will probably not consent to autopsy.[87]
Improved knowledge and confidence will enable clinicians to ignore their '*expectation not to get consent from next-of-kin*' and to always request consent for autopsy properly, or even motivate next-of-kin to have an autopsy performed. As a result, the next-of-kin are probably more willing to give consent.[82,83]

Improving the provided information about autopsies by clinicians and in the media may positively influence the attitude towards autopsy, and next-of-kin's willingness to consent to autopsy. On a professional level, dedicated information forms could support clinicians' requests for autopsy, especially if next-of-kin want to know what will be visible after an autopsy and whether they will be able to ritually prepare the deceased's body for the funeral.

From a different angle, changing the conventional, invasive autopsy technique may be the remedy for next-of-kin's concerns about '*mutilation of the deceased's body*'. Nowadays, non-invasive and minimally invasive autopsy methods are being developed for adults[56,80] fetuses, and children.[88] The minimally invasive methods include post-mortem angiography and/or tissue biopsies, suitable for histology and/ or molecular diagnostics.[26] Higher autopsy rates may be achieved with these alternatives[89] although our study suggests a minor effect in view of the on average low percentage of next-of-kin refusing autopsy because of '*fear of mutilation of the deceased's body*'. However in certain ethnical or religious subgroups non-invasive or minimally invasive procedures might significantly increase the acceptance of post-mortem investigation.

CONCLUSIONS

Our study is the first to report that the main reason for not requesting or allowing an autopsy is the assumption that the cause of death is known. This is a dangerous premise because it is a self-fulfilling prophecy, and it ignores the value of the autopsy as a tool for quality control in medicine. Clinicians should be reminded that autopsies still disclose unexpected findings, which are significant for future patients.

Remarkably, mutilation of the body of the deceased seems a minor consideration of the next- of-kin, suggesting that minimally or non-invasive alternatives for the autopsy might not significantly alter autopsy rates. However, only if these alternatives are really offered will it be possible to study how they will affect autopsy rates in particular among populations with fundamental objections against the conventional autopsy, which thereby miss the benefits of post-mortem investigation.

ACKNOWLEDGEMENTS

Thanks to Jaap Bongers, Jaap Slooff and the other mortuary staff, for always checking if the questionnaire was filled in, and, if not so, asking clinicians to complete them.
Thanks to Tip Stille for assisting in the registration of the questionnaires and endeavoring to collect as many of the required information as possible, by contacting clinicians (repeatedly) and searching the EPR.
Thanks to the clinicians and the next-of-kin, for their willingness to participate in this study.

TABLE

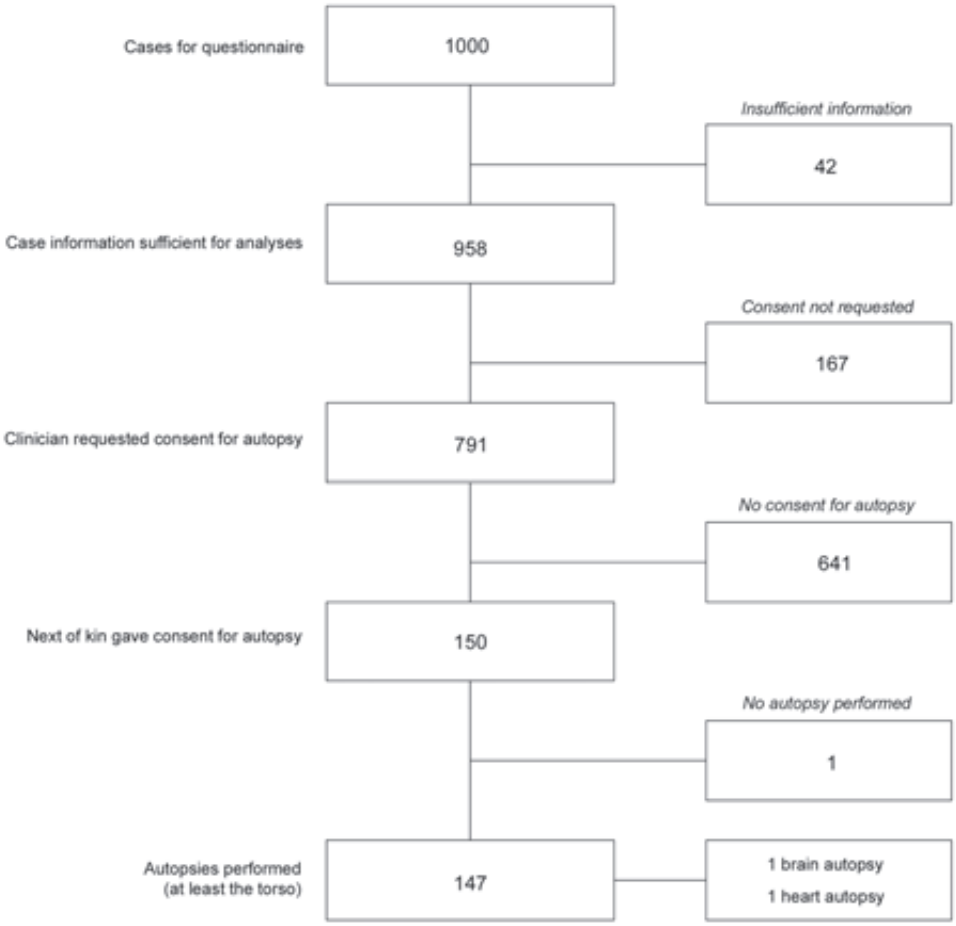
Table 1. Available patient characteristics and clinical aspects

	Autopsy rate	Autopsy torso performed	Autopsy torso not performed	Autopsy not requested	P-value X^2-test
	N = 958	N = 147	N = 644	N = 167	(df)
Sex					
male	14.5%	85	399	102	P = 0.650 (2)
female	16.7%	62	245	65	
Age group					
18-29 years	8.3%	2	13	9	
30-39 years	22.2%	6	15	6	
40-49 years	20.6%	14	40	14	P = 0.004* (12)
50-59 years	14.2%	24	116	29	
60-69 years	20.9%	58	178	41	
70-79 years	13.9%	32	162	36	
80-99 years	6.7%	11	120	32	
Marital status/ Partner					
Not/ Never married	19.4%	27	84	28	
Partner	15.5%	9	37	12	
Married	16.3%	92	385	87	P = 0.328 (10)
Widow(er)	11.1%	6	39	9	
Divorced	10.0%	3	21	6	
Not registered/ Unknown	8.8%	10	78	25	
Ethnicity					
European	17.4%	137	524	127	
Dutch Antilles, Aruba and Suriname	12.2%	6	35	8	P = 0.005* (8)
Arabic	0%	0	25	7	
Asian	7.7%	2	17	7	
Other/ Unknown	3.2%	2	43	18	
Religion					
Christian	16.8%	28	116	23	
Muslim	0%	0	28	11	P = 0.080* (8)
Other	7.1%	1	10	3	
None	18.3%	24	90	17	
Unknown	15.5%	94	400	113	
Way of dying					
Sudden/ Unexpected	15.8%	75	302	97	P=0.033 (2)
Long illness	14.9%	72	342	70	
Donation					
No donation	14.3%	129	620	152	P <0.001* (2)
Any donation	31.6%	18	24	15	

* Expected frequencies did initially not meet criteria for valid Chi Square test, significance level was similar with combined subgroups

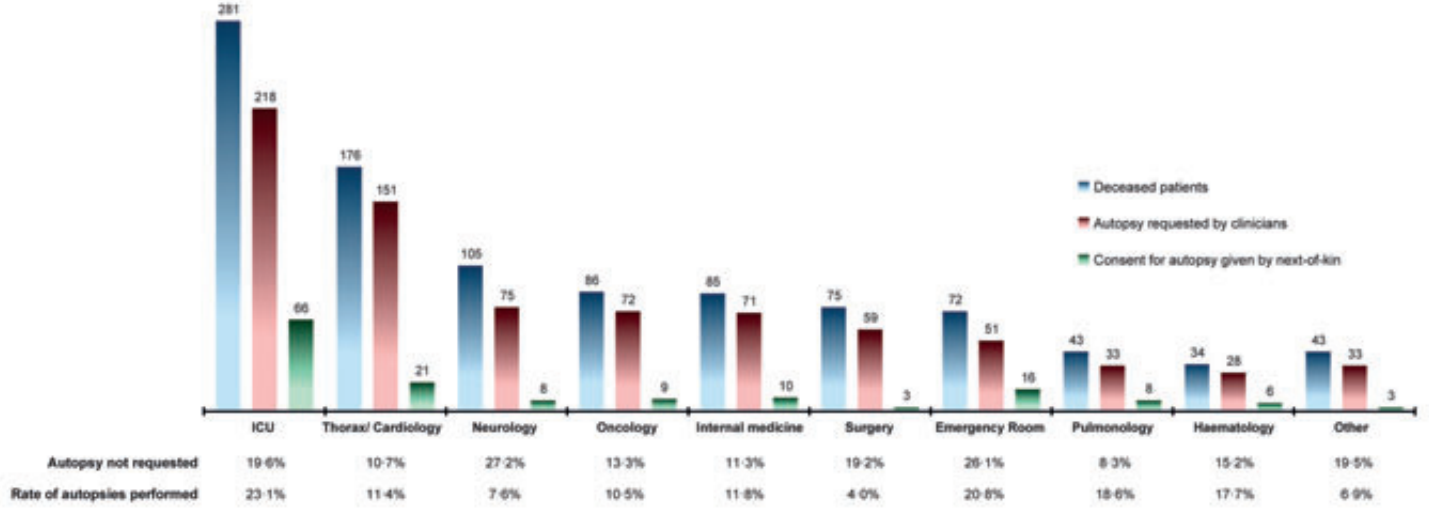
FIGURES

Figure 1. Flowchart survey



Legend: Based on the mortuary logbook 1000 consecutive cases of adult patients who had died in our academic hospital were included in this prospective observational study using a questionnaire. Information was deemed insufficient for further analyses if the clinician had neither reported in the questionnaire, nor in the electronic patient record, whether or not they had discussed autopsy with the next-of-kin and requested consent. Three consent procedures had to be discarded: two on restricted autopsies, and one because the next-of-kin were unable to sign the consent form.

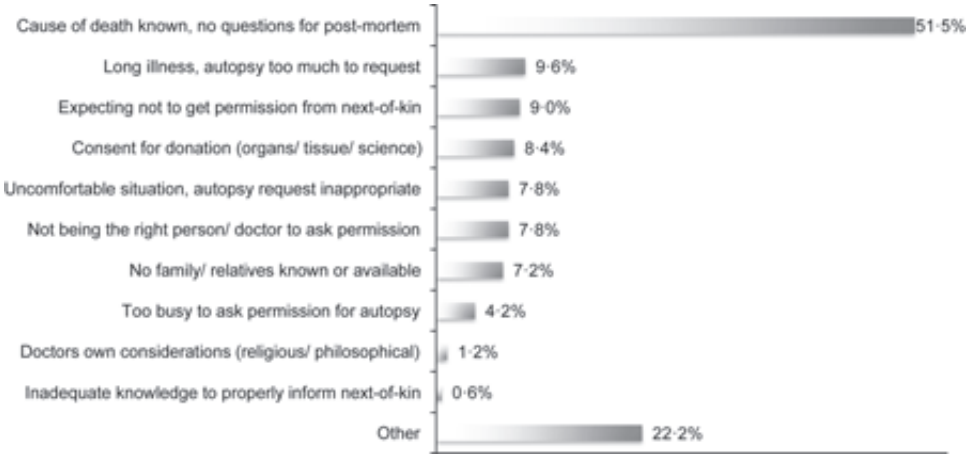
Figure 2. Autopsy requests and consents per hospital ward.



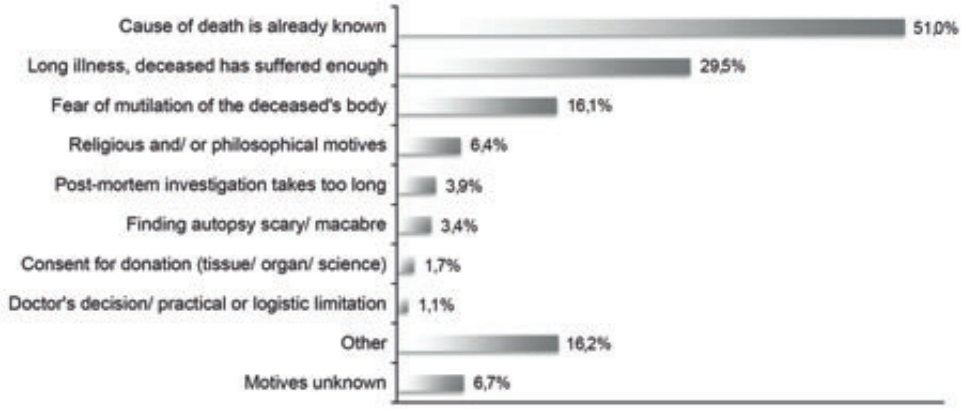
Legend: For some of the deceased patients it is unknown whether the clinicians had requested consent for autopsy: from the Intensive Care Units (ICU) 10, Thoracic surgery/Cardiology and its ICU 7, Neurology/Neurosurgery 2, Medical and Surgical Oncology 3, Internal Medicine 5, Surgery (general and all subspecialties) 2, the Emergency Room 3, Pulmonology 7, Haematology (including haemato-oncology) 1, and the other wards 2.
For the overall rates of performed autopsies per ward we divided the number of autopsies actually performed by the total number of deceased patients. Autopsies had to include at least examination of thorax and abdomen. Two autopsies, respectively from the ICU and Cardiology were restricted to a single organ. One autopsy on a case of the emergency room was cancelled, because the next-of-kin were unable to sign the consent form.

Figure 3. Considerations behind the decisions made in the consent process.

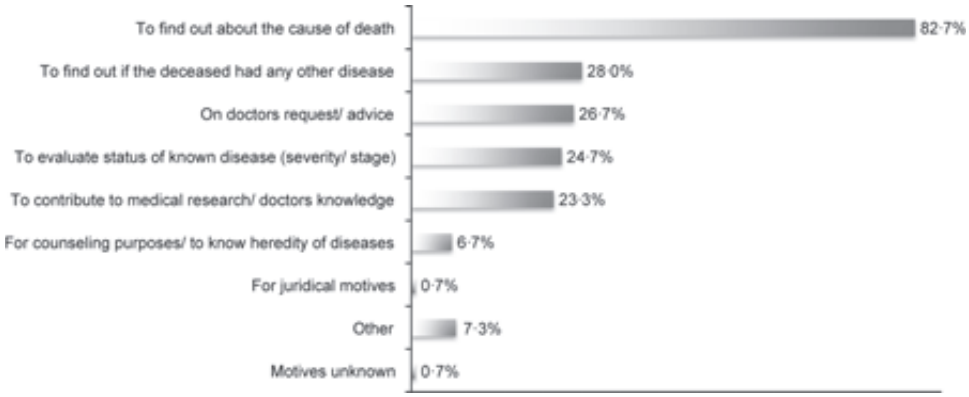
A. Clinician's motives to not request consent for autopsy (N = 167).



B. Next-of-kin's motives to not give consent for autopsy (N = 641).



C. Next-of-kin's motives to give consent for autopsy (N = 150).



Legend: Motives of clinicians and next-of-kin were investigated using a questionnaire with multiple-choice questions and a slot for free text. Per multiple-choice question one or more motives could be given

APPENDICES

Not included in this thesis:

S1 Figure. Questionnaire translated into English
S2 Table. Database in Excel

4

Non-invasive or minimally invasive autopsy compared to conventional autopsy of suspected natural deaths in adults: a systematic review

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Eur Radiol. 2016 Apr;26(4):1159-79.
Epub 2015 Jul 27. Review.

ABSTRACT

Objectives: Autopsies are used for healthcare quality control and improving medical knowledge. Because autopsy rates are declining worldwide, various non-invasive or minimally invasive autopsy methods are now being developed. To investigate whether these might replace the invasive autopsies conventionally performed in naturally deceased adults, we systematically reviewed original prospective validation studies.

Materials and methods: We searched six databases. Two reviewers independently selected articles and extracted data. Methods and patient groups were too heterogeneous for meaningful meta-analysis of outcomes.

Results: Sixteen of 1538 articles met our inclusion criteria. Eight studies used a blinded comparison; ten included less than 30 appropriate cases. Thirteen studies used radiological imaging (seven dealt solely with non-invasive procedures), two thoracoscopy and laparoscopy, and one sampling without imaging. Combining CT and MR was the best non-invasive method (agreement for cause of death: 70%, 95%CI: 62.6; 76.4), but minimally invasive methods surpassed non-invasive methods. The highest sensitivity for cause of death (90.9%, 95%CI: 74.5; 97.6, suspected duplicates excluded) was achieved in recent studies combining CT, CT-angiography and biopsies.

Conclusion: Minimally invasive autopsies including biopsies performed best. To establish a feasible alternative to conventional autopsy and to increase consent to post-mortem investigations, further research in larger study groups is needed.

INTRODUCTION

Current problem and background

Autopsy is an age-old method for identifying the underlying pathology leading to death, and/ or for detecting unnatural deaths. It is an important tool for both criminal investigations and for healthcare quality control. In clinical practice, autopsy contributes to medical knowledge, medical training, accurate mortality statistics, epidemiologic databases, and therapeutic and diagnostic improvements.[17-19]
Despite continuing development of innovative new diagnostic techniques, there are substantial discrepancies between ante-mortem and post-mortem diagnoses.[8-10,13,14,86,90,91] Hence, autopsy continues to provide medical professionals with valuable feedback on provided care and possibly new insights for future decision-making. In some cases it also leads to counselling advice for family members.

Clinical autopsy rates are rapidly declining worldwide.[22,23,32] To perform clinical autopsies, consent from next-of-kin is obligatory in most countries. Unfortunately, consent may not be requested or recommended by physicians (who are often junior staff members) and is often refused by bereaved families.[30,38,39,41,81-85,87,92] Public resistance to autopsies has increased over the years, due to negative press attention,[24] funeral delay, religious or cultural beliefs, and fear of mutilation of the deceased's body. For the latter reason, non-invasive or minimally invasive autopsy methods, which were already implemented in forensic medicine, are currently being developed to substitute clinical invasive autopsies.[22,24,32]

Over the last decades, MR imaging has been introduced in clinical medicine for perinatal and neonatal autopsy.[93,94] Many other clinically established imaging techniques have emerged for broad post-mortem use in forensic medicine. Among these are image-guided tissue biopsies, and CT- or MR- angiography.[48,95-99] Forensic specialists have optimized them for post-mortem settings. However, despite high diagnostic performance in that field, hardly any of the new post-mortem techniques have been implemented in clinical medicine.

Purpose

In this systematic review we investigate whether non-invasive or minimally invasive autopsy methods could replace conventional autopsy in adults with a suspected natural cause of death. We calculate the sensitivity and agreement of non-invasive and minimally invasive autopsy methods using conventional autopsy as reference standard, and discuss if any method may be appropriate for a clinical setting.

MATERIALS AND METHODS

For this systematic review the methods of Cochrane and PRISMA were used to the extent possible.[100,101]

Database search

Together with a biomedical information specialist we searched the Embase, Medline, Web of Science and Cochrane databases. We defined search terms for Embase and, from those, we derived search terms for the other databases. The search terms included the following elements: autopsy, imaging, cause of death and validation (see appendix 1). Case reports, studies on children and animal studies were excluded. The search was performed on the 16th of July 2013 and, to see if any eligible articles had been published since the previous searches, repeated on the 1st of April 2014 and on the 27th of June 2014. The second and third time we also searched PubMed publisher and Google Scholar. EndNote software was used to collect all articles matching the search terms and to remove duplicate records of the same study.

Article selection

The following inclusion criteria were used for article selection: (1) original prospective studies comparing the diagnostic performance of non-invasive or minimally invasive autopsy methods to that of the reference standard (conventional autopsy, not necessarily including brain autopsy); (2) outcomes defined in agreement and/or sensitivity and/or specificity of cause of death and/or detected overall, major and/or minor diagnostic findings; (3) the alternative autopsy methods covered at least an investigation of the deceased's thorax and abdomen; (4) more than five adult cases (≥ 18 years of age) were studied; (5) more than five presumed natural deaths were studied.

Two reviewers excluded the articles outside the scope of this review, based on the article titles and abstracts. Subsequently, they retrieved and evaluated the available full texts of the remaining articles and selected the articles that fully met the five inclusion criteria. A third reviewer was consulted in case the two reviewers disagreed on study eligibility.

Data extraction and analysis

Four reviewers were involved in the analyses, of which two were already for decades involved in research and scientific publication. Two reviewers independently performed the data extraction. Their interpretation was different with respect to one or two minor

data points per table and these differences could easily be resolved. A third reviewer was consulted for advice on structuring the data extraction tables. Per article the reviewers extracted data on study population, number of cases eligible for this review, study design/ methods, cost of the methods, and, if possible, data for outcomes in 2x2 tables. From these 2x2 tables on cause of death and/or (overall, major and/or minor) diagnostic findings both reviewers independently calculated the percentage of agreement, the sensitivity and if possible the specificity. If they were not able to extract any false positives and/ or true negatives, the reviewers only calculated sensitivity. If the reviewers could not extract any data from the original article for a 2x2 table, the reported outcome measures were quoted.
If necessary the reviewers contacted the authors, requesting additional information in order to exclude individual cases (based on age or suspected forensic cause of death) in the articles,[102-107] or to identify multiple reports of the same cases.[105,106,108,109] Unfortunately, only one author responded.[104]

The alternative autopsy methods applied and the case characteristics in the included studies were very heterogeneous, precluding meaningful meta-analysis of the study outcomes.
The outcomes of only two studies were pooled, since these studies seemed to be performed by the same research group, investigating the same alternative autopsy method, and even including some of the same cases.[108,109]

RESULTS

All database searches together provided us with 1538 articles that matched the search criteria (see figure 1), of which 51 were considered potentially relevant (see appendix 2). One of these articles could not be obtained via our hospital library. Of the remaining 50 articles 34 were not eligible for this systematic review upon reading the full text.

Study design and quality appraisal

Sixteen articles, published from 1996 to 2014, met the five inclusion criteria (see tables 1-3). Eight studies included just cases of adult deaths,[57,59,80,102,110-113] and in seven studies the cases were only included if the cause of death was suspected to be natural.[57,104,107,109-111,114] Among the studies that registered a male-female ratio, the majority of cases was male. The available mean ages differed from 22.7 years to 74.0 years.

Seven studies examined the accuracy of non-invasive autopsy methods, and nine studies the accuracy of various minimally invasive methods. In twelve studies a conventional autopsy (reference standard) was performed on all cases within the examined group.[57,80,102,105-112,114] In only eight studies the description of the comparison between new method and reference standard could be interpreted as blind.[57,59,80,105,106,110,111,114]
Complete 2x2 tables for cause of death were extracted from two articles,[103,107] for overall findings from another article,[57] and for new major findings and cultures from yet another article.[107] In addition, partial 2x2 tables could be extracted from eleven studies.[59,80,104-106,108-112,114]

For each available agreement percentage and sensitivity for cause of death in table 3, we calculated the exact binomial confidence interval. We plotted these confidence intervals in forest plots (see figure 2). They were often very wide, due to small study groups.
We plotted both agreement and sensitivity in a funnel plot (see figure 3), and could not detect any signs of publication bias.

Agreement and accuracy of non-invasive autopsy methods

As a potential alternative to the conventional autopsy, the earliest two studies examined the use of magnetic resonance imaging (MRI).[110,111]
Three other studies used (multi detector) computed tomography, (MD)CT.[59,103,104]
The two most recent studies performed both MRI and CT,[102,114] but only one of them combined the results to define a cause of death.[102] This latter study included more cases than all other non-invasive studies together.

The results of these studies, except Puranik et al.,[114] suggest that the non-invasive autopsies using CT perform somewhat better than those using MRI. The highest sensitivity achieved with CT was 70.8%.[104] The one study combining MRI and CT achieved an agreement of 70% in cause of death.[102]

Other outcomes, such as sensitivity for major findings, could only be extracted from three studies. These outcomes could not be compared, due to the heterogeneity in study methods.
The criteria for case selection were various, and studies with similar patient groups investigated different imaging methods.

Agreement and accuracy of minimally invasive autopsy methods

The oldest minimally invasive autopsy study applied a combination of tissue biopsies and post-mortem cultures, without any kind of imaging and showed an agreement and sensitivity of (almost) 60% for the cause of death.[107] They also showed a reasonably good agreement and sensitivity for new major findings.
Two studies performing a combination of post-mortem laparoscopy, thoracoscopy and (if indicated) tissue biopsies showed very high agreement percentages for the cause of death.[112,113] However, one of these studies included very few cases (n=7) and the other selected cases to maximize the benefit of the studied method.
There were two studies, by the same author, examining ultrasonography and (ultrasound-guided) biopsies in comparison to autopsy.[105,106] It is unknown if any of their cases were reported twice. The second study appeared to have worse outcomes than the first, but the agreement was still higher than in all non-invasive methods.

Weustink et al.[57] evaluated a combination of MRI and CT, and ultrasonography-guided tissue biopsies, and showed agreement for cause of death in 76.7%. They were the only investigators who calculated specificity for overall findings, which was 99%.
In their most recent study Wichmann et al. performed native CT and multiphase CT-angiography (no tissue biopsies).[80] With the addition of the CT-angiography the sensitivity of new major diagnoses had improved from 71.4% (MDCT only) to 92.9%.
Two studies combined CT, CT-angiography and (CT-guided) tissue biopsies as alternative to conventional autopsy,[108,109] resulting in high sensitivities for cause of death: 94.7% and 89.5%. Both studies included twenty cases of which six appeared to be duplicates, so together they actually included 34 cases, of which 33 were eligible for this review, leading to a pooled sensitivity of 90.9% (95%CI: 74.5; 97.6).

Further analyses or comparison between these studies was difficult, because of the heterogeneity in studied methods.

Cost of alternative autopsy methods

Although several studies mentioned costs, only one of them compared the actual cost of the two methods investigated. Weustink et al.[57] calculated a mean cost of $1497 ±148 per minimally invasive autopsy, and $2274 ±104 per conventional autopsy. Wichmann et al.[80] stated that the addition of angiography increased cost with $300 per case. Roberts et al. mentioned that alternative autopsies using MRI are more expensive than conventional autopsy.[102,110] Alternative autopsies using CT[59,102,103] or ultrasonography,[105,106] on the other hand, appear to be less expensive than conventional autopsy.

DISCUSSION

This is one of few systematic reviews to analyse the accuracy of alternatives to the conventional methods of autopsy in natural deaths, and the first to focus on naturally deceased adults. Although none of the alternative methods performed as well as conventional autopsy, higher agreement and sensitivity percentages demonstrated that minimally invasive autopsy methods were more accurate than non-invasive autopsy methods, especially those including tissue biopsies.

Comparison with the literature

A similar systematic review has been performed by Thayyil et al.,[88] who found better overall pooled sensitivity and specificity of post-mortem MRI in foetuses (69% and 95%) than in children and adults. As an alternative to conventional autopsy, however, its diagnostic accuracy was insufficient in all patient groups.

Since then, more studies have been published, and the diagnostic performance of alternative methods has improved significantly, as our study shows. With the introduction of minimally invasive autopsy methods, including imaging and tissue biopsies, remarkable improvements in accuracy were achieved. The merit of histological examination of vital organ tissue, in particular obtained under image-guidance, is also addressed in forensic studies.[115]

When comparing cost, minimally invasive autopsy may be less expensive than conventional autopsy. According to the reviewed studies, a minimally invasive autopsy including both biopsies and CT-angiography costs $1649 to $1945, whereas autopsy costs $2170 to $2378. In Switzerland each autopsy is preceded by at least CT, and Flach et al.[116] recently calculated a cost of $820 to $1150 per post-mortem examination including CT, CT-angiography, MRI, and forensic expert opinion.

Even though post-mortem endoscopic methods (thoracoscopy and laparoscopy) appear to be very accurate alternatives to conventional autopsy,[112,113] we hesitate to draw conclusions. Both studies included a fairly small number of cases and did not report whether the examiners were blinded to the conventional autopsy findings. One study states that it induced selection bias by selecting cases in order to maximize the benefit of the alternative autopsy.[112] Avrahami et al.[117] support our doubts, and state that findings from an endoscopic autopsy are insufficient to establish a definite cause of death. They recommend performing endoscopic autopsy only in cases in which there are objections to conventional autopsy and in order to rule out or identify major thoracic or abdominal pathology leading to death.

Several studies have shown that post-mortem whole-body CT-angiography visualizes pathological changes in blood vessels, such as stenosis, occlusion and injuries, and improves the accuracy of a minimally invasive autopsy method.[48,80,108,109,118] As these whole-body angiographies tend to be expensive, for heart-lung machines and large volumes of special contrast agents are required, either out-dated and therefore inexpensive equipment, or newly developed low-cost targeted angiography methods are being used. For instance, a post-mortem coronary CT-angiography was designed to improve the accuracy of a minimally invasive autopsy method in sudden natural death cases.[119,120] For findings in the coronary arteries, Roberts et al. achieved a correlation of 80% between autopsy and CT-angiography. Moreover, Saunders et al. were able to reduce the time for whole-body CT-scanning and a coronary CT-angiography to an average of 48 minutes.

Another interesting technique, which was not performed in any of the reviewed studies, is post-mortem ventilation. In clinical practice, the detection of small lung lesions is improved by having patients hold their breath when the scan is made. To achieve a similar effect in post-mortem imaging, forensic examiners simulated expiration and inspiration scans by ventilating the lungs.[121-123]

When searching for validation studies of alternatives to autopsies, we also found articles about verbal autopsy. This is a WHO-method used in populations lacking vital registration and medical certification, to determine the probable cause of death based on questionnaires and/ or narratives from next-of-kin or other reliable informants (such as caregivers). The method is not based on any post-mortem physical examination of the body, and not accurate for attributing cause of death at the individual level. Therefore, verbal autopsy was excluded from this review.

Limitations

We found very few validation studies on non-invasive and minimally invasive autopsy methods performed on adults with a non-suspicious and supposedly natural cause of death. We therefore chose not to exclude studies that did not provide sufficient data for composing complete 2x2 tables. As a result, the agreement on cause of death could not always be calculated, as it should be based on the combined true positives and true negatives whereas the latter was frequently missing. As we could neither extract true negatives nor false positives, we calculated only sensitivity percentages, even though the results had originally been reported as being agreement percentages.

Due to insufficient data, we were also unable to test whether the agreement percentages on cause of death were any better than chance, since, with incomplete 2x2 tables, the chance-corrected proportional agreement (κ-statistic) could not be correctly calculated.

Also, variability of the investigated study groups and study methods, and the information that was reported in the articles was too large to combine study outcomes in a meta-analysis.

For example, in studies using radiological imaging, one, two, four or six (specialized) radiologists reviewed the images. Previous experience in post-mortem imaging was mentioned in 6 studies: it varied from no experience to 5 years of experience, and was not comparable between studies. Though Roberts et al.[110] found that previous experience did not result in more correctly diagnosed causes of death. Moreover, only two studies calculated an inter-observer agreement (kappa): Weustink et al.[57] reported kappas of 0.85 for CT and 0.84 for MRI, and Ross et al.[108] reported a kappa of 0.94.

In addition, when comparing a new method to the reference standard in a validation study, the investigators performing one method should ideally be blind to outcomes of the other. This might not have been the case in eight of the studies reviewed, in which the agreement or sensitivity percentages may have been influenced, possibly biasing their value.
Controversially, blinding induces failure to detect false positive and false negative results. Christe et al.[124] reported that both gas and fractures were better detected at imaging than autopsy. To prevent these imaging findings from being registered as false positives, they had the findings confirmed after a second look at the autopsy. The same way, taking a second look at the radiologic images after autopsy could rectify false negative results. However, in both situations the findings were not originally reported, so they may be missed again in the future.

Another limitation, which is almost inevitable due to the kind of studies investigated, is knowledge of the medical histories prior to performing autopsy. None of the reviewed studies reported, that the investigators of conventional autopsy and its potential alternative were uninformed about the case circumstances. Therefore, this prior knowledge may have influenced the outcomes of agreement between the two methods, for known pathologies are more likely to be identified than unknown ones.

Advantages and disadvantages of the non-invasive and minimally invasive autopsy methods

When comparing radiological techniques for non-invasive and minimally invasive autopsy methods, CT and MRI are likely to be preferred over ultrasound. Both have their strengths and shortcomings, and may ideally complement each other. Table 4 gives an overview of the advantages and disadvantages of the radiologic techniques.[80,97,103,105,106,119,120,125-128]

Both radiologic techniques and scopic techniques are generally used in medical practice for the living. Hence, they are not available for autopsy cases during busy working hours. If a technique were to be purchased for post-mortem investigations only, the costs may not outweigh the benefits. The more advanced an alternative autopsy technique is, the higher is it's price, but, in general, the better are it's diagnostic capabilities (if the reviewed studies had used all available techniques, their results would inevitably have been better). Yet, those capabilities are not always required for each individual autopsy case. For example, MRI should preferably be used to examine congenital abnormalities or neurologic pathology in neonates, infants and children, whereas CT is required to examine lung pathology in adults.

Without reliable criteria for selecting those techniques or protocols required based on individual case characteristics, it is impossible to minimize cost and enable investigators to identify or rule out specific pathologies. In order to determine an adequate strategy, that is not unduly expensive, more studies should be performed on large study groups that represent patients with all causes of death.

According to the articles reviewed difficulties remain, even with the advanced minimally invasive autopsy techniques The main difficulties are in detecting small metastases[80]; in diagnosing cardiovascular disease, such as (localized or massive) acute myocardial infarction and endocarditis[57,59,108,109]; and in distinguishing post-mortem clotting from true thromboembolic material, especially in the pulmonary arteries.[59,108] On the other hand, in certain cases post-mortem imaging has a diagnostic advantage, since some death related findings are better depicted on imaging than with conventional autopsy. For example, a pneumothorax was diagnosed on imaging only and missed at autopsy.[57,129]

To achieve the highest diagnostic accuracy we think an alternative autopsy method should at least be minimally invasive. Even though the minimally invasive autopsy method is not yet as accurate as conventional autopsy, some of its other features favour this alternative method.

The first is that imaging data can easily be stored and subjected to second reading, and used for clinical feedback and teaching purposes, whereas macroscopic autopsy findings have to be photographed or organs have to be preserved in order to do so.

Another benefit of a minimally invasive autopsy is the possibility to take tissue biopsies under precise CT-guidance from very small lesions. It is known that in patients who died from metastatic disease, scarcely enlarged lymph nodes could be detected at conventional autopsy.

Just as in conventional autopsy, one could collect extra tissue biopsies that can be frozen and stored in a tissue bank. Such frozen samples could be used for further diagnostic analyses on a molecular level, and be used for medical research.[26]

A logistic advantage of minimally invasive autopsy is that a specialised radiologist is able to read the images from another location and even plan the exact coordinates of the biopsy trajectories for a robot to precisely place the introducer needles.[96,130] If multiple biopsies are routinely obtained, certain advanced techniques will minimize procedure time and eventually help reducing cost. From a technological point of view, Lundström et al.[131] see no obstacles to introducing minimally invasive autopsies on a larger scale.

Fryer et al.[129] emphasize the benefit of using a minimally invasive method for screening prior to conventional autopsy in cases with high-risk infections. Among a group of suddenly deceased drug users with a known category 3 infection (such as Human Immunodeficiency Virus or hepatitis-C virus), they identified the cause of death through a minimally invasive examination in a considerable percentage of cases, thereby achieving a two-thirds reduction in the number of high-risk invasive autopsies.

Last but not least, clinicians would gain more information from an alternative autopsy than from no post-mortem investigation at all.

Conclusion

Non-invasive or minimally invasive autopsy methods could serve as an alternative to conventional autopsy. However, it should be remembered that these alternative methods are still less accurate than the reference standard, and that taking image-guided tissue biopsies for histologic examination (and therefore performing a minimally invasive autopsy) is essential for achieving the best possible diagnostic accuracy.

To improve the technical aspects of minimally invasive autopsy methods and to test their potential in larger study groups, including patients who died in hospital with a broad spectrum of diseases, there is a need for more extensive studies. Such studies should not just examine the practical use and accuracy of the alternative autopsy method, but also take into account the cost of implementing the alternative method. If possible, an alternative to conventional autopsy should be developed that is suitable for implementation in academic and non-academic hospitals. Such alternatives to conventional autopsy should ultimately contribute to increasing autopsy rates, improving medical feedback to clinicians, and better overall healthcare quality control.

ACKNOWLEDGEMENTS

Wichor Bramer, biomedical information specialist in our medical library, who helped perform the literature searches.
Nikola Vitlarov, MD, who assisted with the data extraction.

TABLES

Table 1. Article details and study population

	First author	Year of publ.	Journal	Country	Inclusion via	Overall N included/ of which with autopsy	Age mean, range (years)	Sex ratio, male:female (n)	Postmortem interval (hours/days/ minutes)
Non-invasive methods	Puranik[114]	2014	J Cardiovasc Magn Reson	Australia	Department of Forensic Medicine	17/ 17	22.7, 1.5-35	13:4*	Mean time to autopsy: 56.1h
	Roberts[102]	2012	Lancet	United Kingdom	Coroner	182/ 182	n/r	n/r	n/r
	Takahashi[103]	2012	Eur Radiol	Japan	Emergency Department	494/ 20	Median: 74, 0-101 among autopsied: 46.5	306:188 among autopsied: 16:4	Mean PMI for imaging: 21.7m CA within 4-24h after CT
	Westphal[104]	2012	Virchows Arch	Germany	Hospital, Department of Pathology	29/ 28	59, 0-91	19:10	Mean PMI for imaging: 50h Mean PMI for CA: 62h
	Wichmann[59]	2012	Ann Intern Med	Germany	9 Intensive Care Units	162/ 47	among autopsied: 63	among autopsied: 26:21	n/r
	Roberts[110]	2003	Histopathology	United Kingdom	Coroner	10/ 10	n/r	n/r	Median time from death to imaging: 2d
	Patriquin[111]	2001	J Magn Reson Imaging	USA	n/r	8/ 8	64	4:4	Imaging within 12h of death. CA within 12h of imaging.

Minimally invasive methods									
With radiological imaging									
Wichmann[80]	2014	Ann Intern Med	Germany	Department of Intensive Care Medicine	50/ 50	70, 27-84	38:12	Median interval between death and CT: 4d Median interval between death and autopsy: 6d
Ross[108]	2012	Radiology	Switzerland	Institute of Forensic Medicine	20/ 20	56, 15-80	16:4	Mean PMI for imaging: 19h
Bolliger[109]	2010	Am J Roentgenol	Switzerland	Institute for Forensic Autopsy	20/ 20	56.4, 15-82	14:6	n/r
Weustink[57]	2009	Radiology	The Netherlands	Hospital (wards)	30/ 30	65.7, 46-79	19:11	Mean PMI for imaging: 9.6h CA after additional mean of 15.1h
Fariña[105]	2002	Virchows Arch	Spain	n/r	100/ 100	n/r	n/r	n/r
Fariña[106]	1998	J Echogr Med Ultrasons	Spain	n/r	130/ 130	n/r	n/r	n/r
Without								
Fan[112]	2010	Forensic Sci Int	China	Coroner	22/ 22	74.01, 32-96	11:11	n/r
Cacchione[113]	2001	Surg Endosc	USA	Hospital	25/ 9	71.6, 44-94	n/r	All procedures completed within 24h after death
Huston[107]	1996	Mod Pathol	USA	n/r	20/ 20	13-84	n/r	Range: 3-72h

* originally reported as 71% men

Table 2-A. Study methods and design

	First author	Year of publ.	Inclusion criteria	Exclusion criteria	Post-mortem techniques			Reference Standard	Blinded study*
					Imaging	Biopsies	Other		
Non-invasive methods	Puranik[114]	2014	Consecutive patients aged 1-35 years. Referred to dept. of forensic medicine. Death was identified to be sudden. Available scanning time and autopsy delay <24 hours. Verbal and written consent	Patients with trauma, suicide or known drug overdose	1st comparison – 1.5 Tesla MRI: (3D) T1, T1 FFE, T2 DE STIR, FLAIR, FFE and IR of the brain; cardiac balanced FFE and T2 STIR short-axis; T2 STIR multiple lung axes 2nd comparison – 64-slice CT from head to pelvis	No	No	Conventional autopsy	Yes
	Roberts[102]	2012	First case each study day and study days according to availability of staff	Failure to obtain consent Severe obesity (100kg)	8 or 16-slice CT from vertex to symph. pubicus. MRI: T1, DE or FLAIR, and STIR of the brain; T1, STIR and T2 FSE from neck to pelvis; fT2 short-axis of the heart	No	No	Full autopsy	Unknown
	Takahashi[103]	2012	Subjects for whom emergency physicians could not determine COD by an external examination. Permission obtained	Undoubtedly traumatic deaths Cases that had undergone only head CT	6-slice (155 cases), 16-slice (303 cases) or 64-slice CT (36 cases) from the head to the iliac bone	No	No	16 conventional autopsies (6 excluding brain); 4 forensic autopsies	Radiologists were blinded to autopsy findings. Pathologist were not blinded
	Westphal[104]	2012	Deceased persons delivered for conventional autopsy (randomly selected). Manner of death due to natural cause	n/r	64-slice, dual source CT from head to toe	No	No	Conventional autopsy (1 limited to cardiac autopsy)	Unknown
	Wichmann[59]	2012	Patients died at an Intensive Care Unit Informed consent	Funeral scheduled early CT scanner maintenance Medical autopsy performed before CT Eligible for organ donation Body weight too high for CT scanner	Multislice CT from head to abdomen	No	No	Conventional autopsy	Yes
	Roberts[110]	2003	Sudden unexpected adult deaths in the community	Suspicious, violent or potentially drug-related deaths	1.5 Tesla MRI: T1, GE, FLAIR and T2 of the head; T1, FSE, FLAIR with SPIR fat suppression from neck to pelvis	No	No	Full autopsy	Yes
	Patriquin[111]	2001	Consent for both MRI and (limited) surgical dissection	Medical examiner cases Pediatric cases	1.5 Tesla MRI: turbo STIR from vertex to the knees using coronal body coil; T2 FFE or T2 SPIR from thorax to pelvis	No	No	7 conventional autopsies (1 excluding head); 1 percutaneous biopsy technique	Yes

Table 2-B. Study methods and design

Minimally invasive methods	*With radiological imaging*	Wichmann[80]	2014	Hospitalized patients who died unexpectedly or within 48 hours of an even necessitating CPR. Oral informed consent	Maintenance work on the CT scanner. Funerals scheduled before autopsy could be done	4-slice and 16-slice native CT from head to hip joint?	No	Multiphase CTA via femoral vein and artery, from head to hip joint, using heart-lung machine	Full autopsy	Yes
		Ross[108]	2012	Sheduled forensic autopsy. Antemortem reports of chest pain	n/r	6-slice unenhanced CT . from head to pelvis	CT-guided 14G needle biopsies of heart, lungs and suspected areas	Arterial and venous CTA from head to pelvis, via femoral vein and artery, using heart-lung machine	Conventional autopsy	No
		Bolliger[109]	2010	Consecutive cases of unclear COD. No signs of mechanical trauma. Case circumstances indicative of a natural death	n/r	6-slice CT. . Scanned body parts not mentioned	CT fluoroscopic controlled 14G needle biopsies of heart, lungs and suspicious regions	CTA via femoral vein and artery, using heart-lung machine	Full forensic autopsy	Unknown
		Weustink[57]	2009	Age 18 years or older. Weight less than 100kg	No informed consent. Forensic COD. Known or suspected "high-risk" infections. Open abdominal wounds. Cases referred from Friday to Sunday	16-slice CT from the calvarium . to the pelvis. 1.5 Tesla MRI: T1 SE, T2 TSE and T2 FLAIR of the head using head coil; T1 SE, T2 TSE, T2 spectral fat saturation IR of thorax and abdomen	12G needle biopsies: Unguided biopsies of heart and lungs.. US-guided biopsies of liver, kidneys and spleen. If indicated additional.	No	Conventional Autopsy	Yes
		Fariña[105]	2002	n/r	n/r	Ultrasound of the internal organs, GI and urogenital tracts, pleural and peritoneal cavities and superficial structures	Ultrasound guided 14G needle biopsies of the internal organs (incl. brain) and lesions	No	Conventional autopsy	Yes
		Fariña[106]	1998	n/r	n/r	Ultrasound of all organs	14G needle biopsies/ liquids	No	Conventional autopsy	Yes
	Without radiological imaging	Fan[112]	2010	Cases reported to coroner. Cases ordered for autopsy examinations. Cases were selected to maximize benefit of method	n/r	No	If indicated biopsies of the abdominal organs	Laparoscopy and thoracoscopy . in 18 cases;. Endoscopic arterioscopy . in 2 cases	Full autopsies. 4 limited autopsies	Unknown
		Cacchione[113]	2001	Patients who died in their institution	n/r	No	Needle biopsies of the internal organs	Laparascopy, thoracoscopy and. review of patients hospital records	Conventional autopsy	Unknown
		Huston[107]	1996	Patients with permission for complete post mortem examination	Medical examiner cases. Fetal cases	No	Percutaneous 15G needle biopsies of heart, liver, lungs and kidneys. If indicated additional.	Appropriate tissues and fluids for post mortem cultures	Full autopsy	Unknown

* Unknown if not explicitly mentioned

Table 3. Results.

		First author	Year of publ.	Cases for review	Cause of death		Major diagnoses		Minor diagnoses	
					Agreement (95%CI)	**Sensitivity** (95%CI)	**Agreement** (95%CI)	**Sensitivity** (95%CI)	**Agreement** (95%CI)	**Sensitivity** (95%CI)
Non-invasive methods		Puranik[114]	2014	11	MRI: 11/11 = 100%. (71.5; 100). CT: 3/11 = 27.3%. (6.0; 61.0)	MRI: 10/10 = 100%. (69.2; 100). CT: 2/10 = 20%. (2.5; 55.6)	n/r	n/r	n/r	n/r
		Roberts[102]	2012	182 (-6)[1]	"70%". (62.6; 76.4)	n/r	n/r	n/r	n/r	n/r
		Takahashi[103]	2012	16 (-2)[1]	10/16 = 62.5% . (35.4; 84.8)	8/14 = 57.1%. (28.9; 82.3)	n/r	n/r	n/r	n/r
		Westphal[104]	2012	24	n/r	17/24 = 70.8%2. (48.9; 87.4)	*death related diagnoses* 23/44 = 52.3%. (36.7; 67.5)	*death related diagnoses* 23/43 = 53.5%. (37.7; 68.8)	21/69 = 30.4%. (19.9; 42.7)	21/45 = 46.7%. (31.7; 62.1)
		Wichmann[59]	2012	47	n/r	n/r	n/r	*new major diagnoses.* 10/14 = 71.4%. (41.9; 91.6)	*new minor diagnoses.* 26/88 = 29.5%. (20.3; 40.2)	*new minor diagnoses.* 26/61 = 42.6%. (30.0; 56.0)
		Roberts[110]	2003	10	n/r	6/10 = 60%2. (26.2; 87.8)	n/r	n/r	n/r	n/r
		Patriquin[111]	2001	7	n/r	3/7 = 42,9%2. (9.9; 81.6)	13/34 = 38.2%. (22.2; 56.4). *death related diagnoses* 6/15 = 40%. (16.3; 67.7)	13/23 = 56.5%. (34.5; 76.8). *death related diagnoses* 6/11 = 54.5%. (23.4; 83.3)	8/39 = 20.5%. (9.3; 36.5)	8/23 = 34.8%. (16.4; 57.3)
Minimally invasive methods	*With radiological imaging*	Wichmann[80]	2014	50	n/r	n/r	*new major diagnoses.* 13/16 = 81.3%. (54.4; 96.0). *overall diagnoses.* 405/590 = 68.6%. (64.7; 72.4)	*new major diagnoses.* 13/14 = 92.9%. (66.1; 99.8). *overall diagnoses.* 402/474 = 84.8%. (81.3; 87.9)	*new minor diagnoses.* 140/238 = 58.8%. (52.3; 65.1)	*new minor diagnoses.* 140/190 = 73.7%. (66.8; 79.8)
		Ross[108]	2012	19*	n/r	18/19 = 94.7%2. (74.0; 99.9)	n/r	n/r	n/r	n/r
		Bolliger[109]	2010	19*	n/r	17/19 = 89.5%2. (66.9; 98.7)	n/r	n/r	n/r	n/r
		Weustink[57]	2009	30	23/30 = 76,7%. (57.7; 90.1)	n/r	129/140 = 92.1%. (86.4; 96.0). *overall diagnoses.* 2019/2056 = 98.2%. (97.5; 98.7)	129/137 = 94.2%. (88.8; 97.5). *overall diagnoses.* 255/273 = 93.4%. (89.8; 96.1)	n/r	n/r
		Fariña[105]	2002	81	64/81 = 79.0%. (68.5; 87.3)	n/r	n/r	n/r	n/r	n/r
		Fariña[106]	1998	130 (-29)[1]	120/130 = 92.3%. (86.3; 96.3)	n/r	n/r	n/r	n/r	n/r

Minimally invasive methods									
Without									
	Fan[112]	2010	16	15/16 = 93.8%. (69.8; 99.8)	based on 18 cases. "90%" (65.3; 98.6)	n/r	n/r	n/r	n/r
	Cacchione[113]	2001	7	combined with review of patients' hospital records. "100%" (59.0; 100)	n/r	n/r	n/r	n/r	n/r
	Huston[107]	1996	20 (-1 or more)[1]	12/20 = 60%. (36.1; 80.9)	11/19 = 57.9%. (33.5; 79.8)	*new major diagnoses* 20/24 = 83.3%. (62.6; 95.3)	*new major diagnoses* 12/16 = 75%. (47.6; 92.7)	*cultures*. 39/46 = 84.8%. (71.1; 93.7)	*cultures*. 10/15 = 66.6%. (38.4; 88.2)

[1] Some cases should still be excluded, for these deceased were younger than 18 years of age or died from an unnatural cause of death. [2] Originally reported as being agreement
* Some of the included cases seem to overlap

Table 4. Advantages and disadvantages of non-invasive and minimally invasive autopsy methods using radiological techniques

	Advantages	Disadvantages
Ultrasound [105,106]	**Logistics** High availability. Operator friendly	**Image quality and diagnose** Inferior to MRI and CT in image quality. Inferior to MRI and CT in biopsy guidance. Limited visualisation of the vascular system (no flow). Operator dependent
	Cost Inexpensive	
CT [103,125,132]	**Logistics** High availability. Rapid whole-body examination. Repeated scanning possible. Possibility of biopsy guidance	**Logistics** Limited availability during regular working hours (interferes with scanning of the living)
	Cost Relatively inexpensive (compared to MRI)	**Image quality and diagnose** Limited visualisation of pathology in soft tissues and organ parenchyma. Limited differentiation of normal postmortem changes (e.g. clotting, sedimentation) and pathology (e.g. pulmonary thromboembolism). Limited ability to diagnose cardiac causes of death (e.g. patency of coronaries, acute myocardial infarction). Image artefacts (e.g. metal from dental filling, prosthetic valves)
	Image quality and diagnose Good visualization of bone (e.g. fractures), lung parenchyma disease, calcifications (stones, atherosclerosis), acute haemorrhage, air/ gas (e.g. pneumothorax, pneumatosis intestinalis, free air). High in-plane resolution (e.g. small lung nodules). Isovolumetric multi-planar and 3D reconstructions	
MRI [103,126,132]	**Logistics** Possibility of biopsy guidance	**Logistics** Limited availability during regular working hours (interferes with scanning of the living). Relatively time consuming examination (depending on scan protocol). Requires dedicated postmortem scan protocols (e.g. adjusting scan parameters for the body temperature). Requires MRI compatible body bags (e.g. no metal)
	Image quality and diagnose Good, detailed visualization of organ parenchyma (e.g. brain, heart and myocardial infarct age), soft tissue (e.g. muscle injury), fluids (e.g. pleural/ pericardial), nervous system (e.g. spinal canal disorders), bone marrow disorders, metabolic diseases (e.g. hemochromatosis), large vessels (e.g. aortic dissection). Good differentiation between postmortem changes and pathology	**Cost** Relatively expensive. Requires dedicated training of technicians
		Image quality and diagnose Image artefacts (e.g. metal from dental filling)
CT Angiography [80,97,125,128,132]	**Image quality and diagnose** Good detection (of the origin) of haemorrhages (e.g. aortic rupture). Good detection of cardiovascular conditions (e.g. coronary stenosis)	**Logistics** Limited availability of dedicated equipment and contrast agents. Time consuming and complicated examination (e.g. achieving optimal contrast timing and full enhancement is difficult)
		Cost Expensive (longer procedure time, contrast agents, dedicated equipment). Requires dedicated training
		Image quality and diagnose Differentiation between post-mortem clotting and embolus is difficult. Lack of circulation and insufficient mixing of blood and contrast
Targeted CT (coronary) angiography [97,119,120]	**Cost** Relatively inexpensive equipment (compared to whole-body angiography). Cannulation and scanning can be performed with minimal training	**Logistics** Time consuming examination (e.g. positioning catheter, turning the corpse)
	Image quality and diagnose Less expensive than whole-body angiography	**Image quality and diagnose** Images restricted to coronary arteries. Limited visualisation of internal mammary grafts, due to balloon position in the ascending aorta

FIGURES

Figure 1-A. Flowchart article selection: Initial literature search

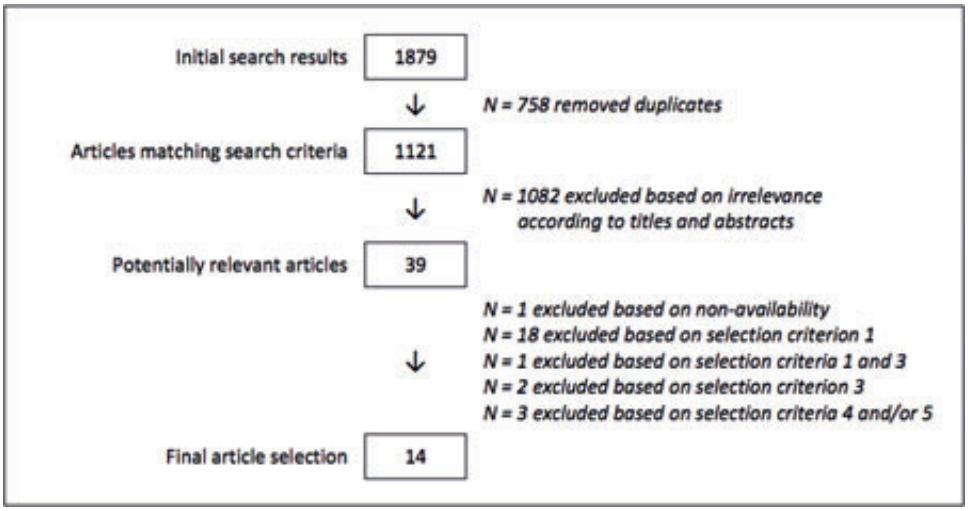


Figure 1-B. Flowchart article selection: Second literature search

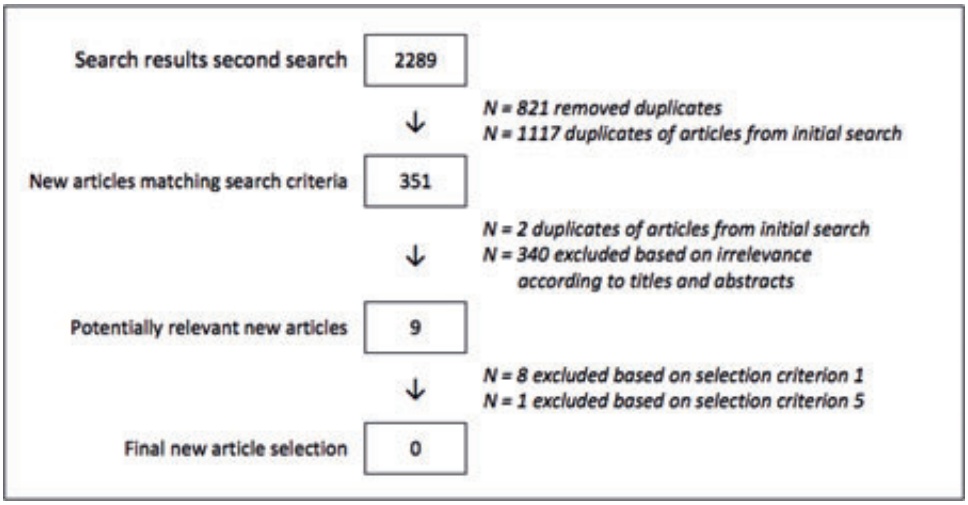


Figure 1-C. Flowchart article selection: Third literature search

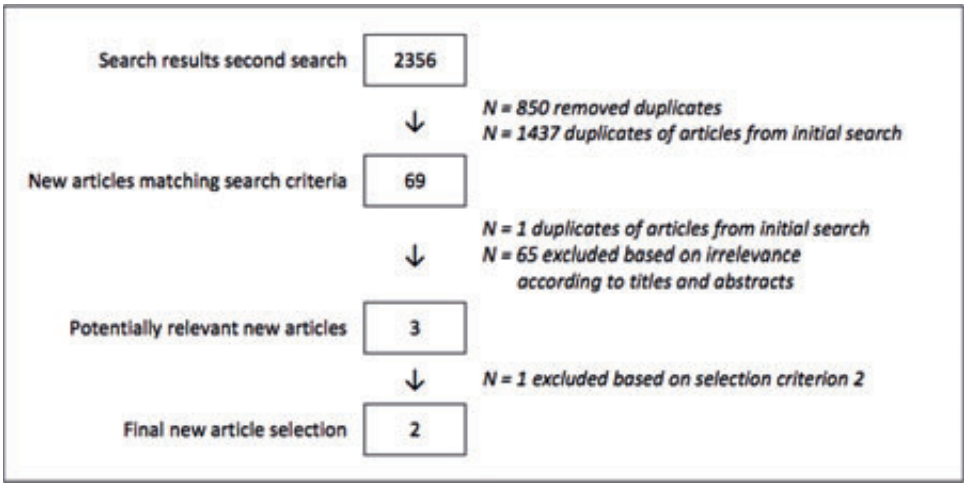


Figure 2-A. Forest plot: Agreement in cause of death

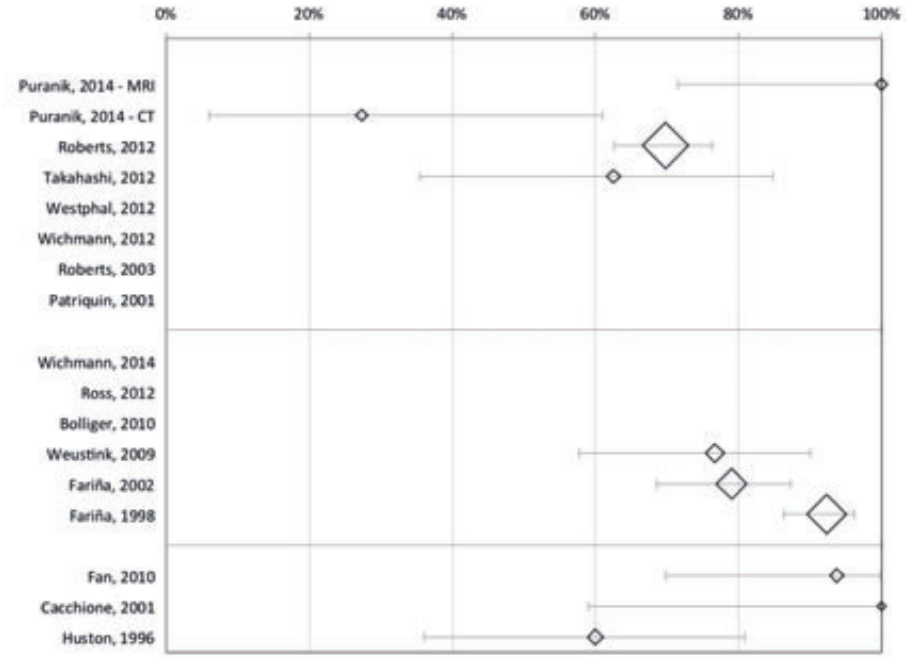


Figure 2-B. Forest plot: Sensitivity cause of death

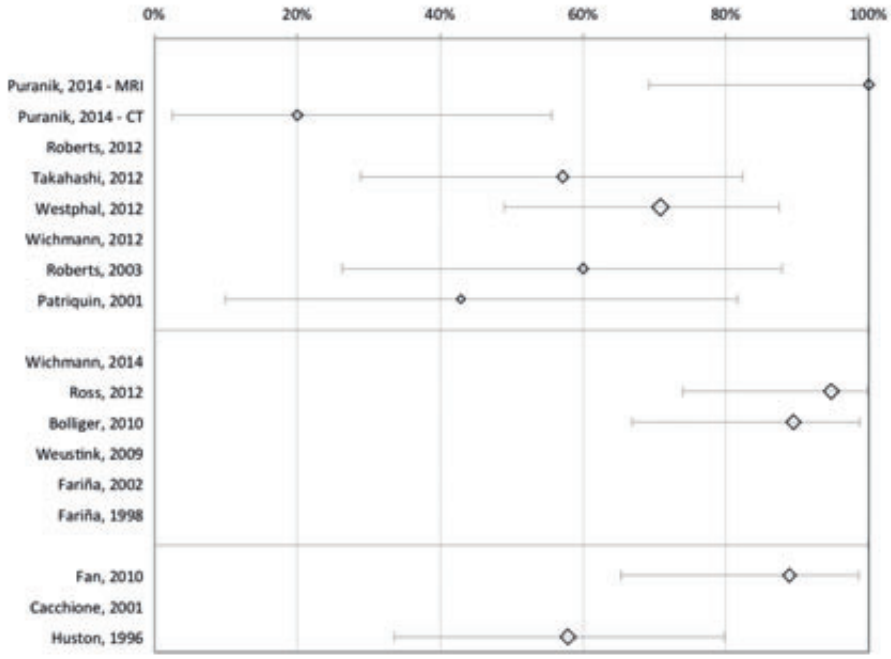
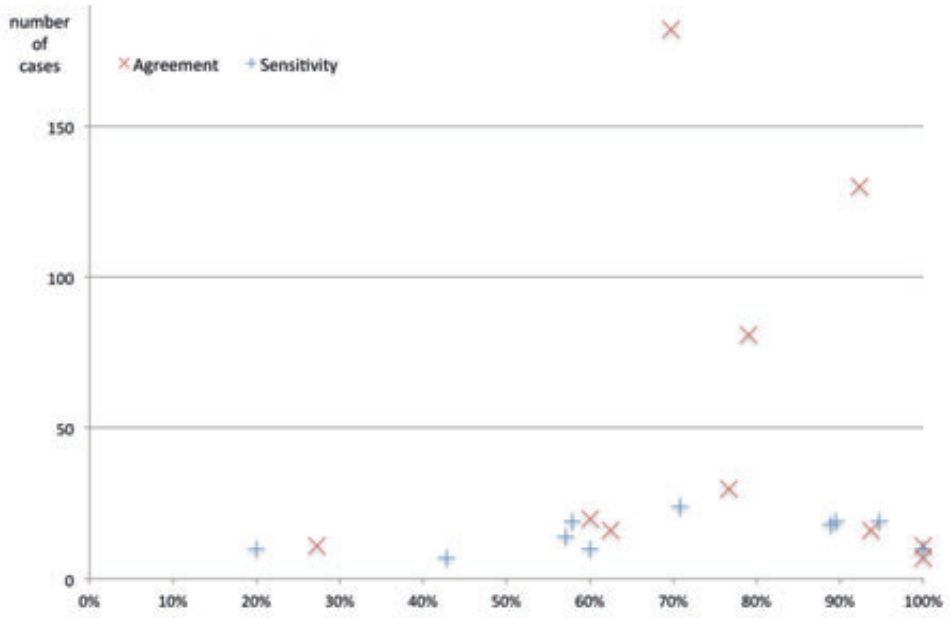


Figure 3. Funnel plot: Validation scores for defining cause of death



APPENDICES

Not included in this thesis:

Appendix 1. Search terms for systematic review on non- or minimally invasive alternatives to autopsy
Appendix 2. Overview of the number of results in literature searches per database (supplementary tables)
Appendix 3. List of potentially relevant articles in literature searches

5

Agreement between Minimally Invasive Autopsy and Conventional Autopsy for Cause of Death: a cross-sectional study

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Submitted

ABSTRACT

Background: The worldwide decline of clinical conventional autopsies (CA), among others due to its invasiveness, means a loss for healthcare quality control, calling for development of alternative methods. Objective of this study was to compare diagnostic performance of a minimally invasive autopsy (MIA) and CA.

Methods: Prospective, single center, cross-sectional study in an academic hospital. The MIA procedure, combining MRI, CT and image-guided biopsies of main organs and pathological lesions detected on imaging, was followed by CA on the same case. MIA and CA were performed by separate teams, blinded to each other.

Primary outcome measure: performance of MIA and CA in establishing cause of death. Secondary outcome measures: diagnostic yield of MIA and CA for all, major and grouped major diagnoses, frequency of clinically unsuspected findings, and percentage of answered clinical questions.

The different setting of MIA (research) and CA (routine) precluded the use of CA as gold standard.

Results: Out of 2197 adult patients, who died in 2012 through 2014, 295 underwent CA; for 139 cases there was also consent for MIA; 99 could be included in the study cohort.

Agreement between MIA and CA for cause of death existed in 91/99 cases, agreement with consensus cause of death in 96/99 for MIA, and 94/99 for CA (P=0.727). 288 grouped major diagnoses were directly related to consensus cause of death, MIA diagnosed 259 (90%) and CA 224 (78%), 200 (69.4%) were found by both MIA and CA. 17% of cause of death, and 43% of grouped major diagnoses were clinically not suspected. MIA answered 189 (86·3%) and CA 182 (83·1%) of 219 clinical questions.

Conclusions: MIA and CA performed equally well in detecting cause of death. MIA resulted in a higher yield of diagnoses. MIA appears a valid alternative for CA, resulting in a permanent, auditable record of the deceased. The frequency of clinically unsuspected findings underscores the lasting importance of post-mortem investigation.

INTRODUCTION

Conventional autopsy (CA) is a valuable tool, particularly for quality control in healthcare.[9,13,15] Nevertheless, autopsy rates have been rapidly declining,[22,23,46] One of the reasons is reluctance of clinicians to request and unwillingness of next-of-kin to consent to autopsy, because of fear for mutilation of the deceased's body or organ retention.[22,24,38,81] Hence, non-invasive or minimally invasive alternative autopsy methods are being developed.[56]

Using post-mortem CT (PMCT) and MRI (PMMR), the entire body can be visualized [59,102,104] and, with minimal damage to the body, image-guided tissue biopsies can be obtained for histological examination.[57] In addition post-mortem CT-angiography (CTA) can be performed.[80,108,109,132-135] Some of these methods are already used to support or even substitute the forensic autopsy.[48,97] In the clinical setting, non-invasive or minimally invasive autopsies of fetuses, newborns and infants[136] have gained acceptance with parents and physicians, and political and public interest.[137] However, they are still rarely applied in adult patients.

The main aim of this study was to compare cause of death (COD) established with CA and minimally invasive autopsy (MIA) in adults deceased in a clinical setting, under the a priori hypothesis that MIA and CA would perform equally well.

METHODS

Study Design

We performed a prospective, single center, cross-sectional study in an academic hospital comparing diagnostic performance of MIA and CA. The institutional review board approved of the study prior to data collection. MIA was followed by CA on the same case. Those involved in MIA and CA were blinded to each other. Primary outcome measure: performance in establishing cause of death (COD) by MIA and CA. Secondary outcome measures: diagnostic yield for all diagnoses, major diagnoses and grouped major diagnoses (GMD); frequency of clinically unsuspected findings; percentage of answered specific clinical questions.

Participants

From January 2012 through December 2014 all hospitalized patients aged 18 years and older who died at Erasmus University Medical Center were eligible, if written informed consent was obtained from next-of-kin for MIA and CA.

Exclusion criteria were (suspected) unnatural cause of death; body size exceeding height of 16 inches in supine position (limitation for PMMR); known or suspected transmittable disease, such as HIV, tuberculosis, hepatitis-B and hepatitis-C; open abdominal wounds that could not be completely closed or taped to prevent leakage of body fluids. Examination of the brain, either biopsies and/ or autopsy, was not compulsory. Excluded cases are shown in Appendix A.

Preparation for Autopsy Procedures

For each case the clinically assumed COD, specific clinical questions, and a comprehensive medical history of the deceased, including pre-mortem imaging, were collected. This file was made available to the MIA and CA team.

Minimally Invasive Autopsy

PMMR and PMCT scans were made according to standardized protocols (Appendix B). A MIA radiologist (ACW, MdV, AEO, MJPR) performed the initial read of the PMMR and PMCT scans, according to protocol, compared these to the available pre-mortem imaging, and marked suspected pathological lesions for biopsy. The MIA researcher (BMB, IMW, JWO) obtained CT-guided biopsies (12 Gauge) from heart, lungs, liver, kidneys, spleen, and radiologically suspected pathology as indicated. Assisted by a neurosurgeon (RD, JSSvH, RKB) stereotactic biopsies were taken from the brain (Appendix C). The MIA pathologist (JWO) and researcher (BMB) examined the microscopic slides of the biopsies; when in doubt they consulted pathologists with specific expertise, not involved in the matching CA, to reach a conclusion.
A general radiologist (NSR) independently performed a second read of the PMCT and PMMR scans. A cardiovascular radiologist (AP) performed a second read of the PMMR scans of the heart. Both were blinded to the initial radiological read. In case of disagreement between the initial and second read, consensus was reached in joint sessions.
Radiological and histological findings were combined in the MIA report featuring clinical history, post-mortem diagnoses, a presumed COD and answers to specific clinical questions.

Conventional Autopsy

The day after MIA, a resident in pathology, supervised by the attending pathologist, performed CA according to the departmental protocol. The CA report included clinical history, post-mortem diagnoses, a presumed COD, and answers to specific clinical questions, and was authorized by the attending pathologist.

Data Analysis

Agreement on Cause of Death

COD determined respectively by MIA and CA were compared; in cases of disagreement between the two methods a consensus COD was reached for each case in three successive reviews by independent experts, as described in Appendix D.

Diagnoses

The MIA researcher (BMB) extracted and coded all different post-mortem diagnoses from the final MIA and CA reports using the International Classification of Diseases, Tenth Revision[138] with minor modifications. Diagnoses were sorted by 20 organ/tissue categories and one category of general diagnoses (Appendix E).

Per case, a MIA researcher (IMW) extracted and coded all pre-mortem diagnoses, using the prepared ICD-10 list. The MIA pathologist and radiologist jointly scored all post-mortem diagnoses per MIA and two independent pathologists (KHL, VSM) together scored all post-mortem diagnoses per CA. Per case, independently for MIA and CA, they scored a post-mortem diagnosis as "certain" according to established radiological and/or pathological criteria, or as "probable", if there was any uncertainty. Also per case, for MIA and CA independently, a post-mortem diagnosis was classified as major if it was directly related to COD.

In retrospect, these major diagnoses were, per organ/tissue category, grouped to combine related diagnoses (e.g. necrosis of the lungs, plus infection of the lungs, plus acute pneumonia). In the final step of our analysis GMD were per case included only if they were directly related to the consensus cause of death.

Diagnostic Errors

Retrospectively the diagnostic errors of MIA and CA were classified. A perceptual error was defined as an abnormality that, though present, was not reported or as an abnormality that, though absent, was reported.[139-141] A cognitive error was defined as an abnormality that, though reported correctly, was not correctly interpreted. An error was defined as a sampling error when a biopsy of a suspected radiological finding, confirmed with CA, was negative.

Analyses

All data were sealed in an OpenClinica electronic database. Agreement on COD between MIA and CA was calculated. Furthermore COD established by MIA and CA, was compared to the consensus COD, and the percentage of cases in which COD was classified as

correct was calculated for each method. Also, the clinically assumed COD, extracted from the clinical data files, was compared to the consensus COD. The performance of PMCT and/ or PMMR in establishing COD was analyzed.
The total diagnostic yield, the contribution of MIA and CA for all diagnoses, major diagnoses and GMD, and the percentage of clinically unsuspected diagnoses were calculated. For GMD we also analyzed overlap between MIA, CA and the pre-mortem clinical evaluation.
The performance of PMCT and PMMR in establishing CMD, and the added value of the image-guided biopsies was retrospectively analyzed. Inter-observer agreement for major post-mortem diagnoses made on PMCT and PMMR was calculated using Kappa statistic.

Role of the Funding source

This study was made possible by grants from Erasmus MC Health Care Efficiency (2010-10112), Stichting Coolsingel Rotterdam (255), and Erasmus MC Vriendenfonds (104117). The funding sources had no role in study design, conduct and reporting.

RESULTS

Recruitment

The size of the cohort was mainly determined by the available time for inclusion, i.e. three years. The goal to assemble a substantially larger cohort than in our published pilot of 30 cases(14) was achieved. From January 2012 through December 2014 2197 hospitalized adult patients died, of whom 295 (13.4%) underwent CA; of the latter 99 consecutive cases (33.6%) were included in the study (Appendix F). Among these 61 were men and 38 women. Their mean age was 63 years (range: 25-92).
Thirty-one cases were recruited from the General Intensive Care Unit, 15 from the Emergency Room, ten from the Departments of Internal Medicine/Gastroenterology, eight from Oncology, seven from Cardiology/Coronary Care Unit, six from Neurology, five from Thoracic Surgery/Cardiothoracic Intensive Care, five from Hematology, five from Pulmonology, four from General Surgery, and three from Gynecology/Urology.

MIA Duration and Tissue Sampling

The mean time between death and MIA was 23.2 (SD 15.6) hours. The mean MIA procedure time, including transportation, was 6.28 (SD 1.07) hours. The mean time between MIA and CA was at 9.47 (SD 1.06) hours.

At MIA 1574 biopsies were obtained, targeting 655 organs/tissues: 78.5% (1236/1574) from heart, lungs, liver, kidney and spleen; 21.5% (338/1574) from other organs/tissues, for suspected pathology upon imaging.
Twenty cases underwent both stereotactic biopsies and brain autopsy; four had biopsies only; 18 brain autopsy only. In 22 cases cytological samples were obtained at MIA: 17 routinely from cerebrospinal fluid; five from pathological fluid collections. (Appendix G)

Agreement on COD

MIA and CA agreed on COD in 91 cases (Table 1-A), including one case in which a certain cause of death was established with neither MIA nor CA. Out of the eight discordant cases, MIA diagnosed the consensus COD in five, and CA in three (Table 1-B), resulting in a correct COD in 96 MIA cases and in 94 CA cases (P=0.727).
The performance of imaging (PMCT and PMMR) alone in establishing COD is shown in Table 2. In 11 MIA a certain COD could have been established without the need for biopsy, because of an unequivocal COD at imaging: Tension pneumothorax, massive air embolus (Figure 1), type-A aorta dissection, esophago-pleural fistula, ruptured aneurysm of abdominal aorta, rebleed of cerebral arteriovenous malformation, acute subdural and intracerebral hemorrhages with compression and cerebral ischemia.

Clinical Correlation

In 65 cases the clinically presumed COD was the same as the consensus COD found by MIA and/or CA. In an additional 17 the consensus COD was mentioned in the clinical differential diagnosis, leaving another 17 cases in which the COD was not suspected clinically. The latter were: pneumonia (N=5); myocardial infarction (N=2); type A aorta dissection (N=2); tension pneumothorax, massive air embolus, multiple organ failure, acute cellular (A2) lung rejection, mesenteric ischemia, sepsis, disseminated intravascular coagulation, and subdural hematoma (N=1). Three of the 17 clinically unsuspected COD were only found by MIA (tension pneumothorax; massive air embolus; Type A dissection); two only by CA (severe coronary atherosclerosis causing acute coronary syndrome; mesenteric ischemia).
In 86 cases 219 additional specific clinical questions were asked. MIA and CA answered respectively 189 (86.3%) and 182 (83.1%) of them (p=0.353). Typically, questions regarding pathological changes not visible or not recognized on imaging, and therefore not biopsied, were not answered with MIA.

Diagnoses

Within this study population 347 different post-mortem ICD diagnoses were encountered; of these, 230 (66.3%) were classified as major in at least one case (Table 3). From the MIA and CA reports of the 99 included cases, a total of 3097 post-mortem diagnoses were extracted (Appendix I); MIA identified 79.0% of these and CA 46.0% (Appendix J-1). 1372 (44.3%) of the diagnoses were initially classified as major; MIA detected 72.3% and CA 67.3% (Appendix J-2). After retrospective grouping, 85 different GMD remained. The post-mortem techniques together scored 283 GMD; 91.5% found by MIA and 79.2% by CA (Appendix J-3). In addition, there were five certain diagnoses in pre-mortem clinical evaluation that were not scored certain by either post-mortem method, but nevertheless classified as major diagnosis, because they were directly related to COD and therefore added to the list of GMD.
Of the 288 GMD 124 (43.1%) were clinically unsuspected, 111 (89.5%) of the latter were found by MIA and 92 (74.2%) by CA; 79 were diagnosed by both MIA and CA, 32 only by MIA and 13 only by CA. Agreement between pre-mortem clinical evaluation, MIA and CA for all GMD, for GMD in lungs, heart, brain and vascular system, for pneumonia and myocardial infarction, and for all neoplastic diseases is illustrated in Venn diagrams (Figure 2).
The performance of PMCT, PMMR, biopsies and CA separately in detecting GMD per category is shown in Appendix H. Inter-observer agreement (kappa) for radiological detection of GMD was 0.91 for PMCT and 0.80 for PMMR.

Perceptual, Cognitive and Sampling Errors

MIA made 16 perceptual errors: 12 on imaging and four on microscopic examination. There were seven cognitive errors: all on microscopy. Four diagnoses were missed due to sampling error.
CA made 26 perceptual errors: nine on gross and 17 on microscopic examination. There were six cognitive errors. (Appendix K)

CONCLUSIONS

In this prospective study on a cohort of in-hospital deceased adult patients, MIA combining PMMR, PMCT, and image-guided biopsies, performed equally well as CA in identifying COD and answering specific clinical questions. MIA had a higher yield than CA for post-mortem diagnoses, major diagnoses and GMD, many of which were clinically unsuspected. The standard application of biopsies allowed making specific microscopic diagnoses with MIA, also of diseases not accompanied by obvious gross

and radiological abnormalities, such as acute cellular lung rejection and disseminated intravascular coagulation. The methods here applied for MIA appeared adequate, and are feasible in any larger general hospital.
Post-mortem angiography, an important technical advancement of post-mortem imaging,[80,108,133] appears not necessary for establishing COD in most of the cases if the cardiac coil and specialized cardiac protocols are used during PMMR and coronary calcium scores are examined at PMCT. For subclinical arterial stenoses and localizing the origin of bleeding the technique is indispensable. However, the logistics of post-mortem angiography is more demanding than the methods applied in our MIA. Moreover, it is more time-consuming, in particular the placement of the catheters, and requires expertise not normally available in general hospitals.

There are a number of potential sources of bias in this study. The teams performing MIA and CA were blinded, however, for the pathologist performing CA the biopsy sites could potentially lead to increased suspicion of pathology in the biopsied areas.
A further limitation of this study is the use of consensus COD as gold standard, because errors are not uncommon with CA.[56,57,90] This appeared also true for our study where CA was performed in the daily routine of an academic hospital, whereas MIA was carried out by a dedicated research team. Another argument against using CA as gold standard, is the fact that some diagnoses are more readily made on imaging than with CA. The use of consensus COD as the gold standard prohibited calculation of sensitivity and specificity of the two compared methods. Therefore, only agreement between the MIA and CA for COD, and agreement of the two methods with the consensus COD was calculated.
Also, for diagnoses, we did not calculate sensitivity and specificity. Specificity cannot be calculated in a meaningful way, since it directly depends on the total number of diagnoses in the entire study population, due to the fact that the number of true negative diagnoses per case increases when more diagnoses are encountered in the population. We decided not to calculate sensitivity, because without a related specificity it is not relevant.
The list of ICD codes was composed by the MIA-researcher, based on the autopsy reports of both methods without input from a pathologist involved in CA. As a precaution, this researcher was not involved in scoring diagnoses. The ICD code list appeared accurate because when scoring the diagnoses for MIA and CA all diagnoses could be coded.
This process of objective scoring of all and major diagnoses, using the ICD-list, resulted in a large number of diagnoses, often involved in the same pathological process. It required grouping of the individual major diagnoses to GMD, to adequately describe

the pathological processes causing death, and to allow meaningful comparison of our study with the literature. Grouping of major diagnoses had to be done retrospectively, because only then the consensus COD was known.
Another limitation of this study is that only few of the 2197 patients who died during the study period were autopsied, and even fewer underwent MIA. This was inevitable, because MIA was only possible if next-of-kin after having given consent to CA, which was the case in only 13.4% of deceased, also consented to MIA. Furthermore, a number of consented MIA could not be executed for logistic reasons. However, the COD established in this sample, most often cardiovascular or pulmonary pathology, are a fair representation of the findings at routine autopsies in Western countries with high-income economies.[142]
Since pathological/histological examination of the brain was consented in a relatively small proportion of MIA (24/99) and CA (38/99) cases, and only 20 underwent both biopsies and brain autopsy, we decided to include all histologically examined cases in our comparison of MIA and CA. The analysis of diagnoses on the brain showed no discrepancies as to COD, more diagnoses with MIA (based on imaging), and more major diagnoses with CA (based on histology). These results emphasize the significance of pathological/histological examination of the brain, in agreement with the large contribution of histological examination to diagnoses established with MIA in general.
Finally, this being a single center study, the conclusions may not be generally applicable.

The high performance of MIA, agreeing with consensus COD in 96/99 cases, should not be surprising, as imaging provides a substitute for gross examination at CA, and excellent guidance for sampling for histology and cytology. Technically the present MIA was improved compared to our previous study, in which biopsies were fewer, and either taken randomly, or guided by ultrasonography.[57] Apart from the current study, our previous one is the only reporting on the diagnostic performance of MIA in patients who died in-hospital using the combination of PMCT and PMMR, and image-guided biopsies. Therefore, our results cannot easily be compared to the literature.

In a cohort of 182 cases, Roberts et al.[102] compared PMCT and PMMR to CA in coroner's cases, and found an agreement for COD of 70% (95% CI: 62.6;76.4). Most often missed were ischemic heart disease, pulmonary embolism, pneumonia, and intra-abdominal lesions. Westphal et al.[104], investigating the feasibility of PMCT only in 29 cases, reported an accuracy for COD of 68% and a positive predictive value of 75%. In agreement with these studies, we found that PMCT and PMMR alone could not reliably diagnose common COD such as pneumonia, myocardial infarction, peripheral pulmonary emboli, gastrointestinal ischemia and sepsis without biopsy confirmation.

Recent studies investigated the additional value of post-mortem CT angiography (PMCTA), achieving an accuracy of 80% for cardiac causes of death.[134] In a selected group of 50 cases Wichmann et al.[80] compared diagnoses (not COD) identified by PMCTA or CA. They found 16 new major diagnoses, comparable to GMD in our study, 93.8% of these were identified by PMCTA and 87.5% by CA. These figures are comparable to the clinically unsuspected GMD in our study. Most recently, in a less biased population, Rutty et al.[133] established a correct COD in 92% of cases using PMCTA relative to consensus COD, as in our study. Bolliger et al.[109] and Ross et al.[108] combined PMCT, PMCTA and biopsies. In our systematic review,[56] we calculated for these studies a pooled sensitivity for COD of 90.9% (95%CI: 74.5-97.6). The performance of MIA in our study is even higher with 97.0% of COD correctly identified.

MIA, apart from being minimally invasive, has other advantages over CA: it provides a permanent auditable record of the entire body including the brain that can be consulted repeatedly, objectively, and at any location by pathologists, radiologists, clinicians, scientists, and next-of-kin. It is therefore potentially a more powerful tool than CA for quality control in healthcare and a more dependable resource for health statistics, epidemiology and biomedical research.[56,95,102] In addition, MIA is suitable for providing biomedical research with pathologically changed tissues, which would otherwise be hard to ascertain, such as metastatic tumor tissue from patients who died of cancer for whom consent for autopsy is often difficult to obtain.[26,143] In the future, a robotic system may enable automated needle placement to shorten the procedure time.[96]

MIA and CA appear to have their own strengths and weaknesses,[56] and future post-mortem examination will probably utilize combinations of the two approaches, such as MIA followed by a partial autopsy, and CA preceded by imaging. In the latter scenario CA could be omitted if COD is established by imaging.[102,136]

In summary, there was a high agreement between MIA and CA as to COD; and importantly MIA resulted in a higher yield of diagnoses. Seventeen percent of COD and 43% of GMD was clinically not suspected, illustrating the lasting importance of post-mortem examination for quality control in healthcare. Further studies into acceptance of MIA by next-of-kin and clinicians have to show whether introduction of MIA will indeed increase autopsy rates, and strengthen its role in healthcare quality control. In an individual case for which a clinician desires an autopsy, including the brain, for clinical or scientific reasons, he may be able to get consent for MIA from next-of-kin refusing CA.

ACKNOWLEDGEMENTS

The authors are very grateful to the families of the included patients, and to the professionals who made valuable contributions to this study: JAH Bongers, J Sloof, prof. dr. CMF Dirven, dr. JS Soria van Hoeve, dr. RK Balvers, J de Groot, dr. PA Wielopolski, R Booij, dr. KH Lam, dr. V Soerdjbalie-Maikoe, dr. M de Vries, dr. AE Odink, MJP Rossius.

We also thank Erasmus MC Health Care Efficiency, Stichting Coolsingel Rotterdam, and Erasmus MC Vriendenfonds for their financial support.

OTHER INFORMATION

Registration: Dutch Trial Register: http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=5072.

TABLES

Table 1-A. Cause of Death – Concordant for MIA and CA (n=91)

Main Pathology in Concordant Causes of Death N = 91	**Incidence N** (%)
Pulmonary Pathology	43 (47%)
Cardiac Pathology	30 (33%)
Sepsis/ MOF/ Shock	20 (22%)
Cerebral Pathology	10 (11%)
Intestinal Pathology	8 (9%)
Metastatic Disease	8 (9%)
Vascular Pathology	7 (8%)
Hemorrhage	6 (7%)
Thrombo-embolic Pathology	6 (7%)
Hematologic Disease	5 (5%)
Infectious Disease	4 (4%)
Liver Pathology	2 (2%)
Urological Pathology	2 (2%)
Intoxication	2 (2 %)
Graft Versus Host Disease	1 (1%)
Organ Rejection	1 (1%)
Storage Disease (aceruloplasminemia)	1 (1%)

Legend: The figures add up to more than 100% because of cases where more than one pathological process was involved in COD.

Table 1-B. Cause of Death – Discordant for MIA and CA (n=8)

Case No.	Sex	Age	MIA	CA	Correct
6	M	72	sepsis erythrophagocytosis multiple organ failure intestinal ischemia	diffuse alveolar damage erythrophagocytosis myocardial ischemia	MIA
16	F	63	tension pneumothorax	acute myocardial infarction	MIA
17	M	60	massive air embolus	myocardial infarction thrombo-emboli	MIA
27	F	92	hemothorax with active bleeding	peripheral pulmonary emboli old hematoma in pleural cavity	MIA
58	M	61	type-A dissection	focal (hypertensive) intracerebral bleeding	MIA
64	M	59	aspiration bronchitis	acute myocardial infarction	CA
79	F	80	shock acute aspiration pneumonia paralytic ileus	shock ischemic colitis	CA
81	F	67	(sub)acute cerebral ischemia pneumosepsis	severe atherosclerosis mesenterial ischemia cerebral ischemia	CA

COD = cause of death; MIA = minimally invasive autopsy; CA = conventional autopsy

Table 2. Cause of Death – Diagnostic Value of Imaging (PMCT and PMMR)

Cause of Death	PMCT	PMMR	PMCT + PMMR
Not Detected	26.3%	9.1%	7.1%
Detected but Uncertain Diagnosis, Biopsy Needed	28.3%	33.3%	33.3%
Detected and Probable Diagnosis, Biopsy to Confirm	34.3%	47.5%	48.5%
Detected and Certain Diagnosis, No Biopsy Needed	11.1%	10.1%	11.1%

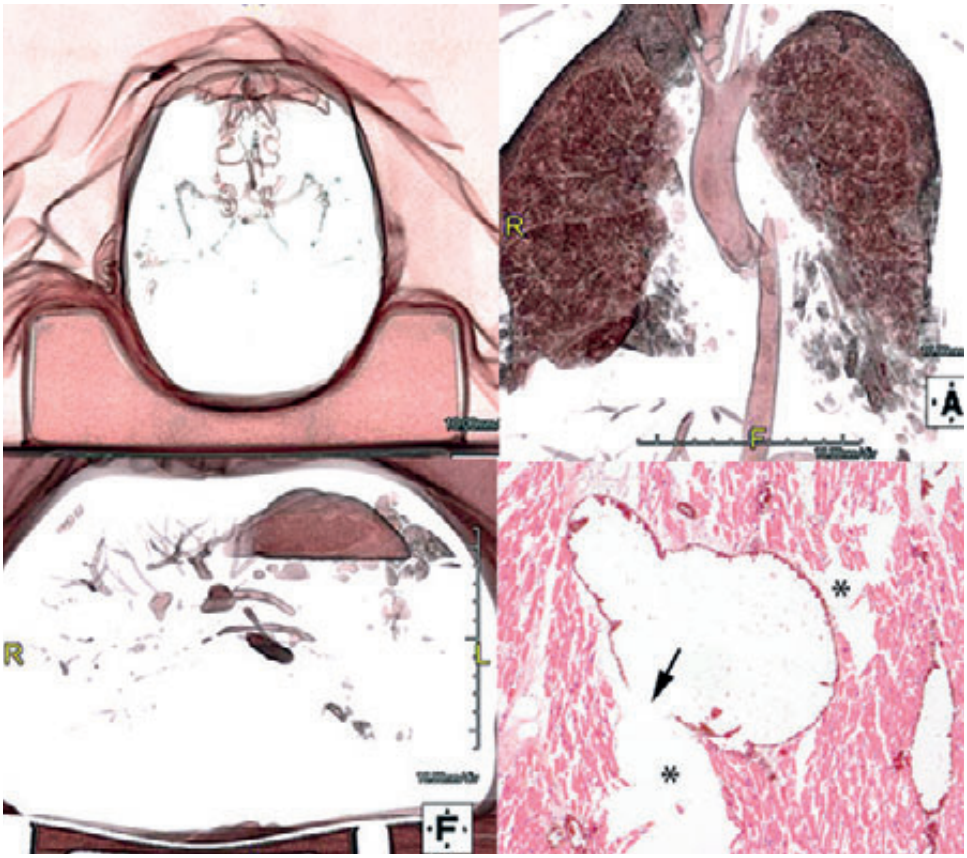
Table 3. Major Diagnoses – Total Number and Unsuspected Diagnoses per Organ/ Tissue Category

Organ/ Tissue Category	Number of Different Post-mortem Diagnoses	Number of Certain Post-mortem Diagnoses in 99 Cases (% of Total)	Clinically Unsuspected Post-mortem Diagnoses (% of Certain Diagnoses)	
			MIA	CA
General	12	82 (6.0)	57 (69.5)	45 (54.9)
Adrenal Gland	2	2 (0.1)	0 (0.0)	2 (100.0)
Bladder	2	2 (0.1)	0 (0.0)	1 (50.0)
Bone Marrow	3	7 (0.5)	3 (42.9)	6 (85.7)
Brain/ Nervous System	20	89 (6.5)	29 (32.6)	51 (57.3)
Female Genital Tract	1	1 (0.1)	0 (0.0)	0 (0.0)
Gastrointestinal Tract	25	73 (5.3)	29 (39.7)	31 (42.5)
Head (ENT/ eye)	3	3 (0.2)	2 (66.7)	2 (66.7)
Heart	25	308 (22.4)	185 (60.1)	199 (64.6)
Kidney	18	74 (5.4)	49 (66.2)	37 (50)
Liver	19	73 (5.3)	44 (60.3)	37 (50.7)
Lung/ Airways	39	430 (31.3)	218 (50.7)	210 (48.8)
Lymph Nodes	3	7 (0.5)	3 (42.9)	3 (42.9)
Male Genital Tract	2	2 (0.1)	1 (50.0)	1 (50.0)
Mediastinum	4	4 (0.3)	3 (75.0)	1 (25.0)
Pancreas	9	12 (0.9)	4 (33.3)	5 (41.7)
Skeleton	1	1 (0.1)	1 (100.0)	1 (100.0)
Soft tissue/ Skin	13	19 (1.4)	7 (36.8)	7 (36.8)
Spleen	8	66 (4.8)	49 (74.2)	24 (36.4)
Thyroid/ Parathyroid	1	1 (0.1)	0 (0.0)	1 (100.0)
Vascular	20	116 (8.5)	60 (51.7)	62 (53.4)
TOTAL	230	1372	745 (54.3)	726 (52.9)

MIA = minimally invasive autopsy; CA = conventional autopsy

FIGURES

Figure 1. Massive Air Embolus as Cause of Death



Legend: 60-year old man who underwent bilateral lung transplantation two months before death. He developed postoperative delirium and was treated for pneumonia. Just prior to death he developed hypotension and agonal breathing. There were signs of elevated jugular venous pressure. Resuscitation during asystole was unsuccessful. The patient died under clinical suspicion of a central pulmonary embolus or cardiac tamponade. CA identified a possible myocardial infarction and thrombo-emboli as the cause of death, whereas MIA explained death by a massive air embolus. The reference standard committee concluded that MIA correctly identified the cause of death.

Volume rendered PMCT image shows diffuse air in the intracranial arteries (left upper panel), in the thoracic aorta (right upper panel), and in visceral arteries of the upper abdomen (left lower panel). Microscopic image of the myocardium showing vessels inflated by air that pushes erythrocytes to the walls, and causes rupture of vessels (arrow), allowing air to escape into the interstitial space, thereby tearing the tissue (asterisks). (Hematoxylin and Eosin, original magnification 50x).

Figure 2. Proportional Venn Diagrams Showing the Concordance of Minimally Invasive Autopsy (MIA), Conventional Autopsy (CA) and pre-mortem Clinical Evaluation (CE): Seven for Grouped Major Diagnoses (All, Specific Organs and Diseases), One for All Neoplastic Diseases

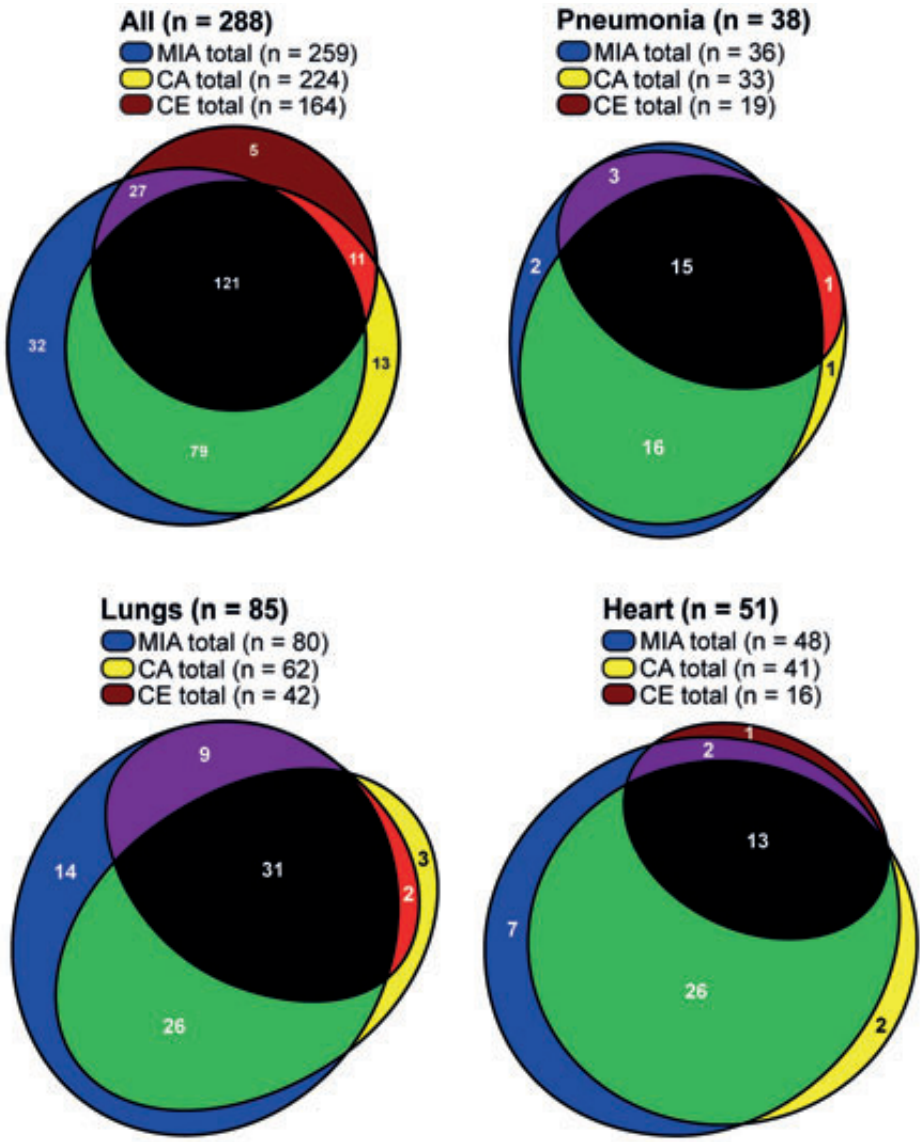
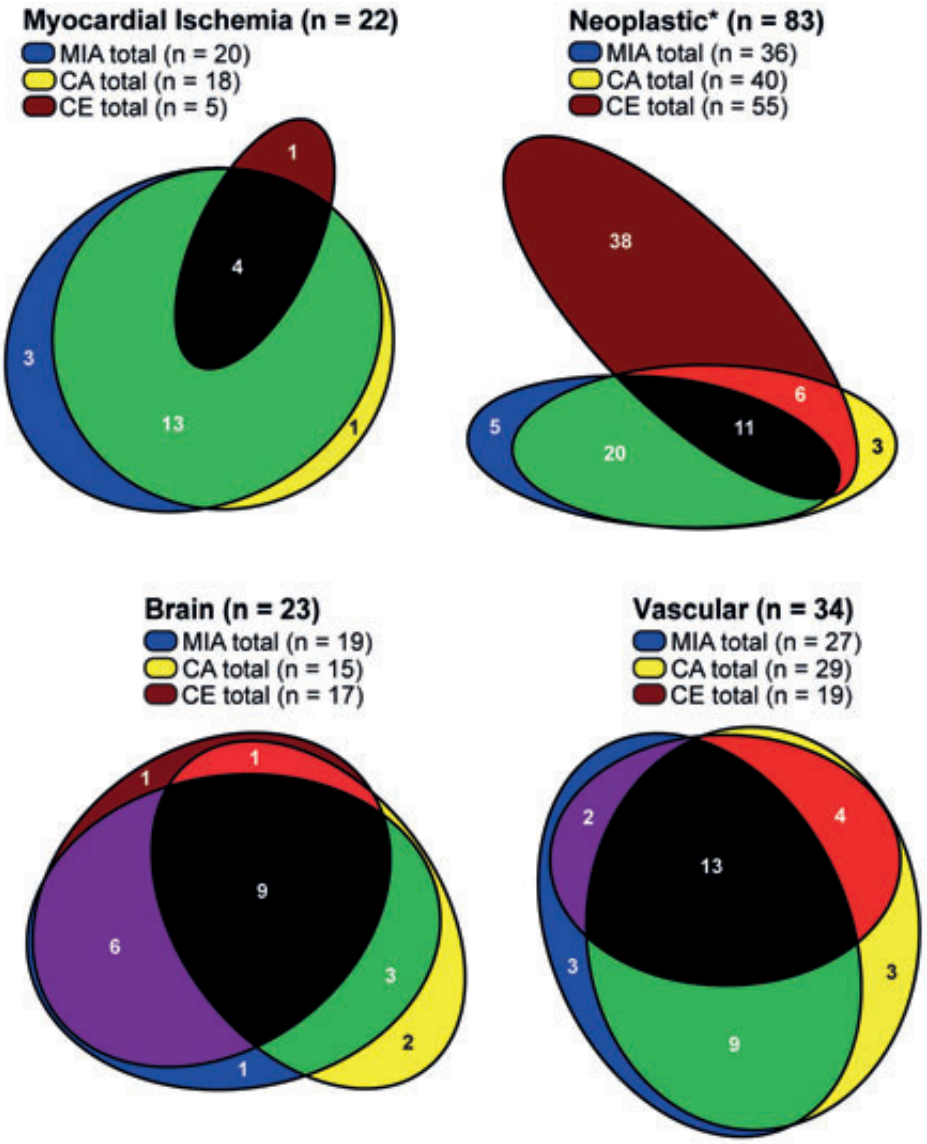


Figure 2. (continued)



* This Venn diagram includes all neoplastic diagnoses (both major and non-major)
MIA = minimally invasive autopsy; CA = conventional autopsy; CE = Clinical Evaluation

Legend: Values represent the numbers of GMD identified by the respective methods. Values in overlapping areas of the circles represent the numbers of GMD made by the methods sharing in the overlap. Diagrams for each organ and disease group are displayed in proportional size to their contribution to the all GMD.
(The Venn diagrams include five certain pre-mortem diagnoses that were not certain at either post-mortem method, but nevertheless scored as major, because of their direct relation to COD.)

APPENDICES

Not included in this thesis:

Appendix A. Excluded Cases
Appendix B. PMMR and PMCT Scan Protocols
Appendix C. Data Acquisition and Processing
Appendix D. Cause of Death – Reference Standard Process
Appendix E. List of Diagnoses Modified to the ICD-10
Appendix F. Flowchart Case Inclusion
Appendix G. MIA Tissue Sampling
Appendix H. Grouped Major Diagnoses - Diagnostic Value of Post-mortem Imaging (PMCT and PMMR) and Biopsy (MIA), and Conventional Autopsy (CA)
Appendix I. All Diagnoses – Total Number and Unsuspected Diagnoses per Organ/ Tissue Category
Appendix J. Cross tables MIA versus CA
Appendix K. Errors

6

Diagnostic accuracy of postmortem CT, MRI and CT-guided biopsies for the detection of ischemic heart disease in a hospital setting

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Accepted for publication in the European Heart Journal - Cardiovascular Imaging

ABSTRACT

Aims: To determine the diagnostic accuracy of postmortem MRI (PMMR), CT (PMCT) and CT-guided biopsy for the detection of acute and chronic myocardial ischemia.

Background: The autopsy rate worldwide is alarmingly low (0-15%). Mortality statistics are important and it is therefore essential to perform autopsies in a sufficient proportion of deaths. The imaging autopsy, noninvasive or minimally invasive autopsy (MIA), can be used as an alternative to the conventional autopsy (CA) in an attempt to improve autopsy rates.

Methods: We included 100 consecutive adult patients who died in hospital, and for whom next-of-kin gave permission to perform both CA and MIA. The MIA consists of unenhanced total-body PMMR and PMCT followed by CT-guided biopsies. CA was used as reference standard.

We calculated sensitivity and specificity and receiver operating characteristics (ROC) curves for PMCT and PMMR as stand-alone test or combined with biopsy for detection of acute and chronic myocardial infarction .

Results: Sensitivity and specificity of PMMR with biopsies for acute myocardial infarction was 0.97 and 0.95 respectively and 0.90 and 0.75 respectively for chronic myocardial infarction. PMMR without biopsies showed a high specificity (acute: 0.92; chronic: 1.00), but low sensitivity (acute: 0.50;chronic: 0.35). PMCT (Total Agatston calcium score) had a good diagnostic value for chronic myocardial infarction (AUC: 0.74, CI: 0.64-0.84), but not for acute myocardial infarction (AUC:0.60, CI: 0.48-0.72).

Conclusion: We found that the combination of PMMR with biopsies had high sensitivity and specificity for the detection of acute and chronic MI.

INTRODUCTION

According to the world health organization cardiovascular disease and stroke are the foremost contributors to worldwide mortality, with ischemic heart disease globally causing almost 9 million deaths per year in 2015. [144] Accurate mortality statistics are important for both policy- and decision-making regarding healthcare funding. For reliable statistics it is essential to perform autopsies in a sufficient proportion of deaths, both in and out of hospital.

Despite available modern diagnostic tests, the conventional autopsy still reveals unexpected findings related to the cause of death in 8.4-24.4% and findings that would have affected patient outcome (class I errors) in 4.1-6.7% of cases. [13,22,145]

Unfortunately today's autopsy rate has dropped to alarmingly low percentages worldwide (0-15%), both for academic and nonacademic hospitals. [146] In the late nineties, the imaging autopsy was introduced as alternative to conventional autopsy as a stimulus to postmortem diagnostics. Since then, a growing number of studies have evaluated the diagnostic value of postmortem CT and MRI with or without image guided biopsies. Two review articles concluded that the imaging autopsy, using a non-invasive or minimally invasive approach, can potentially serve as an alternative to conventional autopsy, but more extensive research in different settings is needed to validate these new autopsy methods. [147,148]

Imaging protocols designed for the living patient differ from postmortem imaging protocols, in particular for cardiac imaging. For example, wall movement abnormalities of the heart cannot be diagnosed. In living patients, contrast-enhanced imaging, either noninvasive or invasive, is the gold standard for diagnosing ischemic heart disease. Postmortem angiographic studies are feasible and not new; since the discovery of X-rays, angiography of organs and tissues has been used as an adjunct to the autopsy procedure. [149] More recently, postmortem total-body angiography, using CT (CTA) or MRI (MRA), has become technically feasible and there is a growing number of studies investigating its diagnostic value. Preliminary results are promising, especially for establishing ischemic heart disease as the cause of death. [150-152]

Interestingly postmortem MRI without the use of contrast agents also shows a sufficient accuracy for detecting both acute and chronic myocardial infarction (MI). The presence and age of MI can be diagnosed by evaluating the signal changes related to morphological alterations in the infarcted myocardium, such as the presence of myocardial edema, fibrosis or fat. [153-156]. Nonenhanced cardiac CT is also useful for detecting coronary artery calcifications.

In this study, we evaluate the minimally invasive autopsy (MIA) approach using both nonenhanced CT and MRI followed by CT-guided biopsies. The aim of this study was to determine the diagnostic accuracy of MRI, CT and CT-guided biopsy for the detection of acute and chronic MI, with conventional autopsy as the reference standard.

METHODS

Study design

This study was undertaken as part of the Minimally Invasive Autopsy (MIA) study; this is a prospective single center cross-sectional study in a tertiary referral hospital comparing diagnostic performance of conventional autopsy and MIA. Conventional autopsy followed MIA on the same case. Pathologists involved with conventional autopsy were blinded to MIA findings as much as possible; however biopsy sites could potentially lead to increased suspicion of the biopsied organs and tissues by the autopsy pathologist. MIA personnel were blinded to autopsy findings.

Patients

From January 2012 through December 2014 all hospitalized patients aged 18 years and older who died at Erasmus University Medical Center were eligible, if written informed consent was obtained from next-of-kin for MIA and conventional autopsy of at least the torso.

Exclusion criteria were (suspected) unnatural cause of death, body size exceeding height of 16 inches in supine position (limitation for MRI), known or suspected "high-risk" infected bodies (tuberculosis, hepatitis B and C, human immunodeficiency virus, methicillin-resistant Staphylococcus aureus, multi-drug resistant Acinetobacter), and open abdominal wounds that could not be completely closed or taped to prevent leakage of body fluids.

Clinical information

All relevant clinical information including medical history and suspected cause of death was recorded and available for both the MIA and the conventional autopsy team. The treating physician decided the most likely cause of death and a differential diagnosis based on the clinical presentation. For the analysis the population was divided into a group with and a group without clinical suspicion for ischemic heart disease.

Minimally Invasive Autopsy procedure

MRI and CT scans were made according to standardized protocols (Table 1-A and 1-B respectively). Total acquisition time was one hour for MRI and around 5 minutes for CT. One radiologist (ACW) with expertise in postmortem radiology, performed the initial read of the MRI and CT scans, compared these to the available premortem imaging, and marked suspected pathological lesions on CT and MRI key images that were used to plan the biopsies.

MRI was performed on a 1.5T scanner (Discovery MR450, GE Medical systems, Milwaukee, Wisconsin USA) and consisted of scans of the brain, neck, thorax, abdomen and pelvis. The MRI total-body protocol consisted of axially-acquired STIR FSE T2w and FLAIR FSE T1w from the cranium to the pelvis. An additional 2D STIR FSE T2w scan and 3D Fatsat FSPGR T1w scan with higher resolution than the total-body scans were acquired of the thorax, using an 8-channel torso array coil. All MRI scans were made in the axial orientation.

After MRI was completed CT scans were acquired from head to feet (Somatom Definition, Siemens Healthcare, Forchheim, Germany). CT data sets of the head, thorax and abdomen were reconstructed with section thickness of 1.0 mm and 5.0 mm in the axial plane and 3.0 mm in the coronal and sagittal planes, by using medium-to-smooth (H31/B31) and very sharp (H70/B70) convolution kernels.

CT-guided biopsies (12 Gauge) were taken from heart, lungs, liver, kidneys, spleen, and radiologically suspected pathology as indicated. In the heart, standard biopsies (5-10 samples) were taken from the lateral wall (mid and basal parts) and apex of the left ventricle. Additional biopsies were taken from MRI signal abnormalities within the myocardium. In those cases where there was a clinical suspicion of myocardial infarction and the MRI showed no signal abnormalities additional biopsies were taken from the septum, anterior and posterior wall. The MIA pathologist (JWO) and researcher (BMB) examined the microscopic slides of the biopsies; when in doubt, they consulted pathologists with specific expertise, not involved in the matching conventional autopsy, to reach a conclusion.

Cardiac imaging evaluation

CT

For each case, CT calcium score was calculated by one observer (IMW) using dedicated software (Syngo.via 3.0 Calcium Scoring®, Siemens Healthcare, Forchheim, Germany) and expressed as total Agatston scores.

MRI

MR images were reconstructed and evaluated in the short axis view. Two radiologists with expertise in cardiac radiology (ACW, APP) independently evaluated MR images and in case of disagreement, consensus was reached in joint sessions. Myocardial infarctions were classified according to a modified classification by Jackowski et al. [153,157,158]

Peracute infarction (within 6 hours after onset) is characterized by T2 hypointense signal in the necrotic center, caused by a state of hypoperfusion. In the acute phase (within 6 hours – 1 week after onset) the marginal areas become edematous and show T2 hyperintense signal, T1 signal in the center is isointense and the edematous marginal regions can show T1 hypointense signal. Subacute infarction (>1 week after onset) shows T2 hyperintense signal in the infarcted area when the area becomes reperfused, while the marginal areas show normal T1 and T2 signal. Chronic infarction (>2 months after onset) shows wall thinning and scar tissue reflected by T1 and T2 hypointense signal and foci of T1 hyperintense signal can be seen due to fatty infiltration.

In our analysis peracute and acute infarctions were grouped into one category and defined as acute MI (<1 week old infarction). Subacute and chronic infarctions were grouped into one category and defined as chronic MI (>1 week old infarction). MRI criteria for determining infarction age are detailed in table 2.

Conventional autopsy

The day after MIA, a resident in pathology, supervised by the attending pathologist, performed conventional autopsy according to the departmental protocol. The autopsy report included medical history, postmortem diagnoses, a presumed cause of death, and answers to specific clinical questions, and was authorized by the pathologist. Macroscopic evaluation consisted of sectioning of the heart in slices of 0.5 to 1 cm and visual inspection of the myocardium. Lactate dehydrogenase (LDH) staining was performed on a mid-ventricular slice. Hematoxylin and Eosin (HE) was used for histologic staining. [159] When there was a discrepancy between the histology of the MIA and conventional autopsy, a pathologist with expertise in cardiac pathology (JHT) reviewed the histology.. The following criteria for myocardial infarction age were used (separately or in combination):

Acute MI: hypereosinophilia and loss of cross striation within myocardial fibers, contraction band necrosis, coagulation necrosis with or without granulocyte infiltration or hemorrhage, and various degrees of nuclear pyknosis, karyolysis, granulocyte infiltration and myocardial edema. Chronic MI: fibroblasts with loose connective tissue formation, angiogenesis (subacute), paucicellular collagenous fibrosis (chronic). [153]

Statistical analysis

Analyses were performed on the patient level. Conventional autopsy was used as the reference standard. We calculated sensitivity and specificity and 95% confidence intervals for the detection of acute and chronic MI for MRI and for MIA (MRI, CT and biopsies). Confidence intervals for sensitivity and specificity are Clopper-Pearson confidence intervals. [160] Inter-observer agreement was calculated using kappa statistics. Calculations were performed using IBM® SPSS® Statistics version 21.

ROC curves

We calculated ROC curves to investigate diagnostic value of CT (Total Agatston calcium score), MRI and biopsies for diagnosing acute and chronic myocardial infarction. In the analysis the diagnostic value (sensitivity and specificity and ROC curves) of biopsies was combined with MRI, because biopsies were taken from radiologically suspect areas, identified at MRI.

RESULTS

Case recruitment

From January 2012 to December 2014, 100 consecutive cases (62 men, 38 women) were included in the study. One case was excluded because autopsy findings warranted a forensic autopsy. The mean interval between death and start of imaging was 23.2±15.6 hours (range: 3.2-71.6). Mean age at the time of death was 62.5 years (range: 25-92). In the group with clinical suspicion of ischemic heart disease 14/30 (46%) patients were admitted to the hospital with out-of-hospital cardiac arrest vs 3/69 (4%) in the group without clinical suspicion of cardiac death.

Agreement between clinical suspicion and autopsy findings

In the group with a clinical suspicion of ischemic heart disease as the cause of death, acute MI was found in 16/30 (53.3%) of cases by conventional autopsy. In the group without clinical suspicion of ischemic heart disease as the cause of death, acute MI was found in 18/69 (26.1%) cases. Twenty-two of the 34 cases with acute myocardial infarction found with conventional autopsy had no known ischemic heart disease during life.

Twenty cases had clinically known ischemic heart disease during life (at least one ischemic episode during life, determined by clinicians). Myocardial infarction, either chronic or acute, was confirmed by conventional autopsy and MIA in 16/20 of these cases (the same cases were identified with MIA and conventional autopsy).

Diagnostic performance

Acute MI was found in 34/99 cases on conventional autopsy and 36/99 cases on MIA. Chronic MI was found in 40/99 cases on conventional autopsy and 51/99 cases on MIA. The diagnostic accuracy of MRI, and biopsies for the detection of acute and chronic MI is shown in table 3 and for CT (Total Agatston calcium score) in table 4, and the ROC curves for acute and chronic MI are shown in Fig. 1 and 2 respectively.

Acute myocardial infarction

Sensitivity of MRI for acute MI was 0.50 (95% CI: 0.32-0.68) and specificity was 0.92 (0.83-0.97). Sensitivity of MIA for acute MI was 0.97 (95% CI: 0.85-1.00) and specificity was 0.95 (0.87-0.99). Fig. 3 shows a case with acute MI.

The area under the curve (AUC) for the detection of acute MI was 0.60 (95% CI: 0.48-0.72) for CT (Total Agatston calcium score), 0.71 (95% CI: 0.60-0.83) for MRI and 0.96 (95% CI: 0.92-1.00) for MRI with biopsy (MIA). Five cases were classified as peracute infarction on MRI, in 2 of these cases no evidence for an infarction was found on conventional autopsy.

Chronic myocardial infarction

Sensitivity of MRI for chronic MI was 0.35 (95% CI: 0.21-0.52) and specificity was 1.00 (95% CI: 0.94-1.00). Sensitivity of MIA for chronic MI was 0.90 (95% CI: 0.76-0.97) and specificity was 0.75 (95% CI: 0.62-0.85). Fig. 4 and 5 are examples of cases with chronic MI.

The area under the curve (AUC) for the detection of chronic MI was 0.74 (95% CI: 0.64-0.84) for CT (Total Agatston calcium score), 0.68 (95% CI: 0.56-0.79) for MRI and 0.82 (95% CI: 0.74-0.91) for MRI with biopsy (MIA).

Inter-observer agreement MRI

The two radiologists were in agreement in 82 of 99 cases, in the remaining 17 cases consensus was reached in joint sessions. The kappa score of inter-observer agreement was 0.85.

DISCUSSION

In this study we investigated the diagnostic accuracy of a minimally invasive autopsy (MIA) consisting of MRI, CT and CT-guided biopsy for detection of ischemic heart disease in a hospital setting. We found that the combination of MRI and biopsies, had the highest accuracy for detecting acute and chronic MI with conventional autopsy as

reference standard. MRI without biopsies showed a high specificity, but low sensitivity for acute and chronic MI. High CT Agatston calcium score (>400) was a good predictor for chronic MI, but not for acute MI.

We found a lower sensitivity of MRI as a standalone test for acute MI (0.50) compared to other studies investigating MRI. Ruder et al. reported that with MRI acute MI (within 3 hours after onset) could be detected in ex vivo porcine hearts in which they correctly detected acute infarctions in all twenty-one cases. [161] Forensic studies showed that with MRI acute and chronic MI (up to 100% sensitivity) could be accurately diagnosed in human subjects. Importantly, MRI could diagnose peracute MI (onset within 3 hours) in cases not yet showing histological changes, but with a matching coronary stenosis at conventional autopsy. [153,154,158]

The differences in sensitivity and specificity among studies can be explained by the differences in studied population and clinical setting; most are forensic studies that investigated subjects who died under the suspicion of an out-of-hospital-cardiac-arrest and as such had a high pre-test probability. Also, these studies often involve high-resolution cardiac imaging at 3T scanners using surface coils and relatively long scan time for imaging only the heart (approximately 1 hour). [157] Conversely, we scanned in a hospital setting and performed total-body imaging to diagnose both cardiac and non-cardiac cause of death. So as not to interfere with the patient workflow at the MR scanner, we were restricted to one-hour scan time for imaging the entire body.

The addition of biopsies to MRI increased the sensitivity substantially. This highlights the importance of extensive sampling, even when no changes are visible yet on MRI. The big difference between sensitivity of MRI and MRI combined with biopsies can be explained by the quantity of sampling. From each biopsy location, at least 5 samples were taken, e.g. from the lateral wall also the mid and posterior segments were biopsied. Furthermore in those cases where there was a clinical suspicion of myocardial ischemia and the MRI showed no signal abnormalities, extra biopsies were taken from the septum, anterior and posterior wall (both mid and posterior segments).

The noninvasive approach (CT and/or MRI) is less expensive than the minimally invasive approach (imaging plus biopsy). CT is now widely used as a stand-alone modality because of its high accessibility, short examination time and robust performance. CT can provide better mortality statistics than the cause of death determined by the clinician, and is useful for excluding certain diagnoses. However, for diagnosing acute myocardial infarction, our results show that the diagnostic accuracy of CT as stand-alone test is insufficient. [162,163]

To improve CT performance, in particular for ischemic heart disease, more recent studies report on the diagnostic value of CT angiography. Grabherr et al. extensively performed feasibility studies on CTA using different contrast agents and perfusion

techniques. There are different CTA approaches; it can be targeted at the coronary arteries by selective placement of the catheter at the level of the coronary ostia, or total-body CTA can be performed including multiphase scanning. [120,150,164,165]
Wichman et al. applied total-body CTA in 50 ICU patients who died unexpectedly or within 48 hours of an event requiring resuscitation and found that CTA confirmed 93% of the clinical diagnoses, and autopsy confirmed 80%. In addition, CTA and CA identified 16 new major and 238 new minor diagnoses. They concluded that in cases of unexpected death CTA was a valuable addition to autopsy.

Rutty et al. performed total-body CTA in 210 cases of natural and non-suspicious unnatural death and found that CTA established a cause of death in 92% of cases. The number of discrepancies with the final cause of death was not significantly different between autopsy and CTA, suggesting that total-body CTA is a feasible alternative to autopsy. [166,167]
A drawback of CTA is that it requires specific training, technical equipment and contrast agents and is time-consuming due to extensive preparation of the body (e.g. intra-arterial and/or intravenous femoral access for catheter placement) prior to scanning.
At the time of the study, the equipment was not available and professional expertise and scanner availability to perform CTA was lacking.
Another noninvasive approach is the use of stand-alone MRI. Diffusion tensor imaging (DTI) is showing promising results in diagnosing myocardial ischemia in situ, correctly predicting MI (either acute or chronic) with an accuracy of 0.73, using fractional anisotropy and mean diffusivity. [168]
First studies show that quantitative MRI can detect and differentiate between early and following stages of myocardial ischemia based on T1, T2 and proton density values. [169-171] They concluded that temperature-corrected quantitative MRI can diagnose early acute, acute and chronic MI, but histological confirmation is required.
In the hospital setting an important part of every postmortem examination should be a thorough evaluation of the medical history and clinical circumstances prior to death. In our patients with known obstructive coronary artery disease, chronic and or acute MI was confirmed in 16 out of 20 cases by postmortem examination (both by MIA and conventional autopsy). Conventional autopsy found 22 new cases of acute MI, highlighting the lasting need for postmortem examinations.
Today the use of postmortem imaging, mostly CT, is widely accepted as adjunct to the medicolegal autopsy. [48,148,166,167,172] In the hospital setting, there is a growing interest in postmortem imaging, however, expertise and logistics (e.g. access to scanners) are still important limitations.

CONCLUSION

We evaluated the diagnostic accuracy of minimally invasive autopsy for the detection of ischemic heart disease in a hospital setting. We found that the combination of MRI with biopsies had high sensitivity and specificity for the detection of acute and chronic MI.

TABLES

Table 1-A. Postmortem magnetic resonance protocol

Scan area	Coil	Sequence	TR/TE/TI (ms)	Slice width (mm)	FOV (cm)	Matrix	Number of slices	Coverage per section (cm)	Number of sections	Scan time per section (s)
Head - Pelvis	Body	FLAIR FSE T1w	2320/9.5/963	4.0/no gap	48x48	384x320	50	20.0	5-8	174
Head - Pelvis	Body	STIR FSE T2w	12000/41/120	4.0/ no gap	48x48	288x224	50	20.0	5-8	168
Thorax	8-channel torso array	3D fs FSPGR T1w	3.3/1.2/14	1.6	40x40	256x256	212	33.9	1	153
Thorax	8-channel torso array	2D STIR FSE T2w	11200/ 94/120	2.0 / no gap	40x40	256x256	170	34.0	1	359

TR, repetition time; TE, echo time; TI, inversion time; FOV, field of view; FLAIR, fluid attenuated inversion recovery; FSE, fast spin echo; STIR, short tau inversion recovery; fs, fat-saturated; FSPGR, fast spoiled gradient echo. All scans were made in the axial orientation.

Table 1-B. Postmortem computed tomography protocol

Scan area	Rotation time (s)	Tube voltage (kV)	Tube current (eff. mAs)	Slice collimation (mm)	Pitch	Scan time (s)	Reconstruction
Head - Neck	1.0	100	750	2 x 64 x 0.6	0.35	21	Filtered back-projection
Thorax - Pelvis	1.0	120	600	2 x 64 x 0.6	0.6	32	Filtered back-projection
Pelvis - Lower extremities	1.0	120	600	2 x 64 x 0.6	0.6	57	Filtered back-projection

Table 2. PMMR criteria for determining infarction age*

		Necrotic center		Marginal regions	
		T1	T2	T1	T2
Acute	Peracute (<6 hours)	=	↓	=	=
	Acute (6 hours – 1 week)	= / ↑	↓	↓	↑
Chronic	Subacute (1 week – 2 months)	=	↑	=	=
	Chronic (>2 months)	↓ / ↑ (fat)	↓	=	=

* Criteria based on Jackowski et al. [153,157,158]

Table 3. Diagnostic accuracy of MRI and MIA

	Prevalence of disease (%)	N	TP	TN	FP	FN	K	Sensitivity (%)	Specificity (%)
MRI									
Acute MI	34	99	17	60	5	17	0.46	50 (32-68)	92 (83-97)
Chronic MI	40	99	14	59	0	26	0.39	35 (21-52)	100 (94-100)
MIA									
Acute MI	34	99	33	62	3	1	0.91	97 (85-100)	95 (87-99)
Chronic MI	40	99	36	44	15	4	0.62	90 (76-97)	75 (62-85)

N, number; TP, true positives; TN, true negatives; FP, false positives; FN, false negatives; K, kappa measure of agreement; PMMR, postmortem magnetic resonance; MI, myocardial infarction; MIA, minimally invasive autopsy.
Values in parentheses represent upper and lower bound for 95% confidence interval.

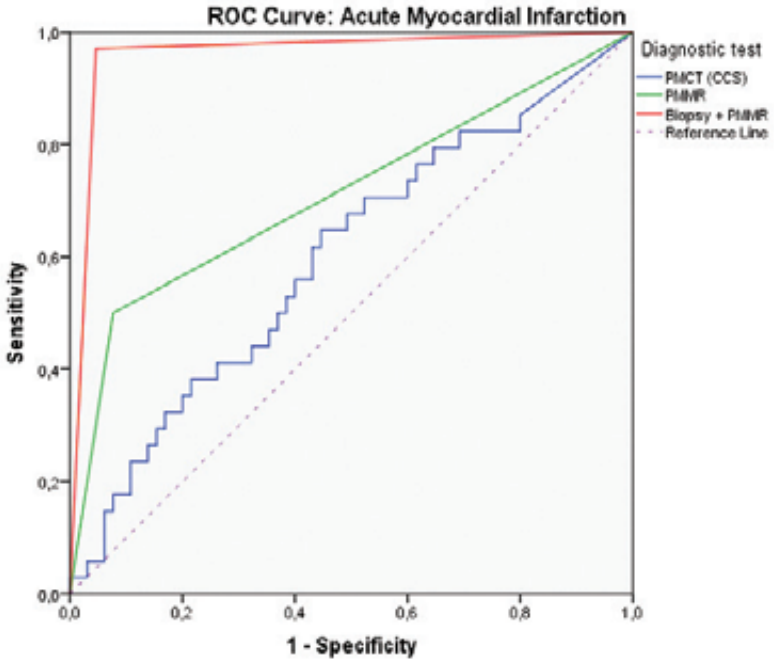
Table 4. Diagnostic accuracy of CT (Total Agatston calcium score)

	Prevalence of disease (%)	N	TP	TN	FP	FN	K	Sensitivity (%)	Specificity (%)
Calcium score >0									
Acute MI	34	99	29	13	52	5	0.04	85 (68-95)	20 (11-32)
Chronic MI	40	99	38	16	43	2	0.19	95 (83-99)	27 (16-40)
Calcium score >100									
Acute MI	34	99	24	30	35	10	0.14	71 (53-85)	46 (34-59)
Chronic MI	40	99	32	32	27	8	0.32	80 (64-91)	54 (41-67)
Calcium score >400									
Acute MI	34	99	17	41	24	17	0.13	50 (32-68)	63 (50-75)
Chronic MI	40	99	25	43	16	15	0.35	63 (46-77)	73 (59-84)

N, number; TP, true positives; TN, true negatives; FP, false positives; FN, false negatives; K, kappa measure of agreement; MI, myocardial infarction.
Values in parentheses represent upper and lower bound for 95% confidence interval.

FIGURES

Figure 1. ROC curve: Acute myocardial infarction

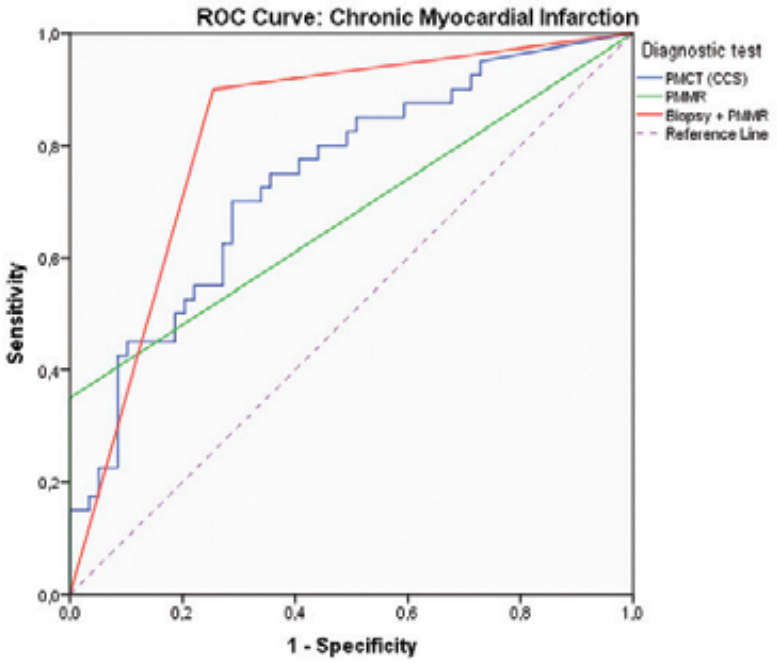


Area Under the Curve (AUC)

Diagnostic test(s)	AUC	95% CI
PMCT (Total Agatston calcium score)	0.60	0.48-0.72
PMMR	0.71	0.60-0.83
PMMR + biopsy	0.96	0.92-1.00

Legend: Receiver operator characteristics curves (ROC) for CT, MRI and biopsy combined with MRI for the detection of acute myocardial infarction. The table details area under the curve for the different diagnostic tests and their corresponding 95% confidence intervals.

Figure 2. ROC curve: Chronic myocardial infarction

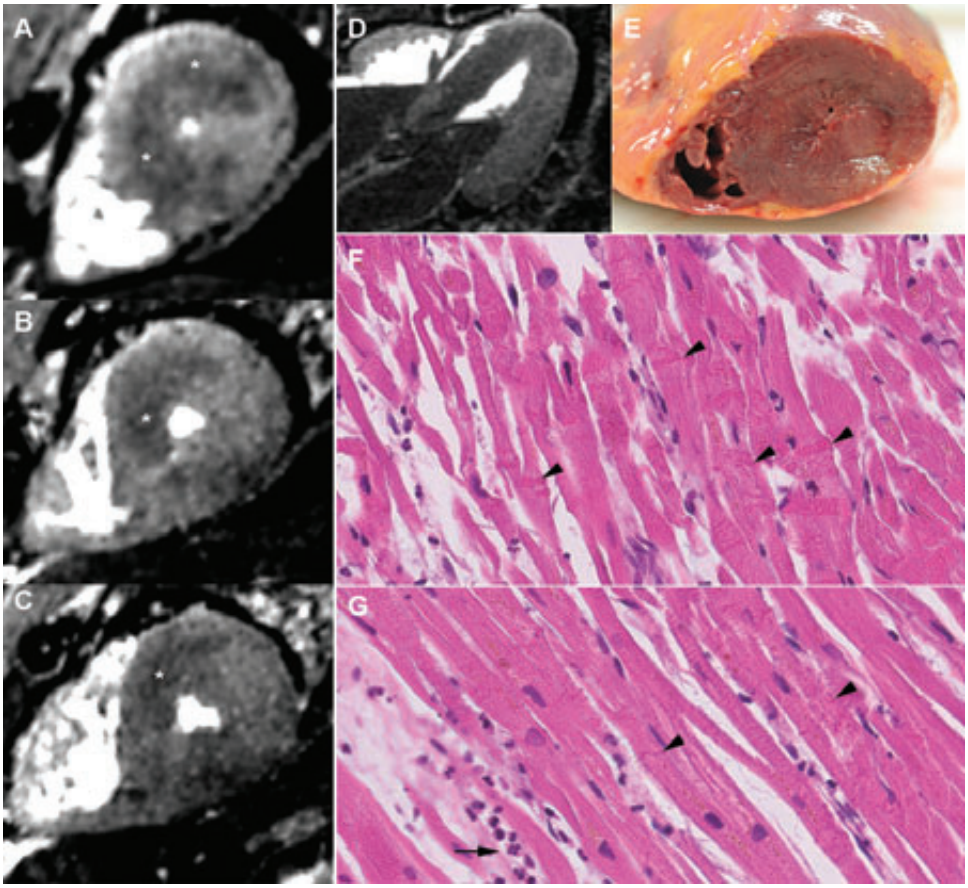


Area Under the Curve (AUC)

Diagnostic test(s)	AUC	95% CI
PMCT (Total Agatston calcium score)	0.74	0.64-0.84
PMMR	0.68	0.56-0.79
PMMR + biopsy	0.82	0.74-0.91

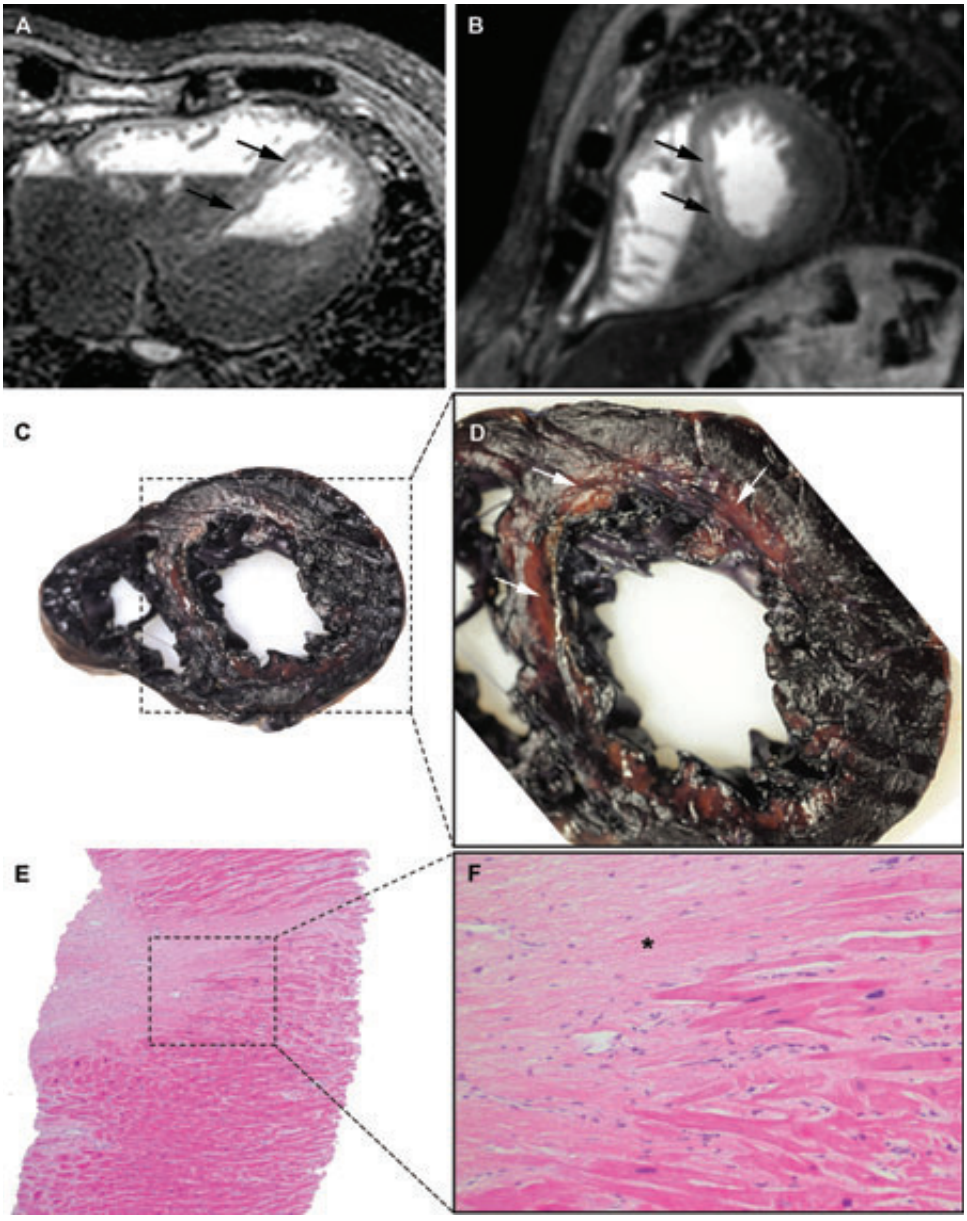
Legend: Receiver operator characteristics curve (ROC) for CT, MRI and biopsy combined with MRI for the detection of chronic myocardial infarction. The table details area under the curve for the different diagnostic tests and their corresponding 95% confidence intervals.

Figure 3. Example of acute myocardial infarction



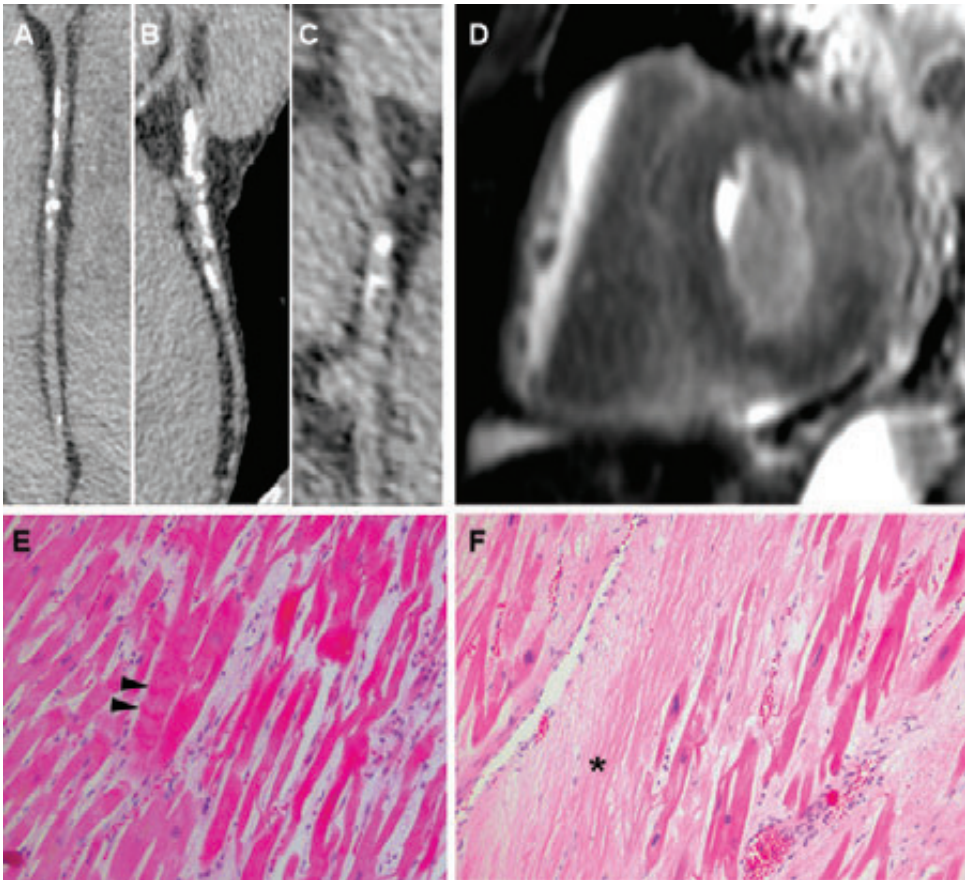
Legend: 45-year old man, who died shortly after requiring cardiopulmonary resuscitation following a period of acute chest pain during sports. T2w MRI short axis (A, B, C) and axial (D) views show diffuse hypointense signal (asterisks) on LAD territory (the entire septum, and anterior and posterior apical wall). T1w MRI did not show any abnormalities. Macroscopy (E) appeared normal. These areas were biopsied and microscopy (HE stain) shows contraction band necrosis (arrows) (F) and infiltration of granulocytes (arrow) (G) confirming acute myocardial infarction.

Figure 4. Example of chronic myocardial infarction

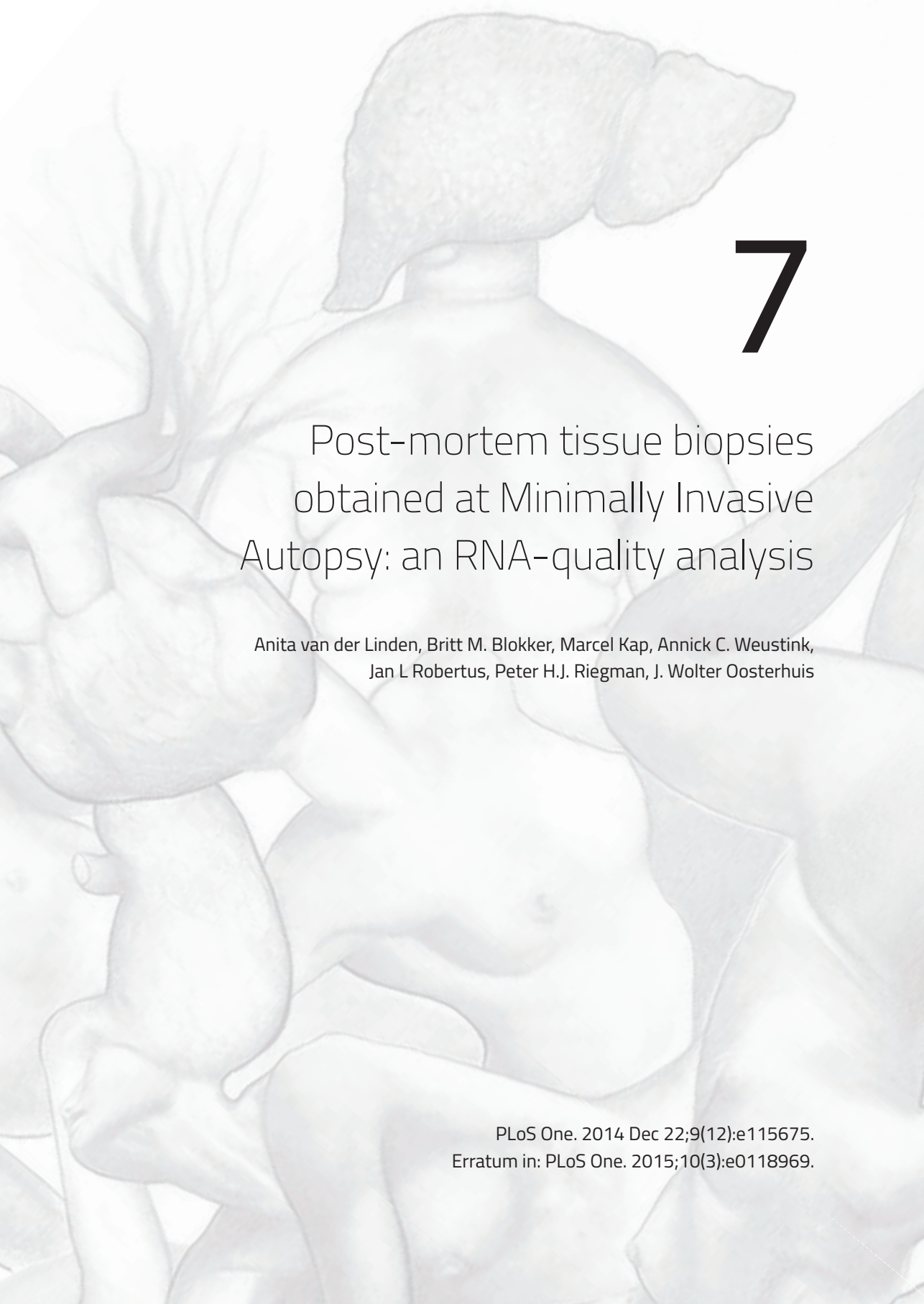


Legend: 66-year old man with a medical history of ischemic heart disease, who died after a period of dyspnea and anemia. MRI STIR FSE T2w MRI (A) axial and (B) short axis showing septal mid and apical wall thinning of the left ventricle with hyperintense T2 signal (black arrows), indicative of a chronic infarction with subacute infarction overlying. (C) and (D) mid ventricular slice stained with LDH, showing discolouration of the corresponding areas of the myocardium (white arrows) indicative of acute infarction. (E) and (F) HE staining of CT-guided biopsy taken from the suspected area in the interventricular septum showing uninflamed replacement fibrosis with viable adjacent myocardium (asterisk).

Figure 5. Example of CT calcium score as a diagnostic test for chronic myocardial infarction



Legend: 54-year old man with no medical history of ischemic heart disease. CT curved MPR of the RCA (A), LAD (B) and LCX (C), showing severely calcified coronary arteries (Total Agatston score: 409). T2w MRI (D): diffuse T2 hyperintense signal indicative of myocardial edema as a sign of acute myocardial infarction. These areas were biopsied and microscopy (HE) shows contraction band necrosis (E: arrow heads) and hypereosinophilia fitting with the diagnosis of acute myocardial infarction and connective tissue (F: asterisk) within a region of chronic myocardial infarction.



7

Post-mortem tissue biopsies obtained at Minimally Invasive Autopsy: an RNA-quality analysis

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PLoS One. 2014 Dec 22;9(12):e115675.
Erratum in: PLoS One. 2015;10(3):e0118969.

ABSTRACT

Introduction: Bereaved relatives often refuse to give consent for post-mortem investigation of deceased cancer patients, mainly because of the mutilation due to conventional autopsy (CA). Minimally invasive autopsy (MIA) may be a more acceptable alternative and, if implemented in clinical practice, creates an opportunity to more often obtain post-mortem tissue samples of (recurred) primary tumors and metastases for molecular research. As a measure for tissue quality for molecular studies, we hereby present a feasibility study, comparing the RNA quality of MIA and CA samples, and fresh frozen samples as reference.

Materials and methods: Tissue samples of heart, liver and kidney were prospectively collected from 24 MIAs followed by CA, and compared to corresponding archival fresh frozen tissue. After RNA isolation and RT-qPCR, RNA integrity numbers (RIN) and *GAPDH* expression (six amplicon sizes ranging from 71 to 530 base pairs) were measured. RIN values and *GAPDH* Cq values were analyzed and compared between all sample groups and post-mortem intervals (PMI).

Results: RIN values in MIA samples were significantly higher than those in CA samples. *GAPDH* was expressed significantly higher in MIA samples than in CA samples and 530bp PCR products could be measured in all cases. *GAPDH* expression was significantly lower in samples with PMI >15 hours. As expected, the samples of the fresh frozen reference standard performed best in all analyses.

Conclusion: MIA samples showed better RNA quality than CA samples, probably due to shorter PMI. Both had lower RNA quality and expression levels than fresh frozen tissue, however, remaining *GAPDH* RNA was still sufficiently intact. Therefore, other highly expressed genes are most likely also detectable. Gene array analysis should be performed to gain insight into the quality of entire post-mortem genomes. Reducing PMI will further improve the feasibility of demanding molecular research on post-mortem tissues, this is most likely more feasible with MIA than CA.

INTRODUCTION

In most cancer patients, only tissues from the primary tumors are biopsied or resected for diagnostic and therapeutic purposes. Outside the context of studies clinicians do not biopsy recurrent or metastatic disease, unless it has therapeutic significance. This hampers the molecular comparison of primary and metastatic disease, despite the fact that it is now evident that there is not just intra-tumor heterogeneity[173,174] but that there are also considerable molecular differences between primary tumors and metastases.[175-178] Chemotherapeutic and other systemic treatments based on genetic characteristics of the primary tumor may not work effectively on metastases due to changes of molecular targets, such as receptor conversion.[179-181] Knowing the molecular characteristics of metastases may help to target them specifically.

It is, therefore, necessary to pursue molecular research, comparing primary tumors and metastases. Post-mortem investigation is an opportunity to obtain tissue samples from (recurred) primary tumors and metastases for comparative molecular studies.[182,183] The so-called "rapid autopsy" is performed soon after death, in order to minimize post-mortem degradation of collected tumor samples and allows for procurement of among others high quality RNA.[184,185]

Unfortunately, autopsies are rarely performed on patients who died of cancer. Bereaved relatives are often not willing to give their consent for conventional autopsy (CA), mainly because they feel that their loved one has suffered enough from the disease and they consider (further) mutilation of the deceased's body undesirable.[38,40,81] Minimally invasive autopsy (MIA) may be an acceptable alternative to CA,[57] because with MIA, the body is imaged by CT and MRI and tissue samples from a (recurred) primary tumor and metastases are obtained through CT-guided biopsies, leaving the body intact.

Here we have investigated RNA in such biopsies as a measure of overall quality of the tissue for molecular studies. We studied whether the biopsies yielded: a) a sufficient amount of RNA and b) RNA of sufficient quality for downstream RNA analysis. By using RNA isolated from MIA, CA and fresh frozen ex vivo tissue in a RT-qPCR amplicon size assay, we were able to determine the levels of post-mortem RNA degradation and quality.

MATERIALS AND METHODS

In this prospective study RNA quality of post-mortem tissues was examined and compared to fresh frozen samples. The post-mortem tissues were obtained from two types of post-mortem examination: MIA and CA. Heart, kidney and liver tissue samples were collected at both MIA and subsequent CA in the same case, thereby excluding inter-patient variation between the two types of post-mortem samples.
Fresh frozen samples of the same three organ types, which had been obtained from living subjects, were culled from our frozen tissue bank. The three tissue types were selected based on accessibility during MIA, different rates of postmortem autolysis, availability in the frozen tissue bank and previous studies, showing acceptable results for basic molecular research with these tissue types.[186,187] The collected tissue samples were not always free from pathological changes.

Subject inclusion and clinical states

This study was approved by the Erasmus MC Medical Ethical Committee (file MEC-2011-055-amendment 002). All cases of in-hospital deceased adult patients whose bereaved relatives have given signed informed consent for both MIA and CA could potentially be included in this study protocol. Samples of fresh frozen residual tissue of heart, kidney and liver derived from surgical specimens or biopsies were provided by the Erasmus MC Tissue Bank and used according to the Dutch Code of Conduct 2011.
The autopsy samples were collected in the period between 11-28-2012 and 11-27-2013 whenever the responsible researcher (AvdL) was available for tissue sampling.
The time of death, entered by the subject's physician, and the time of tissue sampling at MIA and CA were registered. The time elapsed between death and the freezing of the sampled tissue was defined as the post-mortem interval (PMI). PMI is an important parameter known to influence tissue degradation[188] and thus RNA integrity, also taken into account in forensic pathology.[189] The MIA was always performed at the evening before the CA, therefore the PMI was longer in tissues collected at CA. Medical data from the last phase of life (fever, hypoxia, hypertension and diabetes) and patient body mass index (BMI) were obtained from the subject's medical records and the autopsy forms filled in by the subject's physician.

Tissue sampling

During the MIA tissue samples were obtained with CT guided biopsies, using a 12-gauge needle. Immediately after each biopsy the sampled tissue was snap frozen in a 50 ml tube filled with pre-cooled isopentane on dry ice.[190] The samples were then placed in a pre-cooled aluminum vial, stored temporarily in a -80°C freezer and finally transferred into liquid nitrogen storage.
Immediately after MIA the body was returned to the mortuary with an ambient temperature of 4°C. The following day tissue samples of approximately 0.5 cm^3 were collected from the same organs in the same subject during CA. Due to logistics at CA, snap freezing immediately after harvesting was not possible in all cases. In two cases the tissue samples were temporarily stored at 4°C before snap freezing. All collected samples were eventually stored in liquid nitrogen until RNA could be isolated.
Fresh frozen tissues were either derived from surgically resected tissues or from biopsies (heart). These tissue samples were stored in liquid nitrogen in the Erasmus MC Tissue Bank after being snap frozen using pre-cooled isopentane and liquid nitrogen.
Two extra heart samples (1 from MIA, 1 fresh frozen from the Erasmus MC Tissue Bank) were collected for training purposes. To prevent selection bias, these extra samples were both included in the analyses.

RNA extraction, RIN measurement and frozen H&E sections

Depending on the size of the sample, 10 to 20 10 µm thick frozen sections were cut on a cryostat microtome (Microm HM560, Adamas, The Netherlands). The sections were transferred to 700 µL Qiazol (Qiagen, Hilden, Germany) and RNA was extracted from these sections using the miRNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. RNA was eluted in 40 µL RNase-free water and 1 µL of RNase inhibitor (20 U/µL; AB, California, USA) was added to avoid RNA degradation during further handling.
The RNA integrity number (RIN value)[191] of the extracted RNA and the RNA concentration (ng/µl) was assessed by on-chip-electrophoresis, using the BioAnalyzer (Agilent RNA 6000 Nano kit and BioAnalyzer 2100 Expert, Agilent Technologies, USA).

5µm sections were cut and stained with Haematoxylin and Eosin (H&E) for morphological investigation of the samples used for RNA isolation. The morphologic tissue quality assessment comprised of 1) the representativeness of the sample (yes or no); 2) the presence of necrosis (yes or no); 3) scoring the degree of autolysis: no (morphology unaffected), moderate (mild loss of staining of nuclei; some detachment of endothelium in vessels), or severe (severe/complete loss of staining of nuclei; complete detachment of endothelium in vessels).

5 fresh frozen samples and 9 MIA samples contained so little tissue, that it was all needed for the RNA isolation and no histologic slide could be made. For 3 fresh frozen samples, 2 MIA samples and 6 CA samples the necrosis could not be scored, due to freezing artifacts rendering the interpretation of the morphology uncertain. These cases were registered as 'no scoring possible' (n/s), due to severe autolysis.

cDNA synthesis and RT-qPCR

Some smaller tissue samples did not yield the required concentration of 100 ng/µl RNA for cDNA synthesis. In order to achieve a concentration of 100 ng/µl total RNA per sample, a volume containing 1 µg of RNA from all samples (to assure equal treatment of all samples) was transferred to another test tube and air dried by Vacuspin (SpeedVac AES1010, Savant, USA) for 30 minutes at 45°C. 10 µl of RNase free water was then added to reconstitute the RNA samples to the required 100 ng/µl total RNA.

cDNA synthesis of all samples, including a positive control (MCF7 cell line RNA) and a no template control (NTC), was performed as previously described.[177]
Three pools of randomly picked cDNA samples were made by taking 1µl of cDNA of 10 fresh frozen (pool 1), 10 MIA (pool 2) and 10 CA (pool 3) samples and diluting them twenty times in water. A fourfold dilution series was made of the initial pool samples to create a sensitivity curve. This additional step, consisting of fifteen samples in total, was added to each PCR assay to assess the efficiency of the assays.
To determine the quantity (degradation in post-mortem samples compared to the fresh frozen samples) and quality (acceptable length of RNA fragments for demanding downstream RNA based genomic techniques), quantitative real time polymerase chain reaction (RT-qPCR) with the *GAPDH* amplicon size assay (table 1), as previously described by Viertler et al,[192] was performed on all patient and control samples.

Data analysis

Differences in RIN values and Cq values, with respect to tissue types (heart, kidney and liver); sample types (fresh frozen, MIA and CA); PMI; clinical/patient related data and morphology scores were analyzed in SPSS (IBM SPSS Statistics, version 21.0) using the Mann-Whitney U test and the Paired Kruskal-Wallis test.
Since the Cq values were measured for 6 *GAPDH* base pairs per tissue sample, multiple testing needed to be applied.[193,194] Therefore, the usual significance level of P=0.05 was divided by 6, resulting in a significance level of P=0.00833, which was used for the analyses of RT-qPCR outcomes.

RESULTS

Sample collection

A total of 218 tissue samples from three different tissue types were collected. In 23 out of 24 autopsy cases the MIA and CA samples were collected from the same corpse. In 1 autopsy case, the samples had only been collected during MIA and not during the CA; therefore CA samples from another autopsy case were collected from the Erasmus MC Tissue Bank. For training purposes two additional heart samples were collected, one fresh frozen sample and one MIA sample. These were both included in the analyses.
All fresh frozen tissue samples were culled from the Erasmus MC Tissue Bank. Per sample type three tissue types were collected (see table 2).

The collected tissues were categorized based on post-mortem interval (PMI). The post-mortem intervals of our cases ranged from 10 to 59 hours. Six PMI categories were created, starting with the first category of up to 12 hours, each following category consisting of a post-mortem interval of 10 hours, and a last category consisting of 14 hours. An overview of the distribution of PMI categories for the included cases is given in table 3.

An overview of the available (and potentially relevant) clinical variables is shown in table 4 and an overview of the morphologic tissue quality assessment of the samples is shown in table 5.

RNA integrity

RIN values were established for 199 samples. In 6 fresh frozen samples, 3 MIA samples and 2 CA samples the RNA integrity could not be established, because the RNA yield of the sample was too low. These samples were excluded from the analyses, as not being able to establish RIN values was due to the size of the tissue samples and not to the tissue quality.
In 4 MIA samples and 4 CA samples the RNA integrity could not be established, due to extensive RNA degradation (RIN <1.0). These samples were included in the analyses with an RIN-value of 0 (zero), resulting in a total of 207 samples for the analysis.
The median RIN value and interquartile range for all three sample types are shown in figure 1-A. The RIN values in fresh frozen samples were significantly higher than those in post-mortem samples ($P<0.001$, Unpaired Mann-Whitney test, significance level 0.05), and the RIN values in MIA samples were higher than those in CA samples ($P = 0.032$, Unpaired Mann-Whitney test, significance level 0.05).

Figure 1-B shows the median RIN values and interquartile range per sample type and tissue type. For each tissue type the RIN values of fresh frozen samples were significantly higher than those in post-mortem samples (P<0.001, unpaired Mann-Whitney test, significance level 0.05). No significant differences in RIN value per tissue type were found between MIA and CA. The morphological factor autolysis adversely influenced RNA integrity (P < 0.001). Of the patient related agonal factors, hypoxia, BMI, hypertension and dyslipidemia did not influence RNA integrity, but fever and diabetes did have an adverse effect on the RIN value in post-mortem samples (both P = 0.006, unpaired Mann-Whitney test, significance level 0.05).

RT-qPCR

RT-qPCR was performed on all 218 samples and Cq values could be established for 216 samples. In one case the RNA yield was too low, in the other case the Cq value could not be measured due to technical failure. These samples were excluded from the analyses concerning Cq values. Figure 1-C shows that median Cq values are lowest in fresh frozen samples for the *GAPDH* 71 bp assay. The Cq values in CA samples are highest. Figure 1-D show the Cq values per tissue type. Liver tissues have the highest Cq values for all sample types.

Figure 2 shows the Cq values of all *GAPDH* amplicon sizes per sample type. The difference in Cq values between fresh frozen and post-mortem samples (MIA and CA) is significant for all *GAPDH* amplicon sizes (P< 0.001, Paired Kruskal-Wallis test, significance level 0,00833). The difference in Cq values between MIA and CA samples is not significant for all *GAPDH* amplicon sizes, as shown in table 6. When the Cq values were stratified per tissue type, there were no significant differences between MIA and CA samples.

The RNA integrity of all samples decreases with increasing post-mortem interval (see figure 3-A). Figure 3-B shows the RIN values of the three different tissue types per PMI. The fresh frozen samples are represented by PMI category "0". The RIN value in this category was significantly higher than in all other categories, but there were no significant differences in RIN values between PMI categories 1 to 5.

According to figure 3-C, the *GAPDH* 71 bp assay Cq values increase with PMI (i.e. decrease of *GAPDH* expression level). Figure 3D shows the GAPDH 71 bp assay Cq values per tissue type per PMI. Cq values in PMI category 2 are significantly higher than those in PMI category 1.

The morphological factors necrosis and autolysis had an adverse effect on *GAPDH* expression, with *P*-values ranging from <0.001 to 0.008 depending on amplicon sizes (results not shown). The *GAPDH* expression differed between patients who had dyslipidemia or a BMI higher than 25 and those without these symptoms (P ranges 0.001 to 0.008 and <0.001 to 0.015 depending on amplicon size), for patients with and without hypertension only the three longest GAPDH amplicons differed significantly (P <0.001, Paired Kruskal-Wallis test, significance level 0,00833), whereas hypoxia, fever and diabetes did not influence *GAPDH* expression.

DISCUSSION AND CONCLUSION

RIN values were significantly higher in fresh frozen samples than in post-mortem samples. MIA samples showed higher RIN values than CA samples. Also, *GAPDH* expression was significantly higher in fresh frozen samples than in post-mortem samples. There were differences in *GAPDH* expression between MIA and CA samples, but they were significant for only 3 out of 6 *GAPDH* amplicon sizes. Since *GAPDH* amplicon sizes of up to 530 base pairs could be detected well within the limits of the assay's sensitivity, we conclude that the post-mortem samples still contained RNA of reasonable quality.

It is generally believed that post-mortem tissues with poor RNA integrity values (RIN value <5) are not suitable for molecular techniques.[195] In this study we showed that samples with low RIN values could still be used for determining gene expression with RT-qPCR.

The Affymetrix and Illumina gene array platforms respectively use 25-mer or 50-mer probes to detect gene expression. Since it is possible to amplify 530 bp *GAPDH* RNA fragments, this implies that detection of *GAPDH* with gene array technology must be possible. *GAPDH* expression in post-mortem tissue is 4 Cq, approximately 16-fold lower than in fresh frozen tissue (see figure 2). Since *GAPDH* is an abundantly expressed housekeeping gene (i.e. high copy number per cell), the transcript could still be measured in post-mortem tissue derived RNA with qPCR. Assuming post-mortem RNA degradation is a random process, all transcripts (i.e. the entire transcriptome) will be subjected to degradation to the same degree.[196] Therefore, less abundantly expressed genes (i.e. low copy number per cell) may become undetectable after this rate of degradation. Nonetheless, successful gene array analysis of post-mortem heart tissue was previously described.[182] Recently, Romero et al. published that even with low RNA quality RNA sequencing is possible, as long as the RIN values are accounted for during data analysis.[197]

Handling and processing RNA may have major impact on RNA quality and therefore on the outcomes of the study. Inconsistency in executor and work performance, and contamination of RNA (with RNase or other genetic material) must be avoided when working with RNA.[186,198] The experiments were therefore performed by one qualified and experienced researcher (AvL). To all RT-qPCR assays a dilution series was added to assess the performance of both the assay and the researcher's skills. The efficiency numbers (table 1) show that the RT-qPCR assays were all well performed, although the efficiency decreases with amplicon size. The observed (parallel) increase of Cq values in all samples (i.e. post-mortem as well as fresh frozen; figure 2) in relation to the increasing amplicon size may thus be due to decreasing PCR efficiency, rather than increased RNA degradation.

The integrity of post-mortem tissues is subject to various factors.[188,199] Our results suggest that RNA in MIA tissue is less degraded than RNA derived from CA tissue. However, in this study CA was always performed after MIA, so the longer PMI is probably the explanation for this finding. Yet it is possible that differences intrinsic to MIA and CA play a role as well.[181,183,200] More extensive exposure of tissues to air, which occurs during conventional autopsies, could also have a negative influence on tissue quality. It is known that vacuum sealing of tissue specimens has a beneficial effect on RNA preservation.[201]

Regarding patient conditions, our analyses show seemingly contradicting results: whereas hypoxia, BMI, hypertension, and dyslipidemia do not seem to have an effect on RIN values, hypoxia, fever and diabetes did not show significant differences between Cq values. The RNA quality according to the RIN values was significantly lower in cases with reported fever and diabetes, the RNA quality according to the Cq values was significantly lower in cases with dyslipidemia and a Body Mass Index (BMI) of >25, and in cases with hypertension, the Cq values differed significantly for only some GAPDH base pair lengths. The inconsistent results are probably due to the low number of samples from different cases.

Agonal factors, such as hypoxia and fever are known to have a negative influence on RNA quality and can lead to inhomogeneous RNA samples in different tissue types.[202] To our knowledge, the individual influence of hypertension, dyslipidemia and diabetes mellitus on post mortem tissue quality is unknown. Together however, these factors are described as "the metabolic syndrome", which is associated with obesity.[203] The few differences found in RNA quality might therefore be explained by the confounder obesity. Obesity is in fact correlated with post mortem tissue quality, as it causes temperature insulation by fatty tissue, leading to higher core temperatures that are maintained longer. These higher temperatures enhance decomposition (autolysis

and putrefaction)[204] or "warm ischemia" and cause faster degradation of RNA. Since the surrounding temperatures of both the hospital and mortuary have been similar for all cases in our study, they probably hardly affected the course of decreasing core temperatures, especially not to the extent obesity did.
According to Byard et al., obesity also complicates the autopsy, both in a practical manner and, together with the metabolic syndrome, in the diagnostic process.[204] In our experience, the combination of imaging and CT guided tissue biopsies eased these practical issues, and the tissue quality of MIA samples at least equals that of CA samples. On top of that, MIA could also be less expensive than CA.[57] The diagnostic aspects, however, remain to be further investigated.

In conclusion, although RNA integrity is lower in MIA and CA samples than in fresh frozen tissues, MIA and CA samples can be used to detect *GAPDH* PCR products up to 530 base pairs. This implies that tissue obtained by MIA yields a sufficient amount of RNA with a sufficient quality for gene array based research. Therefore, the MIA procedure is a feasible method for researchers to obtain metastatic tumor tissue for molecular translational research.

ACKNOWLEDGEMENTS

The authors would like to thank Martin Rijlaarsdam for advise on statistical analyses and Anieta Sieuwerts for help and advice on RT-qPCR.

TABLES

Table 1. GAPDH primer sequences and base pair lengths used for RT-qPCR analyses

Primer	Primer sequence (5' --> 3')	Amplicon size	PCR efficiency
		Base Pairs	POWER (10(-1/slope))*
GAPDH common fwd	CCA CAT CGC TCA GAC ACC AT		
GAPDH rev	ACC AGG CGC CCA ATA CG	71	1,94
GAPDH rev	GTA AAC CAT GTA GTT GAG GTC	153	1,93
GAPDH rev	TTG ACG GTG CCA TGG AAT TT	200	1,86
GAPDH rev	ACT TGA TTT TGG AGG GAT CT	277	1,81
GAPDH rev	AAG ACG CCA GTG GAC TCC A	323	1,82
GAPDH rev	ACG ATA CCA AAG TTG TCA TG	530	1,71

* based on a 4 fold dilution series of pooled fresh frozen tissue derived cDNA samples

Table 2. Number of tissue samples per sample type per tissue type

Tissue type	Sample type			Total
	Fresh Frozen	MIA	CA	
Heart	25*	25*	24	74
Kidney	24	24	24	72
Liver	24	24	24	72
Total	73	73	72	218

* extra samples collected

Table 3. Distribution of tissue samples per sample type over post-mortem intervals

PMI	Sample type			Total
	Fresh frozen	MIA	CA	
0h	73	-	-	73
≤12h	-	12	-	12
13h-24h	-	21	6	27
25h-34h	-	31	18	49
35h-44h	-	6	36	42
≥ 45h	-	3	12	15
Total	73	73	72	218

Table 4. Available patient conditions (in agonal phase) that could influence RNA quality

Condition	MIA and CA
Fever	40%
Hypoxia	57%
BMI > 25	60%
Hypertension	21%
Diabetes	17%
Dyslipidemia	8%

Table 5. Morphologic tissue quality assessment per sample type

		Sample type		
		Fresh frozen n = 73	**MIA n = 73**	**CA n = 72**
Representative	**yes**	88%	88%	100%
	no	5%	0%	0%
No H&E-slide*		7%	12%	0%
		n = 68	**n = 64**	**n = 72**
Autolysis	**severe**	3%	11%	21%
	moderate	16%	50%	44%
	no	81%	39%	35%
Necrosis	**yes**	3%	3%	6%
	no	93%	94%	86%
	n/s**	4%	3%	8%

** No H&E-slide, all tissue used for RNA isolation*
** n/s = no scoring possible

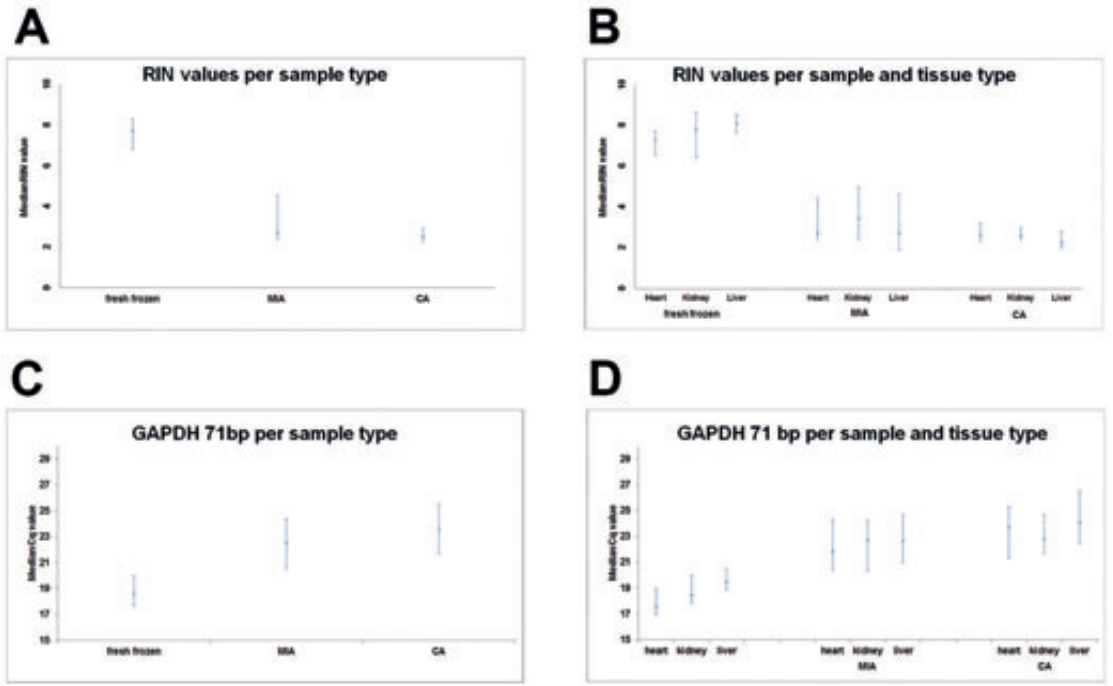
Table 6. P-values comparing sample types per GAPDH size assay

Sample type	***P*-value**					
	71 bp	**153 bp**	**200 bp**	**277 bp**	**323 bp**	**530 bp**
Fresh frozen vs. MIA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Fresh frozen vs. CA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
MIA vs. CA	0.009	0.003	0.005	0.009	0.003	0.016

Kruskal Wallis, significance level 0,00833

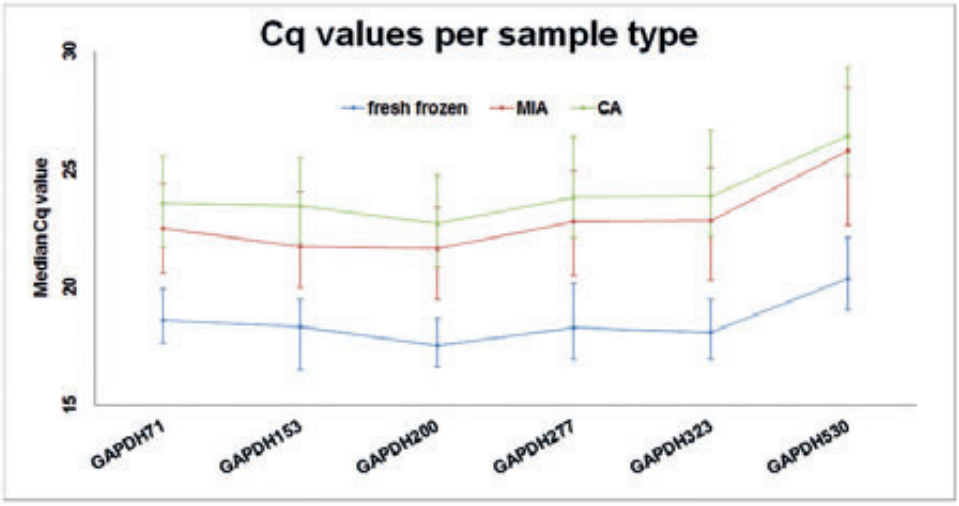
FIGURES

Figure 1. RNA integrity and GAPDH expression in fresh frozen, MIA and CA samples.



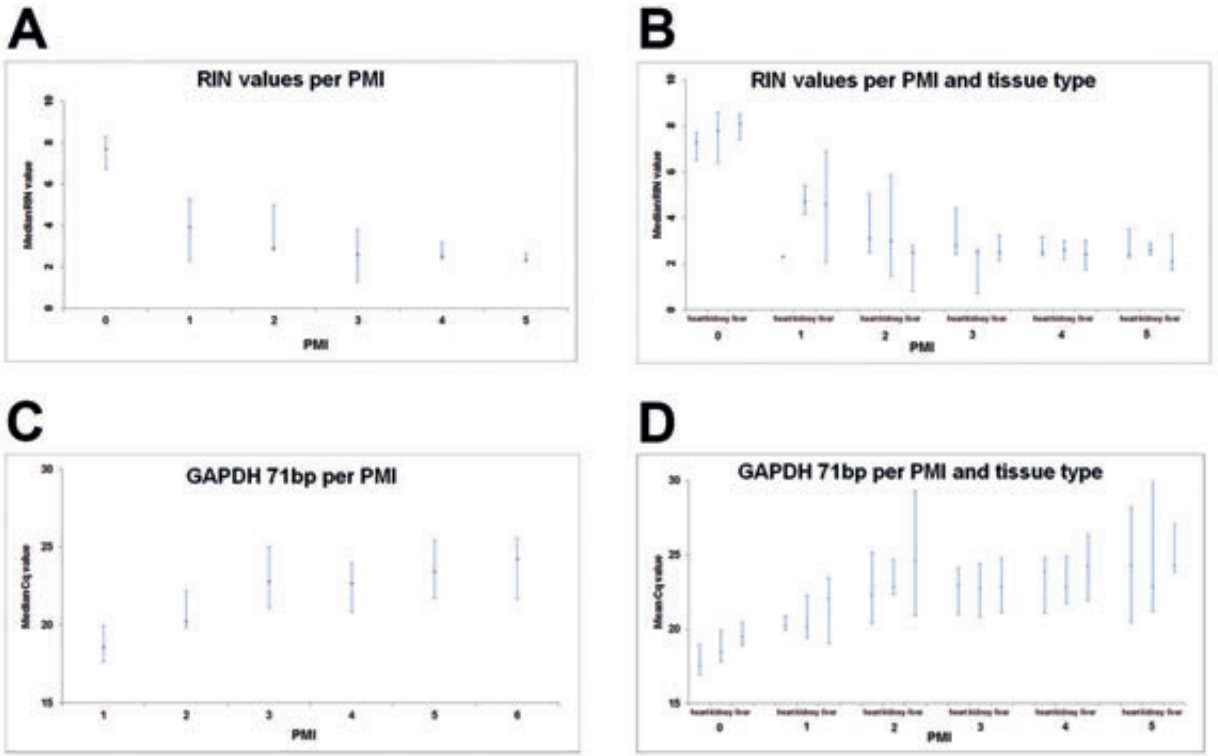
Legend: Figure 1A shows median RIN values (X-axis) of fresh frozen, MIA and CA samples (Y-axis). The fresh frozen samples, taken from surgical specimens, yielded high quality RNA. Both types of post mortem samples MIA and CA yielded RNA of lower quality. In figure 1B the samples are divided into 3 tissue types; heart, kidney and liver respectively. In figures 1C and 1D RT-qPCR results of the same samples are shown, in the same order. Since all GAPDH RT-qPCR assays in the 6-amplicon size assay showed similar results, only the results of the 71 bp assay are depicted here. The Cq value (Y-axis) obtained in fresh frozen samples is low, i.e. the GAPDH expression level is high. In the post mortem samples MIA and CA, GAPDH RNA is partly degenerated, resulting in higher Cq values corresponding with lower GAPDH expression levels.

Figure 2. GAPDH size assay in fresh frozen, MIA and CA samples.



Legend: The median Cq values (X-axis) of RNA derived from fresh frozen, MIA and CA samples obtained by RT-qPCR with various PCR product lengths (Y-axis) are shown. The fresh frozen samples always show lower Cq values, whereas the MIA samples show intermediate and the CA samples show high Cq values. This indicates that RNA in MIA samples is less degraded than RNA in CA samples. The parallel increasing Cq values between GAPDH323 and GAPDH530 indicate suboptimal RT-qPCR performance, rather than decreased GAPDH expression levels.

Figure 3. RNA integrity and GAPDH expression versus post-mortem intervals in MIA and CA samples.



Legend: In figure 3A and B the median RIN value (X-axis) decreases with the post mortem interval (PMI; Y-axis). The fresh frozen specimens (PMI = 0) show the highest RNA integrity. PMI 1 is exclusively comprised of MIA samples. PMI 2 to 5 are comprised of both MIA and CA samples. The actual PMI intervals are given in table 3. In figures 3C and D the results of RT-qPCR with GAPDH 71 bp are shown in the same context. Here we can appreciate that RT-qPCR is a more sensitive method of RNA integrity measurement, since it was possible to find significant differences not only between PMI 0 and PMI 1, as was also measured with RIN values, but also between PMI 1 and PMI 2.
Potential advantages of MIA over CA for obtaining metastatic tumor tissue are the higher chance of getting consent from bereaved relatives, and the better feasibility to reduce PMI, which is the most crucial factor for high quality post mortem tissue for molecular analyses.

8

Total-body CT and MR features of postmortem change in in-hospital deaths

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PLoS One. 2017 Sep 27;12(9):e0185115.

ABSTRACT

Objectives: To evaluate the frequency of total-body CT and MR features of postmortem change in in-hospital deaths.

Materials and methods: In this prospective blinded cross-sectional study, in-hospital deceased adult patients underwent total-body postmortem CT and MR followed by image-guided biopsies. The presence of PMCT and PMMR features related to postmortem change was scored retrospectively and correlated with postmortem time interval, post-resuscitation status and intensive care unit (ICU) admittance.

Results: Intravascular air, pleural effusion, periportal edema, and distended intestines occurred more frequently in patients who were resuscitated compared to those who were not. Postmortem clotting was seen less often in resuscitated patients ($p=0.002$). Distended intestines and loss of grey-white matter differentiation in the brain showed a significant correlation with postmortem time interval ($p=0.001$, $p<0.001$). Hyperdense cerebral vessels, intravenous clotting, subcutaneous edema, fluid in the abdomen and internal livores of the liver were seen more in ICU patients. Longer postmortem time interval led to a significant increase in decomposition related changes ($p=0.026$).

Conclusions: There is a wide variety of imaging features of postmortem change in in-hospital deaths. These imaging features vary among clinical conditions, increase with longer postmortem time interval and must be distinguished from pathologic changes.

INTRODUCTION

Hospital autopsy rates today are as low as 0-5%, having decreased from a rate of 30% or higher in the 1990s.[22,32,205] This low rate is alarming, since one in five autopsies show major discrepancies between antemortem and postmortem diagnoses despite improved diagnostic testing.[13] A possible cause for this decline may be the invasiveness of the conventional autopsy procedure.[102] To provide a less invasive alternative to conventional autopsy, imaging based autopsy methods were developed, primarily in forensic medicine. These modern autopsies include total-body postmortem CT (PMCT) and MR (PMMR), sometimes combined with CT angiography (PMCTA) and image-guided biopsies.[206-208] More recently the imaging autopsy is steadily emerging in clinical radiology and there is a growing number of diagnostic studies analyzing the performance of the noninvasive (imaging only) and minimally invasive autopsy (imaging with angiography and / or biopsies).[56,57,209] Combined PMCT, PMCTA and image-guided biopsies appear most sensitive in diagnosing cause of death, however more clinical studies are needed to accurately determine the diagnostic value of the imaging autopsy. [56,209] In forensic centers access to MR scanners is often limited, so PMCT is most commonly performed. In hospitals, MRI is more widely available, and its high performance to visualize organ parenchyma and soft tissues make PMMR a valuable addition to PMCT.
Postmortem imaging is not the same as imaging the living. Directly after death various chemical and physical processes affect the body in ways that can change PMCT and PMMR features of organs and soft-tissues. These processes can generally be divided into gravity dependent changes (including sedimentation of blood and livor mortis; also known as lividity or hypostasis), decomposition (including putrefaction), rigor mortis (muscle stiffness) and algor mortis (cooling of the body).
Livor mortis is caused by blood settling in the dependent parts of the body due to gravity. Livores can be observed both internally, on imaging and autopsy, and externally upon visual inspection. External livores manifest as dark bluish (or livid) areas of the skin within several hours after death. Internal livores are noted as increased attenuation or signal changes of the dependent areas of organs. The combination of postmortem leakage of cell membranes and subsequent increased osmolality of the interstitial fluid, together with the effect of gravity leads to accumulation of fluids in dependent areas, such as the subcutaneous fat, thoracic cavity and abdominal cavity. [116,210,211]
Decomposition consists of many processes that cause organic material to break down into simpler forms of matter. It includes putrefaction, autolysis and insect and animal predation. Putrefaction leads to gas formation, it is found intravascular in an early decomposition stage and in more advanced stages also in soft tissues and organ parenchyma. Rigor mortis leads to muscle contraction after death that results in muscle stiffness.

Rigor mortis is caused by cessation of synthesis of adenosine triphosphate (ATP). ATP is consumed in muscle fibers to separate actin and myosin filaments. Directly after death ATP is still present in the muscle, but it is consumed in the first hours after death. When the ATP reserves are depleted, actin and myosin filaments cannot separate anymore. This state lasts until decomposition leads to the breakdown of actin and myosin filaments. The speed of this process depends on temperature: both the time until rigor mortis starts and reaches its maximum and the time until rigor mortis recedes are longer in colder bodies. [212-215]
Algor mortis can affect tissue contrast on PMMR images. There is a wide variability of T1 values due to higher sensitivity of T1 to temperature differences. [216] T2 values are less temperature dependent.
Radiologists need in-depth understanding of these processes for correct acquisition and interpretation of PMCT and PMMR scans. The aim of this study is to evaluate the frequency of total-body CT and MR features of postmortem change in in-hospital deaths.

MATERIALS AND METHODS

Study protocol

This study was undertaken as part of the *Minimally Invasive Autopsy* (MIA) study. This is a prospective single center cross-sectional study in a tertiary referral hospital comparing diagnostic performance of conventional autopsy and MIA. Approval of the Erasmus MC Institutional Review Board and Ethics Committee was obtained; the study was filed with the Netherlands National Trial Register. Patients aged 18 years and older who died in the Erasmus University Medical Center were eligible for inclusion, if written informed consent was obtained from next-of-kin for MIA and CA of at least the torso.
Exclusion criteria were (suspected) unnatural COD, body size exceeding diameter of 16 inches in supine position (limitation for PMMR), known or suspected "high-risk" infected bodies (tuberculosis, hepatitis B and C, human immunodeficiency virus, methicillin-resistant Staphylococcus aureus, multi-drug resistant Acinetobacter), and open abdominal wounds that could not be completely closed or taped to prevent leakage of body fluids.
All cases underwent total-body PMCT and PMMR followed by biopsies under CT (torso) or stereotactic guidance (brain) according to standardized protocols (Table 1 and 2). Total scan time was approximately 60 minutes for PMMR and 10 minutes for PMCT. First PMMR was performed on a 1.5T scanner (Discovery MR450, GE Healthcare, Milwaukee, Wisconsin USA) and consisted of scans from the head to the pelvis (legs were omitted

on because of MR scanner availability). Directly after PMMR, PMCT was performed on a dual-source CT scanner (SOMATOM Definition Flash, Siemens Healthcare Forchheim, Germany) and consisted of scans from head to feet. Standardized CT-guided biopsies (12 Gauge) were taken from heart, lungs, liver, kidneys, spleen, and additional biopsies were taken from radiologically suspected pathology. All biopsies were stained with hematoxylin and eosin (H&E) and when requested by the pathologist additional stains were performed.

Scoring

We composed a scoring list of PMCT and PMMR features of postmortem change (Table 3-5). The features that were included were based on our radiological expertise [56,57] and were supplemented with features from published postmortem imaging studies. [48,102,108,110,121,126,128,153,210,217-241]

All cases were retrospectively and independently scored by a radiologist (ACW; board-certified with 10 years of clinical expertise in postmortem imaging) and a researcher (IMW with 3 years of expertise). When available, clinical information and antemortem scans were reviewed. Specific clinical conditions were scored; including intensive care unit (ICU) admittance and post-resuscitation status (PRS).
PMCT and PMMR features were – if possible – categorized to a specific chemical and/or physical process; 1. gravity dependent changes; 2. decomposition; 3. rigor mortis; 4. algor mortis. Features that could not be classified to any of these four processes were labeled to a miscellaneous category.

Statistical analyses

We recorded percentage of male/female cases, mean age at death, and mean postmortem time interval (PTI) including standard deviations. PTI was defined as the time from death to the start of MR scanning. For each case, we calculated the frequency of PMCT and PMMR features. Fisher's exact test was used for the association between specific clinical conditions (ICU and PRS) and frequencies of PMCT and PMMR features. Linear discriminant analysis was used to evaluate the correlation of PTI and PMCT and PMMR features. ANOVA was used to test the correlation of PTI and a combination score for all decomposition and all gravity dependent changes. The inter-observer agreement was calculated using kappa statistics (agreement <0.2: poor, 0.2-0.4: fair, 0.4-0.6: moderate, 0.6-0.8: good, and 0.8-1.0: very good). Furthermore we calculated inter-observer agreement for the group of pathological mimics (those postmortem changes that were most likely to be confused with real pathologic changes) and a group of postmortem changes that does not correspond to a pathologic process with similar radiological features.

RESULTS

We scanned 100 cases from January 2012 to December 2014. The mean age was 62.7 (±13.0), 62% were male (Table 6). Inter-observer agreement was very good, with a kappa of 0.84 for PMCT and 0.83 for PMMR. The kappa score for the group with pathological mimics was 0.79 for PMCT and 0.76 for PMMR, the kappa score for the non-pathologic mimics was 0.89 for PMCT and 0.88 for PMMR.

Total-body CT and MR features of postmortem change – general overview

PMCT and PMMR features of different organs and observed frequencies are presented in Table 7-9.

Brain

Sedimentation led to increased attenuation of the posterior sagittal sinus (96%), cerebral veins (54%) and cerebral arteries (35%) in a symmetric distribution (Fig 1A-B). Putrefactive gas in the brain was seen in only a few cases (8%). Liquefaction of the brain was not observed. PMMR showed high T1 signal of the basal ganglia in one third of cases (Fig 1C). Effacement of sulci (Fig 1D) and loss of grey-white matter differentiation was seen in the majority of cases (85%) (Fig 1E-1F).

Heart and large vessels

The right atrium and ventricle were dilated in 25% (Fig 2A). The thoracic aorta showed clotting in 38% of cases and was detected best on PMMR (Fig 2B). Air in the heart chambers was seen in 44% (Fig 2C-D). No air was observed within the myocardium. T2 signal decline in the myocardium from the epicardial to endocardial regions was seen in 12% (Fig 2A).We observed a collapse of the thoracic aorta in 30% (Fig 2E). Sedimentation of blood was often present in the heart (84%) (Fig 2A) and large thoracic vessels (Fig 2F). The thoracic aortic wall showed increased attenuation in a majority of cases (90%) (Fig 2G). The abdominal aorta was collapsed in 67% and the abdominal vena cava in 53% (Fig 2H). Air in the vertebral venous plexus was seen less frequently (11%), and usually in cases with extensive intravascular air.

Lungs

Livor mortis affected the lungs frequently (86%); it appeared as areas with increased density or high T2 signal in the dependent areas of the lungs. In these parts of the lung it is challenging to distinguish livores from pneumonia (Fig 3A-I) or other interstitial diseases. Liquid in the trachea and bronchi was very common (78%). Pleural effusion was seen in only 38% of cases.

Liver, spleen, kidneys, gallbladder, pancreas, adrenals

Internal livores of the spleen and kidney were noted by two layers of different T1 and T2 signal reflecting blood settling in the parenchyma. In the liver, three layers can be seen: an upper layer with small amounts of putrefactive gas, a middle layer with intermediate signal and a lower layer that together with the middle layer reflect settling of blood (Fig 4A-F). Gravity can cause sedimentation of the gallbladder content and this is best seen on PMMR as vertical signal gradients. Livor mortis in organ parenchyma (spleen 31%, kidneys 6% and liver 74%) was also best depicted on PMMR and presented as different layers of T1 and T2 signal. In general, livores of the organ parenchyma were not clearly detectable on PMCT. Periportal edema was found on PMMR in 27% (Fig 5A). Putrefaction gas in the liver vasculature was seen on PMCT in 37% (Fig 5B). The imaging features of the pancreas and adrenal glands were not notably affected by postmortem change.

Stomach, intestines, abdominal cavity

Sedimentation in the stomach and intestines was seen in only a few cases (15%). Fluid in the abdomen was present in 35%. Bowel distension (14%), gas in the intestinal wall (8%) and free abdominal air (7%) were less common features (Fig 5C-F).

Soft tissues

On PMCT superficial internal livor mortis was manifested as increased densities of the dependent subcutaneous areas (37%). [210] Putrefactive gas in subcutaneous tissue was not observed.

Total-body CT and MR features of postmortem change – in relation to clinical conditions and postmortem time interval

Intensive care unit admittance

In our cohort 38/100 patients died in the ICU. Livores of the liver was seen significantly more often in ICU patients than in non-ICU patients (92% vs. 62%, p=0.001) (Table 10). High T1 signal of the basal ganglia was significantly less frequently observed in ICU patients (44% vs. 13 %, p=0.001).

Post-resuscitation status

Forty-three patients underwent unsuccessful resuscitation just prior to death. Pleural effusion (p<0.001) and periportal edema (p=0.001) were seen significantly more often in patients that had undergone resuscitation (Table 10). Postmortem clotting occurred

significantly less frequently in patients that had underwent resuscitation (p=0.002). Intravascular air (both arterial and venous) was visible in 58% of patients and more frequently present in PRS patients than in non-PRS patients (72% vs. 47%, p=0.013).

Postmortem time interval

The mean PTI was 23.0 (±15.6) hours. PTI showed a significant correlation with internal livores of the lungs (p=0.038), distended intestines (p=0.001) and loss of grey-white matter differentiation in the brain (p<0.001) (Table 11). PTI showed a significant correlation with postmortem changes related to decomposition (p=0.026).

DISCUSSION

This is the first study evaluating the frequency of PMCT and PMMR features of postmortem change in a large cohort of adult patients. Similar imaging studies on postmortem change in fetuses and neonates have been published. [211,242]. We observed a wide variety of PMCT and PMMR features of postmortem change. Particularly livor mortis and decomposition have great impact on the imaging features. Algor mortis and rigor mortis lead to only minor changes. Our results indicate that PMCT and PMMR appear to be complementary for correct interpretation of postmortem changes. Some changes are more clearly seen on PMMR such as livores of organ parenchyma or blood clotting, while others such as the presence and distribution of putrefaction air is better noted on PMCT.

Clinical conditions may influence imaging features of postmortem change. Importantly, postmortem changes may mimic or even mask real pathological changes related to the cause of death: e.g. gravity causes sedimentation of blood contents within the first hours after death. On PMCT the upper (plasma) and lower layer (blood cells) shows decreased and increased attenuation respectively. As a result the upper part of the aortic wall shows relatively high attenuation compared to the plasma content and may mimic aortic wall hematoma (Fig 2G).

Bacterial infections can speed decomposition processes and increase gas and fluid formation in the body. Hypovolemia causes the heart cavities and vessel lumen to decrease in size.

Medical treatments can also change imaging features; e.g. intravascular lines and surgical wounds can be accompanied by air in the surrounding soft tissues and bloodstream.

Resuscitation can cause rib fractures, pneumothorax, lung contusions, hemothorax, and intravascular air. We found that the majority of PRS patients showed significantly more intravascular air as opposed to non-PRS patients, suggesting that air was introduced during resuscitation. [210,222,243,244] Intravascular air after resuscitation is caused by pneumatization of dissolved gas in the blood as a result of compression and expansion of vessels and direct mechanical force to the chest allowing air from the lungs to enter the bloodstream. [245,246] Likewise resuscitation attempts may introduce free abdominal air that should not be confused with free air caused by intestinal perforation. [211] Pleural effusion, periportal edema, and distended intestines were also more frequently observed after resuscitation. Postmortem clotting occurred less often in PRS patients and we hypothesize this is caused by anti-coagulation given during resuscitation attempts. [210,222,243,244]

Lack of oxygenation in the brain was noted by loss of grey- white matter differentiation, edema, swelling of the brain and effacement of sulci. [218,247,248] These features involve the entire brain and are symmetrical. [116,218] Patients with elevated intracranial pressure prior to death may show similar features, and comparison to antemortem scans is recommended. In living patients a dense-artery-sign in the cerebral arteries is often asymmetric and indicative for cerebral ischemia, a postmortem mimic of this sign is usually symmetrical and is non-pathological (Fig 1A). The cessation of cardiac output and fall in blood pressure causes the arterial wall to collapse directly after death. [225,249] This change may obscure an aortic aneurysm or dissection. Within 2 hours after death blood clots form in the heart and large vessels.

Postmortem clots are best detected on PMMR; the clot shows low T2 signal relative to the high T2 signal of the serum. A postmortem clot can often be distinguished from a central pulmonary embolism that shows a more homogeneous high T1 signal (Fig 6A-J). Other distinctive features of postmortem clots are that they are seen in the dependent areas of the vessel, usually fill only part of the lumen and do not expand the lumen. With pulmonary embolism the thrombus follows the blood stream until it reaches a point where the lumen becomes too narrow or the vessel branches. The shape of a postmortem clot is often more irregular than a thrombus (Fig 6C-D).[250] If clinically relevant, a CT-guided biopsy may help differentiate between postmortem clotting and pulmonary embolism.

In this study we investigated in-hospital deceased adult deaths. The mean PTI was relatively short and bodies were stored in a protected environment after death, PTI seem to have an impact on the occurrence and extent of specific changes.

Autolysis occurs early after death. It leads to significant changes that can be noted at microscopic examination of tissues obtained at biopsy, in particular of the pancreas and adrenal glands. [210,251] However, in our cohort imaging features of these organs seem less affected by autolysis. Imaging features related to decomposition were seen more frequently. There was tendency to more extensive livores of the lungs with longer PTI and the livores can become so extensive as to completely consolidate the lung parenchyma. In such cases, accurate diagnosis of underlying parenchymal disease can be challenging. In such cases we highly recommend to biopsy both normal and suspected parts of parenchyma to reliably differ postmortem changes from infection (Fig 3A-I), hemorrhage, or tumor. [116,211,252,253]
The distribution of putrefactive gas also differs with a different PTI, first occurring in the heart cavities and large vessels and with longer intervals in the smaller vessels, organ parenchyma and soft tissues. Putrefactive gas must be differentiated from pathological air collections, such as soft tissue emphysema, free air or gas in the intestinal wall. Putrefactive gas usually has an intestinal origin and travels through the mucosa to the portal veins in the early stage. It may mimic air embolism, however the latter will show a more equal distribution throughout the vascular system. [116] Intestinal bacteria continue to produce gas after death causing bowel distension. The amount of intestinal air significantly increases with longer PTI. This may look similar to a bowel obstruction or paralysis, and should be carefully evaluated. With longer PTI, putrefaction can also lead to formation of subcutaneous air.

Our study had several limitations. We composed a scoring list of postmortem imaging features that may not be complete and some features may be missing. We did not measure body temperature during scanning. Ideally, body temperature should be monitored to allow adaption of MR scan parameters to temperature variations to achieve optimal tissue contrast. However all bodies were stored at the morgue at a constant temperature of 5 degrees Celsius prior to scanning and the transit time from the morgue to the MR scanner was equal for all cases. We optimized MR sequences for scanning of cold corpses. Furthermore the scan time was maintained approximately the same for all scanning sessions while the temperature in the scanning room was kept constant.

CONCLUSION

There is a wide variety of imaging features of postmortem change in in-hospital deaths. These imaging features vary among clinical conditions, increase with longer PTI and must be distinguished from pathologic changes.

TABLES

Table 1. Postmortem imaging protocol
A. Postmortem magnetic resonance protocol

Scan area	**Coil**	**Sequence**	**TR/TE/TI** (ms)	**Slice width** (mm)	**FOV** (cm)	**Matrix**	**Number of slices**	**Coverage per section** (cm)	**Number of sections**	**Scan time per section** (s)
Head-Pelvis	Body	FLAIR FSE T1w	2320/9.5/963	4.0	48	384x320	50	20.0	5-8	174
Head-Pelvis	Body	STIR FSE T2w	12000/41/120	4.0	48	288x224	50	20.0	5-8	168
Thorax	8-channel	3D fs FSPGR T1w	3.3/1.2/14	1.6	40	256x256	212	33.9	1	153
Thorax	8-channel	2D STIR FSE T2w	11200/ 94/120	2.0	40	256x256	170	34.0	1	359

B. Postmortem computed tomography protocol

Scan area	**Rotation time** (s)	**Tube voltage** (kV)	**Tube current** (eff. mAs)	**Slice collimation** (mm)	**Pitch**	**Scan time** (s)	**Reconstruction**
Head-Neck	1.0	100	750	2 x 64 x 0.6	0.35	21	FBP
Thorax-Abdomen	1.0	120	600	2 x 64 x 0.6	0.6	32	FBP
Extremities	1.0	120	600	2 x 64 x 0.6	0.6	57	FBP

Table 2. Image-guided biopsy protocol

Organ	Targets aimed at
Brain	Normal parenchyma and suspected pathology
Lungs	Both lungs, normal parenchyma and suspected pathology
Heart	Left ventricle: lateral wall, apex, normal myocardium and suspected pathology. Right ventricle: on indication
Kidneys	Both kidneys, normal parenchyma and suspected pathology
Spleen	Sub-capsular parenchyma and suspected pathology
Liver	Normal parenchyma and suspected pathology
Other	Region of interest

Table 3. Scoring list of PMCT and PMMR features of postmortem changes in the brain

Postmortem imaging feature	Category	Postmortem process
1. Hyperdense superior sagittal sinus	Gravity dependent changes	Blood sedimentation
2. Hyperdense veins	Gravity dependent changes	Blood sedimentation
3. Hyperdense arteries	Gravity dependent changes	Blood sedimentation
4. Thickened / irregular falx	Gravity dependent changes	Blood sedimentation
5. Liquid in paranasal sinus	Decomposition	Cell wall leakage
6. Intracranial air	Decomposition	Putrefaction
7. High T1 signal basal ganglia	Algor mortis	Temperature change
8. Low T2 signal basal ganglia	Algor mortis	Temperature change
9. Diffusion restriction	Algor mortis	Temperature change
10. Insufficient suppression of liquor on Flair	Algor mortis	Temperature change
11. Loss of grey-white matter differentiation	Miscellaneous	Cerebral hypoxia
12. Sulcal effacement	Miscellaneous	Cerebral hypoxia
13. Compression cisterns	Miscellaneous	Cerebral hypoxia
14. Cerebellar tonsillar herniation	Miscellaneous	Cerebral hypoxia

Table 4. Scoring list of PMCT and PMMR features of postmortem changes in the thorax

Postmortem imaging feature	Category	Postmortem process
1. Hyperdense aortic wall	Gravity dependent	Blood sedimentation
2. Sedimentation of blood aorta	Gravity dependent	Blood sedimentation
3. Sedimentation of blood large vessels	Gravity dependent	Blood sedimentation
4. Livores heart	Gravity dependent	Livor mortis
5. Sedimentation of blood heart	Gravity dependent	Blood sedimentation
6. Livores lung	Gravity dependent	Livor mortis
7. Groundglass opacification	Gravity dependent	Livor mortis
8. Increased density dependent areas skin and subcutis	Gravity dependent	Livor mortis
9. Edema dependent areas subcutis	Decomposition / Gravity dependent	Cell wall leakage / Livor mortis
10. Intravascular air	Decomposition	Putrefaction
11. Gas formation heart	Decomposition	Putrefaction
12. Gas formation myocardium	Decomposition	Putrefaction
13. Susceptibility artifacts heart (gas)	Decomposition	Putrefaction
14. Pericardial effusion	Decomposition	Cell wall leakage
15. Pleural effusion	Decomposition	Cell wall leakage
16. Gas formation lung parenchyma	Decomposition	Putrefaction
17. T2 signal decay from subepicardial to subendocardial	Rigor mortis	Rigor mortis
18. Dilated vena cava inferior	Miscellaneous	Loss of blood pressure
19. Postmortem clotting large vessels	Miscellaneous	Clotting
20. Collapse large vessels	Miscellaneous	Loss of blood pressure
21. Dilated heart	Miscellaneous	Loss of blood pressure
22. Dilated right atrium	Miscellaneous	Loss of blood pressure
23. Postmortem clotting heart	Miscellaneous	Clotting
24. Collapse of aorta	Miscellaneous	Loss of blood pressure
25. Postmortem clotting aorta	Miscellaneous	Clotting
26. Dilated vena cava superior	Miscellaneous	Loss of blood pressure
27. Gas formation subcutaneous areas	Decomposition	Putrefaction
28. Liquid trachea / bronchi	Decomposition	Cell wall leakage

Table 5. Scoring list of PMCT and PMMR features of postmortem changes in the abdomen

Postmortem feature	Category	Postmortem process
1. Intestinal sedimentation	Gravity dependent	Sedimentation
2. Livores liver	Gravity dependent	Livor mortis
3. Sedimentation gall bladder	Gravity dependent	Sedimentation
4. Livores spleen	Gravity dependent	Livor mortis
5. Livores kidneys	Gravity dependent	Livor mortis
6. Free air	Decomposition	Putrefaction
7. Fluid in the abdomen	Decomposition	Cell wall leakage
8. Gas in the intestinal wall	Decomposition	Putrefaction
9. Distended intestines	Decomposition	Putrefaction
10. Gas liver parenchyma	Decomposition	Putrefaction
11. Air liver vessels	Decomposition	Putrefaction
12. Gas bile ducts	Decomposition	Putrefaction
13. Periportal edema	Decomposition	Cell wall leakage
14. Gas spleen parenchyma	Decomposition	Putrefaction
15. Gas kidney parenchyma	Decomposition	Putrefaction
16. Intravascular air	Decomposition	Putrefaction
17. Collapse aorta	Miscellaneous	Loss of blood pressure
18. Collapse vena cava	Miscellaneous	Loss of blood pressure
19. Dilated vena cava	Miscellaneous	Loss of blood pressure

Table 6. Patient demographics

Patient demographics (n=100)				
Men/women (n)	62/38			
Mean age (SD, min-max)	62.7 (±13.0, 25-92)			
Mean PTI (SD, min-max)	22.6 (±15.4, 3.1-71.5)			
PRS (n)	43			
Mean image acquisition time	MRI: 59 minutes, CT: 3-4 minutes			
Hospital ward	**n**			
ICU	38			
ER	15			
Internal medicine / gastroenterology	11			
Oncology	8			
Neurology	6			
Thoracic surgery	5			
Hematology	5			
Pulmonology	5			
General surgery	4			
Gynecology/urology	3			
Antemortem imaging	**Not available** (n)	**CT** (n)	**MR** (n)	**CT and MR** (n)
Brain	65	26	5	4
Thorax	30	70	0	0
Abdomen	31	67	0	2

Legend: SD = standard deviation; PTI = postmortem time interval (hours); PRS = post-resuscitation status; ICU = intensive care unit; ER = emergency room; CT = computed tomography, MR = magnetic resonance

Table 7. Frequencies of postmortem PMCT and PMMR features of the brain

PMCT and PMMR features of brain (n=100)	PMCT	PMMR
Loss of grey-white matter differentiation	85%	85%
Hyperdensity superior sagittal sinus	96%	NA
Hyperdensity veins	54%	NA
Sulcal effacement	44%	41%
Hyperdensity Willis' circle and cerebral arteries	35%	NA
Liquid paranasal sinuses	32%	32%
High T1 signal basal ganglia and thalamus	NA	32%
Thickened / irregular aspect falx	20%	NA
Intracranial air cerebral vasculature	8%	1%

Legend: PMCT = postmortem CT; PMMR = postmortem MR; NA = not assessable

Table 8. Frequencies of postmortem PMCT and PMMR features of thorax

PMCT and PMMR features of thorax (n=100)		
Heart and large vessels	**PMCT**	**PMMR**
Air heart	44%	22%
Sedimentation of blood heart	62%	84%
Dilatation right atrium and ventricle	25%	25%
Pericardial effusion	26%	27%
T2 signal decay epi- to endocardial	NA	12%
Postmortem clotting heart	4%	8%
Air pericardial space	4%	2%
Air coronaries	18%	12%
Hyperdense aortic wall	90%	NA
Sedimentation in blood vessels	71%	88%
Postmortem clotting vessels	25%	38%
Intravascular air	31%	8%
Collapse of thoracic aorta	30%	29%
Lungs	**PMCT**	**PMMR**
Internal livores	86%	85%
Pleural effusion	31%	38%
Liquid trachea / main bronchi	78%	78%

Legend: PMCT = postmortem CT; PMMR = postmortem MR; NA = not accessable

Table 9. Frequencies of postmortem PMCT and PMMR features of the abdomen

PMCT and PMMR features of abdomen (n=100)		
Liver, gallbladder, spleen and kidney	**PMCT**	**PMMR**
Gas liver vasculature	37%	26%
Internal livores liver	NA	74%
Sedimentation gallbladder	8%	14%
Periportal edema	11%	27%
Internal livores spleen	NA	31%
Gas spleen parenchyma or vessels	5%	1%
Internal livores kidneys	NA	6%
Gas kidney parenchyma or vessels	9%	1%
Stomach, intestines, abdominal cavity	**PMCT**	**PMMR**
Intestinal sedimentation	6%	15%
Gas in the intestinal wall	8%	1%
Distended intestines	14%	14%
Free air	7%	2%
Fluid in the abdomen	20%	35%
Abdominal vessels	**PMCT**	**PMMR**
Intravascular air	21%	5%
Collapse abdominal aorta	67%	67%
Collapse abdominal vena cava	53%	53%
Dilated abdominal vena cava	2%	2%
Air vertebral venous plexus	11%	2%

Legend: PMCT = postmortem CT; PMMR = postmortem MR; NA = not assessable

Table 10. Postmortem CT and MR features in relation to clinical conditions

Clinical condition	Modality*	Yes	No	Total	P-value
Intensive care unit admittance		**n=38**	**n=62**	**n=100**	
Hyperdense cerebral arteries	PMCT	17 (45%)	18 (29%)	35 (35%)	0.133
High T1 signal basal ganglia	PMMR	5 (13%)	27 (44%)	32 (32%)	0.002
Postmortem clotting	PMMR	21 (55%)	23 (37%)	44 (44%)	0.098
Subcutaneous edema	PMMR	17 (45%)	20 (32%)	37 (37%)	0.286
Fluid in the abdomen	PMMR	17 (45%)	18 (29%)	35 (35%)	0.133
Livores liver	PMMR	35 (92%)	39 (63%)	74 (74%)	0.001
Livores spleen	PMMR	10 (26%)	21 (34%)	31 (31%)	0.507
PRS		**n=43**	**n=57**	**n=100**	
Pleural effusion	PMMR	25 (58%)	13 (23%)	38 (38%)	<0.001
Periportal edema	PMMR	19 (44%)	8 (14%)	27 (27%)	0.001
Distended intestines	PMCT/PMMR	9 (21%)	5 (9%)	14 (14%)	0.144
Postmortem clotting	PMMR	11 (26%)	33 (58%)	44 (44%)	0.002
Dilated right atrium / ventricle	PMCT/PMMR	15 (35%)	10 (18%)	25 (25%)	0.063
Intravascular air	PMCT	31 (72%)	27 (47%)	58 (58%)	0.015

*Specifies the modality that has the highest detection of the postmortem changes
Legend: PMCT = postmortem CT; PMMR = postmortem MR; PRS = post-resuscitation status

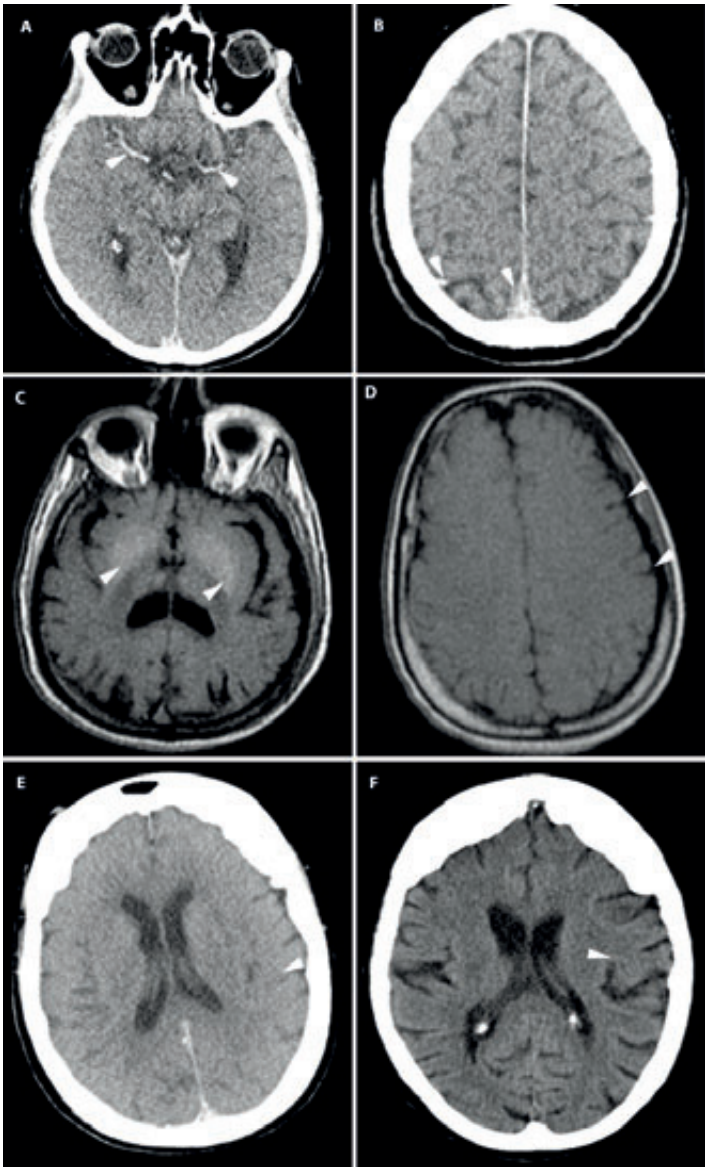
Table 11. Postmortem CT and MR features in relation to postmortem time interval

Postmortem time interval	Modality*	<12 hours	12-24 hours	24-48 hours	>48 hours	Total	P-value
		n=25	n=37	n=28	n=10	n=100	
Intravascular air	PMCT	14 (56%)	23 (62%)	16 (57%)	5 (50%)	58 (58%)	0.905
Sedimentation blood	PMMR	24 (96%)	35 (95%)	28 (100%)	10 (100%)	97 (97%)	0.416
Loss of grey-white matter differentiation	PMCT/ PMMR	14 (56%)	33 (89%)	28 (100%)	10 (100%)	85 (85%)	<0.001
Distended intestines	PMCT/ PMMR	0 (0%)	4 (11%)	7 (25%)	3 (30%)	14 (14%)	0.001
Postmortem clotting	PMMR	6 (24%)	20 (54%)	12 (43%)	6 (60%)	44 (44%)	0.197
Livores lungs	PMCT/ PMMR	20 (80%)	30 (81%)	26 (93%)	10 (100%)	86 (86%)	0.038
Livores liver	PMMR	17 (68%)	27 (73%)	25 (89%)	5 (50%)	74 (74%)	0.805
Livores spleen	PMMR	5 (20%)	11 (30%)	10 (35%)	5 (50%)	31 (31%)	0.062
Gravity dependent changes	PMCT/ PMMR	50%	47%	51%	56%	50%	0.094
Decomposition	PMCT/ PMMR	23%	30%	29%	34%	28%	0.026

*Specifies the modality that has the highest detection of the postmortem change
Legend: PMCT = postmortem CT; PMMR = postmortem MR

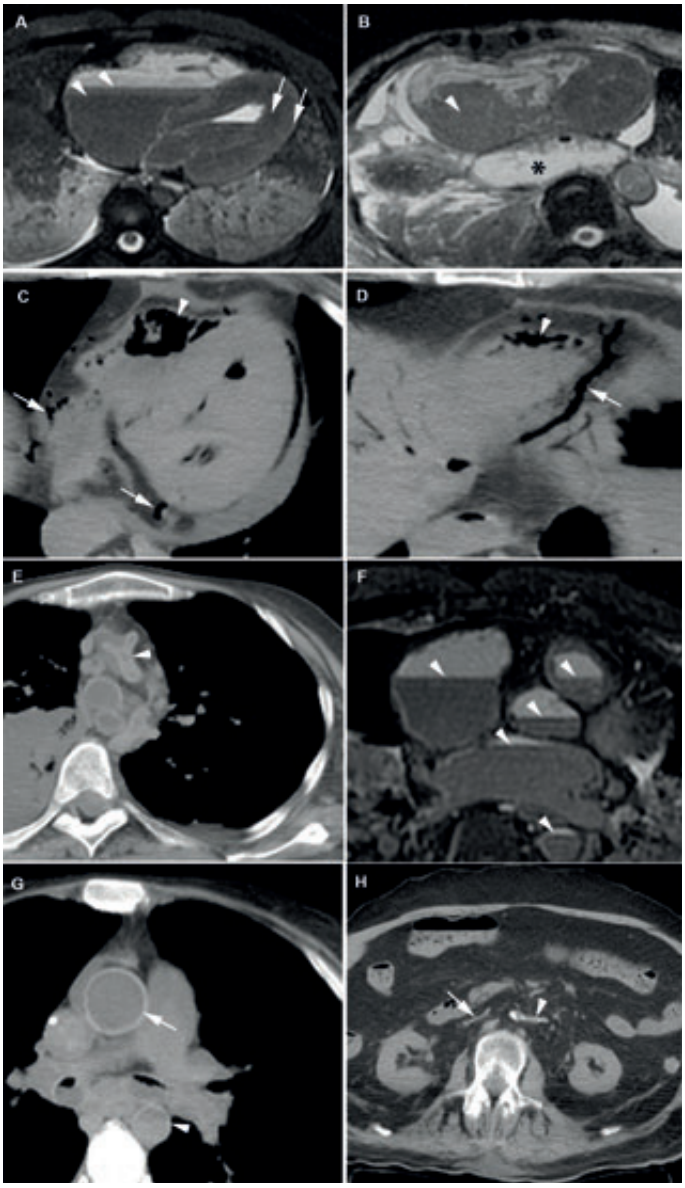
FIGURES

Figure 1. Postmortem imaging features of the brain



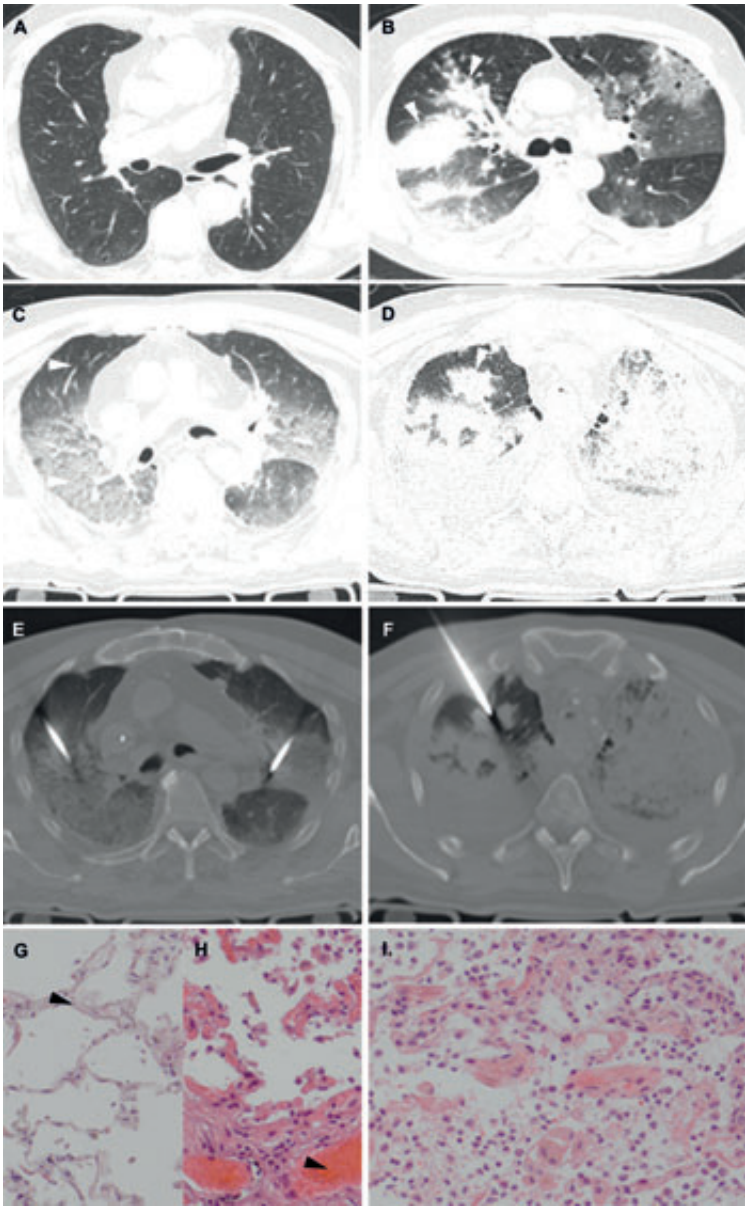
Legend: (A) PMCT: Symmetrical hyperdense cerebral arteries (arrowheads). (B) PMCT: Hyperdensity in the dependent cerebral veins and sagittal sinus (arrowheads). (C) T1w PMMR: High signal of the basal nuclei of the brain (arrowheads). (D) T1w PMMR: Sulcal effacement (arrowheads). (E/F) PMCT (E) and antemortem CT (F) of the same patient. The antemortem CT scan shows normal grey-white matter differentiation (arrowhead). PMCT shows complete loss of grey-white matter differentiation (arrowhead).

Figure 2. Postmortem imaging features of the heart and large vessels



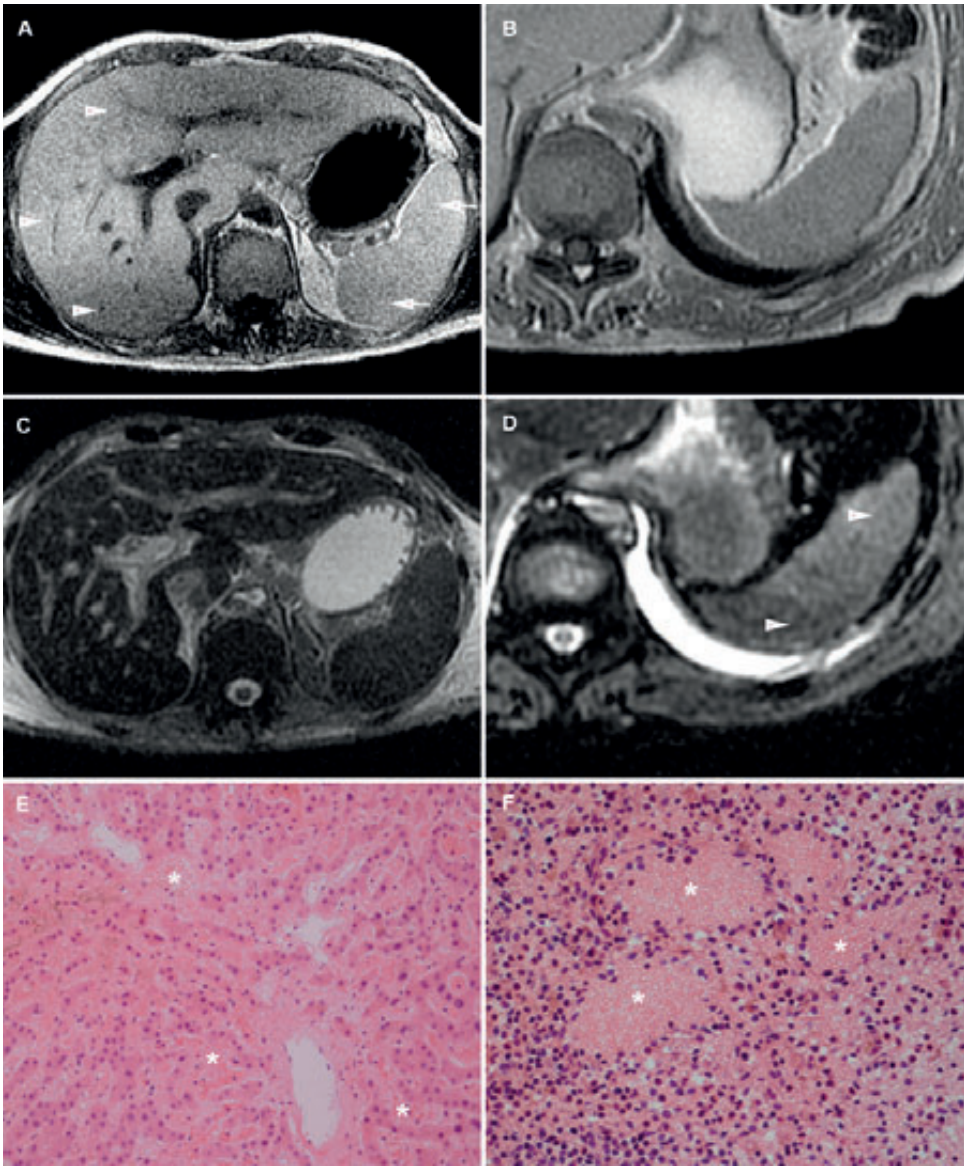
Legend: (A) T2w PMMR: Sedimentation of blood in the heart chambers (arrowhead). T2 signal decay from subepicardial to subendocardial myocardium (arrows). (B) T2w PMMR: Postmortem clotting in the right atrium (arrowhead). Additional finding: a mediastinal herniation of the stomach (asterisk). (C/D) PMCT: Extensive air in the right and left ventricle (arrowheads) and coronary veins (arrows). (E) PMCT: Collapsed ascending aorta (arrowhead). (F) T2w PMMR: Sedimentation of the blood (arrowheads), the plasma layer becomes hyperintense and the dependent layer becomes hypointense. (G) PMCT: Relatively hyperdense aortic wall (arrow) as a result of sedimentation. This is best seen in the ascending aorta. The descending aorta shows a sedimentation level with a hyperdense aspect of the anterior vessel wall (arrowhead). (H) PMCT: Complete collapse of the abdominal aorta (arrowhead) and vena cava inferior (arrow).

Figure 3. Internal livores of the lungs versus pneumonia



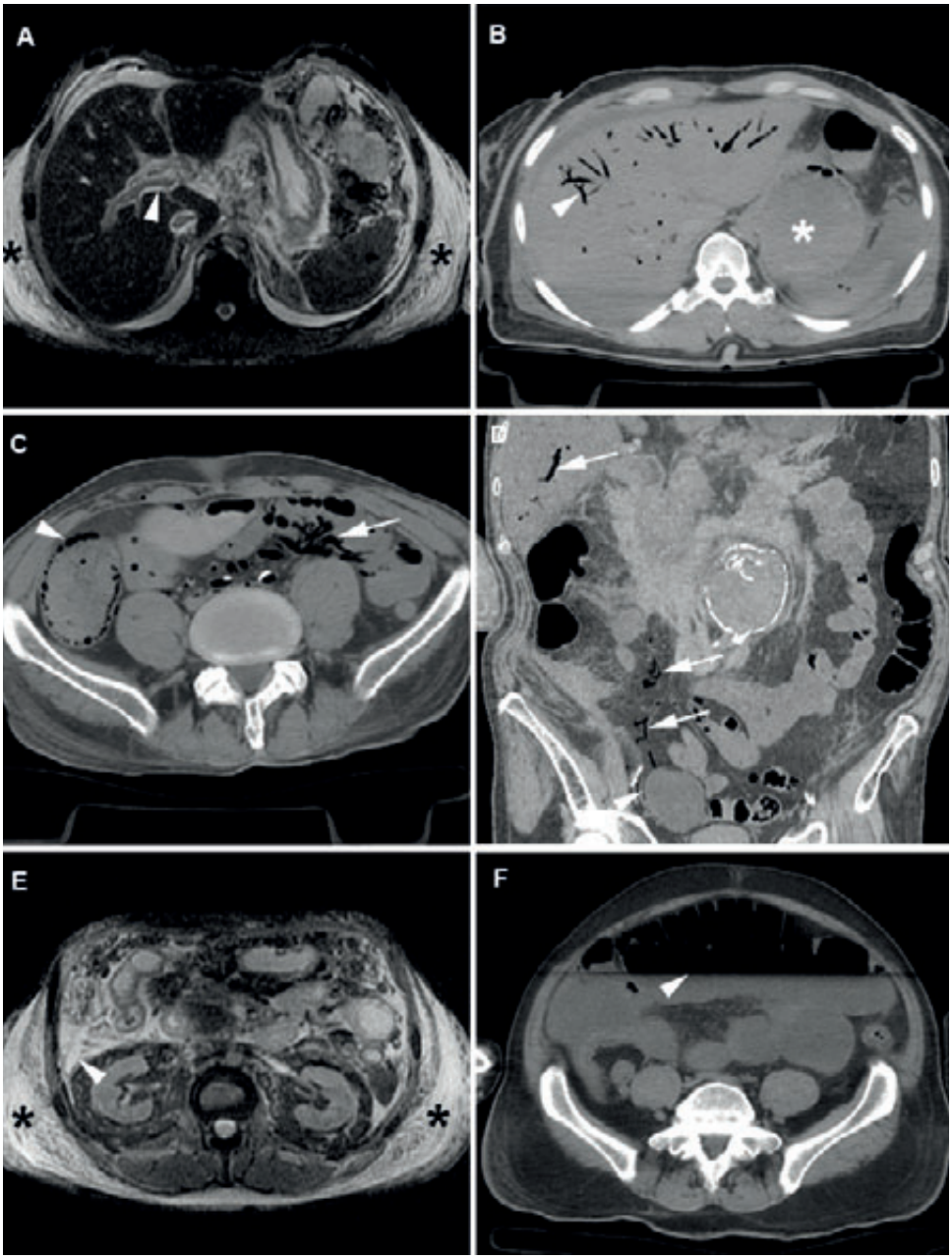
Legend: (A/C) antemortem CT (A) and PMCT (C). Normal (A) and internal livores (C) (arrowheads). (B/D) antemortem CT (B) and PMCT (D). Patient with a pneumonia (arrowheads). (E) CT-guided lung biopsies in the same patient as A/C. (F) CT-guided lung biopsy in the same patient as B/D. (G) HE, x100 original magnification. Lung parenchyma non-dependent: capillaries in alveolar walls practically devoid of blood (arrowhead). (H) HE, x100 original magnification. Same patient as G, lung parenchyma dependent, capillaries in alveolar walls congested with blood (arrowhead). (I) HE, x100 original magnification. Same patient as B/D/F, lung parenchyma, resolving pneumonia with thickened alveolar walls with mainly lymphocytic infiltrates, and hyaline membranes and extravasations of erythrocytes in the alveolar spaces.

Figure 4. Internal livores of the liver and spleen



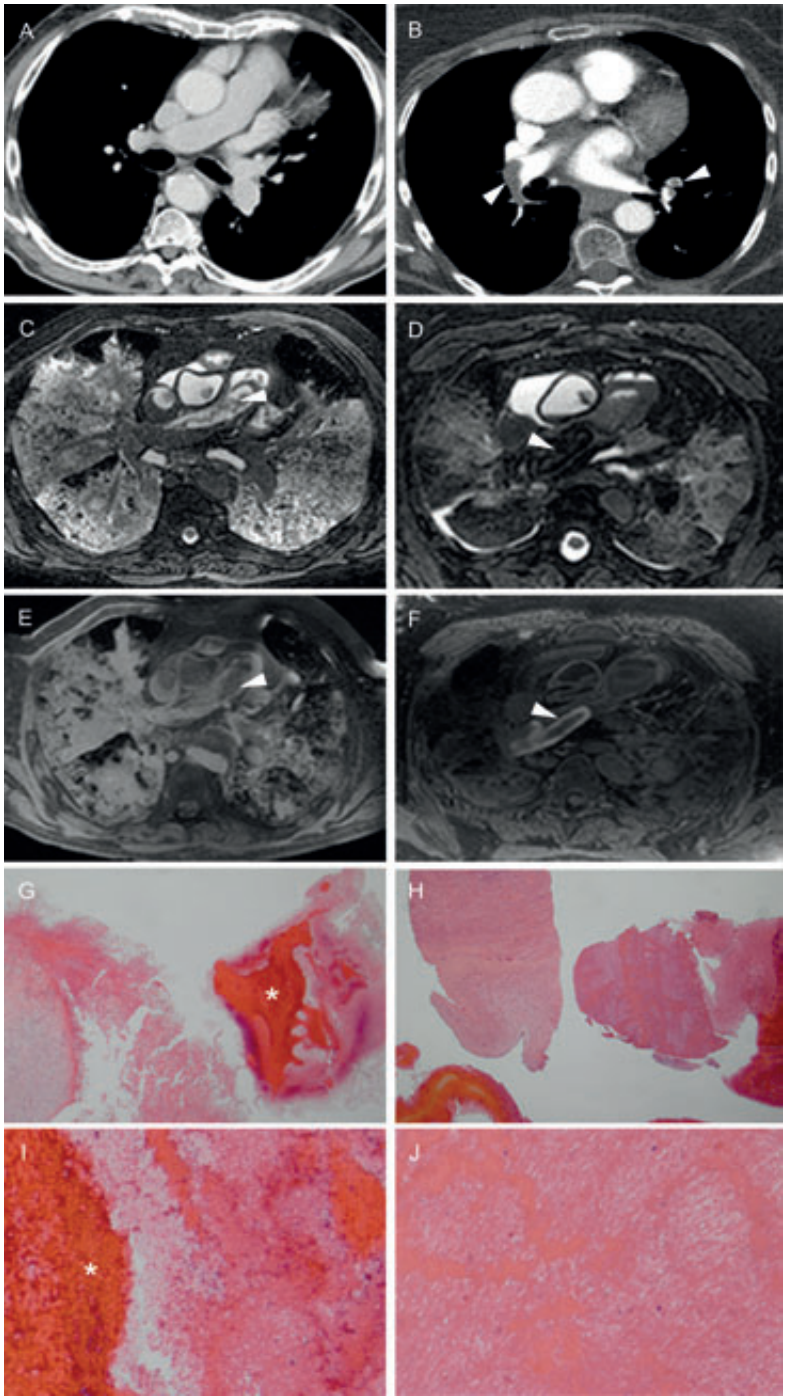
Legend: (A/C) T1w (A) and T2w fs (C) PMMR. Internal livores. In the liver 3 distinct layers (arrowheads) of different T1 signal can be seen and in the spleen 2 layers (arrows). A low T1 signal layer on top, relatively intermediate-to high signal layer in the middle and a low T1 signal layer in the dependent part of the liver. On T2w fs internal livores are not clearly seen. (B/D) T1w (B) and T2w fs (D) PMMR. The spleen shows 2 layers of different T2 signal (arrowheads). T1w show no clear livores in this patient. (E) HE, x100 original magnification. Centrilobular area of the liver with wide sinuses extended by blood (asterisks). (F) HE, x200 original magnification. Congested spleen with lakes of blood (asterisks).

Figure 5. Postmortem imaging features of the abdomen



Legend: (A) T2w PMMR: Periportal edema (arrowhead) and subcutaneous edema (asterisks). (B) PMCT: Putrefactive gas in the liver vessels (arrowhead) and distended stomach (asterisk). (C/ D) PMCT: Gas in the intestinal wall (arrowhead) and air in the mesenteric and portal veins (arrows). (E) T2w PMMR: Subcutaneous edema (asterisks) and fluid in the abdomen (arrowhead). (F) PMCT: Distended intestines with a sharp fluid-air level (arrowhead).

Figure 6. Pulmonary thrombo-embolism versus postmortem blood clot



Legend: (A/C/E) Antemortem CT (A) and T2w (C) and T1w (E) PMMR in the same patient. Antemortem CT (A) shows no abnormalities in the pulmonary arteries. PMMR (C/E) shows an irregularly shaped clot in the right pulmonary artery (C: arrowhead), with low T1 signal (E: arrowhead). (B/D/F) Antemortem CTA (B) and T2w (D) and T1w (F) PMMR in a patient with a thrombo-embolus. Antemortem CTA (B) shows a thrombo-embolus in the right and left pulmonary artery (arrowheads). PMMR (D/F) shows a thrombo-embolus in the right pulmonary artery (arrowheads). (G) HE, x16 original magnification, corresponding to image A/C/E. Wall of right pulmonary artery with a postmortem blood clot (asterisk). (I) HE, x50 original magnification, corresponding to image A/C/E. Higher power of blood clot showing blood with loosely arranged depositions of fibrin. (H) HE, x16 original magnification, corresponding to image B/D/F. Wall of pulmonary artery and thrombus. (J) HE, x50 original magnification, corresponding to image B/D/F. Wall of pulmonary artery and thrombus with dense depositions of fibrin and platelets, alternating with degenerated erythrocytes and white blood cells, thus constituting so-called lines of Zahn.

APPENDIX

Not included in this thesis:

S1 File. Dataset PM changes.

9

General discussion

THE MINIMALLY INVASIVE AUTOPSY

The aim of this thesis is to evaluate the current status of the conventional autopsy and its alternatives. We evaluated the trends of nationwide and in-hospital autopsy rates in the Netherlands, and the attitudes towards autopsy among clinicians and next-of-kin in our own hospital. We reviewed studies investigating the accuracy of non-invasive or minimally invasive autopsy methods for naturally deceased adults.
Furthermore, we improved our own minimally invasive autopsy method, tested its performance in a large study, and explored its limitations, possibilities, and value. In this chapter I will further discuss our findings and compare them to the literature.

Representativeness of our study cohort

Our study cohort had only a few exclusion criteria, to ensure that the studied population would be representative for the patients dying and autopsied in our hospital. Besides the cases not included for logistic reasons, there were four cases with known infectious diseases; two cases with too large abdominal wounds; and one case with a body size that did not fit into the MRI. In one of the 100 included cases the findings at conventional autopsy (CA) after minimally invasive autopsy (MIA) necessitated a forensic autopsy and thus exclusion of the case from the cohort.
Like in many other autopsy studies,[32,33,56,58,78] the ratio of male and female cases in this cohort was in favour of men. Not surprisingly, most cases came from the ICUs and ER, where often critically ill patients are treated.
According to the WHO,[142] in high-income countries death is most often caused by cardiovascular disease, followed by brain (e.g. stroke) and pulmonary pathologies. In this study cohort the same organ systems were involved, however, in a different order with most patients dying from pulmonary pathology, like in our pilot study.[57] Studies performed in Finland, Denmark and Germany (Berlin) found cardiovascular pathologies as the main cause of death.[10,74]
The clinically unknown major diagnoses were most often in the respiratory system. Wichmann et al. found most clinically unknown diagnoses in the cardiovascular system, however, their results are probably biased, because they enrolled cases selected towards cardiac pathology.[80] The 43% of clinically unknown diagnoses that were related to cause of death are similar to those found by autopsy in a study from 1989, although diagnostic techniques were not as advanced as today.[37]

Overall, the in-hospital deceased undergoing MIA in our cohort are a fair representation of common pathology in high-income Western societies.

Practical limitations and considerations

The current method had complex logistics due to the use of both MRI and CT scanners and the image-guided biopsies from the corpse, if consented, including the brain. The bodies were transported from the morgue through the hospital to the radiological department and back, they had to be properly covered in order not to scare personnel, patients or visitors. Because fluids may leak when taking tissue biopsies, the body was put in a body bag. This bag should not contain any metal to be suitable for the MRI scanner.

Because MRI scans are time consuming, and (therefore) relatively expensive, we chose to scan from the head to the pelvis, to include all vital organs, and skip the legs. The lower extremities were scanned with CT only, which took only a few seconds.

During regular working hours the scanners were occupied for scanning patients, so the MIA scans had to be done in the evenings. In our cohort 17 cases were excluded due to logistic reasons (including two cases with another MIA scheduled on the same day), and in six cases next-of-kin did not accept further delay of CA.

Though MIA is a joint effort between radiologists and pathologists, the accuracy of the procedure heavily depends on the availability of skilled radiologists, because they have to identify the pathologies that have to be biopsied, as imaging is the substitute for gross examination. Currently there are only a few clinical radiologists with experience in post-mortem imaging.[254] In particular they have to be familiar with, often subtle, post-mortem changes on both CT and MRI, which may be overinterpreted as pathological changes. [255]

The biopsies of the torso were performed by MIA researchers, who had limited experience in obtaining CT-guided biopsies. Nevertheless, sampling errors occurred in only 17 of the 655 biopsies (2.6%), and just four of these led to a missed diagnosis.

Like for CA, histologic examination of the brain was only possible if next-of-kin had given explicit consent for brain biopsies. These biopsies were taken under stereotactic guidance in cooperation with neurosurgeons on call.

If MIA were to be clinically implemented as a routine, scanners should be available for MIA during regular working hours. However, having an MRI and CT scanner for post-mortem use only, would probably be too expensive. 'Second-hand' scanners, on the other hand, might not be.

Moreover, more radiologists have to be properly trained and professional guidelines have to be developed.[256] The same applies to radiology technologists, and especially for the ones operating the MRI scans, because those require more dedicated protocols. Also, the MIA biopsies are probably best taken by those who are experienced already based on their clinical work. Radiologists are the most likely candidates.

Along the same lines, pathologists have to be trained in the interpretation of CT and MRI scans, and in the collaboration with radiologists: they have to learn their language. They also have to get confidence in reading the MIA biopsies, avoiding both overinterpretation, and being too cautious. In fact, like the specialized pathologist proposed for CA,[43] MIA will require dedicated, specialized teams of radiologists and pathologists.

Diagnostic accuracy

Deaths are usually not the result of a single isolated disease or event.[37] Especially among elderly with multiple medical problems, death often occurs due to a coexistence and possible interaction of several diseases, and (side) effects from given therapies.[257] Therefore, in most cases, CA is a complex investigation, and so is the MIA. As a consequence, different observers may interpret the results of both techniques differently, a situation that is not uncommon in clinical practice. In fact, this is the main reason why the cause of death determined by CA and MIA had to be compared to a consensus cause of death.

As several studies[56,90,258] and previous experience[57] demonstrated, we could not assume that the diagnostic accuracy of CA in our study would be infallible. This was another reason for using consensus cause of death, and consensus diagnoses, rather than CA-results as gold standard in the comparison of MIA and CA.

To determine the consensus cause of death, we set up a 'reference standard process' containing three review steps with different professionals independently deciding per case whether the presumed cause of death was concordant, discordant or undecided. If cases were discordant, the cause of death reported by either MIA or CA was defined as the consensus. The first review was based on the 99 MIA and CA reports only. Two parties had to agree that the two methods reported the same cause of death, in order to accept it as the consensus cause of death. The remaining 40 cases were subjected to the second review, which included both the reports and the radiological and macroscopic images and histological slides, and, if necessary, a correction of erroneous interpretation of the CA histology (to compensate for bias due to the research setting of MIA versus the daily routine of CA). The third review required a "reference standard committee" (RSC) of several specialists discussing the ten remaining cases and jointly defining their causes of death. In the end, there was concordance for cause of death between MIA and CA in 91 cases and discordance in eight cases. For the latter, the consensus cause of death was based on the MIA report in five cases, and on the CA report in three cases. Thus, CA reported the correct COD in 95% of cases and MIA in 97%. chapter 5

Because we could not rely on CA as the gold standard for individual diagnoses either, we created a reference standard that was based on pathologies diagnosed with certainty by either method.[chapter 5] Since the MIA and CA were compared to this reference standard, MIA could have an even better accuracy than CA for some pathologies.

Missed diagnoses

For our MIA we used both MRI and CT, and, by that, benefited from both their specific imaging qualities (e.g. good visualization of organ parenchyma as well as bone and air) and had to deal with both their shortcomings (e.g. image artefacts due to metal parts).[52,103,125,126,132] Even when we evaluated the MRI and CT images together, the detection was not faultless. We sometimes were unable to detect the same lesion on the corresponding images of the other imaging modality. This has occasionally caused difficulties when we aimed to obtain CT-guided tissue biopsies from lesions detected at MRI. In some cases, this problem may have influenced the diagnostic accuracy of the MIA.

That some of the CA diagnoses in our cohort were missed macroscopically (e.g. a kidney infarction, an active cystitis, a spleen infarction and a necrotizing hepatitis) may be due to the fact that macroscopy was mainly performed by residents rather than by the more experienced supervising pathologists. If lesions are not recognized on macroscopy, they will not be sampled for histological examination, whereby some diagnoses will be missed. However, in our cohort, there were also diagnoses that had been missed even though the organs had been adequately sampled. Five out of six acute myocardial infarctions and five out of six pneumonias were originally missed at CA, but they turned out to actually be present on the available histological slides. Only one of each was truly missed, and, by contrast, two acute myocardial infarctions were falsely diagnosed. The false negative and false positive cases were corrected during a secondary evaluation, which was initiated based on discrepancies with the MIA findings.

Cardiovascular pathologies

Cardiovascular diseases (e.g. unsuspected pulmonary emboli and myocardial infarctions) are the leading cause of death in many countries, and not easily detected by MIA.

Localized or massive acute myocardial infarction, endocarditis and thrombo-emboli are considered blind spots of the imaging techniques.[57,59,108,109] However, new techniques are being developed to improve accuracy, such as state-of-the-art high resolution MR of the heart,[233,259,chapter 6] coronary optical coherence tomography[43,260] and either PMCT-angiography[118,120,127,133,165,261-263] or, less frequently used, PMMR-angiography.[99] These angiographic techniques either aim to visualize all the vessels

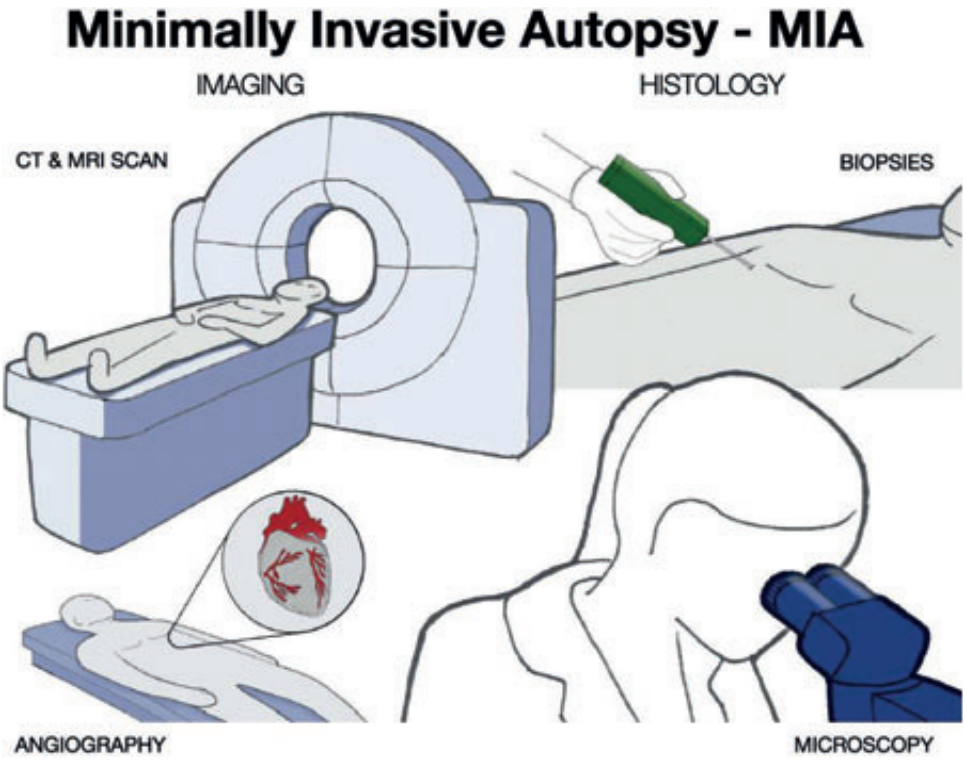
of the body,[99] using a modified heart-lung machine,[264] or target mainly the coronary arteries.[118,120,127,133,165,261-263] The latter approach is cheaper and requires less training than the former. Both angiography methods are time consuming and may invoke difficulties for the differentiation between technique-related or post-mortem artefacts and true pathological findings.[128,152] A common difficulty within the cardiovascular system is distinguishing post-mortem clotting from true emboli, especially in the right side of the heart and the pulmonary arteries.[59,108,109,265] With PMCT-angiography this clotting may even be mistaken for a dissection[266] or a fat embolism.[267] For this latter diagnosis and, generally, for any diagnosis of pulmonary thromboembolism, the radiological images have to be combined with targeted biopsies from the suspected clots.[108,109,265,267] Besides the detection of occluded vessels, PMCT-angiography appears to improve soft tissue imaging,[265] and it helps to identify bleeding as the cause of death,[268] even small branches may be identified as bleeding source.[265] The attitude of next-of-kin towards PMCT-angiography seems positive.[89]

In our cohort, MIA missed one myocardial infarction and in only one case the cardiac findings were misinterpreted (acute myocardial ischemia versus ischemia secondary to abscesses in the heart). This is a significant improvement compared to our pilot study,[57] which is mainly due to the application of multiple CT-guided biopsies from different regions of the myocardium, partially directed by the positive MRI findings, whereas in the pilot a few biopsies of the heart were taken under ultrasound-guidance or even without guidance. In another case the biopsy findings were suspicious for bilateral peripheral pulmonary emboli, but the diagnosis was not certain at MIA.

As a pilot study within our cohort, we explored the feasibility of post-mortem CT-angiography for the visualization of the coronary arteries, targeted via the left carotid artery. However, we planned not to use this technique in every case, for it would be too time consuming and expensive. Only in those cases with a possible cardiac cause of death and no other obvious cause of death present at the unenhanced MRI and CT scans we performed the CT-angiography to further examine the coronary arteries. Using this criterion, we needed angiography in a quarter of the study population.

We mainly experienced difficulties with the cannulation of the carotid artery in cases of obesity; difficulties with positioning and inflating the balloon of the catheter correctly, and occluding the ascending aorta but not the openings of the coronaries; and difficulties with the interpretation of the images, especially if the contrast did not reach the distal parts of the coronary arteries or if there had been surgical interventions in the past (e.g. bypass surgery, or valve replacement).

Figure 1. Cover image patient information folder



Though in some cases we were able to create detailed 3D reconstructions of presumably complete coronary branches, we did not feel confident to rely on the coronary CT-angiography for the diagnosis of stenosis. A narrowing of an artery might as well have been an artifact due post-mortem clots[127] (we could not flush the arteries before introducing the contrast agent) or pressure of the surrounding tissue due to gravity (we could not turn the body on its side). Moreover, the presence of stenosis does not in itself imply acute myocardial ischaemia.

For the detection of ischemic heart disease, the importance of histological examination cannot be stressed enough.[90,269-271] There should be a standard histological examination of the heart included in the MIA-protocol, like in the CA-protocol.[159] We advocate that CT-guided tissue biopsies should preferably be targeted based on MR images and at least obtained from both left and right ventricle (anterior, lateral, and posterior wall), and the septum.

Pathologies related to air/ gas

MIA outperforms CA in pathology where air/ gas is involved, especially in cases of (massive) air embolus and pneumothorax.[57,129, chapter 5] Conversely, the lungs are difficult to examine due to livor mortis in the dependent parts of the lungs. To improve the diagnostic accuracy for small pulmonary lesions, CT scans may be combined with mechanical ventilation, which inflates the lungs to expand their volume and reduce the lung density.[121-123] In our cohort, however, we did not miss any small lesions in the lungs. MIA did falsely diagnose one acute pneumonia, and one aspergillus infection in the lungs was missed upon histological examination.

Collections of air may also be present in the case of intestinal pathologies. On imaging, bubbles of gas within the intestinal wall (pneumatosis) and air collections around the portal vein are a sign of ischemia.[103,111,210] They may, however, also represent the beginning of post-mortem putrefaction. To differentiate the two, it may be helpful to compare the post-mortem scans to those made just before death, if available.
In this cohort, we also had difficulties in identifying or confirming ischemic colitis as the cause of death with MIA, and some intestinal pathologies were difficult to distinguish: fungi in the small intestine versus an infectious enteritis, and radiation mucositis versus typhlitis.

Cerebral pathologies

The main issue regarding neurological diseases was the fact that consent from next-of-kin was required to obtain brain biopsies for histological examination. MIA brain biopsies were permitted in 24 cases and conventional brain autopsy in 38 cases, among these there were only 20 cases with consent for both MIA brain biopsies and conventional brain autopsy. However, with or without consent for brain biopsy or dissection, MIA always included specific imaging of the brain, which was better than no examination at all. Generally, large infarctions and haemorrhages are clearly visible at imaging, but small subdural haemorrhages may be hard to detect, for they may be pushed away by the post-mortem swelling of the brain.
In our cohort were several cases, in which the imaging findings of the brain alone could not establish *certain* diagnoses, while these diagnoses could not be confirmed by brain biopsy or brain autopsy. Due to the lack of histological correlation, the accuracy of MIA for brain pathology cannot be exactly determined in this cohort. Nonetheless, in 11% of the cases cerebral pathology, often based on radiological evaluation only, was related to the cause of death. It was thereby the fourth most common cause of death in our cohort, emphasizing the importance of post-mortem brain examination.

The MIA brain biopsies usually did not interfere with the execution and interpretation of the conventional brain autopsy. However, in one case the brain autopsy detected a focal intracerebral bleeding that was probably caused by the biopsies, for it was, even in retrospect, not present on the imaging. We assume that, if MIA were to be clinically implemented in the future, and histological examination of the brain is permitted by next-of-kin, either MIA-biopsies or conventional brain autopsy but not both will be performed. In that situation, the images could be used for either obtaining the brain biopsies or performing the brain autopsy, but the biopsies will no longer cause artifactual findings at brain autopsy.

Small or subtle pathologies

Another important difficulty with MIA is the detection of small lesions, such as (recurrent) tumor processes and metastases[80], or to radiologically distinguish inflammation processes from, for example, hematological malignancies. For such pathologies it is helpful to compare post-mortem images with recent pre-mortem scans whenever available and to obtain tissue samples for further examination. Patients who were known to have cancer, for example, have probably been scanned for follow-up, thus recurrent tumor growth or metastases are likely to be known at time of death.

When small suspicious lesions are identified at the post-mortem images, it may be difficult to obtain representative biopsies, especially if they measure less than 1 cm in diameter, are connected to soft tissue structures that move along with the biopsy needle, and are poorly visible during the biopsy procedure, due to metal artifacts of the biopsy needle.

In our cohort, we were unable to identify or confirm three squamous cell carcinomas of the respiratory tract with MIA. One recurrent squamous cell carcinoma in the area of mouth, trachea, larynx and lung was missed on imaging (perceptual error) although visible on pre-mortem images, and two squamous cell carcinomas in the lung were missed by biopsy (sampling error).

Typically MIA was unable to answer clinical questions that concerned subtle pathological changes not visible with the applied imaging techniques. For example, the status of surgical sutures could not be realisably imaged without the use of contrast (2 cases). Along the same lines, it was impossible to identify intoxication with certainty as the cause of death without toxicological examination.[52]

FUTURE PERSPECTIVES

A MIA including MRI and CT scans, and CT-guided tissue biopsies seems a valid alternative for CA, however, more experience is needed to establish for which pathologies or clinical questions MIA is less appropriate or even not suitable.
If MIA is to be implemented on a larger scale in hospitals, we should strive for the highest possible diagnostic quality at a reasonable price. Like in forensic practice, clinical radiologists and pathologists should be adequately trained for correct interpretation of post-mortem imaging[255] and biopsy findings[116] with the goal to create an integrated diagnostic specialty for post-mortems that is embedded in the routine workflow. Then, aiming for higher overall autopsy rates, both the intramural and the extramural physicians should be encouraged to request consent for autopsy.
During medical training we should stress the importance of post-mortem examination and discuss all forms of post-mortem examination, including MIA, even though many medical schools do no longer require autopsy attendance and favour case-based studies over anatomic pathology.[272,273] Young physicians should be taught how to request an autopsy and discuss the various autopsy methods with the next-of-kin.[205] Medical students and residents should be made familiar with MIA and CA as a means to train themselves in understanding how pathologic processes develop, interact and lead to death. Residents in pathology and radiology should learn how to integrate the post-mortem radiological images and biopsy findings. The database assembled in this study constitutes a valuable teaching set for practicing the MIA.

Costs of the Minimally Invasive Autopsy

In most countries, an in-hospital CA is a free service. However, in some countries or medical institutes, the costs of post-mortem examinations, although they are not that high compared to many clinical interventions, are a reason for the decreasing autopsy rates,[43,274] especially if there are no minimum requirements for the number of post-mortems.[42] Therefore, it is important to keep the price of a MIA as low as possible.
In the UK, the price for an alternative method of post-mortem examination is estimated at around £1000, whereas the autopsy costs little under £100, not taking into account a substantial subsidy from hospital funds.[254] In Switzerland each forensic autopsy is preceded by at least CT, and the reimbursement per post-mortem examination including CT, CTA, MRI, and forensic expert opinion is $820 to $1,150.[116] According to the studies we reviewed, a MIA including both biopsies and CTA costs $1,649 to $1,945, whereas an autopsy costs $2,170 to $2,378.[56] Thus, MIA may be either more expensive or less expensive than CA depending on the context and country. Either way, the costs for MIA are too high for low-income and middle-income countries with even

fewer resources for autopsy. On top of that, this MIA method may unnecessarily be an overly extensive approach, considering that the leading causes of death in low-income countries are lower respiratory tract infections and diarrheal diseases.[142] Therefore, in Mozambique the MIA was reduced to only tissue biopsies (without any guidance) according to a fixed protocol, and blood and cerebrospinal fluid sampling for histological and microbiological examination.[275] This method sufficed to detect infectious diseases and malignancies in around 80% of the cases.

In our study cohort, a preliminary calculation of the costs of a complete MIA gave the amount of €1,300. This includes all personnel time (e.g. preparation of the body, and the time spent by the involved radiologists, pathologists and neurosurgeons), technique costs (e.g. imaging modalities; histological slides and immunohistochemistry) and, in our cohort, brain biopsies in a quarter of the cases. Even though we performed CT (and stereotactic) guided biopsies, explaining the higher costs of MIA than in our pilot,[57] we were able to prevent an excessive increase of costs, by employing medical students to assist with MIA instead of the more expensive radiologic technologists. The costs of imaging equipment are low in this study because the scanners are fully utilized in the clinical program. Dedicated scanners for MIA, e.g. installed at the morgue, would probably be underutilized, making the procedure more expensive to compensate for the higher depreciation and running costs of the equipment.

However, if 'second-hand' scanners would be made available for post-mortems only, the main financial and logistic impediments will be solved. Then, minimally invasive autopsies can readily be performed shortly after death, and more frequently once its workflow has become a routine. With the improved efficiency, there will be a low-threshold for general practitioners to request autopsy for out-of-hospital deceased, and eventually, MIA may become less expensive.

Another way to reduce the costs of MIA in the future would be to shorten the most time consuming step, which is the biopsy needle placement for tissue sampling. This is being explored in forensics, where a robotic system is under development that permits automated needle placement, thereby shortening procedure time.[96] Another approach would be stereotactic guidance of the biopsies, similar to the technique we used for our brain biopsies. This way, it will no longer be necessary to check the needle position for each biopsy by performing new scans.

Inevitably, the more advanced an alternative autopsy technique is, the higher its price, but, in general, the better its diagnostic capabilities. Future studies are needed to explore possibilities to tailor the MIA technically without reducing its diagnostic performance.

Acceptance of the Minimally Invasive Autopsy

Next-of-kin may always have been hesitant to any post-mortem investigation of their deceased beloved one, but nowadays they dare to speak up to "the then-well-known (and almighty) doctor,"[42] who currently seems less motivated to get them to consent to autopsy.

MIA, however, may be more acceptable to next-of-kin, especially for those who have fear of mutilation of their beloved deceased[89] or to those that may have concerns about organ retention or feel a restriction to invasive autopsy due to religious convictions.[22,24,38,40,81] Among the 2197 in-hospital deceased patients in our study period, there were, apart from the 99 included cases, 98 more cases in which next-of-kin gave or, according to our survey, had been willing to give consent for MIA. Yet in 62 cases CA was not permitted on at least the whole torso.

Next-of-kin might be able to choose for a MIA 24/7 with, if necessary, a "partial autopsy" of a specific organ (e.g. the heart for close examination of the sinoatrial and atrioventricular nodes). It seems plausible that introduction of MIA will increase the overall rate of clinical autopsies. However, acceptance of alternative autopsy techniques and its impact on autopsy rates should be formally investigated.

MIA can support biomedical research programs by providing normal and pathologically changed tissues, which would otherwise be difficult to ascertain. For example, providing tissue from cancer metastases for comparison with primary tumor of the same patient,[276] as pursued in rapid ("warm") autopsy programs, could make an important contribution to the development of personalized cancer treatment.[184,185] Next-of-kin of patients who died of cancer rarely allow a CA, because the cause of death is considered known, and the patient has suffered enough. They may more readily consent to a MIA with the primary goal to collect tissue from metastases in different organs of the same patient. The RNA quality of these tissue samples obtained by MIA is of sufficient quality for molecular research.[26]

The MIA procedure, once embedded in the daily workflow, could be carried out within hours after demise, so as to provide optimal tissue for high-end molecular studies.[26] MIA may also be more acceptable and thereby more useful in counselling families on hereditary diseases.

Practical advantages

MIA provides a permanent auditable record that can be repeatedly and objectively consulted by pathologists, radiologists, clinicians, scientists, and next-of-kin. The images can easily be stored and examined at any time or place, the biopsy targets may even be indicated by an off site radiologist.[130] As soon as the histological slides will be digitally available,[277] the complete MIA could be subjected to second reading.

MIA can supplement epidemiological databases with large amounts of data (e.g. mega imaging and histological data), especially if it were to be used as the objective end-point measurement in the documentation of subjects included in life-long cohorts,[257] like the so-called Rotterdam Study.[278] By improving the accuracy of epidemiological databases, MIA also provides relevant information for the policy makers, who have to decide on how to optimally spend the recourses for healthcare.
As the Royal College of Pathologists stated, MIA may be preferably used to complement autopsy, rather than to replace it. From a technological point of view, there should not be too many obstacles to implement MIA on a larger scale.[131] Some suggest performing a tailored rather than routine approach that is based on the case context, to employ each technique to its maximal advantage and reduce costs.[279,280] We, on the other hand, advocate performing a fully flagged MIA, including histological examination, after which a "partial autopsy" may be all there is needed to complete the investigation, or no further autopsy procedure at all, if the diagnosis is certain and the clinical questions are answered.
Whereas it is mainly introduced to boost autopsy rates, MIA may also provide an opportunity for screening prior to CA in cases with high-risk infections, and thereby help to reduce the number of required high-risk autopsies[129] without loss of information about the cause of death.

To maintain a good quality of the post-mortem investigations, including the MIA, pathologists and radiologists should work together constructively. Their departments should preferably join forces regionally, by providing an efficient infrastructure and sufficient availability of techniques, required facilities, and skilled physicians. The application of MIA should be supported with a guideline for post-mortem radiology in clinical practice, which is currently under development in the Netherlands.[256] Perhaps, autopsy pathology should become a recognised subspecialty.[42,43] Or, if pathologists and radiologists truly cooperate and post-mortem radiology is integrated into autopsy guidelines, post-mortems might even become a shared subspecialty?[281]
Either way, autopsy should be revived, and, evidently, any post-mortem examination provides more information than no post-mortem investigation at all. If we can manage to provide clinicians with quick and useful information, our improved services may change their attitudes towards post-mortems, and they may become more motivated to request consent.

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A

Addendum

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SUMMARY

The autopsy is a post-mortem investigation that is mainly used for identifying the cause of death. Besides that, it also fulfills other important roles in today's clinical practice. For example, by providing feedback on diagnostics and therapies, the autopsy helps us to improve these and thus, keep up the high quality of modern medicine. Nevertheless, autopsy rates have been declining in many Western countries.

To objectify the autopsy rates in the Netherlands and, by that, the severity of the decline in our country, we performed a retrospective study. In **chapter 2**, we describe 35 years of Dutch national death counts and autopsy rates among adults, covering the years 1977 through 2011, and including trends per sex, age, and hospital type. We found that each year more people had died, but relatively fewer people had died in hospitals, and that even fewer deceased had been autopsied. The autopsy rates were highest among men, young patients, and patients dying in academic hospitals. The overall autopsy rates in the Netherlands declined by 0.3% per calendar year and the autopsy rates of in-hospital deceased declined even more, 0.7% per calendar year, from 31.4% to 7.7%.

We wondered why the autopsy rates had declined so rapidly, and found several explanations in the literature: among others, there was overconfidence of clinicians in the advanced diagnostic techniques used clinically, and the autopsy causing disfigurement of the deceased's body. To further investigate this, we performed a prospective observational study based on thousand questionnaires. In **chapter 3**, we describe that 17.4% of our clinicians did not even request permission for autopsy, mainly because they were convinced that it would not show anything other than what was already known through pre-mortem diagnostics. Apparently in many of the other cases, clinicians did not feel the necessity of performing autopsy either, because 51% of next-of-kin did not permit autopsy for this very same reason: cause of death was already known. Only in 16.1% of cases the next-of-kin feared for disfigurement of the deceased's body, which is less than we expected. To increase autopsy rates, the above named assumption, that the cause of death is known, which is a self-fulfilling prophecy, should be addressed. Moreover autopsy methods should be developed that require less mutilation of the deceased's body.

To evaluate which methods have already been developed to substitute the conventional autopsy (CA) in adults, we searched six different databases for original prospective validation studies on this topic, which we systematically reviewed. In **chapter 4**, we compare sixteen different studies, of which thirteen studies investigated radiological imaging techniques, either non-invasive or minimally invasive methods. The minimally invasive autopsy techniques used CT-angiography and/or biopsies in combination with unenhanced MRI and/or CT scans, and usually performed better than the non-invasive

methods. None of the alternative methods performed as well as CA, but the highest sensitivity for cause of death was reasonably good: 90.9% (two studies combined, suspected duplicates excluded). This was achieved with a combination of CT, CT-angiography and biopsies. An important limitation of our review was, apart from the few studies with a blinded comparison and many studies with only few cases, that the data presented in the sixteen studies was too heterogeneous for meaningful meta-analysis of outcomes. We decided that further investigation was required in order to establish a feasible alternative to CA in clinical practice, preferably in a larger study group.

In **chapter 5**, we present our own prospective, single center, blinded cross-sectional study that compares the diagnostic performance of a minimally invasive autopsy (MIA) and CA. For this, 99 deceased adult patients were included, for whom next-of-kin gave consent to perform both MIA and CA. Our MIA comprised unenhanced whole-body CT and MRI scans, and image-guided biopsies of vital organs and pathological lesions detected on imaging. MIA and CA agreed on cause of death in 91 cases; MIA established the right cause of death in 5 and CA in 3 of the remaining cases. Of the 288 diagnoses directly related to the cause of death, MIA diagnosed 259 and CA 224; 200 diagnoses were found by both methods. Finally, MIA and CA performed equally in answering the specific questions of clinicians. The method we applied for MIA, which is feasible in any larger general hospital and generates a permanent and auditable record, appeared to be a valid alternative for CA. Importantly, no less than 17% of the causes of death and 43% of the 288 diagnoses related to cause of death were clinically unsuspected, emphasizing the necessity of performing post-mortem examination for the purpose of healthcare quality control.

The study in **chapter 6** is a sub-analysis of this same cohort, analyzing the diagnostic accuracy of MIA for the detection of ischemic heart disease, which forms a major burden of disease in Western countries. In this analysis, where CA served as the gold standard, the combination of specialized MRI scans and targeted heart-biopsies reached a sensitivity of 97% for the detection of acute ischemia and a specificity of 95%. However, without the heart-biopsies the MRI scans had low sensitivity for both acute ischemia and chronic ischemia, emphasizing the value of histological examination in the post-mortem. Whereas other studies stress the need of post-mortem angiography for establishing a cardiac cause of death, we find that our MRI scans combined with targeted heart-biopsies suffice.

Through the feasibility study described in **chapter 7**, we found that post-mortem tissue biopsies taken at MIA yield a sufficient amount of RNA, which is also of sufficient quality, especially if the biopsies are obtained shortly after death. With such good RNA quality, these biopsies are useful for molecular translational research, including gene

expression analyses. If MIA were to be implemented in clinical practice, it creates an opportunity to obtain more tissue samples of (recurred) primary cancer processes and their metastases, which are required to examine intra-tumor heterogeneity, to further develop cancer therapies.

Because post-mortem imaging is quite a new field in clinical radiology, we evaluated the occurrence of common post-mortem changes (due to chemical and physical processes in the deceased's body), which presented on the MRI and CT scans within our cohort. The study in **chapter 8** shows that decomposition-related changes increased with the post-mortem time interval. Intravascular air, pleural effusion, periportal edema, and distended intestines occurred more frequently after resuscitation, and post-mortem clotting less frequently; distended intestines and loss of grey-white matter differentiation in the brain were correlated with post-mortem time interval; and hyperdense cerebral vessels, subcutaneous edema, fluid in the abdomen and internal livores of the liver were more frequently seen in ICU patients.

In the future, for MIA to be implemented in clinical practice, radiologists need in-depth understanding of these processes for correct acquisition and interpretation of the post-mortem scans.

SAMENVATTING

De autopsie (ofwel obductie) is een postmortaal onderzoek dat vooral wordt uitgevoerd om de doodsoorzaak te vinden of te bevestigen. Daarnaast vervult het ook andere belangrijke functies in de hedendaagse geneeskunde. Denk bijvoorbeeld aan de terugkoppeling naar de clinici over gestelde diagnosen en gegeven behandelingen, waarmee deze kunnen worden geëvalueerd en waar nodig verbeterd, om zo de kwaliteit van onze medische zorg hoog te houden. Helaas blijken de obductiepercentages al jaren dalende te zijn in veel Westerse landen.

Om de situatie in Nederland en de ernst van de daling in ons land goed in beeld te brengen, hebben we een retrospectieve studie uitgevoerd. In **hoofdstuk 2** evalueerden we de Nederlandse sterftecijfers en obductiepercentages onder volwassenen tijdens een periode van 35 jaar (van 1977 tot en met 2011). Hierbij keken we ook naar verschillen in daling per geslacht, leeftijd en type ziekenhuis. We vonden dat elk jaar gedurende deze periode meer volwassenen stierven, maar dat relatief steeds minder mensen overleden in de ziekenhuizen en dat nóg minder overledenen werden geobduceerd. De obductiepercentages waren het hoogst voor mannen, voor jonge patiënten, en voor patiënten die stierven in academische ziekenhuizen. Het landelijke obductiepercentage daalde elk jaar met 0,3% en het obductiepercentage van mensen overleden in ziekenhuizen daalde met 0,7% per jaar (van 31,4% naar 7,7%).

We vroegen ons af waarom de obductiepercentages zo snel gedaald zijn en vonden diverse verklaringen in de literatuur, waaronder het (té) grote vertrouwen dat clinici hebben in de geavanceerde diagnostische technieken van tegenwoordig en het feit dat door de obductie het lichaam van de overledene beschadigd wordt. Om de mogelijke verklaringen verder te kunnen onderzoeken, hebben we een prospectieve observatie studie uitgevoerd, met behulp van duizend enquêtes. In **hoofdstuk 3** beschreven we dat in 17,4% van de gevallen onze clinici niet eens om toestemming voor obductie hadden gevraagd, hoofdzakelijk omdat zij ervan overtuigd waren dat dit onderzoek, naast de tijdens het leven gestelde diagnosen, geen nieuwe feiten aan het licht zou brengen. Omdat 51% van de nabestaanden geen toestemming voor obductie gaf om diezelfde reden, namelijk dat de doodsoorzaak toch al bekend was, vermoeden wij dat de clinici in een groot deel van die casus, waarbij zij dus wél om toestemming vroegen, ook niet de noodzaak van obductie inzagen. Slechts in 16,1% van de gevallen gaven nabestaanden aan, dat zij bang waren voor de schade aan het lichaam die de obductie zou kunnen aanbrengen. Dit is veel minder dan we verwachtten.

Om het obductiepercentage te verhogen, moet de bovengenoemde misvatting, die nooit kan worden tegengesproken als obducties niet worden uitgevoerd, worden bestreden. Bovendien zou een obductie-methode ontwikkeld moeten worden, waarbij het lichaam van de overledene minder beschadigd wordt, zonder verlies van informatie vergeleken met de conventionele obductie.

Om na te gaan welke methoden al ontwikkeld en getest waren ter vervanging van de obductie, zochten we in zes verschillende databanken naar reeds gepubliceerde prospectieve validatiestudies over dit onderwerp. De artikelen die we vonden, hebben we systematisch beoordeeld. In **hoofdstuk 4** vergeleken we zestien verschillende studies, waarvan er dertien gebruik maakten van radiologische technieken, een deel niet-invasief en een deel minimaal invasief. Deze minimaal invasieve methoden, die de blanco MRI en/of CT scans combineerden met CT-angiografie (contrast in de vaten) en/of biopten, hadden vaak betere resultaten dan de niet-invasieve methoden. Geen van de alternatieven die waren onderzocht functioneerde even goed als de obductie, maar de hoogste sensitiviteit die werd gevonden voor het aantonen van de doodsoorzaak was behoorlijk goed: 90,9% (resultaten van twee vergelijkbare studies samengevoegd, exclusief dubbele casus). Dit resultaat was bereikt met een combinatie van CT, CT-angiografie én biopten. In het algemeen viel kwaliteit van de zestien studies tegen (weinig blinde vergelijkingen en veel studies met weinig casus) en ze verschilden te veel van aanpak en uitkomstmaten, waardoor er geen goede meta-analyse van de gepresenteerde data mogelijk was. Wij concludeerden dat verder onderzoek nodig is naar de uitvoerbaarheid van een alternatieve obductie methode in de kliniek, en het liefst ook in een grotere studiepopulatie.

In **hoofdstuk 5** presenteerden we onze eigen prospectieve, "single center", blinde, "cross-sectional" studie, die de diagnostische prestatie van een minimaal invasieve autopsie (MIA) vergeleek met die van de conventionele autopsie (CA). Hiervoor werden 99 overleden volwassen patiënten geïncludeerd, bij wie de nabestaanden toestemming hadden gegeven voor zowel een MIA als CA. Onze MIA bestond uit blanco MRI en CT scans van het gehele lichaam en beeld-geleide biopten van de vitale organen en pathologische haarden die gezien werden op de blanco scans. De MIA en CA vonden in 91 casus dezelfde doodsoorzaak; MIA vond in 5 en CA in 3 van de overige casus de juiste doodsoorzaak. Van de 288 diagnosen die direct gerelateerd waren aan de doodsoorzaak vond MIA er 259 en CA 224; 200 van deze werden door beide methoden gevonden. Daarnaast presteerden MIA en CA gelijkwaardig in het beantwoorden van de specifieke vragen die gesteld waren door de clinici. De methode die wij gebruikten voor onze MIA, die in elk groot ziekenhuis kan worden uitgevoerd en waarvan de beelden altijd opnieuw kunnen worden bekeken, lijkt een valide alternatief voor de CA.

Maar liefst 17% van de doodsoorzaken en 43% van de 288 daaraan gerelateerde diagnosen waren onbekend voor de kliniek. Deze getallen benadrukken het grote belang van postmortaal onderzoek, in welke vorm dan ook (iets is beter dan niets), voor de kwaliteitscontrole van de medische zorg.
Het onderzoek in **hoofdstuk 6** was een subanalyse binnen hetzelfde cohort. Daarin analyseerden we de diagnostische prestatie van de MIA voor de detectie van ischemische hartziekten, die een belangrijke ziektelast vormen in de Westerse wereld. In deze analyse beschouwden we de CA als de gouden standaard en vergeleken daarmee een combinatie van specifieke MRI sequenties en gerichte hartbiopten. We bereikten een sensitiviteit van 97% voor de detectie van acute ischemie en een specificiteit van 95%. Daarentegen, zonder de biopten, werd met de MRI een lage sensitiviteit bereikt voor zowel acute als oude ischemie. Deze bevindingen benadrukken het belang van gerichte biopten bij een MIA, en, in het algemeen, histologisch onderzoek bij postmortaal onderzoek. Hoewel andere studies beschrijven dat een postmortale angiografie nodig is voor het aantonen van een cardiale doodsoorzaak, vonden wij dus dat een combinatie van specifieke MRI scans en gerichte biopten voldoet.
In de zogenaamde feasibility studie, die we beschreven in **hoofdstuk 7**, vonden we dat het postmortale weefsel dat verkregen wordt via de MIA-biopten voldoende RNA bevat met toereikende kwaliteit voor het uitvoeren van moleculaire diagnostiek. Het RNA was vooral goed van kwaliteit als de biopten kort na het overlijden waren afgenomen. Zodra de MIA kan worden verricht als reguliere diagnostiek, wordt hiermee een extra mogelijkheid gecreëerd voor het verzamelen van weefsels voor medisch wetenschappelijk onderzoek. Vooral weefsel van tumor-recidieven en tumor-metastasen (uitzaaiingen) is nuttig. Dit wordt gebruikt voor het onderzoek naar intra-tumor heterogeniteit, waarmee zogenaamde kankerbehandeling op maat verder kan worden ontwikkeld.
Omdat de postmortale radiologie een compleet nieuw veld is binnen de klinische radiologie, hebben we de MRI- en CT-beelden in ons cohort opnieuw beoordeeld. In **hoofdstuk 8** beschreven we (veel voorkomende) veranderingen, die het gevolg zijn van chemische en fysische processen die in het lichaam op gang komen na het overlijden. De veranderingen, gerelateerd aan ontbinding, verergerden naarmate de patiënt langer geleden overleden was. Intravasculair lucht, pleura effusie, periportaal oedeem en uitgezette darmlissen werden vaker gezien na reanimatie; postmortale stolsels juist minder. Uitgezette darmlissen en verlies van grijs-witte stof differentiatie in de hersenen waren gecorreleerd met de tijd sinds het overlijden. Hyperdense hersenvaten, subcutaan oedeem, abdominaal vocht en uitzakking van bloed in de lever werd vaker gezien bij IC patiënten.

In de toekomst, als de MIA wordt geïmplementeerd in de klinische praktijk, hebben radiologen diepgaande kennis en begrip nodig van deze processen om de radiologische beelden goed te kunnen interpreteren.

LIST OF ABBREVIATIONS

CA	Conventional Autopsy
CE	Clinical Evaluation
COD	Cause Of Death
CTA	CT Angiography
DE	Dual Echo
DHD	Dutch Hospital Data
EPR	Electronic Patient Record
ER	Emergency Room
FLAIR	Fluid-Attenuated Inversion Recovery
FFE	Fast Field Echo
FSE	Fast Spin Echo
G	Gauge
GE	Gradient Echo
GMD	Grouped Major Diagnoses
ICU	Intensive Care Unit
IR	Inversion Recovery
CT	Computed Tomography
MDCT	Multi Detector Computed Tomography
MIA	Minimally Invasive Autopsy
MRI	Magnetic Resonance Imaging
N	Number
NA	Not Accessable
NFI	Netherlands Forensic Institute
n/r	not reported
PMCT	Post-mortem CT
PMMR	Post-mortem MRI
PMI	Post-Mortem Interval
PRS	Post-Resuscitation Status;
PTI	Post-mortem Time Interval (a.k.a. PMI)
SD	Standard Deviation
SE	Spin Echo
SN	Statistics Netherlands (a.k.a. CBS Statline)
SPIR	Spectral Presaturation with Inversion Recovery
TSE	Turbo Spin Echo

CURRICULUM VITAE

Britt Blokker was born in Rijswijk, the Netherlands, on the 2nd of July 1986. She finished grammar school (gymnasium) at the Christelijk Lyceum Delft in 2004 and started medical school that same year, at the Erasmus University in Rotterdam. Medical school included scientific research projects, two of which Britt performed abroad: one in Wisconsin, USA, and one in Western Australia. Through these experiences she has become interested in doing medical research.

Another of Britt's interests, already from an early age, has been pathology. Therefore, at career choice days during both grammar school and medical school, she arranged for herself to shadow pathologists. Her final internship in medicine she spent at the Department of Pathology of the Erasmus University Medical Centre and at the Department of Forensic Pathology at the Netherlands Forensic Institute.

After Britt finished medical school in 2011, she became a pathology resident at the Erasmus University Medical Centre. During her residency she was enabled to do research on the Minimally Invasive Autopsy, the results of which are presented in this thesis.

PH.D. PORTFOLIO

Courses/ Workshops

Title	Institute	Year	ECTS
Conceptual Foundation of Epidemiologic Study Design (ESP 38)	NIHES	2011	0,7
Cohort Studies (ESP 39)	NIHES	2011	0,7
The practice of Epidemiologic Analysis (ESP 65)	NIHES	2011	0,7
Health Economics (ESP 25)	NIHES	2011	0,7
The why and how of readable articles (ESP 60)	NIHES	2011	0,7
Multi-phase postmortem CT-angiography (Virtopsy-workshop)	IAFS	2011	0,5
MRI Veiligheidsniveau 2 (intern afd. radiologie) / MRI scannen in de praktijk	EMC	2011	0,3
Basis Opleiding Pathologie: Pathofysiologie	NVvP	2011	1
Classical Methods for Data-analysis (CC02) Biostatistical Methods I: Basic Principles	NIHES	2011	5,7
Stralingshygiëne op deskundigheidsniveau 5A (zorgacademie)	EMC	2011	1
Implementatietraining MMV: Pathologie	Desiderius	2011	0,3
LPAV-cursus: melanocytaire laesies	NVvP	2012	0,5
EWP - Diagnostic reseach (EWP 05)	NIHES	2013	0,7
Molecular Diagnostics VIII	MolMed	2013	1
Meta-Analyses (ESP 15)	NIHES	2013	0,7
Workshop on Photoshop and Illustrator CS6	MolMed	2013	0,3
Biomedical Research Techniques XII	MolMed	2013	1,5
Acute Hartdood in de Klinische Pathologie	AMC	2013	1
English Biomedical Writing and Communication	EMC	2014	3
Intermediate level course: Medical decision making	EMC	2014	0,7
Basis Opleiding Pathologie cursus: Oncologie	NVvP	2015	1
Basis Opleiding Pathologie cursus: Moleculaire pathologie	NVvP	2015	1
Waardenmanagement	EMC	2015	0,3

Title	Institute	Year	ECTS
Cytology Slide Session (Hologic)	PAL	2015	0,3
DOO: Evidence Based Medicine	EMC	2015	1
DOO: Medische Ethiek	EMC	2015	0,5
LPAV-cursus: long	NVvP	2015	0,5
Basis Opleiding Pathologie cursus: Immunologie en ontsteking	NVvP	2015	1
The Renal Pathology Crash Course	AMC	2016	1
DOO: Ziekenhuismanagement	EMC	2016	1
Veldhuizencursus: Cervix en Vochten	EMC	2017	0,5
Rosai sessions: variable subjects	EMC	2010-17	15
Total			**44,8**

Scientific Meetings

Title	Institute	Year	ECTS
Pathologendag: GE & schildklier	NVvP	2011	0,5
24th European Congress of Pathology 2012	ESP	2012	2
Masterclass Sean McCarthy: Horizon 2020	EMC	2013	0,3
Pathologendag: lever	NVvP	2013	0,5
Molecular Pathology Approach to Cancer	EACR-OECI	2013	1
Wetenschapsmiddag "Thema: Verleiding"	AAV EMC	2013	0,3
26th European Congress of Pathology 2014	ESP	2014	2
Wetenschapsmiddag	ASZ	2015	0,3
Pathologendag: long	NVvP	2017	0,5
Postmortem Radiologie Symposium	Radboud UMC	2017	0,5
Total			**7,9**

Student Training/ Education

Coaching medical students in their 21 (+) week research (master) projects:

- Anita van der Linden - " Post-mortem tissue biopsies obtained at Minimally Invasive Autopsy: an RNA-quality analysis"
- Nikola Vitlarov - "Comparative cost analysis of MIA and CA"

Training fellow PhD student in the MIA technique

Multiple teaching sessions/ practical essays in the medical school curriculum provided by the department of Pathology.

Presentations

Multiple in-hospital presentations for the clinical staff of several wards (2011-2013):

- Introduction of the new minimally invasive autopsies, explaining the procedures
- Follow-up during the inclusion period of the study cohort
- Results of the validation cohort (2016)

Poster presentation + pitch at pathologendagen 2014:

- Reasoning behind autopsy consents for in-hospital deceased adults

Presentations at oral free paper sessions during ECP London 2014:

- 35 years of Dutch adult autopsy rates
- Reasoning behind autopsy consents for in-hospital deceased adutls

LIST OF PUBLICATIONS

2017 *Autopsy rates in the Netherlands: 35 years of decline.*
Blokker BM,* Weustink AC,* Hunink MGM, Oosterhuis JW.
PLoS One. 2017 Jun 15;12(6):e0178200. doi: 10.1371/journal.pone.0178200. eCollection 2017. PMID: 28617835

2016 *Autopsy of Adult Patients Deceased in an Academic Hospital: Considerations of Doctors and Next-of-Kin in the Consent Process.*
Blokker BM, Weustink AC, Hunink MG, Oosterhuis JW.
PLoS One. 2016 Oct 13;11(10):e0163811. doi: 10.1371/journal.pone.0163811. eCollection 2016. PMID: 27736974

2015 *Non-invasive or minimally invasive autopsy compared to conventional autopsy of suspected natural deaths in adults: a systematic review.*
Blokker BM, Wagensveld IM, Weustink AC, Oosterhuis JW, Hunink MG.
Eur Radiol. 2016 Apr;26(4):1159-79. doi: 10.1007/s00330-015-3908-8. Epub 2015 Jul 27. Review. PMID: 26210206

2014 *Post-mortem tissue biopsies obtained at minimally invasive autopsy: an RNA-quality analysis.*
van der Linden A,* **Blokker BM**,* Kap M, Weustink AC, Robertus JL, Riegman PH, Oosterhuis JW.
PLoS One. 2014 Dec 22;9(12):e115675. doi: 10.1371/journal.pone.0115675. eCollection 2014. Erratum in: PLoS One. 2015;10(3):e0118969. Robertus, Jan L [added]. PMID: 25531551

2017 *Total-body CT and MR features of postmortem change in in-hospital deaths.*
Wagensveld IM, **Blokker BM**, Wielopolski PA, Renken NS, Krestin GP, Hunink MGM, Oosterhuis JW, Weustink AC
PLoS One. 2017 Sep 27;12(9):e0185115. doi: 10.1371/journal.pone.0185115. eCollection 2017. PMID: 28953923

2010 *Referral patterns of patients presenting with chest pain at two rural emergency departments in Western Australia.*
Blokker BM, Janssen JH, van Beeck E.
Rural Remote Health. 2010 Jul-Sep;10(3):1558. Epub 2010 Sep 5. PMID: 20815655

* Co-First/Equal authorship

THESIS COVER

Organic danse macabre - *by Kim Deja*

For the cover of this thesis illustrator Kim Deja has created a series of designs for the publication's cover and chapter headings. In dialogue with Britt, and inspired by the notion of the human body at the heart of Britt's research, Kim Deja's artworks show human silhouettes and organs interwoven into a delicate, flowing collage.
The drawing combines bodily fragments in coherent, surreal compositions where anatomical elements grow into new organisms. These images are indicative of how the illustrator, like many of Britt's friends and family, understands the intimate knowledge developed by Britt's stories about her research project: she visualizes the body and it's organs by the language of film, books and art. Combined with these drawings, the reader of this thesis is inducted into a fascinating visualization of an illustrated autopsy or an anatomical art class; science fuses with the imagination as the biological becomes almost fantastical.
Kim Deja describes these works as 'a dance macabre, where images appear almost fluid' like a momentarily coherent mirage before its dissipation into disembodied nothingness. Analogous with Britt's research interests, these fleeting, ephemeral images, never becoming sinister or dark, retain an ethereal beauty.

Biography
Kim Deja's work is balanced on the edge of reality and fantasy. Her mostly large-scale drawings form an intricately detailed world of imagination, mystery and symbolism. Always looking for parallel worlds, Kim Deja is an adventurous dreamer armed with a pencil. Born in 1985, she has studied at The Hague Royal Conservatoire, and now lives and works in Zoetermeer, The Netherlands. Instragram.com/kim_deja .

PROEFSCHRIFT OMSLAG

Organic danse macabre - *door Kim Deja*

Illustrator Kim Deja maakte voor dit proefschrift de tekening voor de voorpagina en de fragmenten die als schutbladen de hoofdstukken van elkaar scheiden. Gesprekken met Britt inspireerden haar om menselijke organen, die de basis van Britt's onderzoek vormen, te combineren met de silhouetten van het menselijk lichaam in zijn diverse verschijningen.
Deze tekening is de belichaming van hoe ze beide elementen op bijzondere wijze, maar toch samenhangend en vanzelfsprekend liet samenkomen: ze vormen een surreële compositie die doet denken aan de verhalen die Britt de afgelopen jaren met familie en vrienden deelde. Zeker de niet ingevoerde luisteraar zal haar fascinatie voor het lichaam en de organen met de nodige fantasie hebben moeten visualiseren, omdat we deze beelden alleen van afstand kunnen waarnemen via boeken, televisie, of door de anatomische lessen uit de kunst.
Kim Deja omschrijft het als volgt: "Ik zie het als een soort dans macabere, een door koffie verwekte mirage, een beeld waarbij de stoom transformeerde in lichamen." De speelse combinaties zijn tegelijkertijd ook eigen aan Britt. Met een niet aflatend doorzettingsvermogen werkte zij aan dit onderzoek, waar het nooit macaber werd, maar waar met gepaste afstand en relativering, naast de bron van informatie, ook de schoonheid van het lichaam een plek heeft.

Biografie
Kim Deja maakt werken die balanceren op het raakvlak van werkelijkheid en fantasie. De veelal tekeningen van formaat zijn een gedetailleerde schijnwereld waar verbeelding, mysterie en symbolisme de toon voeren. Kim Deja is altijd op zoek naar de parallelle werelden; een escapist op avontuur met potlood in de hand. Kim Deja (1985) studeerde aan het Koninklijk Conservatorium in Den Haag en werkt momenteel in Zoetermeer. U kunt haar creatieve uitspattingen volgen op www.instragram.com/kim_deja .

DANKWOORD

R	I	T	S	E	C	R	E	T	A	R	I	A	A	T	P	N	E	F	M	I	N	A
E	A	U	B	I	S	E	I	L	R	A	M	N	O	L	K	I	J	A	N	J	U	R
D	Z	Y	T	A	S	Y	L	V	I	A	L	C	U	U	N	O	M	D	O	A	E	A
E	S	K	B	E	R	F	I	O	R	N	A	H	O	J	D	A	E	Y	N	O	T	P
J	N	C	V	E	F	B	M	I	E	N	B	L	O	L	R	A	C	N	R	N	S	P
A	A	I	R	W	R	A	A	N	I	K	O	L	A	T	L	E	I	H	C	I	M	R
A	K	N	O	T	A	N	F	R	A	M	R	P	I	B	T	E	I	O	L	C	A	O
P	R	N	P	S	N	L	A	T	A	D	A	J	E	T	N	H	G	J	S	H	S	M
H	A	A	E	E	K	H	J	R	A	P	N	R	S	N	E	R	U	A	L	T	U	O
D	F	E	L	M	T	A	L	J	D	N	T	A	C	F	I	N	T	A	S	A	M	T
N	A	I	D	A	N	E	E	I	E	I	E	A	L	E	P	I	I	P	R	G	S	O
T	E	I	K	D	T	D	R	P	N	W	N	E	G	O	L	O	H	T	A	P	A	R
S	L	N	C	T	M	D	T	E	P	I	E	E	N	A	J	L	H	B	R	L	R	E
C	O	E	E	I	L	U	A	N	A	L	I	S	T	E	N	U	R	X	A	A	E	E
H	C	E	K	A	N	P	F	I	T	L	G	R	R	N	R	I	T	E	A	A	M	M
A	I	F	N	U	O	I	O	L	H	E	O	I	E	E	E	E	R	L	G	M	D	A
T	N	O	S	S	A	J	L	C	A	M	L	Y	F	L	I	D	H	A	A	T	C	E
H	R	A	A	D	I	U	K	C	N	I	O	V	I	E	N	N	U	T	K	I	U	T
R	C	E	R	J	L	T	E	S	N	E	I	C	N	A	R	F	I	T	R	O	M	R
U	G	A	T	S	I	V	R	I	E	N	D	E	N	H	A	N	S	E	S	A	U	I
B	R	N	O	L	D	D	T	M	K	I	A	N	E	C	A	K	S	I	R	A	M	T
E	O	N	I	C	O	R	I	N	E	O	R	E	J	I	N	E	T	E	D	E	I	D
N	Z	G	P	K	N	W	P	V	R	I	N	D	S	M	R	E	T	E	P	A	I	M

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Bedankt!

ALBERTINE	DON	JASSON	MARCEL	NICO	REINIER
ALEX	DRIES	JENNIFER	MARET	NICOLE	RONALD
ANALISTEN	EFI	JEROEN	MARIANNE	NIKOLA	RUBEN
ANDREA	ERASMUS	JNI	MARISKA	NOMDO	RUTGER
ANITA	ERICA	JOHAN	MARLIES	ODILIA	SCHAT
ANNELIES	FAMILIE	JOHN	MARTHE	OMA	SECRETARIAAT
ANNICK	FOLKERT	JOLANDA	MARTIJN	OPA	SUZE
ANNIE	FRANCIEN	JOYCE	MARTIN	(OUD)AIOS	SYBE
ARLETTE	FRANK	JUT (2x)	MAX	PAL	SYLVIA
ARTHUR	FRE	KARIN	MIASTUDENTEN	PAPA	TEAM
BARBARA	GABRIEL	KATHARINA	MICHAEL	PARANIMFEN	TIP
BERNARDINE	HANNEKE	KATJA	MICHIEL	PATHAN	VIDIJA
CLINEPI	HANS	KIMDEJA	MORTALITAS	PATHOLOGEN	VRIENDEN
CLINICI	HERENEEN	KING	MYRIAM	PETER	VRIND
COACH	IAN	KOEN	NADIA	PIEN	WILLEMIEN
COLLEGAS	IVO	LABORANTEN	NEEF	PIOTR	WIM
CORINE	JAAP (2x)	LAURENS	NEPTUNUS	PROMOTOR	WOLTER
DAMESTWEE	JAN (3x)	LEPO	NFI	RADIOLOGIE	
DIEDE	JANPETER	MAMA	NICHT	RAFAEL	*en allen die ik mis..*

voor deze KANS,

HULP, RAAD *&* REDE,

ZORG *&* STEUN,

KOUT, LOL, ETEN *&* MEER

tijdens MIA's (2x) *en mijn* PHD *traject!*

The research described in this thesis was carried out at the Departments of Pathology, and Radiology and Nuclear Medicine of the Erasmus University Medical Center Rotterdam, and financially supported by Erasmus MC Health Care Efficiency, Erasmus MC Vriendenfonds, Stichting Coolsingel Rotterdam, The Dutch Cancer Society (Muntendamprijs 2011, awarded to J.W. Oosterhuis), and BRAINLAB, Munich.

MIA
The Minimally Invasive Autopsy

ISBN:	978-94-6299-867-4
Author:	Britt M. Blokker
Cover design:	Kim Deja | www.instagram.com/kim_deja
Cover patient information folder:	Jeroen Sluijter | www.studiomoni.nl
Layout:	Design Your Thesis | www.designyourthesis.com
Printing:	Ridderprint B.V. | www.ridderprint.nl