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Introduction

Imaging for postmortem diagnostics, for instance in forensic medicine, has been used since 1895, and include conventional X-rays, computed tomography (CT) and magnetic resonance imaging (MRI). Generally, ultrasound (US) was never considered to be of particular value for postmortem torso imaging because of tissue gas formation [1]. However, as US is a readily available and inexpensive imaging modality, it might be of value for specific postmortem evaluations.

Clinical autopsies are important to determine a precise cause of death but have been declining worldwide [2]. Several factors contributed to this shift, including increasing accuracy of modern imaging and molecular diagnostic procedures, rising costs of autopsies, cultural or religious beliefs prohibiting autopsies.

Aside from macroscopic evaluations, post-mortem microscopic tissue examination also has an important role in medical diagnosis and research. Few examples are tissue sampling from organs that would be problematic in living patients (brain, heart, large vessels); evaluation the effects of medical interventions, such as end-organ lung damage as a consequence of mechanical ventilation or therapeutic agents; studying oral anatomy and diseases by comparing imaging and histology of post-mortem samples; investigating new infectious diseases; or improving the accuracy of cause of death determination in areas devoid of advanced diagnostic systems.

In this paper we review the literature published on US postmortem examinations and we describe several cas-
es in which US enabled physicians to obtain answers to open diagnostic questions in postmortem examinations. In addition, we will illustrate how US can assist in minimally invasive post-mortem tissue sampling (MITS) and how several investigators used cadavers to evaluate diagnostic accuracy of certain ultrasound indications.

**Review of the literature**

A review of the available literature showed that US appears to be particularly important in fetal and neonatal postmortem diagnostics [3,4]. However, overall CT and MRI or X-ray dominate the forensic literature. Heine-mann et al reported on the use of postmortem CT and CT angiography between 2004 and 2014 [5]. CT is considered the standard method in postmortem imaging. It is less susceptible to interference from postmortem gas formation than US. The biggest advantage over clinical autopsy is the detection of free air. Skeletal changes and retained foreign bodies can also often be better detected. The strength of CT angiography lies primarily in the detection of bleeding sources, even in postmortem examinations that are lacking an intrinsic blood pressure. Agreement between postmortem CT (PMCT) and clinical autopsy is nearly 90% if the reporting radiologist has sufficient experience in post-mortem imaging [5].

Grabherr et al [6] reported on the use of X-ray in forensic medicine dating back to 1896, the year an X-ray of a hand was performed on a mummified Egyptian princess. Today, many forensic departments have mobile X-ray machines to examine corpses for radiopaque foreign bodies or bony lesions. X-rays are also used to identify unknown deceased with the help of dental findings or locating osteosynthetic material. The most common techniques used today are PMCT, CT angiography and MRI. A new development is the 3-D surface scanner. Blood and fluids can be well visualized in CT, even postmortem, using this technique. Free air is also imaged without difficulty. However, interpretation is complicated by gas formation, which begins a few hours after death. Free air can therefore only be detected up to a few hours after death. The value of CT in cases of natural death is limited. For example, ischemic heart disease in acute cardiac arrest cannot be detected by CT without angiography [6].

MRI is also used in post-mortem imaging. Limitations include the occurrence of gas, lack of circulation and low body temperature, which influences the behavior of MR contrast agents. Gas leads to a complete loss of signal in MR. Gases formed by autolysis can significantly interfere with imaging. On the other hand, the detection of myocardial infarction is as good in postmortem MRI as in living patients [7]. MRI is currently the method of choice for postmortem imaging of neonates and pediatric cadavers [3]. By contrast, Roberts et al consider CT to be the method of choice in postmortem imaging because discrepancies between autopsy and MRI are more common than between PMCT and autopsy [8].

Simonds et al [9] reported on the use of conventional radiography in cadavers to detect fractures and foreign bodies, positron emission tomography to detect tissue changes especially in Alzheimer’s disease and Lewy body dementia and various uses of CT with stationary and mobile equipment. The working group reported that US has so far been underrepresented in cadaver diagnostics. MRI provides excellent images, but its availability and expense are significant limiting factors. The vastly superior imaging provided by MRI has placed post-mortem US in a subordinate status in forensic medicine, despite cost and availability advantages. This is mainly due to the formation of gas on the corpus after few hours and the resultant limitation to US wave propagation in the body.

Egger et al [10] examined 119 cadavers for gas formation. They found gas formation mainly in the heart and liver. Gas was initially detectable after 5-84 hours after death. In all cases of natural death, gas was found in the liver vessels and the heart simultaneously; in cases of death by gas embolism, gas was noted only in the heart. Spaienza et al [11] reported on seven victims of a flood whom they examined with CT for postmortem intrahepatic gas formation. They found that gas first formed in the portal vessels and then appeared in the hepatic veins. Fischer et al [12] analyzed intrahepatic gas formation in five male cadavers with non-traumatic causes of death using a longitudinal study with CT scans at hourly intervals over a 24 hour period. They found an increase in gas formation between the fourth and seventh hour after death, after which conditions remained constant until the end of the study period. Intrahepatic gas is predominantly caused by mesenteric gas formation [13]. Detection is possible by both CT and US. The first description of portal venous gas was made in 1955 by Wolfe et al in six newborns that had died of intestinal necrosis [14].

The interference by gas formation and its rapid development raises questions regarding what potential significance post-mortem US could have in clinical adult medicine. A search of the literature on this topic revealed a paucity of publications to date. Duarte-Neto et al [15] described ten US-guided multiorgan punctures in patients with COVID-19 induced death. The first published description of this method was in 2002 by Farina et al [16]. They compared US-guided puncture biopsies with conventional autopsies on 100 cadavers and found a concordance rate of 83% for final diagnosis. An easily
overlooked location and timing for postmortem US performance is in the emergency department, where deaths may occur suddenly and unexpectedly. Even in older, chronically ill patients, immediate postmortem US evaluations can provide vital clues as to the cause of death and critical education to clinicians. Some catastrophic etiologies can be assessed with US and include proximal thoracic aortic dissections, pericardial effusion and pneumothorax. Abdominal aortic aneurysm presence can raise its potential role in a death, even if intraabdominal or retroperitoneal fluid is not detected. US examination so quickly after death is unlikely to be encumbered by gas formation. Evaluation of pleural cavities is easily performed in search of fluid collections and assessment of the lower extremity deep venous system may reveal the presence of deep venous thrombosis, thus suggesting pulmonary embolism as a potential cause of death if the clinical scenario is supportive. However, more direct evidence of massive pulmonary embolism is unlikely to be reliable as blood can quickly gel in the cardiac chambers and lack of intracardiac pressure means acute signs of right heart strain will not be identified [5].

Postmortem US in the pathology department has been used to validate US findings in 20 patients to delineate small organ structures, e.g., the adrenal glands [17] and perihilar lymph nodes [18,19]. Recently three cases in which post-mortem US within three hours of death enabled the clarification of a previously unclear cause of death have been reported [20]. In all three cases, an autopsy could not be performed for various reasons. The examinations were performed with the GE S7 (GE Medical Systems Information Technologies, Freiburg i. Breisgau, Germany). Multifrequency transducers were used: a phased array (3-5 MHz), a multifrequency linear array (6-15 MHz) and a sector transducer (2-4 MHz). A Mindray M7 with a phased array (2-5 MHz) transducer was also utilized for evaluation.

The transducers were protected during the examination with sterile probe covers from Civco Medical Solutions. The US device and the transducers were reprocessed after use according to the local hygiene recommendations.

Use of US in postmortem diagnosis

Case 1

An 87-year-old female patient presented via paramedics due to a one-day increase in dyspnea, now at rest. She denied thoracic pain, cough, fever or chills, as well as sweating and palpitations. The patient had been discharged from the geriatric ward only 5 days ago. On physical examination the patient was alert and oriented, Glasgow Coma Scale 15, reduced general status body habitus was obese, slight bilateral leg oedema. Her respirations were equal on both lungs, percussion sound resonant; heart sounds were regular and rhythmic without murmurs, rub or gallops, norm frequent. Hemoglobin and white blood cell count, inflammatory parameters, renal values, electrolytes and urine status were unremarkable. The patient was diagnosed with heart failure, treated conservatively and monitored overnight. After a stable course, the patient was transferred to the ward the following day. That next evening, the patient rapidly deteriorated and had to be resuscitated. Cardiac resuscitation was unsuccessful. A post-mortem US was performed given the lack of clinical diagnosis and the unsuspected clinical course. There were no signs of pulmonary embolism (fig 1).

Case 2

A 71-year-old male undomiciled and living alone in the woods is brought in by the ambulatory emergency service for decreased responsiveness. Prehospital transport time was approximately 40 minutes and the patient expired on the way, despite aggressive and constant resuscitative efforts by paramedics. The family and the l-
cal coroner both declined an autopsy by phone, with the coroner planning to list the death as natural. The deceased patient was placed in an empty patient room shortly after EMS arrival and a point of care US was performed showing an 8.1 cm abdominal aortic aneurysm with retroperitoneal fluid (fig 2).

**Case 3**

An 81-year-old patient with multiple medical comorbidities including prior cerebrovascular accident with residual dysphagia, dementia was admitted to the hospital for several days of gradual decrease in mental status, high fevers, and hypotension. The clinical team felt the most likely diagnosis was urosepsis and started treatment with broad-spectrum antibiotics. Her inflammatory parameters improved, but the patient’s condition continued to deteriorate. Five days after hospitalization the patient died due to cardiovascular failure. Since the exact cause of death was unclear, an immediate postmortem US was performed and clear evidence of significant pulmonary artery embolism was detected (fig 3).

**Use of ultrasound for minimally invasive post-mortem tissue sampling**

Minimally invasive post-mortem tissue sampling (MITS) may be an alternative to overcome the obstacles to conventional autopsy. This type of procedure has been employed since the mid 1930’s in Brazil, when infectious diseases were extensively studied by post-mortem tissue sampling with the use of a simple viscerotome [21,22]. MITS possibilities were substantially expanded under imaging guidance [23]. In 2015, one medical institution built a minimally invasive autopsy (MIA) facility composed of a 7T MRI, a 16 channel CT, as well as diagnostic US devices (PISA project at the Faculty of Medicine, University of São Paulo https://pisa.hc.fm.usp.br/). Over time, it was noted that US became the most frequently used instrument to guide MIAs because of its low cost and transportability, allowing the conduction of MIAs in other institutes of the medical complex [15,24,25].

MIAs played a major role in COVID-19 from March 2020 up to submission of the paper and São Paulo was the epicenter of the present pandemic, allowing a team of examiners to investigate more than 200 cases of a highly contagious disease in an autopsy facility without level three biosafety needs [15,24-26]. In the course of the present pandemic, over 180 autopsies were performed, including on COVID-19 patients and patients admitted to the intensive care units for other causes. A portable Sonosite M-Turbo R (Fujifilm, Bothell, WA, USA) ultrasound equipment with a C60x (5-2 MHz Convex) multi-frequency broadband transducers and DICOM® standard images has been used. Tissue sampling was performed either using Tru-Cut semi-automatic coaxial needles of 14G, 20 cm long or by doing scalpel dissections guided by US, through small incisions over the area of interest (mainly lungs and heart). Some illustrative cases are presented.

**Case reports demonstrating the use of US in MIAs**

**Case 4**

A 71-year-old male patient with a history of HIV diagnosed in 2014 and poor adherence to anti-retroviral...
therapy was admitted in 2017 to investigate an episode of fever, dyspnea and diarrhea. Investigation for tuberculosis was negative, but the patient received treatment for syphilis and hepatitis B. In late 2019 the patient completely abandoned anti-retroviral therapy. Following medication cessation, he lost 12 kg and in October 2020 was admitted for progressive dyspnea and diarrhea. X-ray revealed diffuse bilateral pulmonary infiltrates and CT showed ground glass infiltrates with predominance in basal portions of the lungs. The patient’s respiratory status worsened, and he was intubated requiring progressively higher levels of inspired oxygen. The patient rapidly deteriorated with worsening hemodynamic parameters despite broad-spectrum antibiotics and vasoactive drugs. The patient then developed massive respiratory hemorrhage, which leads to cardiac arrest refractory to resuscitation efforts. The autopsy service received the patient’s body with evident signs of weight loss. US of the lungs revealed an irregular, discontinuous pleural artefact, small supleural consolidations and diffuse pulmonary infiltration (white lung). Bowel US examination showed marked thickening of intestinal walls. Needle pulmonary tissue sampling revealed foci of pulmonary hemorrhage and the presence of larvae of Strongyloides stercoralis perforating the pulmonary capillaries in the lungs and intestinal mucosa (fig 4). The final diagnosis was established as systemic angioinvasive Strongyloidiasis in a patient with acquired immune deficiency syndrome and COVID-19 was excluded.

**Case 5**

A 52-year-old female with a history of type I diabetes and immunosuppression due to a kidney pancreas double transplantation performed in 2008, now back on hemodialysis after renal transplant rejection, presented to the hospital with progressive dyspnea and one episode of haemorrhagic diarrhea. She eventually developed respiratory failure and was intubated and placed on mechanical ventilation. CT scans revealed bilateral ground glass opacities affecting more than 50% of the lungs. RT PCR for Sars-CoV-2 was positive in material sampled from her trachea. She rapidly developed multiple organ failure and septic shock refractory to broad-spectrum antibiot-
ics. During MIA, US imaging disclosed discontinuous thickened pleural line and small sub pleural consolidations. US guided Tru-Cut tissue sampling was conducted from different organs, evidencing acute fibrin thrombi in alveolar capillaries, fibrotic foci suggestive of organization of previous acute pulmonary damage and rare cells with aberrant nuclei, compatible with SARS-CoV-2 cytopathic effects. Final diagnosis was COVID-19 in fibroproliferative phase in a patient with immunosuppression due to pancreas kidney double transplantation (fig 5).

**Case 6**

A 46-year-old male was admitted due to an episode of loss of consciousness while in the outpatient thoracic surgery offices. Family reported severe weight loss during the preceding 4 months (14 kilograms) and the appearance of enlarged lymph nodes in the supraclavicular and cervical regions. He was immediately transported to the emergency ward, where he had a sudden cardiac arrest refractory to resuscitative efforts. Postmortem US images confirmed the presence of diffuse cervical lymphadenopathy. Pulmonary images were not adequate because the massive loss of weight promoted a retraction of intercostal spaces, making proper transducer apposition on thoracic surfaces difficult. An irregular mass was identified in the abdomen in the area corresponding to the transverse colon. US guided right pneumectomy was performed, through a 3 cm incision in the right intercostal space. Macroscopic evaluation of the resected lung showed massive pulmonary thromboembolism and microscopic examination showed the presence of multiple foci of undifferentiated adenocarcinoma in the pulmonary lymphatics (fig 6), lymph nodes and in the mass adjacent to the transversal colon. Final diagnosis was pulmonary thromboembolism due to diffuse carcinomatosis secondary to advanced intestinal (probably colonic) cancer.

**Postmortem US imaging to establish new diagnostic approaches for US use**

Dental US is a virtually non-existing field except for research efforts. There is no widespread clinical use of US imaging technology other than for oral surgery. Imaging technology has recently seen an increase in center frequency and also an increase in point of care solutions, i.e., smaller and more portable systems [27]. This sets the stage for an attempt on the initiation of facilitating ultrasonic imaging with proper spatial resolution, practical scan head size and meaningful clinical applications [28]. These technological advancements could be especially helpful to monitor oral wound healing and evaluate periodontal (gum) and peri-implant tissues longitudinally. The presented images here were recorded in human cadavers and were compared to cone-beam CT (CBCT) as well as to direct caliper measurements to validate dental US as proof of principle studies when we piloted US imaging in dentistry a few years ago. These efforts and materials significantly enhanced our understanding of US imaging of various anatomical structures in the oral cavity, enabling us to comfortably scan live humans with an off-the-shelf US imaging system at the University of Michigan Graduate Periodontal Clinic now. The images were _not_ taken with the intent of performing an autopsy. Since dental procedures currently do not involve US, it is not surprising that forensic investigations also do not involve US.

**Case 7**

In a study in 2015 to 2016 we have investigated the ability of US to depict soft and hard-tissue structures in the oral cavity [29] (fig 7). A Zonare/Mindray scanner (ZS3) with off-the-shelf imaging transducers (L14-5w and L14-5sp) was employed for scans in human cadaver specimens (Study ID: HUM00107975). Findings were...

compared to CBCT and photographs. The intent was to seek US as an additional imaging modality, i.e., to complement CBCT, X-ray and optical scans, among others, to harness the power of US soft tissue contrast and its spatial resolution. Soft- and hard-tissue imaging is of interest. While soft-tissues mostly provide omnidirectional visualization, due to mostly angle independent scattering, hard-tissues predominantly show strong angle dependence and thus require specific spatial adjustment of the ultrasound transducer for satisfactory visualization.

In Figure 8 tooth #9 is shown by means of CBCT and US. The former has excellent hard-tissue contrast though lacks soft-tissue contrast. For US a second harmonic imaging mode, i.e., SH12, was used here. The Mindray scanner offers an optimize function/button which adjusts the assumed speed of sound. In the presented case a speed of sound correction of +20 m/s resulted and is indicated as Zonare Speed Index (ZSI): 20. The overlay of the CBCT and US in panels (c) and (d) illustrates the spatial resolution that can be obtained with off-the-shelf imaging technology. In addition, one may appreciate the soft tissue contrast of the gingiva in panel (b), left side of the ultrasound scan, which is not obtainable via CBCT. The left-most thickest gingiva is 4.4 mm from the jawbone to the epidermis. Relevance to forensics is not straightforward. But subdermal tissue changes may be visible on ultrasound and not be apparent visually or visually evident yet quantified ultrasonically, both in geometry and gray scale appearance. Figure 9, shows the greater palatine nerve. Morphometric parameters have been defined for the dimensions of the greater palatine foramen, which differ between male and female gender [30,31]. It may therefore be possible that similar features can be found for ultrasonographic assessment of the greater palatine to also find either gender differences or other forensic information. It should be noted that with the introduction of the L30-8 linear array by Mindray, three classes of US
transducers exist that might be helpful in the oral cavity. At first the forward looking endocavitary probe, second the sideways looking (transducer cable in the lateral direction), often intra-operatively used, hockey stick probe, as well as third, the also sideways looking but transducer cable in the elevational direction L30-8 array. This allows the transducer to image sagittal, transverse and coronal slices in the oral cavity (fig 8, fig 9).

**Case 8**

The above mentioned soft-tissue imaging qualities were demonstrated in a study concerned with posterior mandible pertinent to clinical dentistry, e.g. oral, periodontal and implant surgery [32]. While oral surgeons may need to be informed about soft-tissue, so may need to be a forensic investigator or a forensic clinician. Figure 10 demonstrates how well connective tissue, muscle, nerve, and glands can be distinguished by US. While such is known for medical US, it has previously been a challenge in dental due to probe dimensions and spatial resolution. Of note is also the observed gas bubble formation in cadaver tissues. Figure 11 shows frames recorded during an elevational sweep from retro-molar to molar region. A significant number of bubbles are seen, most dominantly in the muscle tissue. These might either be the result of previous freezing of the specimen or are originating from the known postmortem gas formation during the process of decomposition [1] (fig 10, fig 11).

**Discussion**

We presented the use of postmortem US under very different conditions. Postmortem US has been compared with CT/CBCT and MRI in forensic medicine reference. In previous studies, US was considered to be of limited value because of gas formation due to tissue autolysis and the regular occurrence of pneumatosis intestinalis due to bacterial transmission [33]. The advantages of post-mortem imaging include potentially valuable findings that may help determine cause of death at low cost [34]. In paediatrics, there are increasing reports of post-mortem echocardiography and US after stillbirths with meaningful findings [35]. In adult medicine, too, postmortem US can yield further findings. Advantages are the ubiquitous availability and the low organizational and financial costs compared to CT or MRI. Postmortem gas formation in the abdomen and tissue maceration present known challenges for US. This has been described as a major limiting factor for use of US in forensic medicine. Fetal US, on the other hand, can be performed weeks postmortem without these impediments and shows good results. We performed the first three described examinations in the first three hours after death and found good conditions. The last two were performed at 20 and 10 minutes after death and also yielded valuable information that would have otherwise been undiscovered. It was even possible to provoke flows in the leg veins by compression. In all cases, sonography made it possible to clarify the cause of death. The next two examinations were performed at 20 and 10 minutes, respectively, after death and also yielded valuable information that would have otherwise been lost, as neither patient would have entered the hospital nor had an autopsy. We therefore recommend that post-mortem US be performed as soon as possible after death. Developments in high-frequency US as well as miniaturization allow for visualization of regions previously

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**Fig 10.** Labeled soft tissue structures demonstrating the wide range of possible soft-tissue imaging and their locations, i.e., as far posterior as molar. (a) Image at right maxillary molar showing muscle attachment (arrow) to jawbone. (b) Image at mandibular left premolar showing the mental (nerve) foramen.

**Fig 11.** Gas bubble or other hyperechoic formation in cadaver tissue. Image frames shown are picked from an elevational sweep from the left retro-molar to molar region.
not of interest due to poor spatial resolution or poor access, such as the oral cavity. The last two cases were not recorded with the intent of autopsy, but rather technology development and for dental imaging and should be seen as feasibility demonstration for future autopsies. Other cases would include difficult to reach abdominal or endocavitary spaces where a side-looking ultrasound array would be advantageous.

US-guided post-mortem tissue sampling played a pivotal role in the study of infectious and non-infectious diseases and significantly helped our group to produce information about the pathogenesis of COVID-19. In a situation in which conventional open autopsies were forbidden in Brazil, US-guided minimally invasive autopsies made it possible to obtain tissue samples from different organs such as lungs, heart, kidneys, liver spleen, gastrointestinal tract, testis, salivary glands, thyroid and lymph nodes. In addition, US imaging guided tissue dissection and sampling conducted via small incisions in the thoracic and abdominal cavities. Finally, US-guided tissue sampling increased the acceptance of post-mortem studies by families and clinicians, opening new possibilities to investigate diseases in which in vivo tissue sampling is not possible. MITS has been long recognized as a technique that could substitute or even offer the unique option to perform autopsies in low- and middle-income countries, where the shortage of resources makes conventional open autopsy very rare. MITS has also been used to orient public health policies, such as occurs in Africa, with a special emphasis on the diagnosis of infectious diseases, to improve efforts on the investigation of child mortality [36]. In our series, US guidance expanded the accuracy and possibilities of targeting different organs at low cost. Indeed, although we have in our pathology office a 16 channel CT and a 7T MRI, US was the imaging modality of choice to plan tissue sampling because it facilitates multiple samplings within a short period of time. In addition, in some circumstances, US guided MITS allowed us to conduct autopsies outside our autopsy office in the Intensive Care Units of our Institution, minimizing time and increasing the efficiency of post-mortem studies.

Currently dental US is at an early stage and has not been used routinely for forensics. Intra-oral US is an emerging technology that has been validated using human cadaver specimens as a transition and learning tool to patient care. In the near future, it may be used to explore cause of death that may be related to oral diseases, e.g., intraoral infection leading to sepsis.

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