Seminars in ULTRASOUND CT and MRI

A Practical Guide to Virtual Autopsy: Why, When and How

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Postmortem imaging is considered a routine investigative modality in many forensic institutions worldwide. Because of its ability to provide a quick and complete documentation of skeletal system and major parenchymal alterations, postmortem computed tomography (PMCT) is the imaging technique most frequently applied in postmortem forensic investigations. Also postmortem magnetic resonance has been implemented in postmortem setting, but its use is mostly limited to focused analysis (eg, study of the heart and brain). PMCT presents some limits in investigating "natural" deaths, particularly related to its poor ability in differentiating soft tissue interfaces and in depicting vascular lesions. For this reason, PMCT angiography has been introduced. A major limitation of these postmortem imaging techniques is the absence of body samples for histopathologic, toxicologic, or microbiological analysis. This limit has been overcome by the introduction of postmortem percutaneous biopsies. The aim of this review is to provide a practical guide for virtual autopsy, with the intent of facilitating standardization and augmenting its quality. In particular, the indications of virtual autopsy as well protocols in PMCT examinations and its ancillary techniques will be discussed. Finally, the workflow of a typical virtual autopsy and its main steps will be described.
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Introduction

Currently, imaging techniques are considered a routine investigative modality in many forensic institutions worldwide. The United States, Switzerland, the United Kingdom, Germany, Sweden, Denmark, Australia, and Japan have introduced dedicated imaging facilities for the forensic examination process.[1]

The potential of imaging techniques in forensic investigations was clear soon after the discovery of X-rays by Roentgen in 1895. Even in the same year, X-rays were used for the documentation of projectiles retained within the body and in 1896 these imaging methods entered the courtrooms in the United States and the United Kingdom as evidence for investigations related to cases of gunshot wounds. Moreover, in 1986 this imaging technique was used for age estimation and in 1920 for identification purposes based on paranasal sinuses morphology.[2]

However, the real advance toward a more extensive and effective use of imaging techniques in forensic medicine was prompted by the discovery of computed tomography (CT) in 1971, and particularly with the introduction of spiral CT technology in 1989, due to its capability to provide 3-dimensional (3D) representation of the body structures.

Magnetic resonance (MR) technologies were discovered by Lauterbur and Mansfield in 1973, but were not clinically employed for the first time until the early 1980s. Only in 1990, MR technology entered forensic investigations with the study of Ros et al,[3] who proposed this imaging technology as a preautopsy technique, with promising results.

Generally, the major problem preventing routine usage of imaging techniques in forensic investigations is due to the costs related to the purchase, installation, and maintenance of a dedicated modern imaging technology (CT and/or MR)

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Figure 1 Three-dimensional volume rendering of the entire skeleton of a 3-year-old child (anterior view).

in forensic institutions. This is truer when MR scanning is considered, because such technology is not always available for the even in clinical setting. In addition, postmortem imaging examinations on the whole body are generally performed before classical autopsy. Because CT imaging is a technique able to provide in a few minutes a complete representation of the whole body with detailed depiction of skeletal system and major parenchymal alterations (Figs. 1—9), it is the most frequent imaging modality used as postmortem imaging in the routine practice of forensic institutions worldwide.

Although MR is able to provide an almost perfect representation of soft tissue pathology, because of its length of

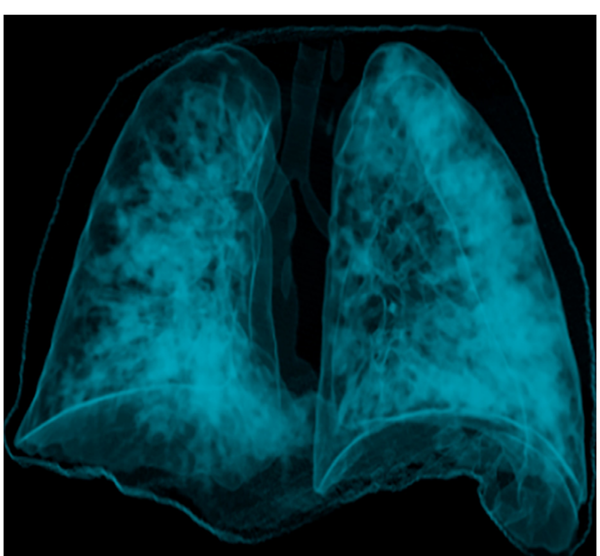

Figure 2 Three-dimensional volume rendering of the lungs in a subject who died due to drowning. Note the diffuse presence of areas of increased density, prevalent in the perihilar regions.

execution and related costs, it is not available inside forensic institutions with a few exceptions such as Zurich and Bern.[4-7] As a consequence, its employment in postmortem settings is actually limited to specific fields of investigations, such as the study of the heart[6-10] and the brain.[5,11-14]

Although postmortem CT (PMCT) is very useful in analyzing violent deaths due to its excellent visualization of skeletal traumatic lesions (Fig. 10), intravascular gas (Figs. 11—13), and foreign bodies (Fig. 14), it is somewhat limited in investigating "natural" deaths, because of its poor ability to differentiate soft tissue interfaces and to depict vascular lesions.[15]

Thus, in 2005, Jackowski et al[16] introduced PMCT angiography (PMCTA) as a new supplementary technique to PMCT to overcome these limitations (Figs. 15—17). Since then, this

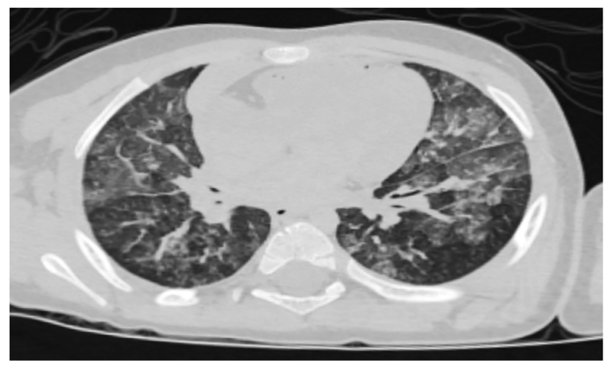

Figure 3 Axial PMCT image at the level of the thorax in a subject who died due to drowning. Note the smooth thickening of interlobular septa, the patchy areas of ground glass opacities, with mosaic distribution, prevalent in the perihilar regions. After consideration of circumstantial data, these findings were attributed to water aspiration from the upper airways.

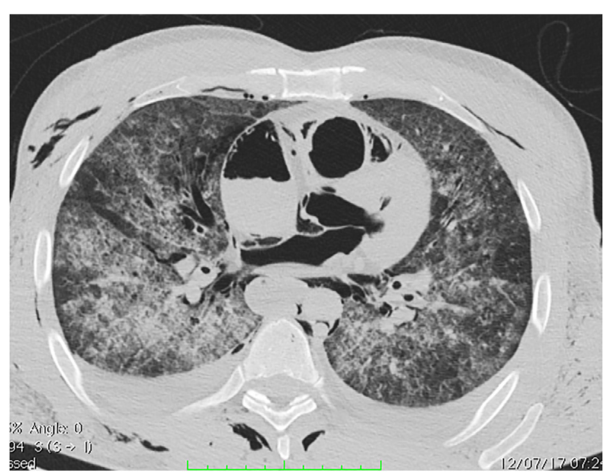

Figure 4 Axial PMCT image at the level of the thorax in a subject who died due to drowning found in the water. Note the smooth thickening of interlobular septa, the patchy areas of ground glass opacities, with mosaic distribution, prevalent in the perihilar regions. After consideration of circumstantial data, these findings were attributed to water aspiration from the upper airways. Bilateral pleural effusion is also documented. Soft tissue emphysema and gas in the cardiac cavities are detectable, probably due to putrefaction.

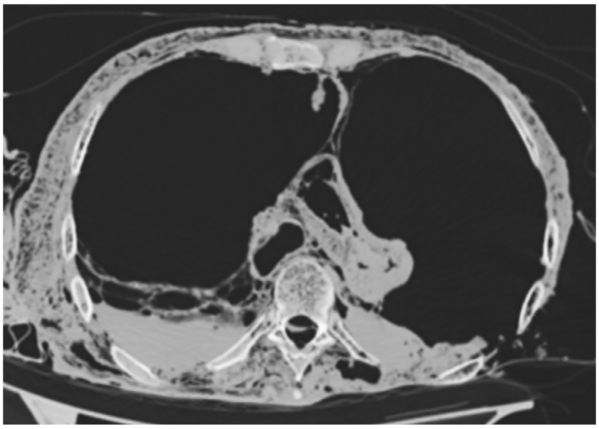

Figure 5 Axial PMCT image at the level of the thorax in a subject found in water in advanced state of putrefaction. Note the complete collapse of the lung parenchyma bilaterally, substituted by air, and of the mediastinal structures. These findings were attributed to decomposition.

technique was improved[17-19] and other whole-body PMCTA techniques were proposed.[20,21] These techniques differ in the type of the intravascular contrast media and the contrast media injection and/or propulsion method used.

Although postmortem CT (PMCT), eventually implemented with PMCTA, and postmortem MR are considered very useful tools in forensic investigations, a major limit of these techniques is the absence of documentation of histopathologic findings or the availability of body samples for toxicologic or microbiological analysis. In fact, image resolution of these modalities is not sufficient to demonstrate pathologic alterations at a cellular, microscopic level.

Since 2007, many studies have been published by the Swiss Group of Thali,[22-25] demonstrating that postmortem biopsy can provide tissue specimen suitable for histologic examination and for developing forensic conclusions with a minimally inva-

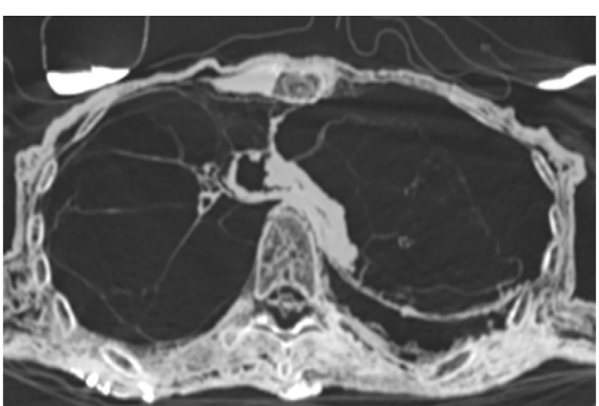

Figure 6 Axial PMCT image at the level of the thorax in a mummified subject. Note the disruption of the lung parenchyma, which is represented mostly by fibrous laciniae, pneumothorax, and of the mediastinal structures. These findings were attributed to mummification.

Figure 7 Axial PMCT image at the level of the thorax in a subject who died due to acute myocardial ischemia. Note the smooth thickening of interlobular septa, the patchy areas of ground glass opacities, more represented in the dependent regions. After consideration of circumstantial data, these findings were attributed to pulmonary edema and pulmonary hypostasis.

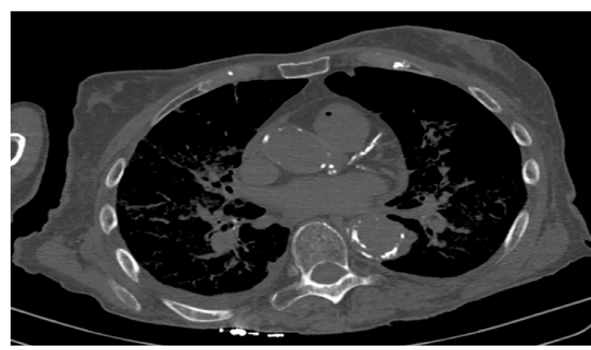

Figure 8 Axial PMCT image at the level of thorax in a subject who died due to myocardial ischemia. Note the extensive parietal calcification of the anterior descending coronary artery and of the descending thoracic aorta.

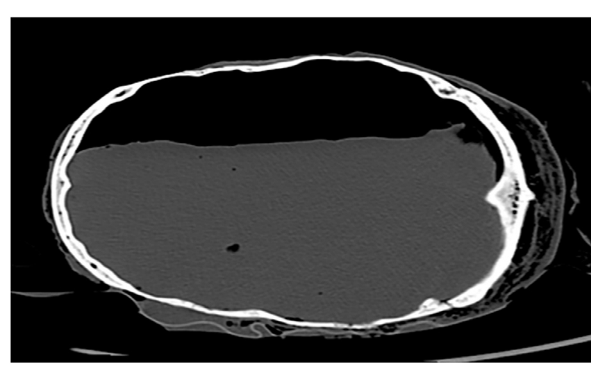

Figure 9 Axial PMCT image at the level of the head in a subject found dead in the water, in advanced state of putrefaction. Note the almost liquefied encephalic tissue with an "air level."

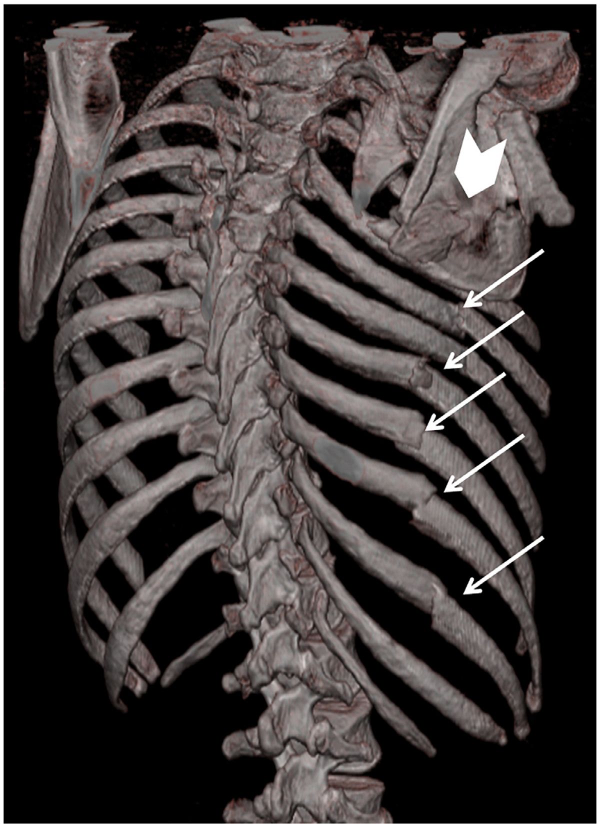

Figure 10 Three-dimensional volume rendering reconstruction of skeletal structures of the thorax in a trauma case. The figure shows multiple right rib fractures on the posterior arches (arrows) and transversal fracture of the right scapula (head arrow).

sive technique. Thus, postmortem biopsy has been suggested as a valuable method in adjunction to PMCT and eventually PMCTA as a useful technique supplementary to virtual autopsy.

The aim of this review is to provide a practical guide for virtual autopsy, basically performed with whole-body PMCT. In particular, there will be discussions about the scope of virtual autopsy and its main indications, protocols in PMCT examination and consideration of some ancillary techniques such as postmortem percutaneous biopsy and PMCTA. In addition, the workflow of a typical virtual autopsy procedure and its main steps will be described. The main intent of this paper is to facilitate standardization of this forensic investigation method, for augmenting its quality and power in the courtrooms.

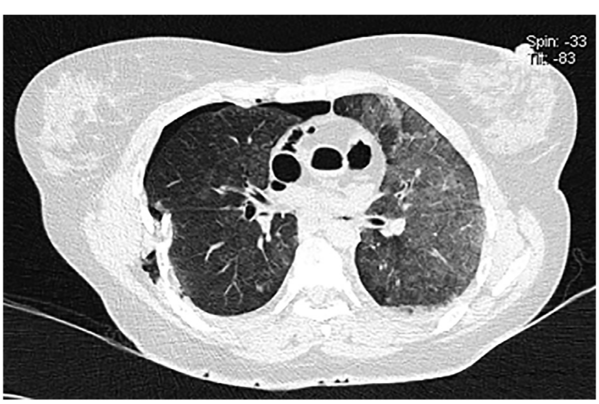

Figure 11 Axial image in the same case as in Figure 10. The figure shows the presence of right pneumothorax caused by rib fractures, right lung contusion adjacent to a rib fractures, ground glass gradient density in the left lung probably largely due to hypostasis (the body lied in the left lateral position). In the mediastinum, gas embolism is evident within the right atrium and ventricle included in the image, in ascending aorta, and superior vena cava.

Figure 12 Axial PMCT image at the level of the superior abdomen in a subject found in water. Note the presence of intravascular gas in the left portal venous system in the liver, due to putrefaction phenomena. A small amount of subcutaneous emphysema in the pericostal soft tissues is also present.

Why

The main objectives of virtual autopsy are of course completely coincident with those of classical autopsy.[15] In fact, both classical and virtual autopsy aim to determine the cause, the nature, and the manner of death, the dynamic of

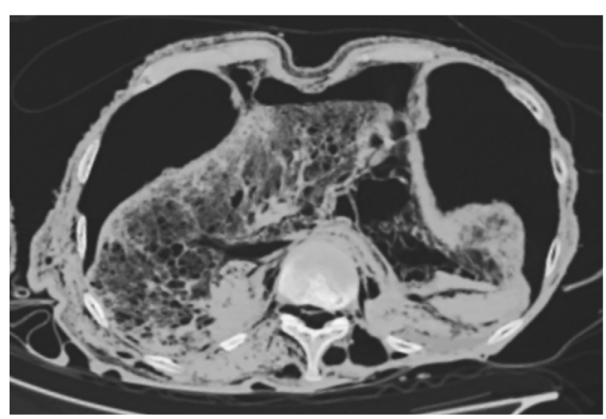

Figure 13 Axial PMCT image at the level of the upper abdomen in a subject found in the water in advanced state of putrefaction. Note the diffused alterations of liver parenchyma, with air bubbles. The kidneys are hardly visible as 2 parenchymal bands in the retroperitoneum of the flanks.

Figure 14 Three-dimensional volume rendering of the region of the left wrist in a subject who died due to gunshot injuries. Note the hyperdense foci in the carpal region (in blue), interpreted as small projectile fragments. (Color version of figure is available online.)

the damaging event leading to death, and to ascertain the vitality of eventual lesions. Another specific objective might be the identification of the deceased (Fig. 18) and other anthropology-related aspects. Another important characteristic of a classical forensic report is that it should be comprehensible to nonmedical individuals such as laymen and other experts in a court of law, allowing for the objective and correct evaluation of each specific case.

Virtual autopsy with PMCT has been demonstrated to be a formidable method for quickly and effectively identifying and describing skeletal lesions in trauma cases, bone characteristics for identification purposes,[26-30] gas embolism as potential cause of death, and other similar findings.

However, the key question is why choose virtual autopsy by PMCT with the additional costs of possible ancillary

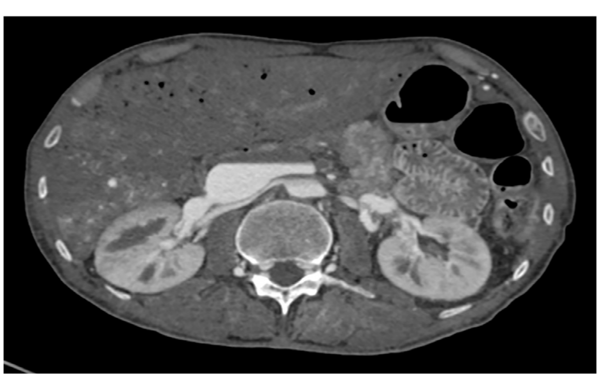

Figure 15 Axial PMCT angiography image at the level of the upper abdomen in a subject who died of cardiac tamponade. Note the presence of contrast media leveling in the inferior cava and abdominal aorta as well as the renal veins and arteries. Note the "enhancement" of the kidneys and the liver.

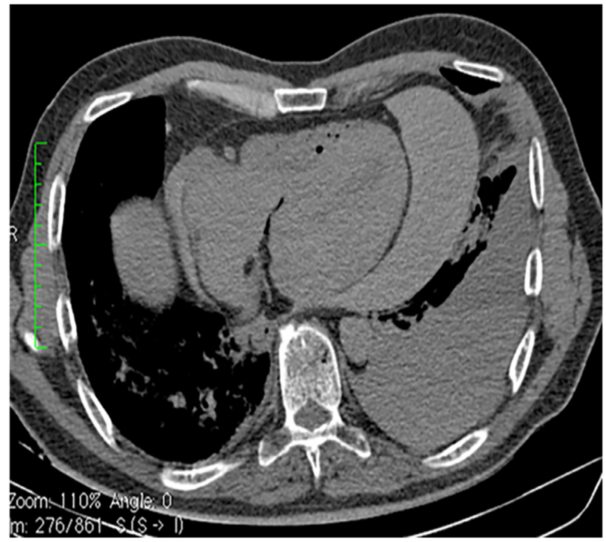

Figure 16 Axial PMCT image at the level of the thorax in a subject who died due to aortic dissection rupturing into the pericardial sac. Note the presence of dense material in the pericardial sac, interpreted as hemopericardium, and the fluid-fluid level in the left pleural space, attributed to sedimented hemothorax.

techniques as an adjunct to or even instead of classical autopsy, the actual gold standard for forensic examinations.

It is well known that there are clear advantages of virtual autopsy as compared to classical autopsy. First, virtual autopsy provides a means of obtaining data from the cadaver with a noninvasive or minimally invasive, absolutely nondestructive approach. Classical autopsy, on the other hand, while still the actual gold standard in forensic examinations, consists in the opening of the corpse after external findings

Figure 17 Axial PMCT angiography image at the level of the thorax in a subject who died due to aortic dissection rupturing into the pericardial sac (same subject as Fig. 16). Note the presence of contrast media in the pericardial sac and in the left pleural cavity. A pericardial defect was hypothesized.

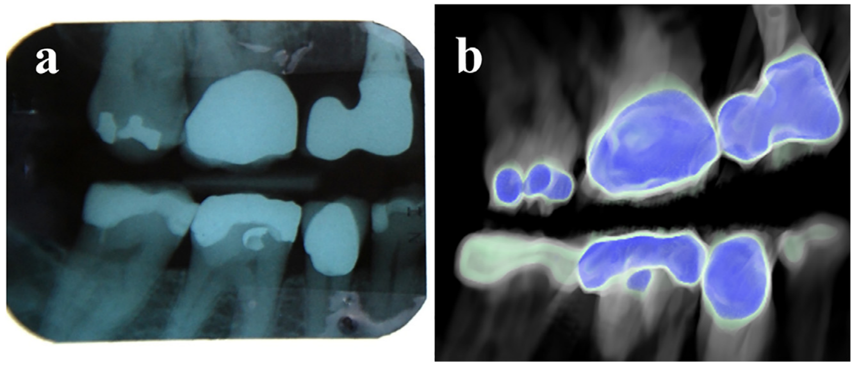

Figure 18 (a) Ante-mortem dental RX and (b) postmortem CT reconstruction of the same dental elements of (a) in a suicidal case with traumatic lesions due to the impact against a train. A perfect correspondence of dental implants is evident, utilized for identification purposes.

documentation. All external and internal findings are reported in a written format and 2D photographs are used for documenting relevant findings. This examination process can be highly standardized to guarantee a certain interpretational reproducibility of the findings. However, it necessarily depends on the skills of each specific examiner, resulting an observer-dependent and relatively subjective procedure that unfortunately is destructive and often unrepeatable, particularly if cremation is considered.

Furthermore, in PMCT examinations, the entire body volume should be covered including body regions that are not usually dissected during classical autopsy such as the extremities, soft tissues of the back, the spine, and where potentially relevant findings might be unrecognized.

Moreover, this noninvasive or minimally invasive approach is better accepted by certain religious or cultural communities, and may be desirable in particular cases such as those involving children.[31] The reduction of risk from infection to the operator should also be considered among the advantages.[23,25]

Another advantage of modern imaging techniques is the digitalization of the images allowing for the storage of raw data for a theoretically unlimited time, with the possibility of recalling this data providing other ways of scrutinizing it depending on the needs of the examiner.[15] It is not infrequent, in fact, that new investigative attempts arising years later reopen the case and focus on different details of the body examination.

Another important advantage of virtual autopsy is the possibility to provide a clear visualization and 3D documentation of the forensically relevant findings in a format more suitable and intelligible for nonmedical experts, or for students in an academic context.[15]

When

As mentioned above, virtual autopsy with PMCT and eventually its ancillary techniques of postmortem biopsy and PMCTA is widely considered a useful and effective method complementary, and even in selected cases a substitute to classical autopsy.

However, the routine introduction of these techniques is prevented by certain drawbacks related mainly to costs, and above all to logistic problems. It is not infrequent that CT scanners for virtual autopsy and its ancillary techniques are not available inside the forensic medicine department for dedicated usage. Therefore, PMCT examinations are performed on CT scanners situated in the radiology department, often after daily routine working hours. This means additional difficulties related to the transportation of the cadaver to different locations that are not always near that of forensic medicine, and also related to the difficulties in engaging qualified personnel for after-hours work.

Even when PMCT scanner is available inside the institute of forensic medicine, the indications of virtual autopsy vary from institution to institution. There are institutes such as the Institute of Forensic Medicine of the University of Zurich where PMCT examination on a dedicated CT unit is performed routinely on all the bodies delivered for forensic investigation, prior to autopsy and even if an autopsy is not performed thereafter.

In other forensic institutions equipped with a dedicated scanner, PMCT is performed as a screening method to establish if a classical autopsy is necessary, for example, by excluding cases where postmortem imaging ascertained natural cause of death.[1,32] In certain countries, such as Japan, where the next of the kin has the authority to object classical autopsy, virtual autopsy with PMCT might represent the only investigative modalities other than external examination.[33,34]

The potentials of virtual autopsy with PMCT examination are well recognized particularly in fields of investigation where they are most valuable. For example, as mentioned above, CT technology is very valuable for the detection of skeletal alterations and for the documentation of skeletal characteristics. According to Flach et al,[35] whole-body PMCT should be performed in cases involving various types of trauma, such as gunshot wounds, blunt or sharp trauma, in cases of accidental deaths where a reconstruction of the event is desirable, in cases of train fatalities, in decomposed bodies, or for identification purposes.

Regarding PMCTA as an adjunct to whole-body PMCT in virtual autopsy, its usefulness is particularly valuable when natural causes of death are supposed (eg, acute aortic dissection, ruptured aortic aneurysm, etc.) or in traumatic deaths, where parenchymal lesions are suspected and where unenhanced PMCT often fails to correctly document and identify such pathologic alterations.[36] Postmortem biopsy through percutaneous sampling is a desirable adjunct to PMCT if virtual autopsy remains the only investigative method performed for a given case.

Moreover, it is important to underline that, in selected cases, whole-body PMCT with postmortem percutaneous biopsies and/or PMCTA was considered sufficient for closing the forensic case without proceeding with classical autopsy.[37,38]

How

In this section, important, general indications about protocols of whole-body PMCT, postmortem percutaneous biopsy, and PMCTA will be discussed and a typical workflow to a virtual autopsy will be suggested.

Whole-Body PMCT

The PMCT scan protocol suggested here resembles that proposed by the study group guided by Professor Thali at the Institute of Forensic Medicine of the University of Zurich, as reported in the paper of Flach et al.[35]

For a correct PMCT, a whole-body scan from the vertex to the tips of the toes is recommended covering the entire body volume. If the scan range is too short and the entire body volume is not covered by 1 single scan, it may be necessary to rotate the body 180° to obtain images of the entire lower extremities. The ultimate goal is to obtain a panoramic view of the entire skeleton, by 3D volume rendering of the bony structures (Fig. 19).

The body is positioned in a supine position wrapped, if possible, in an artifact-free bag, with the head positioned in the median location, to avoid mistakes related to the sides when reconstructing the images.

After whole-body scan has been obtained, a dedicated scan acquisition on the head and neck reconstructed with a small field of view is recommended to ensure a proper image quality of these regions.

After these 2 acquisitions that can be performed without interruption, with lowered arms, a third scan for imaging thorax and abdomen should be acquired, with the upper extremities elevated, to avoid artifacts.

For detailed scan and reconstruction parameters and other supplementary scan acquisitions, the paper of Flach et al is recommended.[35]

Whole-Body PMCTA

Many PMCTA techniques have been proposed in the recent years. Variations among them are basically related to the type of contrast media injected, the method of injection, and the selection of acquisition phases.

The first to introduce this innovative methodology was Thali's group at the Institute of Forensic Medicine of the University of Bern and by Jackowski et al, and later modified by Ross et al.[17-19] This technique is based on the injection through cannulated femoral vessels of hydrophilic iodinated contrast media mixed with polyethylene glycol by using a modified heart-lung machine.

Later, the group of the Institute of Legal Medicine of the University of Lausanne[20] proposed a different technique named multiphase PMCTA. With this technique, a pressure-controlled perfusion device is used to establish circulation of the contrast agent constituted by a mixture of iodinated contrast media and paraffin oil. Multiphase PMCTA is constituted by multiple acquisitions, in particular, 1 native whole-body scan and 3 angiographic phases. Another whole-body PMCTA technique has been proposed in Japan. With this practically noninvasive technique, the contrast media is injected within a peripheral venous vessel, as it occurs in a clinical setting, and the circulation is guaranteed by using a cardiopulmonary resuscitation technique, immediately after death.[21,39] In France in 2011, in single case report, a slightly different technique for PMCTA was proposed, where catheterization of the femoral artery was obtained under ultrasound guidance, and a mixture of iodinate contrast medium and water was used, propelled with a roller pump, similar to that of Ross et al.[36,40] Recently, in 2016, Schweitzer et al proposed a variation of the abovementioned technique by Jackowski and Ross, which utilizes a relatively economical mobile immersion instead of the standard roller pump of a heart-lung machine, with good results.[41]

For PMCTA, according to Jackowski et al and Ross et al,[17-19] after cannulation of the femoral artery at the inguinal level, a scout view from the head to just above the knees is obtained.

The first enhanced phase is the arterial one, obtained after injection of the contrast media in the cannulated femoral artery, from the head to the thighs, with lowered upper extremities. Immediately after, another arterial scan is acquired on the thorax and abdomen, with upper extremities elevated above the head. To obtain a good visualization of the coronary arteries, a supplementary scan may be necessary and is obtained after rotating the body into a prone position with another arterial injection of contrast. The venous phase follows, first on the thorax and abdomen by injecting the contrast media through the cannulated femoral vein and then after the arms have been lowered, on the head and neck to the thighs.

Postmortem Percutaneous Biopsy

Image-guided core needle biopsies and fluid sampling are minimally invasive approaches for gathering diagnostically relevant information that is otherwise not possible by using conventional PMCT.[22,23] In most cases, internal body samples are an required step for a minimal invasive postmortem examination with virtual autopsy. Moreover, image-guided needle placement facilitates a sampling that is free of contamination.

Image-guided core needle techniques are not time-consuming methods, allowing for fast sampling of the specimen and overcomes the problem of cellular autolysis and

Figure 19 Three-dimensional volume rendering of the whole body in a case of natural death (heart tamponade due to ruptured myocardial infarct) in the anterior (a) and posterior (b) view. Note the absence of recent fractures.

decomposition, especially in situations when the autopsy can only be performed several days after death or in case of religious or cultural circumstances.[22,23]

Recent studies have shown promising results in improving the diagnostic value of virtual autopsies by using those minimally invasive tools.[23,42,43] Postmortem biopsy sampling can be performed manually, if the target is located in an easily accessible part of the body visible to the operator or, more frequently, under CT guidance.

From a technical point of view, percutaneous postmortem biopsy can be performed by using fine needle aspiration, tru-cut or core biopsies with automated biopsy guns, or coaxial techniques with an introducer needle. Fine needle aspiration, due to its thin caliber and its relatively short length, is not

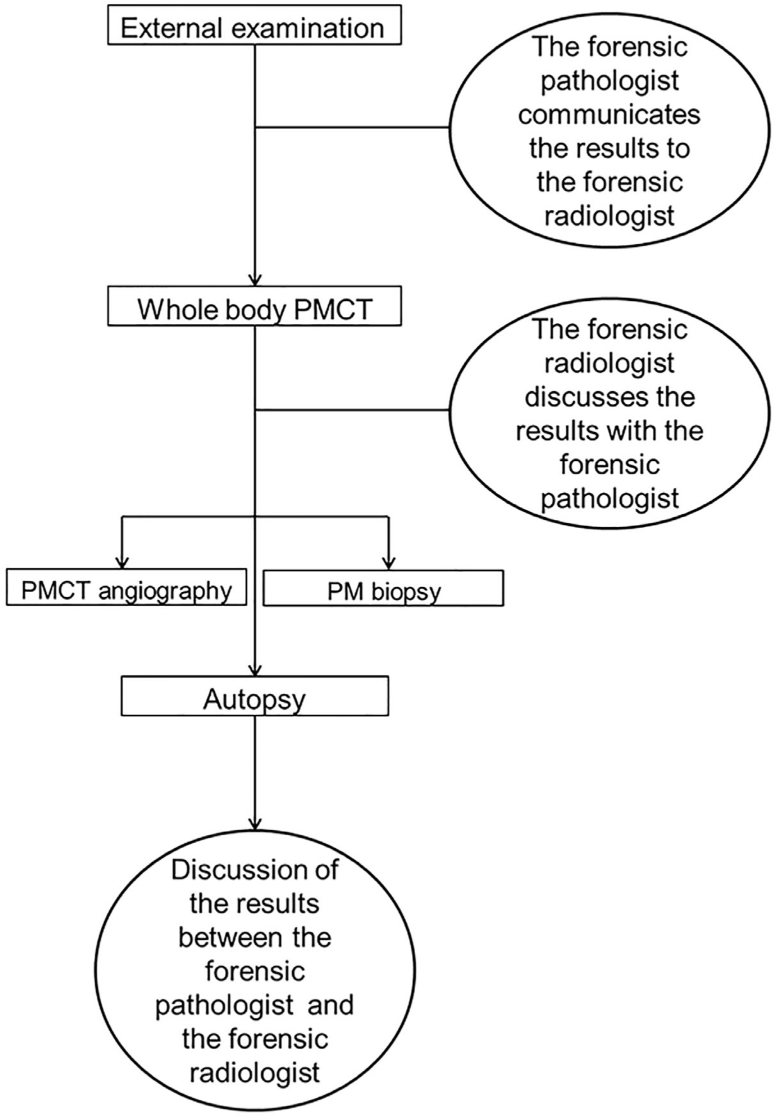

Figure 20 The figure illustrates the general workflow of virtual autopsy. After external examination, the forensic pathologist communicates the results to the forensic radiologist, who performs a whole-body PMCT scanning. The results of this examination are reported by the radiologist to the forensic pathologist and ancillary virtopsy techniques are eventually performed before autopsy. After autopsy, the forensic pathologist and the radiologist discuss their results to reach common preliminary conclusions.

suitable for comprehensive histopathologic examination but can be used for fluid samplings. Tru-cut or core biopsies with automated biopsy guns, devices easy to use and reliable, provide specimens sufficient for histopathologic analysis. Coaxial technique with an introducer needle is the method of choice when it is desirable not to lose the access to the target while performing multiple samplings.

Targeting procedures may vary similar to those used in clinical practice. PMCT-guided biopsy is the most widely used technique because of its high spatial resolution, its speed of use, and the opportunity to follow the needle trajectory in real-time by using CT fluoroscopy. In this context, classic step-and-shot technique can be used, which consists in checking the needle position by scanning the body and the target after every movement of the needle; or CT fluoroscopy technique can be adopted, available on newer CT scanners, that allows one to follow the real-time trajectory of the needle. Thanks to this technique, the procedure time is significantly reduced for the operator who is standing in the room during the biopsy and is subjected to radiation exposure. Moreover, computer-assisted navigated biopsies have been introduced since late 1980s to increase the accuracy and efficacy of surgical procedures. This principle could be applied to biopsy technique as well, by using trackable navigation probes equipped with a needle. Those probes are linked to a 3D gathering system programmed by computer software according to CT data. In this way, the operator can have real-time visual feedback of the probe movements without using X-rays. Navigated biopsies with robotic assistance can be considered an evolution of the computer-assisted navigated biopsy, mainly to eliminate the human factor and make the biopsies reliable and more accurate. In postmortem settings, navigated biopsies with robotic assistance have been introduced by Thali's group in Switzerland, with the creation of the Virtobot.[43]

In this system, the probe equipped with a needle is installed on a robotic arch that moves around the body. Basically, the procedure starts with identification of the region to be sampled. Then the robotic arch moves to the planned position. The tool position is confirmed by the navigation system and corrected automatically if necessary. Finally, the pneumatic actuator, which is the retracted position prior to this point, is fully extended to reach the target point. Once the target point is reached, the needle is released by the gripper and the robot returns to the start position of the cycle. Virtobot, when used, alleviates the need to expose someone's hand under the CT scan to perform the biopsy.

Workflow in Virtual Autopsy

The general workflow of virtual autopsy is illustrated in Figure 20.

Both in the case of forensic institutes with dedicated CT machine and in forensic institutions not equipped with dedicated CT technology, the first important step of every virtual autopsy is to collect circumstantial data and external examination findings that should be provided to the radiologist in charge of the case, before performing the whole-body PMCT examination. At this point, the radiologist may recommend PMCTA and/or postmortem percutaneous biopsy.

First, a whole-body PMCT scan is acquired, preferably within 48 hours from the presumed time of death to avoid pitfalls related to decomposition. Then, postmortem percutaneous biopsies for toxicologic and/or histologic analysis may eventually be performed followed by a PMCTA examination if deemed useful by both radiologist and forensic pathologist in charge of the case.

Before autopsy, the radiologist should provide the forensic pathologist with a report of the PMCT images that have been obtained, so that they as a team can decide if it is appropriate to obtain postmortem percutaneous biopsies and/or PMCTA. Moreover, in this preliminary exchange of information between the radiologist and the forensic pathologist, the former can provide the latter with suggestions about focalized dissection such as, for example, the presence on PMCT of pathologic alterations of the spine, pelvis, or extremities, which are areas generally not dissected.

Alternatively, when the autopsy is concluded, the radiologist should enter the autopsy room to receive information about the principal autopsy findings and to correlate postmortem imaging to autopsy results.

The final radiology report with imaging documentation about the results of virtual autopsy should be made available as soon as possible to allow for the proper management of the forensic case.

Conclusions

This paper intends to provide a practical guide to virtual autopsy. Forensic imaging, thanks to the introduction and the recent advances of virtual autopsy, has become a new radiology subspecialty. As such, it requires special and focused skills by both radiologists and forensic radiologists who adopt these investigation techniques. Most importantly, only a close cooperation between radiologists and forensic pathologists well versed in the standardization of indications and procedures can guarantee the reliability of virtual autopsy.

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