Chapter

Post-Mortem Assessment and Evolutionary Role of the Autopsy

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Abstract

The Chapter is dedicated to the evolutionary role of autopsy, reporting the historical profiles, the state of the art, and prospects for future development of the main related techniques and methods of the ancillary disciplines (like Radiology), involved in historic synergy in the post-mortem assessment, together with the mother discipline Forensic Pathology. A task sustainable through the utilization of the so-called advanced molecular autopsy, a convergence of different skills jointly makes use of the high dimensionality of data generated by new technologies requiring a data mining approach governed by improved bioinformatics and computational biology tools. The evolution of the scientific research and the increased accuracy of the various disciplines will be able to weigh the value of evidence, placed at the disposal of the justice system as truth and proof.

Keywords: medicolegal autopsy, forensic autopsy, post-mortem assessment, forensic pathology, radiology, and imaging

1. Introduction

The autopsy is the dissection of the dead body to determine the cause of death or the nature of the disease [1].

In particular, the anatomical or anatomical-pathological autopsies aims to study the human morphology and to improve the knowledge among students of medicine [2]. The forensic autopsies are requested by police, prosecutor, coroner, or judge, to solve specific problems, not only the cause of death (natural or traumatic), but also the manner of death (homicide, suicide, accidental), the time of death and sometime the identity of the deceased [3].

Unfortunately, in the past half century the rate of autopsies conducted throughout much of the world declined [2]. In the past, usually the pathologist revealed the diseased anatomy of patients. Such activity confirmed or disproved diagnoses, permitted assessment of the effects and appropriateness of treatment, and illustrated the relentless advance of disease.

The experience could be humbling or reassuring, but it was always a deterrent to medical hubris. Autopsies were a form of audit for the whole medical team, including internists, surgeons, and radiologists. With a reduced emphasis on autopsies, the skills required for performing these procedures and reliably interpreting associated histopathology risk being eroded, and a time-honored form of quality control is being lost [4].

Globally, the reasons for decreased reliance on autopsies are diverse. They include an emphasis on reducing health care and educational costs; altered working patterns for pathologists, who are spending more time engaged in surgical pathology; cultural aversion to interfering with cadavers; a belief that advances in imaging and other diagnostic and teaching methods have rendered autopsies obsolete except for medicolegal purposes; and concern among practitioners about litigation if errors are identified during post-mortem examination [5].

Studies have repeatedly demonstrated that rates of identification of diagnostic errors or unrecognized diagnoses have not decreased over time; an oft-cited estimate is that such mistakes are revealed in about 30% of autopsies. Various approaches representing less invasive and more limited investigations into causes of death have been explored, but we generally rely less at present on examining cadavers altogether. Estimates of overall and cause specific mortality are mostly derived from information collected while people are living. But there is much we can learn from the dead, for both clinical and public health purposes [4].

Some epidemiological studies showed in different countries a decline in autopsies for diagnostic purposes in favor of those of a judicial and, even, didactic nature [3].

It is therefore difficult to recognize autopsies conducted solely for medicolegal purposes, especially as the documentation on this subject is often dispersed in works, including those of a non-medical nature, that are difficult to uncover [6].

2. Autopsy techniques

The modern autopsy techniques were developed in the 19th century in Germany, when the observation of single organs was accompanied by removal of organs in four blocks: 1) neck, lungs, heart, and thoracic aorta; 2) liver, spleen, stomach, duodenum and pancreatic gland; 3) intestine and 4) kidneys, adrenals, the abdominal part of the aorta and pelvic organs. Such a "new" technique permitted the investigation of injuries in anatomical relation to the surrounding organs and structures [6]. Today, this method is usually applied and sometime adapted to the requirements of every single case for injury patterns, for example in case of neck trauma, traffic accident, SIDS [7, 8], death of pregnant women [9], death due to air embolism [10], sexual homicide [11] and medical malpractice [12].

Each autopsy should be preceded by an examination at the scene of death and followed, sometimes, by different types of radiological examinations (X-ray, CT, and MRI), to then perform a thorough external examination of the corpse with a complete internal examination, by opening all three body cavities [13].

Indeed, to contribute as much as possible to the prosecution of the guilty and the defense of the innocent, the forensic autopsy should be performed in a completely way (*"the only thing worse than no autopsy is a partial autopsy"*). In fact, an incomplete autopsy may require a subsequent exhumation of the body, because what may seem initially not important could be it for the resolution of the case [3].

An autopsy well performed require different measurements: body weight and length, chest circumference, weights of various organs to describe health state and in children cases the development. Regarding the "report", the first part must describe all the findings (normal or pathological) of the external and internal examination. The second part contains an initial assessment of the results. Additional investigations (eg radiological examinations, which as X-ray, CT and MRI;

chemico-toxicological or microbiological exams), which are deemed necessary for answering questions, must be listed.

Furthermore, should be sampled and stored during autopsy tissue specimens for histological and immunohistochemical exams, sometimes whole organs (e.g., brain, heart, lungs), wounds, body fluids and tissue specimens for toxicology, genetics, and microbiology. All of these must be stored under special conditions, at different temperatures and for varying periods, guaranteeing an adequate chain of custody.

3. Microscopic techniques

microscopic examination is necessary in all cases in which gross examination leads to conflicting results.

Stainings of interest for forensic purposes are: hematoxylin–eosin (HE) for hemorrhages, Prussian blue reaction and Quincke stain for hemosiderin; Hematoxylin-Orange, Weigert stain for fibrin for the thrombosis; Sudan III for fat embolism; Hematoxylin-Orange for inflammatory cells (leucocytes), carmine or Gentian violet for mast cells, Heidenhain iron hematoxylin stain and eosin for fibroblasts.

Concerning forensic histopathology, several experimental studies tried to find new stainings for the identification of metallization on skin electric marks [14], for the study of gunshot entrance wound and gunshot residues (i.e., Giemsa) [15], for the determination of glycogen in cardiac tissue (PAS - periodic acid Schiff).

The cerebral injuries or chronology of lesions were particularly studied with experimental enzymatic studies [16, 17].

In the last few years, the scanner electron microscopy (SEM) has opened new fields of research regarding the heart [18], the detection of gunshot residue [19] or the electric and thermal injuries [20]. Furthermore, transmission electron microscopy (TEM) was able to demonstrate the hemorrhages and of oedema in asphyxia deaths [21], and silicon in lung silicon embolism. The Laser scanning confocal microscope permitted the simultaneous observation of more fluorochromes and multidimensional analysis (2D, 3D and 4D) of the images, allowing to evaluate the activation stage of the cell and the detection of metals in tissues [22].

The environmental scanning electron microscopy has also permitted the analysis of materials or tissues without specimen preparation, to characterize saw or stab marks on bones [23] or detect diatoms in cases of drowning [24], in forensic cases of intoxications [25] or for the estimation of firing distance in gunshot-related deaths [26].

The immunohistochemistry (IHC) is mainly used in the analysis of the vitality and chronology of skin lesions or burned bodies, the chronology of cerebral hypoxia and myocardial ischemia and the diagnosis and duration of asphyxia, hypothermia, or hyperthermia [27].

The double-color immunofluorescence analysis combined with confocal microscopy is used for assessing the chronology of lesions and the cause of death in asphyxia cases [28].

In situ hybridization (ISH) is used in the identification of gender or in the search for viral DNA, for the study of the cases of diagnosis of SIDS [29].

Regarding histology, in the past it was recommended *"sections of normal and any abnormal areas of each organ for microscopic examination"* [30]; more recently all autopsies must performed a sampling of *"specimens from the main organs for* *histology*" [13], because the histology is fundamental for identifying and dating natural or traumatic disease, deaths related to acute or chronic exposure to xenobiotics [31], for reconstructing accidental or criminal dynamics [32] even in cases of medical malpractice [33].

4. Forensic radiology

Forensic Radiology have gained increasing importance in the field of Legal Medicine, but each method has its own advantages and limitations, that depend on the method used, the experience of the "legal radiologist". These kinds of exams were used to estimate the age and height of the victims, mainly for identification purposes [34].

4.1 Virtual autopsy

In 2000 the most famous project (the Virtopsy project) [35] opened the collaboration of the Legal Medicine with the Radiology in new systematic research combining both specialties in this field with the use of different basic techniques (conventional X-rays, CT, MRI) and complementary methods, like imaging-guided sampling, post-mortem angiography, and ultrasound, used to locate blood vessels for cannulation [36].

4.1.1 Conventional X-ray

It is the oldest technique of forensic imaging and has almost been eclipsed by the CT in modern imaging.

4.1.2 Computed tomography

The post-mortem CT (PMCT) is used to the reconstruction of two- and three-dimensional images, especially in examining the skeletal system. This method represents an excellent screening tool and adjunct to the conventional autopsy for: the high special resolution; rapid examination times (in only a few minutes permits the scanning of a whole body); easy handling of the CT unit; the possibility to detect any foreign material (projectiles and/or surgical material); the investigation and identification of victims (age and gender) of mass catastrophes [37]. The simple and clear images present accessible information to judges, police officers, and other individuals operating in the field of legal medicine [38].

However, the low contrast for organ visualization and the limited sensitivity for the detection of organ findings are the major downsides of the method, making it suitable for examining cases of traumatic death, such as firearm cases [39], but not natural death [40].

4.1.3 Magnetic resonance

MRI has the potential to overcome the limitations of PCMT, but it is only rarely used in forensic imaging because is a complex technology requiring specific training and high maintenance costs. The high soft-tissue resolution makes MRI a perfect tool for detecting natural causes of death (e.g., in the heart) and for examining traumatic soft-tissue injuries (e.g., in the brain).

4.1.4 Imaging-guided biopsies

These complements increase the sensibility of the radiological exam: imaging-guided biopsies or liquid aspiration, different matrices, organ tissue or body fluids can be obtained in a minimally invasive way, combining PMCT with histological, toxicological, or micro-biological investigations (e.g. collection and analysis of gas composition, distinguishing gas due to post-mortem decomposition from vital gas embolism or from other origins).

4.1.5 Angiography (PMA)

It is a promising method, that use an injection of contrast agent into the vascular system [41], selective (e.g., coronary arteries) or in a whole-body. PMA in combination with PMCT also leads to an increase of contrast in the soft tissue, allowing the detection of pathological findings. The most widespread technique is the multi-phase PMCT angiography (MPMCTA), that consists in the performance of a CT scan and after an injection of contrast agent (Angiofil®) with a perfusion device (Virtangio®). In this way the sensitivity of PMCT for detecting pathological findings in forensic cases can be increased from 65 to ~81%, very similar to a conventional autopsy, with important advantages in cases of sudden cardiac death (because it allows the detailed investigation of coronary arteries), fatal hemorrhage (because it permits the finding of even the smallest sources of bleeding), stab and gunshot trauma, and suspected medical error. Extra-vascular local application of contrast agent has been used to demonstrate the capacity to reconstruct stab directions and estimate the depth of the inflicted wounds [42].

4.1.6 Conclusion

In conclusion, for the detection of foreign bodies, PMCT or conventional X-ray techniques are most suitable and permit: rapid detection even in putrefied, carbonized, or otherwise highly damaged bodies; guidance for sampling the foreign bodies (e.g., projectiles); investigation of traumatic death, providing an information concerning the biomechanical origins of fractures for forensic reconstruction of the case. In cases of fatal hemorrhage, due to a sharp or gunshot trauma or suspected medical error, PMCT should be extended by adding whole-body PMCTA, that permits the discovery of the source of bleeding and the visualization of the trajectories of knives, needles, and projectiles. In cases of natural deaths, like sudden cardiac death, the methods of choice are MPMCTA, which permits a detailed investigation of stenosis or other lesions of the coronary arteries, and MRI, which shows the myocardium and eventual lesions within it.

4.2 Micro-radiology and future

Micro-radiology may prove to be the bridge that connects histopathology and diagnostic imaging as subspecialty of its own [43] and its use in forensic sciences is growing. The leading application is the structural study of calcified tissues, permitting the measurement and calculation of mineral density, volume, and surface, allowed to identify lesions, fractures, or dislocations of bones or calcified tissues [44]. In this way, this technique could inform on the morphology of tool marks on bone, on vitality and timing of fractures, and on age identification.

Micro-CT and micro-MRI can also help to identification of fire victims and to estimate the age at death and the cremation temperature of burned human remains.

This technique can perform a tri-dimensional spatial distribution of gunshot residue particles, also in wounds covered by textiles or altered by putrefaction, fire, or water, for the diagnosis between entrance and exit lesions and for the estimation of the firing distance.

Micro-MRI imaging can allow the estimation of the neuronal loss related to age, cranial trauma, or degenerative diseases.

5. Specific autopsy procedures

In 2015 the European Council of Legal Medicine (ECLM) published a checklist to identify qualified forensic experts who perform professional services of a high-quality standard, successfully undergoing official accreditation/certification [45, 46].

The ECLM document helps to standardize the medico-legal examination about the determination of the manner and cause of death. In particular, the document provides that the specialists should have a good experience in Legal Medicine, be independent of the police or the prosecuting authorities, and should be in possession of all the necessary equipment to provide high quality reports. The document includes general aspects (administrative space, quality assurance, statistical reports) and specific aspects (scene investigation, external examination, performance of an autopsy, radiological, histological, toxicological exams). accreditation can be request by any institution to the president of the ECLM who then nominates two inspectors to check it. The standards reported in this document [45], which take up the previous recommendations of the Council of Europe [13], are those necessary to qualify the activity of a forensic pathology service.

6. Post-mortem interval (PMI)

The Post-Mortem interval (PMI) is one of the most important items in forensic investigation, above all in case of homicide o traumatic deaths.

We can identify two different objectives of PMI at the scene of violent death (e.g., homicide):

1. to give a preliminary opinion of the time of an assault.

2. To check whether PMI is consistent or inconsistent with the alibi of a suspect.

For estimating the PMI, different sources are used:

1. information from the environment in the vicinity of the body (e.g, date, and hour in a watch).

2. Anamnestic factors concerning the deceased's habits (e.g., work, activities).

3. Post-mortem changes.

All sources of information on PMI always should be kept in mind.

In forensic medicine much research has been carried out on post-mortem changes. The progression of all post-mortem changes is influenced by many internal or external factors, mainly the ambient temperature. The longer the post-mortem interval, the less accurate is the estimation of the time since death based on post-mortem changes.

Various methods are proposed for estimating the PMI, in particular [47]:

1. physical processes, like body cooling and hypostasis.

2. Physicochemical processes, like rigor mortis.

3. Concentration changes of metabolites or substrates.

4. Autolysis with increase or decrease of analytes in various body fluids.

5. Putrefaction due to bacterial processes.

Furthermore, the methods for estimating the PMI are not only different but also have widely varying scientific value in terms of the underlying scientific background, the mode of investigation and the validation of the method.

The chemical and biochemical analysis after death of bodily fluids (and among these Vitreous Humor) is an important approach to assess the PMI.

Vitreous Humor (VH) is a chemically complex aqueous solution of carbohydrates, proteins, electrolytes, and other small molecules present in living organisms; this biofluid is useful tool for its isolated environment, preserved from bacterial contamination, decomposition, autolysis, and metabolic reactions. VH has been used as a biofluid for forensic purposes in different studies. A recent review [48] evaluated the chemical and biochemical advances with particular importance on the endogenous compounds present at the time of death and their modification over time, which are valuable for the PMI prediction and to also identify, sometime, the cause of death. This review confirms that the VH analysis, especially the dosage of its endogenous compounds, is very useful for calculating the PMI (and sometimes also the causes of death), even if further studies are needed. The characteristics of VH make it an optimal biological sample for the determination of PMI, given its aqueous constitution and low cellularity, compared to other biological samples, such as blood, being partly protected from post-mortem alterations and bacterial contamination. $K+$ and $NH3$ significantly increase after death, while $Na +$ and Cl-decrease slightly. The slight increase in magnesium lactate, aminoacids and creatinine needs further study. $K+$, $Na+$, $Cl-$ and aminoacids are therefore strongly related to PMI, while $Mg+$, $Cl-$ and lactate are weakly related to PMI. No correlation was found between glucose decrease and PMI, or between creatinine increase and PMI.

Other Authors [49] reviewed 26 publications that provided substantial evidence on structural changes within the eye in determining the PMI, identifying the following application areas: reduction of intraocular pressure; temperature of the eyeball; changes in pupil diameter; corneal thickness and opacity; alterations of the lens; changes in the retina; segmentation of retinal vessels. The major limitations of the studies examined were represented by the use of small case studies, by the absence of a solid statistical methodology and by the use of mathematical models valid only under ideal conditions and, finally, by the use only for short PMI. Although different ocular alterations cannot be used to reliably estimate the PMI, studies indicate that they may be promising in the future thanks to the use of new technologies.

All the methods described above, currently in use, are still imprecise to provide an authentic estimate of the PMI. It is therefore necessary to study new methods to better estimate the PMI. The post-mortem modifications of the corpse are therefore used as "indicators" of the PMI, providing in fact only a completely indicative time range. With the development of molecular biology, attempts have been made to estimate PMI by evaluating the degradation pattern of biological markers (DNA, RNA and Proteins). DNA is shown to be unshakable in the long post-mortem phases, RNA is much more labile in nature and sensitive to degradation in a tissue-specific way. Thus, recently some Authors provided a review that mainly focuses on potential use of RNA markers in estimation of PMI [50]. For this review, the systematic evaluation of 47 studies were executed according to the default inclusion and exclusion criteria. There have been many attempts made by forensic scholars to find out the methods of estimating the PMI accurately, but those methods were based on conventional and established approach. In this recent review, Sangwan et al. [50] reported the use of RNA, DNA and protein degradation for PMI estimation. Many studies have been carried out to find a more accurate and reliable method for estimating the PMI, but currently none can provide an accurate estimate of the PMI. The degradation of DNA nucleic acids appears to be time-correlated such that it is useful for estimating the PMI over long time periods. However, several ante-mortem and post-mortem factors, including environmental conditions, significantly affect DNA degradation. Studies have so far focused on animal models. Then, the development of the multiparametric mathematical model based on DNA degradation has not been able to evaluate the error with a known PMI. Comparatively, the contribution of RNA degradation with molecular approaches in estimating PMI has revealed great potential but is not yet used in forensic practice. Among the studies on RNA degradation based mainly on animal models, only the multiparametric mathematical model is the most reliable method for estimating PMI.

Another new method for estimating PMI is that based on protein degradation. Experimental studies have been conducted on four animal tissues: lungs, kidneys, bones, and skeletal muscles (the only rare, albeit of high quality, performed on human tissue and in the forensic field).

One of the major limitations, in addition to the almost exclusive use of the animal model and obviously not in the forensic field, is the use of biomarkers that are difficult to use on humans.

Certainly, the future will focus on the combination of different methods, also considering the influence of environmental factors in the estimation of the PMI.

Ultimately, further studies on human samples and tissues are needed to better understand the usefulness of such methods. Despite numerous advances in PMI estimation, the inaccuracies observed in these methods require further research effort. In the future, the development of more reliable and cheaper techniques will change the course of forensic science also in the determination of PMI.

It is essential that researchers turn to innovative techniques that produce fast and reliable results, also to obtain validation and acceptance not only in the forensic scientific community but also (and above all) in the courtrooms.

7. The future

A European survey [51] showed that different countries has national autopsy guidelines; the most common protocols concern violent or sudden deaths, mass disasters, and medical negligence.

In the future innovative robotized and advanced microscopy systems and techniques will be utilized, finding their application in the medico legal field, by perfecting and standardizing the autopsy methodology, and achieving a more precise identification of natural and traumatic pathologies.

For improving accuracy and efficiency, the forensic autopsy will performed with a robotic system, using different methods (photogrammetry, optical surface scanning, tissue, or liquid sampling image-guided).

Atomic force microscopy (a high-resolution scanning probe microscopy with production 3-D images), will allow the study of biological macromolecules for the evaluation of PMI.

The direct tissue analysis by MALDI-IMS and SALDI, used for the identification, analysis, spatial profiling and quantification of drugs, heavy metals, proteins, peptides, and metabolites, will increase diagnostic potential and reduce analysis time in forensic cases.

Furthermore, MSI will enable to define tissue types by chemical composition rather than structure, revealing how macromolecules, such as proteins, peptides, nuclei acids, metabolites and xenobiotics are spatially distributed within a given sample. SALDI will be able to detection of small molecules and MSI will promise more sophisticated extensions to forensic applications, including cause of death and PMI identification, by observing the post-mortem decay of metabolites in cells, and the detection and distribution of xenobiotics in tissues.

8. Conclusions

The technological innovation of the medico legal sciences, begun at the end of the 20th century and continuing into the new millennium, has led to the need for an interdisciplinary approach in the execution of the necroscopic ascertainment. In this way the medico legal sciences will be able to identify and describe injuries that could be difficult to objectify and to respond to specific forensic problems, such as the determination of the post-mortem interval, the identification of wound vitality, the reconstruction of the chronology of injuries, or the timing of natural diseases. In particular, the involvement and the integration of multiple specialists such as the forensic pathologist, geneticist, toxicologist, radiologist, anthropologist, microbiologist, and entomologist has become of fundamental importance.

The traditional autopsy is and will probably remain an essential way to investigate the death, despite enormous technological developments and the consequent future prospect of a robotic or virtual autopsy, with the aid of micro- and nanotechnologies.

Therefore, it remains today valid L.S. King affirmed in 1965: it is a mistake to assume that mere autopsy can advance medical science; in fact, scientific progress in the medical field does not depend on the autopsy but on the person who is performing it; so it is important not only (or not so much) the number of autopsies that are performed, but how they are performed, it being essential that the data provided by the autopsies are profitably used, through *"people who have imagination, originality, tenacity, mental acuity"*, but above all adequate and profound scientific preparation, without which the observed data are completely useless [52].

Then, it will not only be necessary that the Forensic Pathology Services are certified and accredited, but also all the specialists involved in the necroscopic ascertainment, from the forensic pathologist to experts such as the radiologist, the toxicologist, the geneticist, thus ensuring a high-quality of the services in the forensic field.

The critical issues that most affect post-mortem forensic evaluation is above all the lack of evidence in scientific knowledge.

The scientific evolution of forensic research will benefit from the omics, bio-analytical, and imaging sciences. In this way, the increased accuracy of the various forensic disciplines will be oriented towards the elaboration of specific *"algorithms"*, able to weigh the value of *"evidence"* placed at the disposal of the *"justice system"* as truth and proof.

Conflict of interest

The authors declare no conflict of interest.

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