Post-mortem interval estimation: a mini review

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Abstract **—Estimating time since death is critical to investigation as it permits the reconstruction of death scenario and confirmation of testimonies and/or alibis. Various methods of post-mortem interval (PMI) estimation have been developed by the scientific community, based mainly on evaluating intrinsic characteristics of the decomposing cadaver or relying on extrinsic biotic and abiotic factors. These methods are the fruit of scientific findings and extensive research in different disciplines; however, most of these techniques remain at the research stage and have limited value for routine laboratory work. This short paper gives an overview of the main methods developed and discusses some promising techniques for PMI estimation.**

Index Terms— **Post-mortem interval, cadaver decomposition, forensic investigation, time since death.**

I.INTRODUCTION

The fascination with the phenomena related to death dates back to ancient times, and led to speculation and tales among populations and societies throughout history [1]. "Taphonomy" was named by the Russian paleontologist Ivan Efremov [2] to describe the discipline that explored the changes in human cadavers, while it focuses, nowadays, on cadaver decomposition and associated materials [3].

In its quest to develop robust forensic tools for post-mortem interval (PMI) estimation, the scientific community has long been focused on understanding human decomposition. This facilitated the development of various methods for PMI estimation; nonetheless, there are still unknown processes related to cadaver decomposition. PMI estimation is critical to death investigations, particularly, in the absence of witnesses, as it facilitates the reconstruction of a death scenario, provides insight into the circumstances of death and permits the confirmation of testimonies and alibis [4].

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II.POST-MORTEM INTERVAL (PMI) ESTIMATION METHODS

For decades, scientists and forensic practitioners have attempted to develop robust methods for PMI estimation [5]; nevertheless, these have focused, mostly, on relatively fresh cadavers for a higher accuracy in PMI assessment.

Soil related to crime scenes is typically considered as associative evidence. It has long been seen as a passive medium for decomposition [6]. Soil is, in fact, dynamic and sensitive to biochemical and physical environmental disturbances [7] such as animal death and decomposition, hence soil's characteristics have the potential to be used for criminal investigations. For this purpose, tools based on fundamental sciences such as chemistry, biology, and ecology have been developed for the estimation of time since death [8].

Parallel to method development, scientists have emphasized the need to classify PMI estimation tools into: a) temperature-based approaches, applied during the first 24h, and process-based methods, used until late decomposition [9]; b) tools based on noticeable ante-mortem changes (group 1), which permit an estimation of the time of survival, and methods focused on post-mortem changes (group 2), which facilitate PMI estimation [10]. Based on the nature of the post-mortem processes, these tools can be classified as physical, metabolic, autolytic, physicochemical, and bacterial.

Bisker and Ralebitso-Senior [5] recommended a classification that makes a distinction between main (real) and auxiliary PMI estimation methods (Figure 1). Thus, PMI assessment methods can be developed to: assess impacts on the cadaver due to its inherent internal changes; external factors, or to collect auxiliary information. This classification facilitates informed decision-making on which time since death method to use relative to the case being investigated.

A . Methods based on the assessment of cadaveric inner changes:

1 . Lividity (Livor mortis):

This phenomenon starts after the cessation of blood circulation. Minor signs appear on specific parts of the cadaver about 30 minutes after death, become fully noticeable after 3-4 hours and fixed after 8-12 hours [11]. The color shifts, and patterns will usually persist partly even after repositioning of the cadaver. Additionally, these are rarely observable on body areas in contact with solid surfaces due to blood vessels' compression [12].

2 . Rigidity (Rigor mortis):

This natural phenomenon is due to post-mortem ATP degradation, the accumulation of calcium ions and pH decrease [13], it is used for PMI estimation during the early phase after death. Its start and duration vary considerably depending on the environment, the activity and medical condition of the deceased. It is typically accelerated by higher ante-mortem activity [12; 13]. Changes appear after 2 - 6 hours post-mortem in mild climates and attain their maximum around 12 hours. After 24 - 84 hours, the putrefactive alkaline liquids cause the loosening of the cadaver until it regains its flaccidity [3].

3 . Temperature (Algor mortis):

Algor mortis is the gradual decrease of cadaver temperature by convection, conduction, radiation and/or fluid evaporation [14] until it reaches an equilibrium with the surrounding environment usually after 18-20 hours [15]. The cooling rate is determined by the difference in temperature between the cadaver and surrounding environment [16] with bigger differences in temperature leading to faster cooling rates [17]. Body temperature remains stable for up to 1 hour after death. This delay is related to metabolism, heat production, and body surface tissues.

The first reports on the use of algor mortis date back to the first half of the nineteenth century [9], however, it is region-specific and only applicable to temperate and cool weather conditions.

4 . Supravital reactions:

Supravitality is the human tissue survival period beyond resuscitation during which tissues' sensitivity to different stimuli is maintained [18]. Supravital reactions encompass skeletal muscle excitability by electrical or mechanical stimuli and pharmacological excitability of the iris. These reactions result from metabolic activity and/or continuity up to 20 hours post-mortem, primarily due to anaerobic glycolysis and creatine kinase reaction.

5 . Biochemical changes:

Potassium concentration in the vitreous humor (VH) is arguably the most documented biochemical parameter for PMI estimation due to its higher resistance to autolytic changes and location in the body [19; 20]. Rognum et al. [21] suggested measuring hypoxanthine in VH, together with potassium, and ambient temperature for enhanced estimation precision. Also, various parameters: sodium, calcium, glucose, cardiac troponin I, glucagon, lactate, creatinine, and urea were explored in body fluids including - VH, cerebrospinal fluid, synovial fluid or blood, and organs such as the heart, liver, spleen, lung, and brain [5].

Cingolani et al. [22] used an immunohistochemical, ultrastructural and cytochemical approach to evaluate the autolytic changes of the skin for PMI assessment up to 12 hours after death, while the utility of the adenohypophysis immunohistochemical analysis for PMI evaluation up to 20 days post-mortem was recommended by Ishikawa et al. [23]. Cina et al. [24] suggested the assessment of DNA degradation using spleen samples. This was reported to be reliable by Di Nunno et al. [25]. In addition, Sampaio-Silva et al. [26] developed a mathematical model for PMI estimation based on RNA degradation rate in various tissues.

Pittner et al. [27; 28] used muscle protein degradation for PMI assessment, using western blotting and casein zymography. Likewise, Lee et al. [29] explored the use of lateral flow assay to pinpoint and quantify PMI protein markers.

Molecular methods include the study of gene expression of the "biological clock" for PMI estimation [30], and the utility of genetic materials in buried human cadavers profiling for up to 42 years [31].

B. Methods based on the assessment of cadaveric inner changes:

1.Putrefaction/Post-mortem decomposition:

The environment-dependent breakdown of soft tissues' proteins, fats, sugars, and nucleic acids is putrefaction. This phenomenon, led by microorganisms, is identifiable based on the cadaver changes in color, gas, and liquefaction liquids production [32]. Skin marbling is initially apparent on the abdomen 36 - 72 hours before spreading to the rest of the body. Blisters appear on the skin and gas accumulation causes abdominal wall swelling. On land, putrefaction might give an initial PMI estimation; nevertheless, this process is environment-depending and varies significantly. In case of submersion, the constancy of water temperature for long periods [18] might allow a relatively good PMI indication.

2 . Microbiota - mycobiota:

Soil Microbial communities can be used for PMI estimation [33]. Olakanye, Thompson, and Ralebitso-Senior [34] observed a consistent shift in soil microbial communities in reaction to domestic pigs decomposition. Metcalf et al. [35] reported a predictable order of detection of decomposing microorganisms, which might facilitate PMI estimation.

Bisker et al. [36] sequenced fungal 18S rRNA and bacterial 16S rRNA gene amplicons from soil adjacent to decomposing piglets in order to use the fungal and bacterial community shifts to estimate time since death and reported a significant difference in ecological indices that might be used for PMI estimation [37]. These studies are parts of emerging disciplines at the interface of forensics, molecular microbiology and bio-archaeology meant to improve our understanding of decomposition site ecology by measuring the effect of carrion decomposition and surrounding soil on each other for forensic use [5; 6; 38].

The presence of fungi growing on and inside cadavers was reported by Ishii et al. [39] then Tranchida et al. (40, 41), nonetheless, their precise role in cadaver decomposition and species involved are yet to be discerned [41].

3. Forensic entomology:

This widely used technique for the estimation of post-mortem interval relies on the study of the cadaver-eating insects' lifecycles [42]. This well established forensic discipline has been adopted by forensic laboratories globally and facilitates the resolution of criminal cases and unexplained deaths. Nonetheless, it has investigative limitations due to its susceptibility to environmental parameters, type of burial and regional fauna [27] and suffers from inaccuracy related to insect evidence collection and subsequent analysis [43].

4 . Forensic botany:

Plants have an effect on carrion decomposition, as reported by Reed Jr [44], Behrensmeyer [45] and Vass [46]. Roots might accelerate decomposition via physical bone fragmentation and degradation [47; 48], together with the action of acids produced during plant roots decomposition [17]. In general, plants growth rate might help in post-burial interval (PBI) estimation for clandestine graves or in cases of human remains translocation. This method relies on the growth and adherence of plants on bones, which might be delayed by the environment, type of burial, and soft tissue persistence [49; 50]. In addition, plant DNA markers might be used to link the crime scene to materials associated with the suspect or victim [51].

5 . Forensic anthropology:

Forensic anthropology consists in the application of biological anthropology, osteology and archeology principles in a forensic context. Besides determining the deceased sex, age, stature, ancestry, antemortem and perimortem trauma, taphonomic effects, and pathological and anomalous conditions using human remains, namely bones and teeth [52], this forensic discipline helps estimate PMI based on the persistence of tissues on bones after skeletonization. However, decomposition being a variable phenomenon, PMI estimation differs largely depending on the burial setting and environmental parameters, and, therefore, lacks precision.

C . Auxiliary "PMI estimation" methods:

In addition to the aforementioned disciplines and techniques, other approaches might help in the estimation of the survival time and reconstruction of the death scenario, these include:

1 . Gastric contents analysis:

This complementary technique is widely used in death investigations as it facilitates the estimation of the victim's survival time or time elapsed between their last meal and the moment of death [18], techniques based on radiology, ferromagnetic traces analysis, absorption kinetics, radio isotopes and ultrasound have been used for gastric content analysis. Nevertheless, factors such as illness, infections, drugs, stress and type of food ingested affect the analysis heavily and might speed up or slow down gastric emptying [53].

2 . Wounds and trauma:

The regeneration of wounds, bruises and trauma is frequently assessed to determine whether the injuries were ante-, peri- or post-mortem, estimate the victim's survival time, and determine the cause of death, in order to reconstruct the death scenario and estimate PMI. Tissue regeneration is a variable phenomenon affected by the type of injury, its location and severity which makes the assessment of a wound, bruise or trauma age challenging [53].

III. DISCUSSION

PMI estimation is crucial to forensic investigations and necessitates the use of different scientific approaches in conjunction and/or confirmation of laboratory results by means of intelligence. The advances in science and technology led to the advent of new technologies with superior analytical capabilities and higher resolution. Besides the methods aforementioned, and in search of more accurate tools, research has been conducted using approaches such as the use of DNA degradation [54], dental DNA [55], microbiological and histological in tooth pulp, for PMI estimation. Although these methods are still being developed, their future application might help narrow down the error related to current PMI estimation tools and lead to robust techniques, applicable at different weather conditions, geographic regions and stages of decomposition.

IV. CONCLUSION

PMI estimation methods are intended to facilitate the understanding of the circumstances of death, confirm testimonies and help solve crimes. Regardless of the number of methods developed, PMI estimation remains one of the major challenges that face the forensic and scientific community. This is mainly due to the variable nature of decomposition, which is influenced by biotic and abiotic factors related to the environment together with cadaver endogenous factors such as corpulence and cause of death. Recent advances in science and technology should facilitate the optimization of current methods and exploration of parameters that were previously challenging to study in order to develop more robust PMI estimation tools.

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