Scientific Letters

Oral Communication 14

A multiparametric microbial and biochemical model for predicting *postmortem* interval

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Abstract

Background: *Postmortem* interval (PMI) estimation remains a major challenge in forensic science due to the influence of multiple factors. Traditional methods are often limited to early *postmortem* stages and lack precision in advanced decomposition [1,2]. Emerging approaches based on *postmortem* microbiology (thanatomicrobiome) and biochemical alterations (thanatochemistry) show promise [3,4], although their combined application remains underexplored. **Objective:** This study aimed to perform a combined microbial and biochemical analysis of different organs of mice over time since death to develop an accurate mathematical model for PMI estimation. **Methods:** Organs (lungs, heart, kidneys, liver, and brain) from male C57BL/6J specific pathogen-free mice were collected at six PMI (0, 12, 24, 48, 72, and 96 h; n=3 animals/timepoint) under aseptic conditions. For microbial analysis, organs were homogenized in buffered peptone water and cultured aerobically on Blood Agar, MacConkey, or Slanetz–Bartley media to quantify [colony-forming units/g of tissue] total bacteria, *Escherichia coli*, and *Enterococcus faecalis*, respectively. For biochemical analysis, tissue homogenates in phosphate buffer (pH 7.4) were analyzed for urea, lactate, uric acid, glucose, total proteins, magnesium, ethanol, and iron using the Accent MC240 autoanalyzer (Cormay®). For PMI model development, markers were normalized to magnesium, and those with consistent temporal trends and $R^2 > 0.900$ were selected. **Results:** Microbial dynamics were strongly tissue- and time-dependent, with total bacterial loads peaking at 72 h in most organs and later in the brain (96 h). *E. coli* was absent in the liver, heart, and brain, while *E. faecalis* showed consistent colonization in the kidneys, lungs, and the brain, particularly from 24 h onwards. Biochemical markers also exhibited distinct temporal patterns: lactate increased early, whereas uric acid and urea correlated with later PMI stages. Iron showed a progressive tissue-dependent increase. Based on these results, a mathematical model for PMI estimation was developed (Figure 1). **Conclusions:** The integration of culture-based microbiological data with biochemical markers provides a robust multiparametric framework for PMI estimation. In particular, *E. faecalis* colonization and the temporal dynamics of lactate, uric acid, urea, and iron emerged as reliable indicators of *postmortem* progression. Further validation in complex forensic scenarios will be required.

A

$$PMI = \frac{(10.04 - |\bar{Y}x|)}{0.19} \pm 2.78 \times \left[4.89 \times \sqrt{0.19 + \frac{(\bar{Y}x + 17.95)^2}{242.39}}\right]$$

B

$$S_x = \frac{S_y}{m}\sqrt{\frac{1}{K} + \frac{1}{n} + \frac{(\bar{Y}x - \bar{y})^2}{m^2 \sum xx}}$$

Figure 1. Global model for PMI estimation. Equation A allows for the calculation of the PMI from the mean value of the following magnesium-normalized parameters: uric acid and iron in the kidneys; urea in the liver; glucose and urea in the lungs; glucose, lactate, uric acid, and iron in the heart; and lactate, uric acid, and urea in the brain. Equation B estimates the error.

Keywords: thanatomicrobiology; thanatochemistry; *postmortem* interval

Acknowledgments/Funding

This work was supported by FCT - Fundação para a Ciência e Tecnologia, I.P., in the scope of the project UID/04378/2025 (DOI identifier 10.54499/UID/04378/2025), and UID/PRR/04378/2025 (DOI identifier 10.54499/UID/PRR/04378/2025), of the Research Unit on Applied Molecular Biosciences – UCIBIO, and the project LA/P/0140/2020 (DOI identifier 10.54499/LA/P/0140/2020) of the Associate Laboratory Institute for Health and Bioeconomy - i4HB.

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