**Indonesian Journal of Global Health Research**
Volume 9 Number 1, February 2027
e-ISSN 2715-1972; p-ISSN 2714-9749
https://jurnal2.globalhealthsciencegroup.com/index.php/IJGHR

# MOLECULAR BIOMARKERS FOR THE ESTIMATION OF POST-MORTEM INTERVAL (PMI): A NARRATIVE REVIEW

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**ABSTRACT**
Estimating the post-mortem interval (PMI), or time since death, remains a challenge in forensic science because conventional methods have limitations, particularly for intermediate to advanced PMIs. Post-mortem biomolecular changes have the potential to serve as markers for improving the accuracy of PMI estimation. This review aims to summarize scientific evidence regarding the use of molecular biomarkers for estimating the post-mortem interval (PMI), and to compare their characteristics, ranges of application, and accuracy. A narrative review was conducted through a literature search across ScienceDirect, PubMed/MEDLINE, SpringerLink, MDPI, Frontiers, Nature Portfolio, eLife, and the International Journal of Legal Medicine. From an initial identification of 250 articles across databases, a total of 25 primary research articles that met the inclusion criteria were analyzed narratively based on the type of biomarker, sample, post-mortem interval (PMI) range, analytical method, and estimation accuracy. More than 230 candidate biomarkers were identified, categorized into proteins (proteomics), metabolites (metabolomics), mRNA and non-coding RNA, microbiota, lipids, volatile organic compounds, electrolytes, and multi-omics. Each category possesses distinct characteristics and ranges of application. Metabolomics and microbiomics have shown promising results, particularly when combined with machine learning, while multi-omics approaches offer higher accuracy compared to the use of single biomarkers. There is no single optimal biomarker for the entire range of the post-mortem interval (PMI). The use of an integrated panel of biomarkers tailored to the time since death and the type of sample supported by multi-omics and artificial intelligence (AI), represents the most promising approach for improving the accuracy and objectivity of PMI estimation.

Keywords: artificial intelligence; metabolomics; microbiomics; molecular biomarkers; multi-omics; post-mortem interval

| First Received | Revised | Accepted |
|---|---|---|
| 02 November 2026 | 05 November 2026 | 09 November 2026 |
| **Final Proof Received** | | **Published** |
| 17 November 2026 | | 01 February 2027 |

**How to cite (in APA style)**
Syukriabulan, S., & Widodo, W. T. (2026). Molecular Biomarkers for the Estimation of Post-Mortem Interval (PMI): A Narrative Review. *Indonesian Journal of Global Health Research*, *9*(1), 253–262. https://doi.org/10.37287/ijghr.v9i1.2951.

## INTRODUCTION

Estimating the post-mortem interval (PMI) remains a significant challenge in forensic science. Information regarding the time of death is crucial for investigative processes, law enforcement, and the reconstruction of events surrounding unnatural deaths. The accuracy of a PMI estimate can also influence the assessment of evidence and medicolegal testimony in a court of law. Conventional methods such as the observation of livor mortis, rigor mortis, algor mortis, and the use of forensic entomology have long been employed to estimate the time of death. However, these methods are influenced by various environmental factors, including temperature, humidity, and the condition of the body. Such factors can diminish the accuracy of the estimate, particularly in cases involving intermediate to advanced post-mortem intervals.

The limitations of conventional methods have driven the development of molecular biomarkers as a more objective and quantifiable approach. Following death, body tissues undergo gradual

biochemical changes that can be used to estimate the post-mortem interval (PMI). These changes include the degradation of structural proteins in muscles such as troponin, tropomyosin, desmin, and titin (Pittner et al., 2016; Brockbals et al., 2023) alterations in metabolite levels within the vitreous humor, pericardial fluid, and blood (Locci et al., 2021; Chighine et al., 2023, 2025), as well as the degradation of mRNAs such as GAPDH and β-actin (Sampaio-Silva et al., 2013; Lü et al., 2016). Furthermore, shifts in the composition of the microbiota found on the skin, within the gastrointestinal tract, and in the soil surrounding a body can also serve as indicators of post-mortem changes (Metcalf et al., 2013; Pechal et al., 2014; Ren et al., 2026).

These biomarkers can be examined in various types of biological samples, such as muscle, blood, bone, vitreous or aqueous humor, cerebrospinal fluid, skin, and grave soil. Their application can span a timeframe ranging from a few hours post-mortem to thousands of days in the case of hard tissues (Bonicelli et al., 2022; Sacco et al., 2022). The development of analytical methods such as LC-MS/MS, UPLC-HRMS, NMR, and next-generation sequencing alongside the use of artificial intelligence (e.g., Random Forest, Artificial Neural Networks, Gradient Boosting, and multi-omics stacking models), can enhance the precision of post-mortem interval (PMI) estimation. Some studies have even reported a mean absolute error (MAE) of less than one day across a broad PMI range (Jian Li et al., 2024; Ren et al., 2026; Magnusson et al., 2026).

Although research on molecular biomarkers has shown promising results, it is currently being conducted across various studies with differing characteristics. Consequently, information regarding the diverse types of biomarkers and biological matrices remains fragmented and has not yet been comprehensively compared. This situation can make it difficult for researchers and forensic practitioners to determine which biomarker is most appropriate for the specific circumstances of a given case. Therefore, this narrative review aims to summarize and synthesize current research findings on the use of molecular biomarkers for estimating the postmortem interval (PMI), including proteins, metabolites, mRNA, microbiota, lipids, and multi-omics approaches. This review examines the types of samples utilized, postmortem changes in these biomarkers, analytical methods, their applicable timeframes, levels of accuracy, and the limitations associated with each biomarker. The findings are expected to provide a clearer overview of advancements in molecular biomarkers and contribute to the development of more accurate and objective PMI estimation methods suitable for use in forensic practice.

## METHOD

This study employs a structured narrative review methodology, encompassing the stages of literature search, selection, data extraction, and synthesis. A literature search was conducted across ScienceDirect, PubMed/MEDLINE, SpringerLink, MDPI, Frontiers, Nature Portfolio, eLife, and the International Journal of Legal Medicine, using combinations of the keywords “postmortem interval,” “PMI estimation,” “forensic biomarker,” “proteomics,” “metabolomics,” “microbiome succession,” and “mRNA degradation,” integrated with Boolean operators (AND/OR). The search covered literature published up to 2026, with a focus on research from the last 10 years. Inclusion criteria comprised peer-reviewed original research articles that address biochemical, molecular, proteomic, metabolomic, transcriptomic, or microbiomic biomarkers related to the postmortem interval (PMI); are available in full-text English; and provide quantitative data on biomarker changes and/or the accuracy of PMI estimation. Review articles lacking primary data, conference proceedings without complete data, and articles deemed irrelevant to the study’s objectives were excluded.

A total of 25 primary research articles involving animal and human subjects were selected, covering a post-mortem interval (PMI) ranging from a few hours to over 800 days. The extracted data include the types of biomolecules and samples, biomarker alterations, analytical methods,

estimation accuracy, findings, and study limitations. The data were then synthesized narratively based on the type of biomolecule (proteomics, metabolomics, transcriptomics, microbiomics, lipidomics, electrolytes, and multi-omics) as well as the early, intermediate, and late phases of the PMI.

## RESULT

The literature selection process yielded 25 articles that met the inclusion criteria. These studies employed various models, including animal subjects such as Sprague-Dawley rats, mice, pigs, and sheep as well as human samples. A diverse range of biological samples was utilized, encompassing skeletal muscle, blood or plasma, bone, vitreous and aqueous humor, pericardial fluid, cerebrospinal fluid, visceral organs, skin, gingival tissue, textiles, and even grave soil. The post-mortem interval (PMI) ranges examined were extensive, spanning from a few minutes after death to several decades, particularly in the case of bone samples.

Table 1.
*Characteristics of studies included in the narrative review (n = 25)*

| No. | Author (Year) | Sample | Biomolecule Category | PMI Range | Analytical Method |
|---|---|---|---|---|---|
| 1 | Jian Li, et al. (2024) | Skeletal muscle (SD rat) | Metabolomics, proteomics, FTIR, multi-omics | 0-30 days | UPLC-HRMS, protein chip, FTIR, stacking |
| 2 | Battistini, et al. (2023) | Skeletal muscle (pig) | Proteomics | 0-120 hours | LC-ESI-MS/MS |
| 3 | Zhang, et al. (2024) | Liver, spleen, plasma (rat) | Proteomics | 0-240 hours | Western blotting, Bradford |
| 4 | Wang, et al. (2024) | Pig rectum, rat intestines, soil | Microbiomics | 0-96 hours s.d. 30 days | 16S rRNA seq, RF, ANN, GB |
| 5 | Prieto-Bonete, et al. (2019) | Bone, vitreous, blood, muscle | Proteomics, biochemistry, metabolomics | Early PMI - 20 years | LC/MS-MS, imunoasai, HPLC-MS |
| 6 | Brockbals, et al. (2023) | Skeletal muscle (human) | Proteomics (peptides) | ±120 days | Untargeted LC-MS/MS (DIA) |
| 7 | Collins, Maestrini, et al. (2023) | Bone, textile, soil, nail/hair | Isotopes, lipids, microbiomics, metabolomics | >100 years | AMS, GC-MS, NGS |
| 8 | Pittner, et al. (2016–2017) | Skeletal muscles, bones | Proteomics, metabolomics, multi-omics | 0-5 days- late PMI | SDS-PAGE, Western blot, LC-MS/MS |
| 9 | Bonicelli, et al. (2022) | Tibial cortical bone (human) | Multi-omics (proteomics, metabolomics, lipidomics) | 0-872 days | LC-MS/MS, DIABLO |
| 10 | Zhao, et al. (2022) | Rat cecum, soil | Microbiomics | 0-59 days (± 800 ADD) | NGS, Random Forest, k-NN |
| 11 | Teixeira, et al. (2025) | Vitreous, blood, muscle, CSF | Metabolomics, biochemistry, proteomics, electrolytes | 0-10 days | Thanatochemistry |
| 12 | Mojsak, Samczuk, et al. (2025) | Porcine blood/plasma (±EDTA) | Metabolomics | 0-168 hours | GC-MS |
| 13 | Chiappetta, e al. (2025) | Mandibular bone (pig) | Metabolomics | 0-6 month | GC-MS, LC-MS/MS |
| 14 | Marrone, et al (2023) | Skeletal muscle (pig) | Proteomics | 0-24 hours | LC-MS/MS |
| 15 | Sampaio-Silva, et al. (2013) | Brain, heart, muscles, gums (rat/human) | Transcriptomics (mRNA/rRNA) | 0,5-312 hours | RT-qPCR |
| 16 | Marrone, et al. (2023) | Heart blood, muscle, bone (rat) | Metabolomics, proteomics | 0-872 days | UPLC-HRMS, GC-MS, LC-ESI-MS/MS |
| 17 | Szeremeta, et al. (2023) | Porcine plasma (±EDTA) | Metabolomics/lipidomics | 0-24 hours | LC-MS, LC-Q-TOF-MS |

| No. | Author (Year) | Sample | Biomolecule Category | PMI Range | Analytical Method |
|---|---|---|---|---|---|
| 18 | Metcalf, et al. (2013) | Soil/pig rectum, burial ground | Microbiomics, VOCs, metabolomics | 5-579 days | Indeks Shannon, RF, PLS-DA |
| 19 | Marrone, et al. (2023c) | Human skeletal muscle, liver, spleen | Proteomics, metabolomics | 6 hours to years | LC-ESI-MS/MS, LC-MS/MS |
| 20 | Pittner, et al. 2017 | Skeletal muscle, bones (human/pig) | Proteomics, multi-omics, miRNA | Late PMI | SDS-PAGE, integrasi omics |
| 21 | Locci, et al. (2021) | Aqueous humour (sheep) | Metabolomics, electrolytes | 2-24 hours | 1H NMR, Capillary Ion Analysis |
| 22 | Locci, et al. (2023) | Vitreous humour (sheep) | Metabolomics, inorganic ions | 6-86 hours | 1H NMR, CIA, PLS non-linier |
| 23 | Chighine, et al. (2023) | Pericardial fluid (human) | Metabolomics | 16-170 hours | 1H NMR, oCPLS2 |
| 24 | Chighine, et al. (2025) | Pericardial fluid (human) | Metabolomics | 16-199 hours | 1H NMR, oCPLS2 |
| 25 | Magnusson, et al. (2026) | Femoral blood, muscle, vitreous humor | Metabolomics, transcriptomics | 0-67 hours | LC-HRMS, Gradient Boosting, FFNN |

### *Proteomic Biomarkers*

Protein biomarkers exhibit a pattern of gradual degradation following death, with the onset and rate influenced by the type of protein, the tissue of origin, and temperature. In porcine skeletal muscle, ATP2A2 remains stable for up to 24 hours before declining; PDLIM7 undergoes gradual degradation from the outset, while TPM1 begins to decline after 72 hours at a temperature of 21°C (Battistini et al., 2023). Similar patterns are observed in desmin, troponin, and titin, with the latter experiencing 80% degradation within the first seven days (Pittner et al., 2016; Teixeira et al., 2025). In rat liver, β-actin expression and total protein content show a strong negative correlation with the post-mortem interval (PMI) ($R^2$ = 0.81-0.90), whereas GAPDH degrades very rapidly and becomes undetectable after 12 hours (Zhang et al., 2024). For longer PMIs, the peptide ratio in human muscle remains correlated with the PMI for up to approximately 120 days ($R^2$ = 0.50-0.63) (Brockbals et al., 2023). Meanwhile, in cases involving very long PMIs ranging from 1 to 37 years the non-collagenous bone protein CSPG2 ($rs = -0.68$; $p < 0.0001$), as well as proteins associated with bone mineralization (PGS2, COBA2, and MGP), exhibit a slower rate of degradation compared to proteins found in muscle tissue (Bonicelli et al., 2022; Sacco et al., 2022).

### *Biomarker Metabolomik*

Metabolomics represents the category of biomarkers with the largest number of candidates. In the skeletal muscle of rats, 131 metabolites underwent significant changes within the first five days post-mortem. A stacking model yielded a Mean Absolute Error (MAE) of 0.20 days for a post-mortem interval (PMI) range of 0-5 days, and 1.50 days for a range of 0-30 days (Jian Li et al., 2024). In sheep, the metabolomic profile of vitreous or aqueous humor demonstrated superior performance compared to using potassium as a standalone biomarker, with SDEP values of 99 minutes and 210 minutes, respectively, and RMSEP values of 8.5 hours and 11.5 hours (Locci et al., 2021, 2023). In human pericardial fluid, choline, glycine, hypoxanthine, and formate showed a significant correlation with a PMI of 16-170 hours, with an SDEP of 13-15 hours for PMIs under 100 hours (Chighine et al., 2023). Subsequent research expanded this panel to eight or nine key biomarkers, achieving a minimum SDEP of 16.7 hours (Chighine et al., 2025). In porcine plasma, alanine yielded the lowest total prediction error, with a Total Mean Absolute Error (TMAE) of 29.29 hours, although its utility is influenced by the presence of EDTA (Mojsak, Samczuk et al., 2025). Meanwhile, in porcine mandibular bone with a PMI range of 0-6 months, analysis using LC-MS/MS produced an optimal MAE of 0.48 months (Chiappetta et al., 2025), whereas in human blood, a neural network model yielded an MAE of 1.45 days for a PMI range of 1-67 days (Magnusson et al., 2026).

***Transcriptomic Biomarkers (mRNA and Non-Coding RNA)***

The degradation of housekeeping gene transcripts, such as β-actin and GAPDH, is widely used to estimate the post-mortem interval (PMI) during the early to intermediate phases. In mouse muscle and liver tissues, the decline in transcript abundance correlates with a PMI of 1-10 hours, with an RMSE of approximately 51.4 minutes (Sampaio-Silva et al., 2013). In brain tissue, the estimation error rate in humans is higher ranging from 24.6% to 41.0% compared to 4.8% in mice, highlighting interspecies differences (Lü et al., 2016). The combination of β-actin2/GAPDH2 is better suited for estimating an early PMI, whereas 18S rRNA and U6 snRNA can be used for up to 312 hours, although error rates increase at lower storage temperatures. Some biomarkers exhibit patterns of change that do not follow a simple, gradual decline. For instance, Cdc25b mRNA in mouse hearts decreases initially before increasing again, with an error rate of less than 15% for a PMI of 0-36 hours; meanwhile, HIF-1α mRNA in human gingival tissue increases up to the fifth day before becoming undetectable by the sixth day. Furthermore, microRNAs have been reported to be more resistant to degradation than conventional mRNAs, though their application for PMI estimation remains in the research stage.

***Microbiome Biomarkers***

Microbiomic methods show promising results for estimating the post-mortem interval (PMI), especially when combined with machine learning techniques. Actinobacteria serve as a key indicator, yielding a mean absolute error (MAE) of 6.93 ± 1.19 hours with an $R^2$ of 98.76% when analyzed using a Random Forest model (Wang et al., 2024). In the cecal microbiota of rats, an artificial neural network model produced an MAE of 1.5 ± 0.8 hours within the first 24 hours, which increased to 14.5 ± 4.4 hours after 15 days (Liu et al., 2020). Changes in microbial communities within the gut and anus of rats are influenced by both anatomical location and exposure to exogenous chemical substances. Under these conditions, a gradient boosting model provided the most accurate estimates, with an RMSE of 5.6 hours and an $R^2$ of 0.954 for a PMI range of 0-96 hours (Ren et al., 2026). For longer PMI ranges, a Random Forest model applied to cecal microbiota achieved an overall accuracy of 90.48%, with an MAE of 0.580 days during the first seven days, increasing to 3.165 days for a PMI of 9-30 days (Li et al., 2023). A decline in accuracy as the PMI increases has also been observed in other studies (Cao et al., 2021; Cartozzo et al., 2021). Furthermore, the "microbial clock" concept applied to microbial communities on the skin and within body cavities yielded an MAE of 3.30 ± 2.52 days (Metcalf et al., 2013), while microbiota from the nasal and ear cavities resulted in an MAE of approximately 55 accumulated degree-days (ADD) over a range of up to 800 ADD (Johnson et al., 2016).

***Biomarker Lipid, Senyawa Organik Volatil, dan Elektrolit/Ion***

Lipid analysis of textiles from funerary garments has identified 30 types of lipids that correlate with accumulated degree-days (ADD) over a range of 0-105 days (Collins, Maestrini, et al., 2023). The abundance of volatile organic compounds (ethanol, acetone, dimethyl disulfide, and toluene) increases with the post-mortem interval (PMI) (Statheropoulos et al., 2007), while putrescine and cadaverine levels in rat muscle show a gradual increase with putrescine proving to be a more accurate indicator than cadaverine (Teixeira et al., 2025). Although potassium remains the most extensively studied classical electrolyte biomarker, it is consistently less accurate than global metabolomic profiling within the same matrix, particularly for PMIs of less than 24 hours (Locci et al., 2021, 2023). In porcine plasma, hypoxanthine, lactate, histidine, lysophosphatidate (LPA), and lysophosphatidylcholine (LPC) levels increase within the 0-24 hour window; among these, LPA is considered particularly promising as it does not require the use of an anticoagulant (Szeremeta et al., 2023).

### *Multi-Omics Approaches and Machine Learning*

The integration of various types of biomolecules through a multi-omics approach consistently outperforms single-omics approaches. A "Multi-omics Stacking Model" combining metabolomic, proteomic, and FTIR spectroscopic data achieved a Mean Absolute Error (MAE) of 0.07 days (with an accuracy of 0.93 and an AUROC of 0.98), significantly surpassing the performance of single-omics platforms (which yielded an MAE of 0.71-3.14 days) (Jian Li et al., 2024). In human bone samples with a very advanced post-mortem interval (PMI) (219-872 days), the integration of proteomic, metabolomic, and lipidomic data (using the DIABLO approach) revealed that acetamide showed the strongest positive correlation with PMI (Bonicelli et al., 2022). Furthermore, a multi-organ (muscle, liver, lung, kidney) ensemble stacking model demonstrated superior performance ($R^2 \approx 1$) compared to single-organ models for the 0-30 day interval (Lu X. et al., 2023) a finding consistent with the integration of machine learning applied to microbiomic and transcriptomic data by Ren et al. (2026) and Magnusson et al. (2026). These findings underscore a clear pattern: increasing the number and diversity of integrated biomarkers is directly proportional to a reduction in the margin of error for PMI estimation, albeit at the cost of increased computational complexity.

## DISCUSSION

### *Biomarker Kinetic Patterns Based on Postmortem Time Intervals*

The findings of this study indicate that no single type of biomarker is optimal for the entire post-mortem interval (PMI) range; rather, each category of biomolecule possesses a relatively specific "window of applicability." In the very early PMI (<24 hours), electrolyte biomarkers from vitreous or aqueous humor, as well as labile mRNA transcripts such as Cdc25b, exhibit rapid changes though these are sensitive to temperature and individual variability (Locci et al., 2021; Tao L. et al., 2018). For intermediate PMIs (ranging from days to weeks), the degradation of structural muscle proteins and the metabolomic profiles of body fluids prove more informative; this is reflected in the high accuracy of metabolomic models for muscle tissue (MAE 0.20-1.50 days) and pericardial fluid (SDEP 13-17 hours within <100 hours post-mortem). In cases involving advanced PMIs extending even to years only biomarkers inherently stable within hard tissues (such as non-collagenous proteins and bone lipids) remain reliable, as most soft-tissue biomolecules have undergone complete degradation (Bonicelli et al., 2022). This tiered pattern reinforces the argument that conventional methods share similar limitations: they are accurate during the early phase but lose precision as the PMI progresses into intermediate or advanced stages, thereby underscoring the need for a combination of complementary biomarkers tailored to the specific context of each case.

### *Comparison of Accuracy Across Biomarker Categories*

Cross-category comparisons indicate that metabolomics and microbiomics consistently yield the smallest margins of error when analyzed using machine learning models; some studies have reported a mean absolute error (MAE) of less than one day, even for post-mortem intervals (PMI) spanning several tens of days (Jian Li et al., 2024; Li et al., 2023; Magnusson et al., 2026). Potassium historically favored due to its ease of measurement and low analytical cost has repeatedly demonstrated inferior predictive performance compared to global metabolomic profiles (Locci et al., 2021, 2023), suggesting that its use as a standalone marker warrants reconsideration. Proteomic and transcriptomic biomarkers in soft tissues exhibit greater variability in accuracy across studies, partly due to differences in model species, ambient temperatures, and quantification methods as evidenced by the stark contrast in estimation error rates for brain mRNA between mouse models (4.8%) and humans (24.6–41.0%) (Lü et al., 2016).

### *The Role of Multi-Omics Integration and Artificial Intelligence*

One of the most consistent findings in this field is the superiority of multi-omics approaches combined with machine learning over conventional regression-based single-biomarker models. The stacking model developed by Jian Li et al. (2024) achieved a mean absolute error (MAE) of 0.07

days more than ten times more accurate than the best single-metabolomics model on the same dataset. Similar patterns have been observed in the integration of bone-derived data (Bonicelli et al., 2022) and multi-organ data (Lu X. et al., 2023). This advantage is mechanistically sound, as each category of biomolecule possesses distinct degradation kinetics and is influenced by confounding factors that do not entirely overlap; consequently, integrating data allows the models to compensate for the inherent limitations of any single analytical platform. Furthermore, decision tree-based algorithms such as Random Forest and Gradient Boosting have proven superior to linear regression when applied to high-dimensional, non-linear microbiome data (Wang et al., 2024; Ren et al., 2026). This aligns with the broader trend of leveraging artificial intelligence to capture complex patterns within forensic metabolomics and proteomics.

### *Methodological Limitations in the Reviewed Literature*

Despite promising results, most of the studies reviewed have significant methodological limitations. First, the majority of the research (19 out of 27 studies) utilized animal models; therefore, generalizing these findings to humans requires caution, as differences in metabolic rates and burial environmental conditions can significantly influence the degradation kinetics of biomarkers. Second, the sample sizes in many of the studies were small (n = 2 to n = 24), including those in human studies that are the most translationally relevant (Chighine et al., 2023; Brockbals et al., 2023; Bonicelli et al., 2022), which risks reducing the generalizability and statistical power of the model. Third, the variability of environmental conditions particularly temperature has been repeatedly reported as a major confounding factor that alters degradation kinetics, affecting protein biomarkers (Battistini et al., 2023), RNA (Lv Y. et al., 2014), and microbiota (Chen et al., 2025); however, not all studies consistently document storage conditions. Fourth, there is a lack of standardized pre-analytical protocols across blood metabolomics studies as evidenced by the significant impact of using EDTA on the accuracy of alanine and phosphate biomarkers (Mojsak, Samczuk et al., 2025) which complicates direct comparisons between different research studies.

### *Implications for Forensic Practice and Future Research Directions*

In practical terms, the most promising strategy for estimating the post-mortem interval (PMI) in future forensic practice does not rely on a single type of biomarker, but rather on an integrated panel of biomarkers tailored to the estimated time since death, the type of available samples, and the analytical facilities at the forensic laboratory's disposal. For cases with an indication of an early post-mortem interval (PMI), a combination of ocular or pericardial fluid metabolomics and electrolyte biomarkers appears most promising; for an intermediate PMI, a skeletal muscle proteomic panel or an analysis of the gut/skin microbiome may be considered; whereas for cases involving skeletal remains or a very advanced PMI, a bone-based multi-omics approach becomes a more realistic option. Validation studies involving larger human population samples and more diverse environmental conditions are urgently needed, alongside the standardization of pre-analytical protocols and the development of open-access forensic multi-omics databases. These efforts are essential to accelerate the creation of artificial intelligence-based PMI prediction models that are cross-validated and ready for application in medicolegal practice.

## CONCLUSION

A narrative review of 25 primary studies indicates that no single type of molecular biomarker is optimal for estimating the post-mortem interval (PMI) across the entire post-mortem period. Each category of biomolecule has a relatively specific window of utility: electrolyte biomarkers and labile mRNA transcripts are most informative during the very early PMI (<24 hours); the degradation of structural muscle proteins and the metabolomic profiles of body fluids are superior for intermediate PMI (ranging from days to weeks); while non-collagenous proteins and bone lipids remain reliable for advanced PMI, extending up to years. Across all categories, metabolomic and microbiomic approaches analyzed using machine learning algorithms consistently yield the smallest

margins of estimation error. Furthermore, multi-omics integration has repeatedly proven superior to single-biomarker approaches, with some models achieving a mean absolute error of less than one day. Nevertheless, the available evidence still carries significant methodological limitations a predominance of studies based on animal models, generally small human sample sizes, high variability in environmental conditions affecting biomarker degradation kinetics, and a lack of standardized pre-analytical protocols across laboratories. Consequently, the generalization of findings to human populations and their application in medicolegal practice must be approached with caution.

## DECLARATIONS

**Acknowledgment:** The author expresses gratitude to the academic supervisor and all faculty members of the Master's Program in Forensic Science at the Graduate School, Universitas Airlangga, for their guidance, direction, and scholarly input throughout the preparation of this study. The author also thanks the researchers whose work served as the foundation for this review, providing a scientific basis for the development of molecular biomarkers for the estimation of the post-mortem interval. Sincere thanks are also extended to family and fellow students for their support, prayers, and encouragement, which enabled the completion of this manuscript.

**Conflicts of interest:** The authors declare no conflict of interest, whether financial or personal, that could have influenced the preparation, interpretation, or publication of this article.

**Ethical approval:** Ethical approval was not required for this study because it was a narrative review based exclusively on previously published literature. No human participants, animals, or identifiable personal data were directly involved in this review.

**Financial support and sponsorship:** This study received no specific grant or financial support from any funding agency in the public, commercial, or not-for-profit sectors.

**Data Availability Statement:** Data sharing is not applicable to this article because no new datasets were generated during the current study. The information analyzed in this narrative review was obtained from previously published articles, which are cited in the reference list.

**Author Contributions:** Syukriabulan was responsible for the conceptualization, literature search and selection, data extraction, analysis and interpretation of the findings, and drafting of the original manuscript. Wimbuh Tri Widodo contributed to the conceptualization, supervision, critical review, and revision of the manuscript. Both authors reviewed and approved the final version of the manuscript.

**Declaration of the Use of Artificial Intelligence in Scientific Writing:** Artificial intelligence (AI)-based tools, including Claude AI, were used to assist with language refinement, paraphrasing, and improving the clarity and readability of the manuscript. These tools were not used to generate research data or determine the scientific findings. The author reviewed, verified, and takes full responsibility for the accuracy, originality, and final content of the manuscript.

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