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Looking for Post-Mortem Metabolomic Standardization: Waiting for Godot—The Importance of Post-Mortem Interval in Forensic Metabolomics

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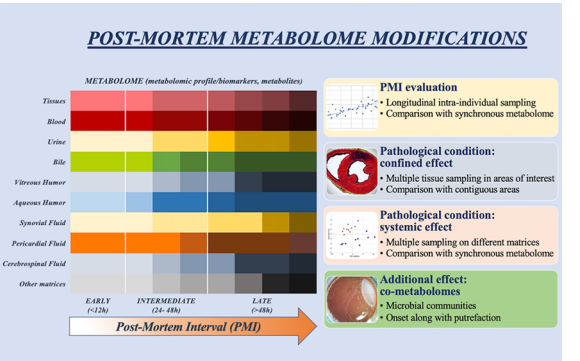
ABSTRACT: A growing body of evidence suggests that the post-mortem interval exerts a strong effect on the metabolome, independently of the biological matrix or the cause of death. A sound and shared approach in standardization is mandatory.

Scientific literature provides evidence concerning death as a dynamic event more than a static one. What was traditionally considered as an on/off event represents the result of multiple, ongoing, and interlinked biological phenomena.[1] Post-mortem modifications are the result of complex endogenous (i.e., pathological or physiological conditions, autolysis) and exogenous (i.e., microbiome) processes. What is more, the variability can be related to both in-life and post-mortem periods. Amid such confounding factors, something emerges as a milestone and appears to act independently: elapsing time. The recent literature shows that multiparametric approaches such as metabolomics, which relies on the study of the global biofluid or tissue metabolome, appear to be more promising than methods based on the study of a single or few parameters to intercept the complex phenomenon of death and to perform a reliable post-mortem interval (PMI) estimation.

In the last years, metabolomics has been increasingly used in the forensic scenario to investigate both the cause of death and the PMI. In designing a forensic experimental investigation, PMI could be partially ignored whenever dealing with a cause of death reasonably characterized by a metabolic effect confined mainly to a single organ such as heart and brain damage secondary to cardiac arrest.[2] In this setting, it may be possible to distinguish lesion-related profiles from the ones induced by PMI, directly comparing the damaged tissue(s) and the physiological control represented by contiguous, although preserved, areas. On the other hand, considering pathologies that can rationally exert a systemic effect on the metabolome, such as pneumonia,[3] PMI evaluation becomes a pivotal factor to be taken into account. To clarify, if the post-mortem metabolomic profile modifications are, or not, the effect of a pathological condition, the analysis should be necessarily performed challenging them with a well-established PMI control group. In such a way, it would be possible to rule out modifications directly induced by the PMI, which, of great note, are far from negligible.

Our research group has recently reviewed the extant literature highlighting past, present, and future potential forensic applications of ^{1}H nuclear magnetic resonance (NMR) metabolomics including PMI estimation.[4] A section was totally devoted to the evidence of post-mortem modifications on several biological fluids (namely aqueous humor, vitreous humor, serum, and urine) and tissues (namely muscle, cornea, heart, liver, kidney, spleen, skin, and adipose tissue) on both animal and human models. A list of the more promising metabolites, whose modifications showed a correlation with PMI, was also provided. Despite our main focus on NMR metabolomic applications, several studies employed a dual analytical approach using both NMR and mass spectrometry.



We recently described the prominent effect of PMI on the post-mortem metabolome in an animal model of aqueous humor.[5] Using a ^{1}H NMR metabolomic approach, a robust multivariate calibration model for PMI estimation was built, offering major hints on the relationship between post-mortem phenomena and metabolome. The highlighted post-mortem

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modifications exerted a stronger effect on the metabolome compared to the variable introduced in the experimental protocol, that is, the alternative withdrawal of aqueous humor from opened and closed eyes. At higher PMIs (>1000 min), it was also possible to detect metabolites, such as acetate and dimethylsulfone, which supported the hypothesis of an overlapping contribution of the microbial with the endogenous metabolome, consistent with the classical macroscopical finding of post-mortem transformation. PMI estimation univariate models based on the single metabolite were also built and, of great note, even the metabolites associated with the best error in prediction showed a lower accuracy than the whole metabolomic profile, underlining a crucial post-mortem effect on the metabolome.

The results were also challenged with the experimental gold standard for PMI estimation on ocular matrices, namely potassium concentration. Intriguingly, the results suggested that a part of the metabolomic profile can explain most of the statistical, and biological, information carried by potassium, indicating a shared post-mortem mechanism. Despite that, the metabolomic profile showed a comparable, if not greater, predictive power than potassium concentration in estimating PMI.

When dealing with post-mortem metabolomics, particular attention should be devoted to the biological matrix on which the experiment will be conducted. As classical pathological knowledge may suggest, certain biological matrices (i.e., blood) are more prone than others to undergo transformation (autolysis) and then putrefaction, while others, like ocular matrices, in consideration of the anatomical and physiological isolation of the eye, appear ideal to intercept the complex modifications occurring after death. At the same time, metabolomic post-mortem modifications have been detected in several biofluids and tissues in both animal and human studies supporting the potential use of this approach in forensic routine.[4]

In the upcoming years, metabolomics will take over in forensic as it already did in clinical medicine. To make it fully applicable to the rigorous criteria required in the forensic setting, a huge effort by the scientific community is needed. As, from a metabolomic perspective, death-related modifications are preponderant compared to any other underlying biological phenomenon, a multicentric, international collaboration is mandatory to fully investigate on a large-scale a specific condition at specific PMIs on specific biological matrices. These results should be implemented in the current available metabolomic databases as for all the other relevant pathological conditions. Two main issues regarding post-mortem metabolome should be addressed: the qualitative and quantitative contribution of microbial cometabolomes and specific time window in which the single metabolite can be detected. A call to action would be advisable.

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Notes

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REFERENCES

(1) Ferreira, P. G., Muñoz-Aguirre, M., Reverter, F., Sá Godinho, C. P., Sousa, A., Amadoz, A., Sodaei, R., Hidalgo, M. R., Pervouchine, D., Carbonell-Caballero, J., Nurtdinov, R., Breschi, A., Amador, R., Oliveira, P., Çubuk, C., Curado, J., Aguet, F., Oliveira, C., Dopazo, J., Sammeth, M., Ardlie, K. G., and Guigó, R. (2018) The effects of death and post-mortem cold ischemia on human tissue transcriptomes. *Nat. Commun. 9*, 490.

(2) Locci, E., Chighine, A., Noto, A., Ferino, G., Baldi, A., Varvarousis, D., Xanthos, T., De-Giorgio, F., Stocchero, M., and d'Aloja, E. (2021) Metabolomics improves the histopathological diagnosis of asphyxial deaths: an animal proof-of-concept model. *Sci. Rep. 11*, 10102.

(3) Elmsjö, A., Vikingsson, S., Söderberg, C., Kugelberg, F. C., and Green, H. (2021) Post-Mortem Metabolomics: A Novel Approach in Clinical Biomarker Discovery and a Potential Tool in Death Investigations. *Chem. Res. Toxicol. 34*, 1496.

(4) Locci, E., Bazzano, G., Chighine, A., Locco, F., Ferraro, E., Demontis, R., and d'Aloja, E. (2020) Forensic NMR metabolomics: one more arrow in the quiver. *Metabolomics 16*, 118.

(5) Locci, E., Stocchero, M., Gottardo, R., De-Giorgio, F., Demontis, R., Nioi, M., Chighine, A., Tagliaro, F., and d'Aloja, E. (2021) Comparative use of aqueous humour ^{1}H NMR metabolomics and potassium concentration for PMI estimation in an animal model. *Int. J. Legal Med. 135*, 845−852.