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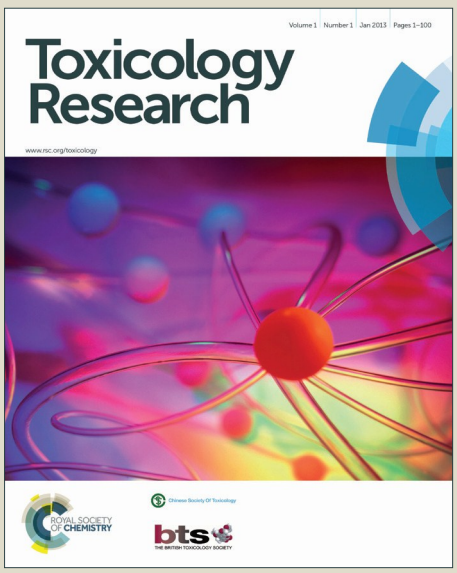

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RESPONSE TO THE COMMENT ON **"Promising blood-derived biomarkers for estimation of the postmortem interval''** *by Joris Meurs, Katarzyna M. Szykuła*

Running Head: Estimation of *postmortem* interval

Authors' names and institutional addresses:
Ricardo Jorge Dinis-Oliveira[1,2,3]*, Félix Carvalho[2], Isabel Costa[1,3], Ricardo Silvestre[4,5], Teresa Magalhães[1,3]

[1]IINFACTS - Institute of Research and Advanced Training in Health Sciences and Technologies, Department of Sciences, University Institute of Health Sciences (IUCS-CESPU), Gandra, Portugal.
[2]UCIBIO-REQUI*M*TE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal.
[3]Department of Legal Medicine and Forensic Sciences, Faculty of Medicine, University of Porto, Porto, Portugal.
[4]Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal.
[5]ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal.

*Corresponding author:
Ricardo Jorge Dinis-Oliveira: ricardinis@med.up.pt
Faculty of Medicine, University of Porto,
Alameda Prof. Hernâni Monteiro,
4200-319 Porto, Portugal
Phone: 00351 225513600

ABSTRACT

In sequence of Meurs and Szykuła comment on our published article entitled **"Promising blood-derived biomarkers for estimation of the *postmortem* interval''** [1], we recognize the importance of the raised issues, but would like to emphasize that these contain some misinterpretations and that most of the points were already discussed in depth in our manuscript specially in the conclusion section. We also aim to highlight further data regarding the difficulties of *postmortem* interval estimation.

INTRODUCTION

A precise estimation of the *postmortem* interval (PMI) is a challenging task and represents one of the biggest concerns in practical casework routine in forensic pathology[2]. *Algor mortis*, *livor mortis*, *rigor mortis*, metabolic alterations (*e.g.*, alterations of metabolites, substrates and enzymes activities), autolysis, physiochemical and bacterial processes comprise the main basis for PMI estimation, especially during the first hours after death. However, these methods present obvious limitations for PMI estimation due to the influence of several variables [2]. Nevertheless, over the last few decades, an important progress in PMI estimation has been made using biochemical parameters, which have been supported by different statistical approaches [3,4]. Some of these methods have been shown to be more accurate in PMI estimation than the traditional methods. Nevertheless, in spite of the extensive literature, most methods failed to enter in forensic routine, since the majority focuses on a single parameter or analyte. Moreover, most methods proposed for PMI estimation are only of academic interest since they merely describe *postmortem* alterations. To circumvent these constraints, combination of several parameters have been proposed to increase the accuracy of PMI estimations [5].

In our study, we quantified the kinetic alterations of 46 biochemical parameters in human *antemortem* blood submitted to a temperature gradual decrease from 37 to 21ºC, mimicking the decline of body temperature after death [6]. Substrates, proteins, enzymes, electrolytes and lipids were targeted, since they may be useful for an accurate PMI estimation. Our data evidenced a significant linear correlation between total and direct bilirubin, urea, uric acid, transferrin, immunoglobulin M (IgM), creatine kinase (CK), aspartate transaminase (AST), calcium and iron with the time of blood putrefaction. These ten parameters allowed us to develop two mathematical models that may have predictive value and become important complementary tools of traditional methods to achieve a more accurate PMI estimation. Moreover, through multiple biochemical parameters, our approach definitely increases the reliability and self-confidence of the researcher in estimating PMI.

SPECIFIC RESPONSE TO RAISED COMMENTS

In their first comment, the authors evoke conflicting results with literature in some evaluated biochemical parameters. Regarding creatine kinase MB, the authors mentioned that we found correlation coefficient above 0.900, while the literature presented intervals from 0.266 to 0.304 [7]. This is inaccurate since our results found linear correlations for CK and not CK-MB, which is another isoform. The authors did not attend to this specific difference. Moreover, the authors claim that our results for urea are much different from recent published data [8]. The study of Palmiere & Mangin [8] quantified urea in *postmortem* serum, vitreous humor, and pericardial fluid for 48h after death and found relatively stable concentrations during this period. Naturally, this fact is highly probable since in this short *postmortem* period, glucose is the main source of energy by anaerobic metabolism. PMI cases beyond 48h were rejected from analysis as mentioned by authors. We performed the *in vitro* study for 11 days. Indeed, during the first 48h we registered very stable values for urea (supplementary Fig. S1; comparable to the Palmiere & Mangin study [8]) but after that time point we found a significant increase of urea concentration. Moreover, stability for biochemical parameters is not to be expected, since nearly all parameters are more or less prone to changing with PMI. In our study, we aimed to determine those analytes from which we can predict their kinetic behavior with PMI and being less affected by internal (or intrinsic) and external (or extrinsic) *antemortem* and *postmortem* variables that can influence PMI estimation.

Concerning the comments that other factors besides temperature can influence the PMI estimation, such as gender, age, cause of death, etc., this issue was already thoroughly discussed in our manuscript. In fact, it is impossible to attain all potential variables in one study and therefore it is quite obvious that further studies are required to assess their influence, which are ongoing. Regarding their comment on pH, we observed a slightly decrease compared to initial values (pH 7.45 to 7.10) and then increased to 7.74 until the end of our study. Curiously, very comparable results were previously reported, since after a decrease, pH starts to rise after approximately four days [9]. The authors closed the second point suggesting that "differences between *in situ* putrefaction and *in vivo* putrefaction are missing", which is an awkward observation.

Finally, as we mentioned before, the anatomic sampling place must be carefully selected due to *postmortem* redistribution. Previously, for toxicological analysis, femoral blood was suggested to be the less affected blood anatomic place [10]. Therefore, we recommend obtaining blood from femoral vein or artery for PMI estimation.

Moreover, the time lag to have an acceptable blood sample will depend on the PMI conditions that may accelerate or reduced the extent of putrefactive changes. Since these alterations are reduced in lower limbs, this may increase the probability to work with a relatively "good sample". Therefore, a clear definition of the sampling site is mandatory. This implies that PMI should be better studied with longitudinal studies.

CONCLUSIONS

The postulate that *postmortem* studies will be much more reliable if tested directly in human samples, is an obvious assumption. Nevertheless, due to law restrictions, *in vitro* studies represent a very useful approach to vector potential biomarkers. Although every considerations made by the authors of this comment are very relevant, they evoke several topics that were already thoroughly discussed in our paper. Finally, selection to literature must be rigorous since comparison with Palmiere & Mangin data can only be applied to our first 48h of blood putrefaction.

Our *in vitro* results were confronted with documented alterations in cadaver where strong similarities were registered, meaning that our basic research approach was useful to select potential biochemical parameters that were then used to develop two mathematical models. However, these models are certainly influenced by several variables such as age, gender, drug administration, cause of death, body mass, duration of agonal state and hypothermia, environment temperature, wind, humidity, rain, clothing, location of the body and insect or animal activity that were previously discussed [1]. These parameters, together with interindividual variability, influence the starting point and slope of the curves and prevents the direct translation to practice. This implies that only an interval can be estimated and not a time point. In addition, forensic researchers must be aware that in death investigation, PMI estimation represents just a single piece of the puzzle and it is not an exact science.

We agree and stress that further studies are required to increase the soundness of our results. We also believe that to acquire practical application, any method for PMI estimation should include, besides quantitative experimental measurements and robust statistical background, the study of the influence of internal and external variables. Progress in this field will certainly require long-lasting research and not a single paper will define the end. As mentioned by other authors, years of research are needed to

validate the accuracy of the method in the forensic field [2]. This represents the major difficulty in solving PMI estimation, which can only be possible through an increased interaction of research groups with forensic and non-forensic expertise [11].

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