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Evaluation of postmortem biochemical markers: Completeness of data and assessment of implication in the field

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ABSTRACT

Throughout the years an increase has been observed in research output on biochemical markers for determining the postmortem interval (PMI). However, to date, a complete overview is missing on the results of postmortem biochemical markers (PBM's) for PMI estimation. In this paper, literature was reviewed in order to identify the knowledge lacunae of PBM research from a practical point of view. A three-step approach was undertaken in order to achieve the set goal. Literature was collected, the PBM's were evaluated for completeness by means of a scorings index based on set criteria, and PBM's were subsequently evaluated in light of the *Daubert* & *Frye* criteria for scientific evidence in court. Seven PBM's were found to be well investigated, from which potassium had the highest completion score. However, none of these PBM's could be qualified as suitable for court evidence. Further, this study revealed that the majority of PBM's (94%) is not well investigated. Consequently, these PBM's did not meet *Daubert* & *Frye* criteria. In order to improve the assessment for use of PBM's as evidence in court regarding PMI estimation, PBM's should be investigated more thoroughly and data should be made readily available.

1. Introduction

The postmortem interval (PMI) is defined as the elapsed time between death and investigation of the body. This interval can provide valuable information for legal purposes. Several approaches exist for estimating the PMI, i.e. (but not limited to) entomological development [1], cooling models [2] and biochemical [3]. The latter approach concerns postmortem biochemical markers (PBM's) in tissue or body fluids that change over time and correlate with the PMI. Several methods have been proposed for estimating the PMI using PBM's, though, none has been implemented due to lack of practical relevance [4,5].

Postmortem biochemistry, also known as thanatochemistry [4], is a growing field of research. Also, a number of literature reviews on PBM's for PMI estimation have been published. For instance, Öhmichen focused on postmortem alterations of brain enzymes [6]. Later, Coe created an overview for numerous PBM's [7]. More recent, Donaldson & Lamont created an updated overview on PBM research [8].

In order to improve PBM research, Henssge & Madea, Madea & Musshoff and Zhou & Byard denoted that age, gender, biological background, lifestyle, cause of death, use of medication, degree of autolysis and putrefaction, temperature and other environmental conditions, and the postmortem interval should be taken in to account when investigating a PBM [3,9,10]. Besides these in- and extrinsic factors, also other variables should be taken into account based on current literature, namely location on the body from which the sample is taken, type of sample (matrix), actual change in the postmortem value (statistical significance) over the PMI, strength of correlation between the measured postmortem value of a PBM and the PMI, survival time, intra- and inter-individual difference in postmortem values, the used analytical methodology, and the sample size (N) [11–17].

Only if the influence of these variables is known, a PBM can be considered to be completely investigated. Besides the completeness of research in to a PBM, it is essential to understand if a marker shows practical potential to be used as evidence in court. In the United States, admissibility of scientific evidence is tested by using either *Frye* or *Daubert* standards [18,19]. The *Frye* standard is based on general acceptance of a technique/method within related scientific fields [19–21]. The *Daubert* standard prescribes a preliminary assessment of the evidence in order to ensure its validity and reliability [22]. In order

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to make this decision, a method has to be empirically tested, peer review published, have acceptable error rates, and generally accepted within related scientific fields [21,22].

To the best of our knowledge, PBM's have never been screened for their potential use to serve as evidence in court, whilst case examples show the use of PBM's for PMI estimation in legal cases [23]. Here, we revisited peer review published research on PBM's for PMI estimation and collected data using set criteria. Based on the collected data, PBM's were scored for their completeness of research on the above stated parameters and were tested against *Frye's* & *Daubert's* criteria to assess the potential for practical use for PMI estimation.

2. Materials & methods

2.1. Collecting literature & postmortem data

Literature was collected using the keywords '*postmortem interval estimation*' in *PubMed* and *Google Scholar*. Additional literature was obtained using reference lists in articles gathered in the primary collection. Literature was included when describing biochemical markers for postmortem interval estimation or describing the influencing factors on PBM values, written in English, and published in or prior to 2016 ($n = 303$). Articles describing in situ methods for postmortem interval estimation ($n = 6$) or animal studies ($n = 55$) were excluded. Data was collected from the included literature using the categories listed in Table 1, and has been added as supplement to this paper (Supplementary File 1).

2.2. Completion score

The data in Supplementary File 1 was used to evaluate the completeness of a PBM. The evaluation was carried out as follows; a score was assigned based on the total data provided (based on all articles) for a certain marker. The scoring index is outlined in Table 1. The minimum and maximum score a PBM could obtain were respectively zero and 17, which corresponds to completely uninvestigated and completely investigated.

2.3. Assessment of postmortem biochemical markers for use in practice

In order to assess biochemical markers for their practical suitability two criteria were set:

- The value of a PBM has to change significantly ($p < .05$) over the investigated PMI.
- The PMB has to meet the *Daubert's* & *Frye's* criteria.

Table 1
Scoring method for determining the research completion of a PBM.

Category	Score	Comment
Postmortem alteration	Not presented = 0 Presented = 1	Significance should be mentioned in text, graph or table
Intrinsic factors	Range: 0–11	No internal factors investigated = 0 +1 for every investigated factor Included parameters: age, gender, biological background, lifestyle, cause of death, medication, autolysis, putrefaction, disease, body condition, survival time Should be mentioned in text, graph or table
Extrinsic factors	Range: 0–2	No internal factors investigated = 0 +1 for every investigated factor Included parameters: temperature, environmental conditions Should be mentioned in text, graph or table
Coefficient of determination (R^2)	Not presented = 0 Presented = 1	
Postmortem values	Not presented = 0 Presented = 1	Values should be presented as mean value including standard deviation for at least one time point/time interval or as mean value including standard deviation over the total investigated postmortem interval
Error of the PMI estimation	Not presented = 0 Presented = 1	

Table 2
Grouping of postmortem biochemical markers based on completion score and assessment results.

Group	Requirements
Well investigated and practically applicable	Completion score ≥ 9 PBM meets *Daubert's* & *Frye's* standard Studied in at least two articles Significant change ($p < .05$) of PBM over investigated PMI[a] No conflicting results between research articles
Well investigated, not practical	Completion score ≥ 9 PBM does not meet *Daubert's* & *Frye's* standard Studied in at least two articles No significant change of PBM over investigated PMI[a] Conflicting results between articles[b]
Poorly investigated, practical	Completion score < 9 PBM meets *Daubert's* & *Frye's* standard Studied in at least two article Significant change (p < .05) of PBM of investigated PMI[a] No conflicting results between research articles
Poorly investigated, not practical	Completion score < 9 PBM does not meet *Daubert's* & *Frye's* standard Studied in at least two article No significant change of PBM over investigated PMI[a] Conflicting results between articles[b]
Unknown	Not enough information provided to assign PBM to group 1–4

[a] Minimum of two articles should support finding.
[b] Minimum of two articles should show conflicting results.

Based on the completion score of a PBM in a specific matrix and the results of the above outlined assessment PBMs were divided into five groups. The method of group division is outlined in Table 2.

3. Results

3.1. Completion score

In total, 388 PBM's were scored for research completion (Supplementary File 2). The average completion score was 3.0 out of 17 (SD: ± 2.2). From all included PBM's, potassium showed the highest completion score, namely 12. All well investigated PBM's (i.e. completion score ≥ 9; $n = 7$) are shown in Table 3.

Table 3
Overview of well investigated postmortem biochemical (completion score ≥ 9).

Rank	Marker	Completion score
1	Potassium	12
2	Sodium	11
3	Urea	10
4	Chloride, Magnesium, Hypoxanthine, CTnT	9

Table 4
Classification of postmortem biochemical markers (N = 388) according to research completeness and potential for practical use.

Group	Number of postmortem biochemical markers
Well investigated and practically applicable	0
Well investigated, not practical	6
Poorly investigated, practical	0
Poorly investigated, not practical	18
Unknown	364

3.2. Assessment of postmortem biochemical markers for use in practice

After scoring, the PBM's were assessed for their potential to be used in practice for estimating the postmortem interval (Table 4). None of the included PBM's showed to be ready for practical use. Furthermore, 24 PBM's were found not to be suitable for PMI estimation based on the provided information in the research articles. In most cases, the significant change of PBM over the investigated PMI is conflicting between research articles. Further, no PBM's were identified as having potential for PMI estimation based on the set criteria. The majority of PBM's (94%) were classified as ‘unknown’ due to a lack of information. Classification of all markers can be found in Supplementary File 2.

4. Discussion

The aim of this study was to investigate the knowledge lacunae on biochemical markers for PMI estimation. The majority of the PBM's show a completion score below 9, which can be considered to be relatively low. It is possible that not all relevant information was provided in the published literature, or that not all relevant information was available, and thus, based on current insights with regard to relevant parameters it is important to provide as much details with regard to the study population as possible, especially with regard to sustainability of scientific research. In order to assess the completeness of future PBM research, the suggested scoring index can be used as a checklist to ensure all, or as much as possible, relevant data are be provided.

Besides standard physical methods, PBM's have been used to estimate the PMI [8,24]. However, in this study, none of the PBM's were found to be ready to use for estimating PMI in practice. In order to meet the *Daubert* & *Frye* standard, empirical testing has to be performed on the PBM [18,22,25]. This is not a straightforward procedure, since a large population is needed to investigate the effect of intrinsic and extrinsic factors individually whilst the rest of the influencing factors is known. In reality, most influencing factors are unknown. Factors as disease or cause of death can increase or decrease the normal values and should therefore be taken into account for PMI estimation [26–28]. In addition, the sample size (N) can be limited due to law restrictions or unavailability of human cadavers [29].

Further difficulties in meeting *Daubert's* & *Frye's* criteria were encountered with the availability of acceptable error rates [21,22]. This can refer to either the error of the PBM measurement or the error of PMI estimation. A number of studies have reported standard errors or confidence intervals for PMI estimation. Nonetheless, it is not clear whether these values are too large to be considered practically relevant, i.e. acceptable to be used as evidence in court. To the best of the authors' knowledge, no maximum error has been defined regarding the practical use of PBM's as scientific evidence. Without a maximum tolerable error, no PBM will meet the criteria and can therefore not be qualified as suitable for use in practice. Thus, for this purpose it is essential that an acceptable estimation error will be defined. Ideally, a PBM should predict to PMI correctly at a rate of 100%. However, due to aforementioned influencing factors and systematic bias such prediction rate (precision) will not be reached easily. Therefore, the goodness-of-prediction (Q^2) for the PMI should be set to a value high enough to be practically relevant but also methodology-wise achievable. Here, a Q^2 value of 0.95 is proposed to be used as cut-off, meaning that prediction models with a 95% precision or higher should be considered as practically relevant for court evidence. This validation step can be performed by using a subset of samples to create a prediction model and calculate the precision of the model using another subset of samples. Another option use the full set of samples and perform a *k*-fold cross validation procedure with sufficient Monte-Carlo repetitions.

Furthermore, for this study, the sampling and the analytical method were not taken into account. It has already been reported that measurements with different instruments and samples obtained from different locations in the body can lead to varying results [30,31]. Therefore, standardised methods are necessary to be able to compare between studies of the same PBM [24]. This will improve the assessment of PBM's based on the outlined method in this paper because influences due to different methods are ruled out. Lastly, not all of the included literature directly investigated markers regarding PMI estimation, though, those articles provide with valuable information on PBM influencing factors such as the effect of cause of death and environmental conditions. That will also lead to an improved assessment of a PBM due to the availability of such data.

5. Conclusion

This paper showed a novel way of assessing PBM's in terms of research completeness and suitability for practical use. This study revealed that the knowledge lacunae on PBM's is large, none have been completely investigated. It was found that to date, no PBM has shown to be implementable as scientific evidence in court.

To reduce the knowledge lacunae on PBM's, it is recommended to investigate and report all essential data as outlined in this paper. Further, criteria regarding acceptable error rates have to be established for the estimation of the PMI. In this way, a PBM can be easily evaluated for its use as evidence in court. Altogether, this should lead to more practical-driven research on PBM's.

Conflict of interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scijus.2018.09.002.

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