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Biochemical markers of time since death in cerebrospinal fluid: a first step towards “Forensomics”

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The accurate estimation of the time of death is a challenge in forensic medicine, as the methods routinely used to assess the post-mortem interval (PMI) are far from being precise. Over the past decades, biochemical methods have been implemented on post-mortem samples to improve the precision of PMI estimation. Studies have focussed on the biochemical profiles of closed compartment body fluids, as they are preserved longer than blood after death and are thus subject to confined post-mortem chemical changes. Cerebrospinal fluid (CSF) has been considered a suitable fluid to investigate these changes, as it is found in large amounts and is easy to sample. Moreover, the main molecules found in CSF have known reference values in living subjects, unlike most other body fluids. In this literature review, we focus on the panel of biomarkers that have been studied in CSF based on their potential of offering information on the time of death. The interest of these biomarkers in casework and the research perspectives in this field are discussed. Integrating data from different methods, including biochemistry, for better estimation of the time of death would represent a step forward in the forensic field, paving the way for an innovative approach that we suggest to call “Forensomics”.

Keywords: biomarkers; time of death; cerebrospinal fluid; biochemistry; forensic medicine

Introduction

One of the most important and challenging tasks in forensic medicine is the estimation of the post-mortem interval (PMI). PMI is defined as the period of time that has elapsed since a person has died, and is thus also called the time since death (TSD). The accurate determination of the PMI is a critical step with obvious medicolegal implications in cases of unwitnessed death. This may be used to establish the timeframe of a crime, to narrow the number of suspects based on their alibi at the time of death, but also to help in identification of a victim in case of a missing person [1–3].

After death, the structure and composition of the human body are considerably altered by a complex series of pathological and biochemical processes [4]. As several of these changes occur sequentially, various methods have been proposed to estimate the TSD based on these processes. In the early PMI, i.e. before the onset of microbial proliferation [5], these methods are mainly based on physical/physicochemical progressive changes, such as body cooling, *livor mortis* (hypostasis) and *rigor mortis*. Supravital reactions, including post-mortem excitation-induced reactions of tissues (e.g., mechanical and electrical excitability of skeletal muscle) and pharmacologically induced excitability of the iris, may also be used [6]. Later on, bacterial processes (referred to as putrefaction) and entomology may be considered [6, 7]. These different methods are still routinely used, although they give only imprecise estimates of the PMI. Indeed, they are influenced by a variety of factors that tend to introduce considerable errors, including external (or environmental) factors, such as ambient temperature and humidity, and internal (or endogenous) factors, such as pre-existing diseases, age, or body mass index [3,8,9].

Post-mortem chemistry, also called thanatochemistry, has been introduced in forensic medicine to assist in more precise estimation of the TSD [2] and in providing useful

information regarding the cause of death [8,10,11]. Coe [5] defined forensic chemistry as “*one of the more important ancillary procedures for the forensic pathologist*”. As a result of significant improvements in biochemical techniques, current methods are now able to provide precise, reliable and rapid results as required by the forensic community [2]. Numerous studies have been conducted considering post-mortem chemical changes in a wide variety of biological fluids, including blood, vitreous humor (VH), urine, CSF, pericardial and synovial fluids [6,7,10,11]. Some of these changes are due to autolysis (process of cell self-digestion from the activity of endogenous enzymes), while others result from metabolic processes, since the metabolic activities continue in the tissues for variable duration after death [6,7].

Emphasis has been placed on the study of concentration changes of chemicals in closed compartment body fluids, as they are neither altered nor contaminated as quickly as blood after death. Because of their isolated and confined topography, autolysis proceeds more slowly in these fluids than in blood [3,12,13]. VH has been the most extensively studied fluid compartment. In particular, the post-mortem increase in potassium concentration in the VH has been widely investigated for estimating the PMI, but this method has not led to the improvement of the accuracy of the TSD estimation [6,7]. CSF may represent a good alternative to VH as it is a closed compartment fluid that is found in large volumes and is easy to sample [6,7]. Due to its closeness to the central nervous system (CNS), CSF has also been considered to reflect the changes in the biochemical composition of the brain tissue after death. Contrary to VH, many analytes found in CSF have reference values in living subjects, which make the post-mortem results easier to interpret.

In this paper, we have reviewed the various biochemical compounds that have been studied in CSF as potential biomarkers of the PMI. Studies written in either English or

French and whose full texts were available online have been included in this review. Book chapters on this topic have also been consulted.

The actual value of these biomarkers in daily forensic practice and the research perspectives in this field are discussed. Integrating data from different methods, including thanatochemistry, could improve TSD estimation through the implementation of an innovative approach that we suggest to call “*Forensomics*”.

Biochemical markers of the post-mortem interval in CSF

1. Electrolytes

a. Potassium

Mason et al. [14] was the first to find a significant relationship between PMI and potassium levels in cisternal CSF. Many studies have since reported a similar trend [3,13,15–22]. Urban et al. even showed a strong linear correlation between the estimated TSD from cisternal potassium concentration and the estimated TSD based on rectal temperature by using the nomogram method of Henssge [1]. He developed a method for PMI estimation based on cisternal potassium concentration with 98% confidence limits, but his results have never been able to be reproduced in any other study.

b. Sodium

Naumann [16,17] was the first to report a slight to moderate decrease of sodium in the CSF after death. This decrease was assumed to be due to autolytic changes of cell membranes resulting in a greater permeability, allowing extracellular ions to diffuse into the cells. Others similarly found a non-significant decrease of sodium with PMI in post-mortem CSF samples [15,20]. More recently, studies showed a statistically significant negative correlation between sodium levels and PMI up to 20-25 h after

death [1,3,13,21], although the ratio of sodium and potassium concentrations may remain a better parameter for PMI estimation compared to ion values alone [13].

c. Chloride

Similar to sodium and other predominantly extracellular ions, chloride was shown to decrease in CSF after death in studies by Naumann [16,17], whereas Paulson and Stickney [20] found that they tend to slightly increase with PMI. More recently, Karkela [21] showed a statistically significant post-mortem decrease in chloride levels after death.

d. Calcium

According to Naumann [16,17], calcium values in post-mortem CSF closely approach those found during life. From 131 post-mortem samples of cisternal fluid, levels of calcium were found to slightly decrease from an ante-mortem value of 5.0 mg/dL to 4.8 mg/dL by 10 h post-mortem. A relative stability of calcium ions was also reported by others [3,15,20].

e. Magnesium

Magnesium concentration may tend to increase in CSF after death as a result of its diffusion out of the cells into the extracellular fluid [16,20]. However no correlation with the TSD has been shown so far [1,15].

f. Phosphates

A marked increase of inorganic phosphorus in cisternal CSF after death was noted by Naumann [16,17] and interpreted as evidence of post-mortem esterase activity. The same author found an increase in phosphorus levels from an ante-mortem value of 1.4 mg/dL to 8.9 mg/dL 10 h after death [17]. Eliakis et al. [23] stated that TSD could be determined within 3 h by measurement of CSF inorganic phosphorus, as the increase in phosphorus tends to be linear during that period. Paulson and Stickney [20] also found

values which tend to be elevated above the normal range for a living patient. According to Schleyer [19], a concentration of inorganic phosphorus exceeding 15 mg/dL in cisternal CSF may suggest a minimum TSD of 10 h.

g. Bicarbonate

Naumann [16] reported a slight to moderate post-mortem decrease of bicarbonate with time in cisternal fluid. However, Karkela [21] did not find any statistically significant correlation between these two parameters.

h. Carbon dioxide

The drop in carbon dioxide concentration in CSF after death has been found to result from post-mortem acidosis [16,20,24], without any correlation with the TSD [20]. Karkela [21] measured the carbon dioxide values in cisternal fluid samples obtained 2, 4, 10 and 24 h after death, and found carbon dioxide levels to increase 2 to 4 h after death, then decline.

2. Carbohydrates

a. Glucose

In his pioneer work, Naumann described CSF as a better material than blood for post-mortem glucose analysis because of the greater reliability of the results obtained in this fluid [24]. He found that glucose was subject to a decrease in CSF after death due to post-mortem glycolysis [17,24]. It was assumed that post-mortem values were related to ante-mortem levels and to the cooling rate of the cadaver [20]. A significant correlation between the decrease in glucose and the PMI has also been reported [3,21].

b. Pyruvate

Only one study found pyruvate to significantly decrease with time in CSF after death [21].

c. Lactic acid

Lactic acid is produced in CSF after death due to local anaerobic metabolism (glycolysis). This lactate increase results in post-mortem acidosis [16,19,20,24]. Post-mortem anaerobic glycolytic metabolism was found to proceed at a high rate during the first 10 h post-mortem. Correspondingly, lactic acid shows its steepest increase within the first 6 to 8 h after death, with the pH showing its steepest decrease during the same time interval [5]. Karkela [21] further showed a statistically significant increase in lactate with time up to 24 h after death.

d. Inositol

Nixon [25] investigated inositol as a biomarker of PMI and found that post-mortem cisternal fluid contained higher inositol concentrations than lumbar fluid during life. The mean value was more than 10 times that of the mean value from the control group. The rapid rise in the CSF inositol concentration after death was thought to be related to an enzymatic breakdown of a combined form of inositol within the CNS with the subsequent diffusion of free inositol into the CSF. Free inositol contained within the cytoplasm may also diffuse through the cell membrane into the CSF as a result of an increase in membrane permeability due to autolysis. Nixon found a statistical relationship between inositol concentrations and PMI. However, the ranges of concentrations were so wide that it precluded the use of inositol as a means of estimating the PMI.

3. Nonprotein nitrogen compounds

Schleyer [19] showed that TSD may be less than 12 h if nonprotein nitrogen levels in cisternal fluid are below 50 mg/dL, while it may be less than 24 h if levels are below 70-80 mg/dL. However, these results have never been confirmed by any further studies.

a. Amino acids

Pucher and Burd [26], Schourup [5] and Schleyer [19] have all demonstrated a rise in free amino acid levels in post-mortem CSF. According to Schleyer [19], amino nitrogen concentrations in cisternal fluid below 14 mg/dL could mean that death occurred less than 10 h before. Coe [5] analysed CSF glutamine levels after death and noted a progressive rise in concentrations with an increasing PMI. However, he mentioned that isolated values were difficult to interpret with regard to the TSD. The concentrations of all essential amino acids (phenylalanine, valine, threonine, tryptophan, methionine, leucine, isoleucine, lysine and histidine), in addition to those of glutamate, glutamine, alanine, glycine, proline, aspartate, serine, GABA, cystine, arginine and tyrosine, were found to significantly increase linearly in cisternal fluid 4 to 24 h after death [21,27]. Girela et al. [28] also attempted to correlate free amino acid concentrations in CSF with the PMI, but the authors did not further evaluate the data due to sample contamination with blood. As noted by Karkela [21], little is known about the pathophysiology of CNS amino acid metabolism in terminal stages of life and in the early PMI, which makes the origin of the different amino acids found in post-mortem CSF difficult to ascertain.

b. Ammonia

According to Schleyer [19], ammonia concentrations below 2 mg/dL in cisternal CSF may suggest a maximum TSD of 10 h. Ammonia was subsequently found to significantly increase linearly in cisternal CSF 4 to 24 h post-mortem [21].

c. Urea

A post-mortem increase of urea in CSF was reported by Naumann [17,24], whereas Jenkins and Fekete showed urea levels in post-mortem CSF to be closely correlated with ante-mortem blood levels [29,30]. Swain et al. [3] found no statistically significant correlation between PMI and CSF urea concentration.

d. Monoamines

Endo et al. [31] measured the concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxy-4-hydroxyphenylglycol (MHPG) and 5-hydroxyindoleacetic acid (5-HIAA) in post-mortem cisternal fluid. They found no relationship between PMI and the levels of HVA, 3-methoxy-4-hydroxyphenylglycol (MHPG) and 5-HIAA, but they showed a significant correlation between PMI and the level of DOPAC, as the possible result of the persistent post-mortem activity of monoamine oxidase (MAO) in brain. However, the large inter-individual variability in DOPAC concentration precluded its use as a measure of the PMI.

Karkela et al. [27] investigated the concentrations of the same four monoamine metabolites in cisternal fluid from 40 cadavers up to 24 h after death. Levels of 5-HIAA, DOPAC, HVA and MHPG were higher in post-mortem samples 2 h after death than in lumbar fluid *in vivo*. Furthermore, 5-HIAA concentrations were higher in lumbar fluid than cisternal or ventricular CSF during life at any time after death. DOPAC, HVA, and MHPG concentrations tended to decline with time thereafter, whereas 5-HIAA levels tended to increase up to 10 h before declining. Nevertheless, the differences between the group means were not statistically significant.

A more recent study by Balbi et al. [32] showed that dopamine and noradrenaline are elevated in post-mortem CSF as compared to CSF from living subjects, with dopamine showing the highest increase. However, he found these increases not to be statistically significant, and wide ranges of concentrations were observed. The same findings were

reported for serotonin (5-HT) and catecholamines, in line with results from other studies [33,34]. A significant correlation between serotonin and TSD was shown by Quan et al. [35].

e. Creatine

Post-mortem CSF creatine values have been shown to increase after death with regard to length of time [19,24], with a significant inter-individual variability for the same PMI [24]. Nonetheless, Schleyer [19] thought that creatine could serve as a rough indicator of the PMI. He showed that the TSD may not be more than 10 h if creatine levels in cisternal fluid are less than 5 mg/dL, while it may not be more than 30 h for levels lower than 10 mg/dL. Furthermore, Takata et al. [36] found a significant correlation between CSF creatine levels and PMI up to 50 h post-mortem.

f. Creatinine

CSF creatinine levels have been found to mirror the blood values after death [5,17,24,26]. Naumann [17,24] showed that the post-mortem levels of creatinine in CSF were more than twice as high as those ante-mortem for an average PMI of 10 h, while Jensen found that the concentration of creatinine in CSF increased in parallel with the increment of PMI [5]. Furthermore, Swain et al. [3] reported a significant correlation between post-mortem creatinine levels and PMI, but with wide 95% confidence limits.

g. Ribonucleosides

Harkness et al. [37] investigated uridine and inosine as biomarkers of PMI in a few post-mortem CSF samples from newborns. He found a rapid increase within the first hour after death, and to a lesser extent thereafter.

h. Oxypurines

i. Uric acid

Naumann [24] reported higher post-mortem levels of uric acid in CSF than ante-mortem for an average PMI of 6 h, confirming preliminary results from Pucher et al. [26]. In post-mortem CSF samples from newborns, Harkness et al. [37] reported uric acid levels of 70 $\mu\text{mol/L}$ within 1 h of death, rising to 136 $\mu\text{mol/L}$ during the first day. As for other oxypurines, no significant differences in the uric acid concentration during the first 3 days after death were reported by Manzke et al. [38], but their samples were probably taken too late to show any rapid increase occurring immediately after death [39]. Nevertheless, these authors found a rise in uric acid levels between 1-5 days after death [5].

ii. Xanthine

Praetorius et al. [40] reported a greater than 100-fold increase in the concentration of xanthine and hypoxanthine during the first 36 h after death. In newborns, Harkness et al. [37] found mean levels of xanthine to increase from 53 $\mu\text{mol/L}$ in the first hour after death to 73 $\mu\text{mol/L}$ during the first day. Although Manzke et al. [38] did not observe any significant differences in the oxypurine concentrations during the first 72 h post-mortem, they showed an increase in xanthine levels between 1-6 days after death [5].

iii. Hypoxanthine

Hypoxanthine has been found to rise exponentially in CSF within the early post-mortem period, with the most rapid rise occurring during the first 15 h post-mortem and equilibration achieved after approximately 20 h [1,37,39,40]. However, Madea et al. [39] showed the 95% confidence interval to be large and increasing with increasing PMI. No significant post-mortem time-dependent increase was observed by Carpenter et al. in children 1 week to 2 years of age [41].

4. Proteins

a. Total proteins

CSF has a low concentration of proteins in healthy individuals, the normal range being 0.20-0.45 g/L [42]. No rise in CSF proteins in the first 24 h after death was reported by Mangin et al [43]. In contrast, Morihara et al. [44] found a three-fold post-mortem increase with an average PMI of 5.7 h. A significant relationship with PMI was found for longer intervals [45]. Finehout et al. [46] performed a proteomic analysis of both ante- and post-mortem CSF from seven individuals with short PMI (1.5-9.5 h) using two-dimensional gel electrophoresis (2DE) and mass spectrometry: 54 protein spots were identified, showing significantly different intensities between ante- and post-mortem CSF. Most of these proteins were intracellular proteins involved in glycolysis or in prevention from oxidative stress, and all but one showed a rise in their spot intensity. This difference in spot intensity was found to be correlated with PMI for 14 proteins.

b. Enzymes

Paulson et al. [20] reported an increase of beta glucuronidase, acid phosphatase, lactate dehydrogenase (LDH) and creatine kinase (CK) in CSF after death, with no relationship with PMI. In contrast, Osuna et al. [45] found a significant correlation between PMI and CSF concentrations of the two latter enzymes, but also of aldolase and alanine aminotransferase (ALT). However, Osuna et al. studied individuals with significantly longer PMI. With 2DE, Finehout et al. [46] found a 12-fold increase in the intensity of the CK-B spot in post-mortem CSF compared with ante-mortem CSF from the same individual. Karkela [21] found that the concentration of total CK, CK-B, LDH and its isoenzymes 1-3, β -D-N-acetylglucosaminidase (NAG), and acid phosphatase

significantly increased linearly with increasing TSD. CK-MB, however, did not show any significant relationship with the TSD across different studies [47–49].

CSF enzymes, including glutamic oxaloacetic transaminase (GOT) [50] and 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNP) activity [51] were also studied. GOT concentration increased with an increasing PMI up to 70 h, while a rise in CNP activity was observed in CSF samples collected within 14 h after death. A subsequent reduction of enzymatic activity was found thereafter, as the result of proteolytic degradation of the enzyme in the CSF and probable lack of further release from the CNS tissue. However, the wide range of the values obtained for both enzymes precluded any accurate assessment of PMI.

c. Albumin

Using bromocresol green dye-binding method, Parmar et al. [52] studied albumin concentration in cisternal fluid samples at different time intervals. He found a significant and linear decrease in albumin levels, but only between 24-72 h post-mortem. These findings are in line with previous studies that showed no correlation between PMI and CSF albumin concentration within the first 24 h after death [43].

d. Tau protein

Morihara et al. [44] showed that tau levels in demented and non-demented subjects were more than 30-fold higher in post-mortem CSF compared with those in ante-mortem CSF. Tau concentrations were also found to increase with PMI. The post-mortem leakage of tau into the CSF was assumed to reflect the same underlying mechanism as in neurodegenerative diseases such as tauopathies (neuronal death).

e. Cardiac troponins

Three studies showed that cardiac Troponin T and I in cisternal CSF were slightly correlated with PMI up to 48 h post-mortem [47–49].

f. Myoglobin

According to one post-mortem investigation, myoglobin in cisternal CSF was found to significantly increase with PMI for various causes of death [48].

g. S-100 protein B

S-100 protein B in cisternal CSF showed a moderate time-dependent increase up to 3 days post-mortem for deaths resulting from acute cardiac failure and asphyxia [53], whereas no significant relationship was found between PMI and the protein level in ventricular CSF in suicide and non-suicide cases [34].

Discussion

The biochemical markers that have been investigated in CSF as potential indicators of PMI are presented in this literature review. Various molecules, such as electrolytes, carbohydrates, nitrogen compounds and proteins, have been studied by means of thanatochemistry in attempts to precisely estimate the TSD, as this estimation still represents a challenge in the forensic field. Unlike post-mortem physical changes whose interpretation is often influenced by subjective judgement [9], biochemical methods have the advantage of being standardised and statistically assessed [2].

CSF has been considered as a suitable fluid compartment to investigate early post-mortem chemical changes, as concentration gradients in this fluid are still effective in the first few hours after death [6]. The exploration of some biomarkers may even remain relevant for a few days after death, as their concentrations have been found to change in CSF longer than 72 h post-mortem [5,36,38,50,52].

Although some of these biomarkers have shown a statistically significant correlation with PMI (Table 1), their values are often highly variable over PMI, with large 95% confidence limits and weak correlation coefficients. Hence, most biochemical components in CSF have been considered to be only of limited value for determining the TSD when used singly, having at best a statistical significance.

The inter-individual variability observed in the biochemical profile of the CSF after death is undeniably one of the explanations for these large margins of error. However, the present review has raised various methodological issues that should be considered in order to improve the design of further studies that need to be performed in this field, including the following:

- Many studies lack statistical power and thus potential significance of results due to a small sample size [18,32,37,46].
- Criteria used for selecting participants greatly differ from one study to another, which makes inter-comparison of results obtained for the same biomarker difficult. For example, some studies have included patients with CNS diseases or trauma [32,46,50] while others have not [3,13,20,52]. This may greatly affect the results as the post-mortem levels of intracellular biomarkers in the CSF from patients with CNS disorders (such as neurodegenerative disease, stroke or infection) or traumatic brain injury may be subject to an increase due to substantial brain cell death.
- PMI should be inferred from the actual time of death. Instead, time of death is sometimes estimated based on autopsy findings or data obtained from police investigation [31,47–49,53], leading to biased estimates of PMI.
- Factors known to influence post-mortem changes are inconsistently considered. Discrepancies in the results might, for instance, arise from body refrigeration before CSF sampling, as cooling slows autolytic and metabolic processes (e.g. glycolysis).

However, only a few studies have included corpses stored at ambient temperature before CSF collection [31,52]. This may be partly due to ethical concerns that this methodology could raise in case of bodies left at room temperature for a long time. In some studies in which samples were taken at different TSD, corpses were stored at room temperature in the first hours, then cooled, probably in order to prevent putrefaction [21,27]. Like the ambient temperature, the agonal time is also assumed to be an important factor. In slow-death patients, accumulation of lactic acid can lead to massive acidosis that may affect, for instance, CSF concentrations of monoamines or amino acids [21,27,54]. The agonal status has, however, only been considered in a few studies [21,27,43].

- The site of CSF collection frequently differs across studies and within the same study. CSF is alternatively withdrawn by lumbar, cisternal or ventricular punctures. Ante-mortem punctures are, in their vast majority, performed in the lumbar region, whereas post-mortem CSF is usually removed from cisternal or ventricular aspirates. However, significant variations in the concentrations of several biochemical parameters have been reported between these sites [55,56]. Reiber [57] found individual protein concentrations in the ventricular region to be up to 3.5-fold higher than those in the lumbar region. A significant rostro-caudal concentration gradient has also been shown for tryptophan, HVA, 5-HT and 5-HIAA [33,58,59]. A recent study showed statistical differences in electrolytes in CSF between the lumbar and the ventricular sites, with the post-mortem samples obtained from the ventricular subarachnoid space having statistically lower sodium and chloride but higher magnesium and potassium [60]. Because of divergent gradients of diffusion, the values obtained from these different sites should not be compared with one another.

It is assumed that the intracranial fluid would better reflect the subtle biochemical changes occurring in the brain compared to the spinal fluid [27,60].

- Pre-analytical and analytical methodologies vary across studies for the same biomarker, although it is known that there are significant differences among the values obtained according to the particular methodologies used [5]. Such variations may be critical to properly evaluate analytes. For instance, particular attention should be paid to the type of container used to collect and store CSF samples, as the concentration of various components may be affected by the characteristics of the receptacle (e.g. decrease due to adhesion to the tube wall). Furthermore, samples should be frozen as soon as possible after collection (if not processed and analysed immediately), and repeated freeze/thaw cycles should be avoided in order to preserve the stability of the analytes [61]. Various analytical procedures have also been combined in some studies without having previously been compared [50,51] or validated by independent research. Comparing values from different studies requires identical methodologies or at least procedures that are assumed to give comparable results [5]. Indeed, variations may occur between laboratories even when using the same assays. Standardised procedures, similar to those applied to the analysis of the proteins associated with Alzheimer's disease, should be established and implemented to address this point [62,63].
- Post-mortem concentrations are rarely compared with reference values from living persons, although the latter are of interest to interpret post-mortem variations and to determine the minimum time necessary for these changes to occur after death. These reference values (Table 2) are mostly obtained from the literature [16,24,27,64,65] or from control groups [20,21,44]. At the individual level, it may be difficult to ascertain that ante-mortem values were comprised within the "normal" range before

death. Moreover, the broad range of some reference values can also make the interpretation of post-mortem concentration changes difficult, especially in the early PMI. To our knowledge, only one study compared ante- and post-mortem values from the same patients, although the samples were collected from two distinct sites [46].

- There is a lack of kinetic studies assessing the time dependence of biomarkers at the individual level, by means of repeated samples performed at different times after death in the same person. Therefore post-mortem fluctuations in the concentrations of most biomarkers over time are not currently known.
- In regard to statistical analysis, most studies do not provide clear data on the precision of methods used. Many authors only consider mean values of the correlation between concentrations of the studied parameter(s) and the TSD or correlation coefficients. Instead, there is a need to refer to statistical parameters of the deviation of single values from the mean correlation, such as confidence limits [1]. It should also be noted that no comparison of the precision of new methods with that of standard methods for estimating PMI could be found in most studies that have been reviewed.

Overall, several factors contribute to the fact that to date, no single CSF parameter has been found to enhance the accuracy and the reliability of PMI estimation. If inter-individual variability is inevitable, following a strict study design could help address most methodological issues discussed above. Madea [6] established several criteria that an estimation method for TSD should meet to be of highest evidence value:

- The method must have been investigated in longitudinal studies on well-characterized samples;

- The method must rely on a mathematical description of the post-mortem change(s) studied and provide a quantitative measurement;
- Influencing factors governing the change of the studied parameter(s) must be quantitatively taken into account, such as ambient temperature and agonal time;
- Clear data on the method precision must be available, with calculation of mean values and confidence limits. It must then be compared with those of standard methods, at the same PMI, to check for improvement.
- The precision and the reliability of the method must be determined using independent case material, including different causes of death, pre-existing diseases and agonal periods;
- The method should have been tested through field studies to check its practical applicability.

When dealing with chemical-based methods, particular attention should also be given to the standardisation of the pre-analytical and analytical procedures, and the post-mortem concentration changes of biomarkers should be assessed by means of kinetic studies in the same individual.

To date, only a few methods have fulfilled Madea's guidelines: the temperature-based methods of Henssge [7,66–70] and Baccino [71,72], as well as various approaches based on potassium concentration in VH [6]. As these latter have not led to a significant improvement of PMI estimation, both methods based on body cooling still remain the most simple, precise and reliable procedures for TSD estimation. Nevertheless, the resulting estimation has, at best, an interval of a few hours around the mean value with these methods.

Improving the precision of PMI by means of thanatochemistry represents a challenging task in the forensic field. Some chemical parameters that were investigated decades ago with traditional analytical methods may hold potential value in estimating the TSD, but have not been further studied by adequate and newer methodologies on larger cohorts since. Thus, attention should now be focused on performing well-designed studies instead of necessarily looking for new biomarkers. As stated by Camps [73], *“our aim should be to obtain a figure with as small a margin of error as possible”*.

Recent developments in biochemical methodologies and techniques now provide the opportunity to analyse multiple biomarkers at once within a short time, based on a multiplex approach [2]. This is of interest as it is very unlikely that the measurement of a single parameter will provide a completely reliable estimate of PMI in the future. Instead, it has been suggested that a combination of chemical determinations may be helpful in estimating the TSD, particularly in the early PMI [2,8]. The focus should be placed on biomarkers whose post-mortem increase is solely the result of diffusion, rather than on parameters that increase in response to metabolic processes [1]. Data from thanatochemistry could then be used together with those provided by scientifically-validated methods by performing a multiple linear regression analysis, for a common result of death time estimation, in an attempt to narrow down the confidence limits and increase the reliability of the estimation of PMI [74].

In the same way as it has been suggested to combine methods based on supravital processes with predominantly physical methods such as Henssge’s nomogram for estimating the TSD in casework [1], we further propose to integrate data from thanatochemistry in an innovative and holistic “forensomic” approach, in reference to “omics sciences” [75]. Consideration of such data has already been shown to be relevant in the determination of the cause of death [10,11]. To the best of our

knowledge, the only author who assessed the combination of physical and biochemical methods for the estimation of TSD obtained a high correlation between death time estimation from cisternal potassium and body temperature [1]. However, further studies are necessary because of discrepancies between his results and those from subsequent research.

Conclusion

For the last decades, many biochemical parameters have been studied in post-mortem CSF as potential biomarkers of TSD. Their single value remains limited in practice, since these parameters lack precision and reliability for estimating TSD. However, biochemical markers still remain of great interest for research purposes, as thanatochemistry could eventually assist in more precise estimation of the interval since death in the future.

Current biochemical techniques have the potential to open up some interesting horizons, as they are more sensitive and specific than traditional ones and allow high throughput approaches. They provide the opportunity for some biochemical markers that have previously been investigated to be “revisited” by means of the most recent technological developments, and for some potential biomarkers never considered before to be identified.

These new methodologies should be implemented in well-conducted, unbiased field studies complying with the aforementioned criteria. Combining the most accurate chemical-based methods with current standard procedures would pave the way for an integrated approach that we suggest to call “*Forensomics*”, that could ultimately

provide sufficient certainty for a reliable medicolegal statement regarding PMI estimation.

Disclosure of interest

The authors report no conflict of interest.

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Table 1. Summary of the studies reporting a statistically significant correlation between biochemical markers in CSF and PMI.

Parameter	Number of cases	Characteristics of the study population	Refrigeration	Puncture site	PMI	Analytical method	Concentration change with PMI	Reference
Potassium	46	Bodies randomly selected at the mortuary, exclusion of meningeal disease and diseases likely to disturb the serum potassium level	N/A	Cisternal	1.5-70 h, punctures at different PMI in eight cases	Flame photometry	Increase	[14]
	40	Autopsy cases, natural deaths except for one case (intoxication), no known neurological disorder	For the 10 and 24 h groups	Cisternal	4 groups: 2, 4, 10 and 24 h	Ion-selective electrode method	Increase	[21]
	100	Autopsy cases, serum electrolyte levels assumed to be normal at the time of death, head trauma and brain pathology excluded	N/A	Ventricular	4 groups: 0-6 h; 6-12 h; 12-18 h; >18 h	Flame photometry	Increase	[13]
	100	Autopsy cases, meningitis, encephalitis, septic shock and intracranial haemorrhage excluded	For long PMI	Cisternal	4.5-78.5 h	Ion-selective electrode method	Increase	[3]
Sodium	40	Autopsy cases, natural deaths except for one case (intoxication), no known neurological disorder	For the 10 and 24 h groups	Cisternal	4 groups: 2, 4, 10 and 24 h	Ion-selective electrode method	Decrease	[21]
	100	Autopsy cases, serum electrolyte levels assumed to be normal at the time of death, head trauma and brain pathology excluded	N/A	Ventricular	4 groups: 0-6 h; 6-12 h; 12-18 h; >18 h	Flame photometry	Decrease	[13]
	100	Autopsy cases, meningitis, encephalitis, septic shock and intracranial	For long PMI	Cisternal	4.5-78.5 h	Ion-selective electrode	Decrease	[3]

		haemorrhage excluded				method		
Chloride	40	Autopsy cases, natural deaths except for one case (intoxication), no known neurological disorder	For the 10 and 24 h groups	Cisternal	4 groups: 2, 4, 10 and 24 h	N/A	Decrease	[21]
Glucose	40	Autopsy cases, natural deaths except for one case (intoxication), no known neurological disorder	For the 10 and 24 h groups	Cisternal	4 groups: 2, 4, 10 and 24 h	N/A	Decrease	[21]
	100	Autopsy cases, meningitis, encephalitis, septic shock and intracranial haemorrhage excluded	For long PMI	Cisternal	4.5-78.5 h	Enzymatic endpoint method	Decrease	[3]
Pyruvate	40	Autopsy cases, natural deaths except for one case (intoxication), no known neurological disorder	For the 10 and 24 h groups	Cisternal	4 groups: 2, 4, 10 and 24 h	Enzymatic endpoint method	Decrease	[21]
Lactic acid	40	Autopsy cases, natural deaths except for one case (intoxication), no known neurological disorder	For the 10 and 24 h groups	Cisternal	4 groups: 2, 4, 10 and 24 h	Enzymatic endpoint method	Increase	[21]
Inositol	25	Autopsy cases, natural deaths except for one case (intoxication), including strokes	Yes	Cisternal	Up to 30h	Paper chromatography	Increase	[25]
Amino acids	40	Autopsy cases, natural deaths except for one case (intoxication), no known neurological disorder	For the 10 and 24 h groups	Cisternal	4 groups: 2, 4, 10 and 24 h	HPLC	Increase	[21,27]
Ammonia	40	Autopsy cases, natural deaths except for one case (intoxication), no known neurological disorder	For the 10 and 24 h groups	Cisternal	4 groups: 2, 4, 10 and 24 h	HPLC	Increase	[21]
Monoamines								
DOPAC	52	Autopsy cases, hanging or ischemic heart failure	No	Cisternal	1-52 h	HPLC	Increase	[31]
Serotonine	351	Autopsy cases (including head	N/A	Intracranial	5-47 h	HPLC	Increase	[35]

		injury and cerebrovascular disease)						
Creatinine	100	Autopsy cases, meningitis, encephalitis, septic shock and intracranial haemorrhage excluded	For long PMI	Cisternal	4.5-78.5 h	Jaffe's method	Increase	[3]
Total proteins	34	Autopsy cases, various causes of death (including head trauma)	N/A	Cisternal	41.1-51.5 h on average (depending on the groups)	Bradford's method	Increase	[45]
	7	Autopsy cases, demented patients	N/A	Ventricular	1.5-9.5 h	2DE, MS	Increase	[46]
Enzymes								
LDH	34	Autopsy cases, various causes of death (including head trauma)	N/A	Cisternal	41.1-51.5 h on average (depending on the groups)	UV spectrophotometry, commercial kits	Increase	[45]
Aldolase								
ALT								
CK								
CK CK-B LDH Acid phosphatase LDH isoenzymes NAG	40	Autopsy cases, natural deaths except for one case (intoxication), no known neurological disorder	For the 10 and 24 h groups	Cisternal	4 groups: 2, 4, 10 and 24 h	Ion-selective electrode method	Increase	[21]
Albumin	100	Autopsy cases, various causes of death, head trauma excluded	No	Cisternal	2-72 h	BCG dye-binding method	Decrease	[52]
cTnI, cTnT	257	Autopsy cases, various causes of death (including head trauma and strokes)	N/A	Cisternal	4-48 h	RPIA (cTnI) ECL (cTnT)	Increase	[47]
Myoglobin	295	Autopsy cases, various causes of death (including head trauma and strokes)	N/A	Cisternal	5-46 h	RPIA (cTnI) ECL (cTnT)	Increase	[48]
	1923	Autopsy cases, various causes of death (including head trauma and strokes)	N/A	Cisternal	3-48 h	RPIA (cTnI) ECL (cTnT)	Increase	[49]
	295	Autopsy cases, various causes of death (including head trauma and strokes)	N/A	Cisternal	5-46 h	RPIA	Increase	[48]

S-100 protein B	216	Autopsy cases, various causes of death (including head trauma)	N/A	Cisternal	Up to 3 days	ELISA	Increase	[53]
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2DE two-dimensional gel electrophoresis, *ALT* alanine aminotransferase, *BCG* bromocresol green, *CK* creatine kinase, *cTnI* cardiac troponin I, *cTnT* cardiac troponin T, *DOPAC* 3,4-dihydroxyphenylacetic acid, *ECL* electro-chemiluminescence immunoassay, *ELISA* enzyme-linked immunosorbent assay, *HPLC* high-performance liquid chromatography, *LDH* lactate dehydrogenase, *MS* mass spectrometry, *N/A* not available, *NAG* β -D-N-acetylglucosaminidase, *PMI* post-mortem interval, *RPIA* radial partition immunoassay, *UV* ultraviolet

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Table 2. Reference values of various biochemical parameters in lumbar CSF in adults.

Parameter	Range of values	Reference
Potassium	2.5 - 3.7 mmol/L	[16]
Sodium	134 - 152 mmol/L	[16]
Chloride	119 - 128 mmol/L	[16]
Calcium	1.1 - 1.3 mmol/L	[16]
Magnesium	0.85 - 1.1 mmol/L	[16]
Phosphates	0.29 - 0.65 mmol/L	[16]
Bicarbonate	25 - 28 mmol/L	[16]
Glucose	2.2 - 3.9 mmol/L	[64]
Lactic acid	1.1 - 2.4 mmol/L	[64]
Urea	1.3 - 4.7 mmol/L	[24]
5-HIAA	86 - 215 nmol/L	[27]
DOPAC	0.43 - 14.6 nmol/L	[27]
HVA	158 - 428 nmol/L	[27]
MHPG	25 - 48 nmol/L	[27]
Creatinine	44 - 106 μ mol/L	[24]
Uric acid	23 - 166 μ mol/L	[24]
Total proteins	0.15 - 0.45 g/L	[64]
Tau protein	< 300 ng/L (21–50 yr)	[65]
	< 450 ng/L (51–70 yr)	
	< 500 ng/L (71–93 yr)	

DOPAC 3,4-dihydroxyphenylacetic acid, *5-HIAA* 5-hydroxyindoleacetic acid, *HVA* homovanillic acid, *MHPG* 3-methoxy-4-

hydroxyphenylglycol