



Machine learning and metabolic signatures of drowning: A pathway to uncovering cause of death in aquatic environments

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ABSTRACT

The postmortem diagnosis of drowning is challenging due to the nonspecific and transient nature of classical autopsy findings. This study aimed to investigate whether postmortem metabolomics can differentiate drowning from other causes of death, offering a potential biochemical tool to support forensic diagnosis in water-related deaths. A total of 503 drowning cases and four control groups were included, matched on sex, age, BMI, and postmortem interval. Three control groups represented alternative causes of death relevant to bodies found in water; chronic heart disease ($n = 510$), intoxication ($n = 516$), and trauma ($n = 497$). Hangings ($n = 511$) were included as a fourth “positive” control group to see how well the model can separate two different asphyxiation processes. Using multivariate modeling, we investigated whether drowning could be discriminated from these competing causes of death based on metabolomic signatures. Four binary OPLS-DA models comparing drowning to each control group showed good performance ($R^2 = 0.61\text{--}0.76$; $Q^2 = 0.40\text{--}0.56$), with sensitivities and specificities ranging from 83 to 87 % and 78–89 %, respectively. Metabolite and pathway analyses identified 52 differentiating features and multiple significantly enriched pathways, including glycerophospholipid metabolism, steroid hormone biosynthesis, and cytochrome P450-related drug metabolism. In conclusion, postmortem metabolomics show promising accuracy for forensic cause-of-death determination of drowning cases, with minimal impact from postmortem submersion times, though further research is needed to fully evaluate the applicability.

1. Background

The postmortem diagnosis of drowning is an overall assessment where the circumstances play an important role, and to make the diagnosis solely from postmortem findings can be challenging. The most indicative signs at autopsy are emphysema aquosum (overinflated lungs), foam in the airways and pulmonary edema, while other “classical” findings such as fluid in the sinuses and watery stomach content can result from the submersion of the body after death and not necessarily the drowning process itself [1]. Although these findings can support a drowning diagnosis, they are not specific. Foam in the airways

and pulmonary edema may be seen in intoxications and different natural deaths, while changes similar to emphysema aquosum can occur in both hangings, mechanical airway obstructions and deaths from natural disease [2,3]. Also, these findings tend to disappear relatively quickly and may not be present in drownings where the dead body has been submersed for more than a couple of days [1].

Metabolomics is the comprehensive study of small molecules, or metabolites, within biological systems, and in recent years, it has proven to be a valuable tool in forensic pathology for uncovering biochemical markers related to cause of death (COD) [4]. The agonal phase prior to drowning could be assumed to affect the metabolome. While drowning

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metabolomics has to our knowledge not been previously studied in humans, a preliminary trial on rats showed promising results. When comparing the metabolome of rats that were drowned with those asphyxiated with CO₂, a clear separation could be made [5]. Both groups were submersed in water for some time after death, so the metabolomic differences could not be explained by postmortem submersion. A small proteomic autopsy study on humans showed promising results with regard to separating drownings from control cases by analyzing the levels of apolipoprotein A1 and alpha-1 antitrypsin in plasma obtained from femoral blood [6]. Another study measured pulmonary surfactant-associated proteins A and D (SP-A and SP-D) in a series of medicolegal autopsies and found elevated levels in deaths from drowning [7]. SP-A and/or SP-D were also elevated in other CODs involving pulmonary injury such as acute respiratory distress syndrome (ARDS) and pneumonia, and the authors concluded that these proteins may be useful for investigating alveolar damage in the death process.

Thus, metabolomics could potentially be used to help differentiate drowning from other competing CODs of bodies found in water. Examples of potential differential diagnoses are chronic heart disease, intoxication, and trauma. If specific metabolic changes occur due to postmortem submersion, it could either involve putrefactive components that are affected by the water environment, or osmotic changes in the blood due to external water that passively enters the lungs or gastrointestinal canal. The aim of this study was to determine if post-mortem metabolomic analysis can differentiate drowning from other CODs.

2. Methods

2.1. Cases and controls

Cases considered for inclusion were deaths due to drowning where the deceased was 18 years or older and had been subject to a forensic autopsy at the Swedish National Board of Forensic Medicine (Rättsmedicinalverket, RMV) during the three-year period of November 2017 – October 2020, and for which a routine toxicological analysis of femoral blood had been performed using high-resolution mass-spectrometry.

For each case, the autopsy report and if needed the police report were reviewed for circumstances and autopsy findings related to the drowning event. The circumstances included circumstantial indication of drowning, water type and cardiopulmonary resuscitation (CPR), see Table 1. The autopsy findings recorded were presence or absence of emphysema aquosum, foam in the airways, pulmonary edema, and pleural effusions, see Table 2.

Four control groups were selected from the same time period as the drowning cases. The control groups consisted of hangings, chronic heart disease (CHD), intoxication, and deaths from trauma, respectively, and where the deceased was 18 years or older. The controls were individually matched as close as possible on sex, postmortem interval (PMI), body mass index (BMI) and age, with matching priority in that order. To test for differences in case characteristics between the drowning cases and the four control groups, Kruskal-Wallis test was used for age, BMI and PMI, and Chi-squared for sex distribution (SPSS, ver. 29.0, IBM). Except for hangings, the control groups were chosen from the perspective that they might constitute a competing COD for bodies found in water. Hangings were included as a “positive” control to see how well the model can separate two different asphyxiation processes.

Hangings with any contributory COD were excluded. The CHD group were cases where the immediate COD was either myocardial hypertrophy, atherosclerotic heart disease, or old myocardial infarction. That is, the immediate COD was a chronic heart disease without any demonstrable acute complication at the autopsy. CHD cases with intoxication as contributory COD were excluded. Intoxication cases were all cases where the immediate COD was intoxication from drugs, narcotics or biological substances. The trauma group consisted of people who died

Table 1

Classification of Drowning Circumstances, Water Type, and Cardiopulmonary resuscitation (CPR) Intervention.

Circumstances	Values	Criteria
Circumstantial indications of drowning	Witnessed	Someone witnessed the drowning incident.
	Clear indication	Indication that the person had been engaged in some water-related activity before being found lifeless in water, e.g. swimming or fishing. Cases where the forensic pathologist had assessed that the circumstances indicated suicide were also included here.
	Other	The body was found lifeless in water but there was no clear indication of water-related activity.
Water type	Salt	Drowning along the Swedish west coast where the salt level is around 15–25 ‰.
	Brackish	Drowning along the rest of the Swedish coastline where the salt level is around 3–8 ‰.
	Fresh	Drowning in lakes, rivers and other freshwater reservoirs.
	Tap Pool	Drowning in tap water, mostly bathtubs. Drowning in larger pools, presumably chlorinated water.
CPR ^a	Yes	CPR was performed by laymen and/or health care professionals, but the person was not hospitalized.
	Inpatient care	CPR was performed and the person was hospitalized.
	No	No CPR by laymen or health care professionals was performed.
	Unclear	CPR is not mentioned and it is not obvious from the circumstances whether it was performed or not.

^a CPR = cardiopulmonary resuscitation.

from either gunshot wounds in the head, being hit by a train (suicides) or traffic incidents, and that died the same day as the trauma.

Since some bodily changes observed in drowning victims can be caused by the submersion in water itself and not the drowning process [1], an ideal control group would be bodies that have died from other causes than drowning, and then been submersed in water after death. However, cases in which this can be ascertained are rare. One way to tackle this problem is to study witnessed drownings where the body was recovered from the water before the submersion-related change in question could reasonably occur.

The following data was retrieved for both cases and controls: date of autopsy, date of death (certain or plausible), date found dead, date last seen alive, age, sex, length, weight, COD, contributory COD(s), manner of death, and date of trauma (if applicable). PMI was calculated as the number of days between death and the performance of the autopsy at which blood samples for toxicology analysis were collected. When there was a certain or plausible date of death, as assessed by the forensic pathologist who performed the autopsy, the calculation was straight forward. In cases where there was neither a certain or plausible date of death, the date of death was set to the mean of the interval between when the person was found dead and last seen alive. For drowning cases, the number of days from when the body was removed from the water until the date of the autopsy was calculated. Since putrefaction can potentially be accelerated post-submersion [8], this time can be relevant besides the PMI for the metabolomic analysis.

This study was approved by the Swedish Ethical Review Authority (Dnr 2019–04530, 2024–05087–02, 2025–02459–02). Due to the retrospective nature of the study, the need of informed consent was waived by Swedish Ethical Review Authority. All methods were carried out in accordance with relevant guidelines and regulations.

Table 2
Autopsy Findings in Drowning Cases.

Autopsy findings	Values	Criteria
Emphysema aquosum (overinflated lungs)	Yes	There was either one or more of the following criteria: 1. Description in the protocol that the lungs were large/inflated/puffy or similar. 2. Description of crackles when the lungs were felt through or incised. 3. Description of acute micro emphysema in the histological examination and mentioning of this in the final assessment. 4. Mentioning of "drowning lungs" in the final assessment.
	No	It was clear from the report that none of the above criteria 1–4 were present.
	Unclear	It was unclear whether any of the above criteria 1–4 were present or not (e.g. the lungs were not described with enough detail).
Foam in the airways	Yes	Description in the protocol of foamy fluid in the nostrils, mouth or lower airways, or if it was mentioned in the background information.
	No	No foamy fluid was described in the protocol or the background information.
	Unclear	The content of the lower airways was not described in the protocol.
Pulmonary edema	Yes	Description of moderate to abundant emission of watery fluid from the pulmonary cut surfaces.
	No	Description of none to mild emission of watery fluid from the pulmonary cut surfaces.
	Unclear	No or ambiguous description of emission of watery fluid from the pulmonary cut surfaces.
Pleural effusion	Yes	Description of increased amount of pleural effusion. If quantitatively described, more than 10 ml in one lung sack was considered as increased.
	No	Negation of increased fluid or a quantity of 10 ml or less in each lung sack.
	Unclear	No or ambiguous description of pleural effusion. If there was an increased amount of pleural effusion and mentioning of putrefaction in relation to this or in the final assessment, the finding was coded as unclear since the effusion could be either antemortem effusion, putrefactive liquid, or a mixture of both.

2.2. Data acquisition and metabolomics analysis

Femoral blood samples, obtained during autopsy for routine toxicological screening, were prepared and analyzed according to a standardized procedure. Each sample was prepared by protein precipitation using an organic solvent mixture of 0.075 % formic acid in acetonitrile: ethanol (90:10), including an addition of three internal standards (amphetamine-D8, diazepam-D5 and mianserin-D3). All samples were injected on a UHPLC-ESI-QToF system (Agilent 1290 Infinity LC with an Agilent 6540 QTOF as detector, Agilent Technologies Sweden AB, Sweden). Separation was performed using gradient elution on a C18 column (150 mm×2.1 mm, 1.8 µm; Waters Acquity HSS T3 column, Water Sverige AB, Sweden). MS-data was collected in positive mode and the total acquisition time for each sample was 12 min. In the beginning and at the end of each analytical run a blank whole blood sample containing the three internal standards were analyzed. For blank and authentic samples an acceptable run showed absolute areas over 1.2×10^6 , 1.4×10^6 and 1.6×10^6 for amphetamine-D8, diazepam-D5 and mianserin-D3 respectively, a retention time deviation of maximum ± 0.1 min and a mass accuracy deviation of maximum ± 5 ppm.

The raw LC/MS data from the selected autopsy cases were exported

to mzData-files using MassHunter (v10.0, Agilent Technologies). The postmortem metabolomics analysis was conducted using the 'XCMS' package in R (4.4.1), which integrates the 'CAMERA' package for feature annotation. In XCMS the centWave algorithm was used for feature detection using the following parameters $\Delta m/z$ of 30 ppm, minimum peak width of 3 s, maximum peak width of 30 s and signal-to-noise threshold of 3 with noise variable set to 500. Retention time correction was performed using the Obiwrap function and for the grouping an m/z width of 0.05, base width of 3 and minimum fraction of 0.6 were used.

After preprocessing, the dataset was split into a training and a validation set. Every fifth sample, in sequential order based on time of registration, was allocated to the validation set, while the remaining samples were included in the training set. This procedure resulted in approximately 80 % of the data being used for training and 20 % for validation. The split was performed strictly at the case level to prevent data leakage between the training and validation sets.

2.3. Data preprocessing and multivariate analysis

The training set was normalized in Excel using the probabilistic quotient normalization, and log-transformed, scaled with unit-variance and subjected to multivariate analysis using SIMCA 17.0.2 (Sartorius AG, Germany). Features with a retention time < 45 s and > 660 s were excluded. Principal component analysis (PCA) was used to give an overview of the data, enabling identification of outliers and observation of trends. In addition, partial least square (PLS) models for PMI, age, sex and BMI were created to investigate systematic differences in the metabolic profiles related to the five CODs. Orthogonal partial least square discriminant analysis (OPLS-DA) was used to identify variables contributing to group classification between drowning and the four control groups. This was done both by modeling drowning against all control groups in one model and in four separate models with drowning against the four controls groups individually. To investigate the influence of submersion in water, we defined *the postmortem submersion interval* as the time that the body had laid in water after death. The models were rebuilt only using cases with a postmortem submersion interval of less than 24 h. In addition, to oversee the possibility that postmortem changes are accelerated after submersion, *the post-submersion interval* was defined as the time from when the body was taken out of the water until the autopsy and blood sampling was performed. All drowning cases were divided into two datasets with post-submersion intervals of ≤ 5 days or > 5 days, respectively. Binary models with drowning cases with different post-submersion interval versus each individual control groups were built and compared.

Experimental reproducibility was assessed by examining the score plots from the PCA, by cross validation in OPLS-DA model of the training set, and by external validation of the OPLS-DA model using a validation set to assess the predictability of the multivariate model. False negatives were investigated in depth.

Features with a VIP > 1.5 and p(corr) above 0.2 together with all features in the final model were identified and annotated by matching molecular weight (± 5 ppm) and retention time against an in-house database and online databases; Human Metabolome Database (<https://hmdb.ca>). All features were also uploaded into MetaboAnalyst module, functional analysis, usable for untargeted metabolomics data. The basic assumption is that putative annotation at individual compound level can collectively predict changes at functional levels as defined by metabolite sets or pathways. For visualization of metabolite differences between the five study groups a selection of representative annotated metabolites was validated through univariate analysis via Kruskal-Wallis test, with subsequent Bonferroni correction for accuracy (SPSS, ver. 29.0, IBM).

3. Results

3.1. Demographic and characteristics of the study population

A total of 514 drowning cases were identified. For 11 of these the LC/MS data was not made available for the current study. Thus, 503 drowning cases were included, with 4 control groups: hangings (n = 511), CHD (n = 510), intoxication (n = 516), and trauma (n = 497). A demographic overview of the drowning cases and the four control groups is provided in Table 3. In summary, there were no significant differences in sex or PMI. However, the trauma group and the CHD group had a significantly different age distribution compared to the drowning cases. When comparing the control groups to each other, some statistically significant differences were observed. The hanging group had a lower BMI than both the CHD and trauma groups, and the CHD group was older than all other groups.

3.2. Circumstances of the drowning event

A review of autopsy and police reports provided information on the circumstances and autopsy findings related to drowning cases, and are summarized in Tables 4 and 5, respectively. Among the cases, 23 % were witnessed drownings, while 54 % had a clear indication of drowning. In 22 % of cases, the body was found in water without clear indication of drowning. Drownings occurred mostly in freshwater (63 %), followed by brackish water (19 %), tap water (11 %), saltwater (6 %), and pool water (1 %). CPR was performed in 36 % of the cases; in 31 % without subsequent inpatient care and 5 % with subsequent inpatient care, while 63 % did not receive CPR. In 1 % of the cases CPR status was unclear, but it was clear that they had not received inpatient care.

3.3. Autopsy findings

Regarding autopsy findings, emphysema aquosum was present in 80 % of cases, absent in 14 %, and unclear in 7 %. Foam in the airways was observed in 35 % of cases, while 65 % had no foam, and 1 % was unclear. Pulmonary edema was found in 61 % of cases, absent in 22 %, and unclear in 17 %. Pleural effusions were present in 39 % of cases, absent in 48 %, and unclear in 13 %.

3.4. Multivariate modeling and model evaluation

Mass spectra data were processed using XCMS, which gives a comprehensive list of chromatographic peaks with specific accurate masses and retention times, so called features. After exclusion of features with a retention time of < 45 s and > 660 s, 2610 features remained for multivariate modeling. All five groups were included in an OPLS-DA model demonstrated statistical significance, with R² = 0.27, Q² = 0.19, along with a CV-ANOVA p-value of < 0.001. An overlap between the groups was observed (Figure S1). For the training set, the model correctly classified 65 % of the 1895 samples, with a sensitivity of 77 % and a specificity of 91 % for the drowning cases. For the false negatives there were an overrepresentation of cases with inpatient care. These cases were often classified as intoxication.

Table 3
Demographic overview with medians and interquartile ranges.

	Drowning	Hanging ^a	CHD ^b	Intox	Trauma ^c	p-value
N	503	511	510	516	497	
Sex (male %)	75 %	75 %	75 %	75 %	76 %	n.s.
Age (years)	61 (43–74)	59 (44–71)	63 (53–74)	60 (41–69)	57 (37–72)	< 0.001
BMI (kg/m ²)	26 (23–28)	25 (22–28)	26 (23–30)	25 (22–29)	26 (23–29)	< 0.001
PMI (days)	6 (4–9)	6 (4–8)	6 (4–9)	6 (4–9)	6 (4–8)	n.s.

^a Hanging has a significantly different distribution in BMI compared to cardiovascular decease and trauma group.

^b The cardiovascular decease group has a significantly different distribution age against all groups.

^c The trauma group has a significantly distribution in age compared to drowning group.

Table 4
Circumstances in Drowning Cases.

Circumstances	Values	%
Circumstantial indications of drowning	Witnessed	23
	Clear indication	54
	Other	22
Water type	Salt	6
	Brackish	19
	Fresh	63
	Tap	11
	Pool	1
Cardiopulmonary resuscitation	Yes	31
	Inpatient care	5
	No	63
	Unclear	1

Table 5
Autopsy Findings in Drowning Cases.

Autopsy findings	Values	%
Emphysema aquosum (overinflated lungs)	Yes	80
	No	14
	Unclear	7
Foam in the airways	Yes	35
	No	65
	Unclear	1
Pulmonary edema	Yes	61
	No	22
	Unclear	17
Pleural effusion	Yes	39
	No	48
	Unclear	13

To further evaluate the model's predictability, the remaining autopsy cases in the validation set were predicted using the same model. The predicted score plot and corresponding ROC curve for the 505 cases in the validation set are shown in Fig. 1. For the validation set, the model accurately classified 53 % of the samples, with an average (macro) sensitivity of 53 % and average specificity of 88 %, see Table S1. Specifically, for the drowning class predictions a sensitivity of 69 % and specificity of 84 % was achieved. In the validation set, four cases had received inpatient medical treatment (i.e. not only CPR). Two were correctly classified as drowning while the other two were misclassified. All four were on the classification border between drowning and intoxication.

As it is unlikely that five hypotheses exist simultaneously, and to investigate the specific metabolic differences between drowning and the four controls groups, four individual OPLS models were built. As expected, model performance increased, giving values for R² between 0.61 and 0.76 and Q² between 0.40 and 0.56. Classification of drowning cases versus the four control groups had sensitivities and specificities that ranged between 83 % and 87 % and 78–89 %, respectively. The predicted score plots of the validation set for the four individual OPLS-DA models are found in Fig. 2 and a misclassification table is found in the supportive material, Table S2. Five cases of drownings that were classified as controls in all four binary model were examined in more

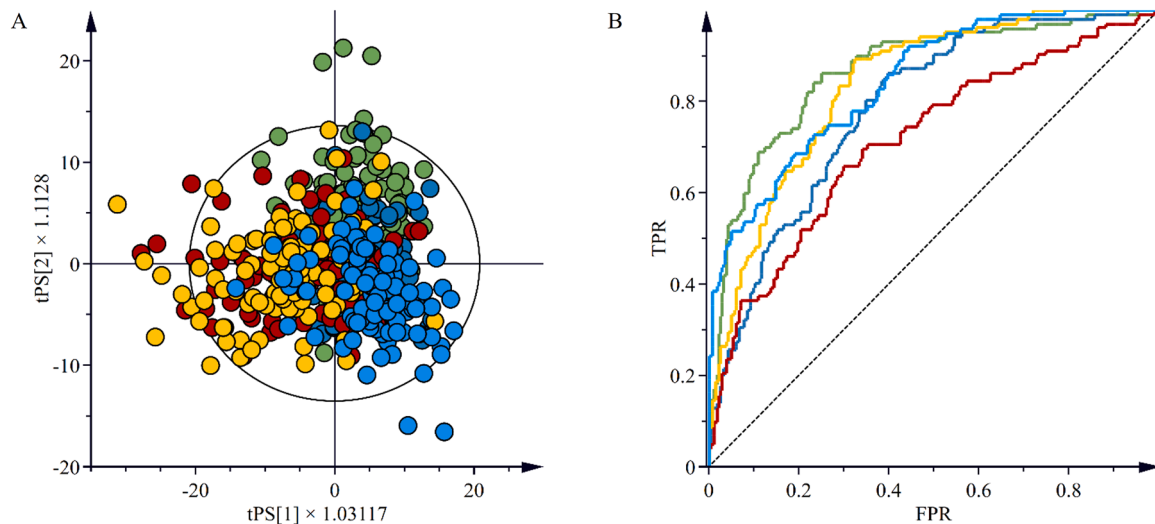


Fig. 1. Predicted scores of the validation set are presented, showing a model performance with $R^2 = 0.27$ and $Q^2 = 0.19$. Model significance was confirmed using cross-validated ANOVA (CV-ANOVA), yielding a p-value of $p < 0.001$. The classification performance for each cause of death, assessed by the Area Under the Curve (AUC) from the Receiver Operating Characteristic (ROC) analysis, was as follows: drowning (AUC = 0.87), hanging (AUC = 0.79), chronic heart disease (CHD) (AUC = 0.72), intoxication (AUC = 0.84), and trauma (AUC = 0.84).

detail. Four of these were found in bathtubs and they all had findings that could suggest an alternative COD-diagnosis; for example, myocardial hypertrophy with sparse signs of drowning or high concentration of sedatives.

To investigate the influence of water, we built models that included only drowning cases with short postmortem submersion intervals ($n = 216$) and compared them with previous models consisting of cases with varying postmortem submersion intervals. Only marginal differences were observed when using models restricted to cases with short postmortem submersion intervals (see [Figure S2](#)). Additionally, we examined models based on different post-submersion intervals. No apparent differences were observed between models using cases with short post-submersion intervals and those using cases with long post-submersion intervals. Furthermore, models trained on cases with short post-submersion intervals were able to predict outcomes for cases with long post-submersion intervals, and vice versa (see [Figure S3](#)).

3.5. Metabolite identification and pathway analysis

In-house and online public database matching led to the identification of 52 features that differentiated the drowning group from the four control groups, see [Table S3](#). The metabolite analysis showed distinct patterns among different CODs, with notable similarities between CHD and intoxication cases, as well as between trauma and hanging cases. Overall some metabolites, such as 1-(beta-D-ribofuranosyl)-1,4-dihydropyridinamide, 5-hydroxy-L-tryptophan, methylguanosine, and dimethylguanosine, show significantly higher values in CHD and intoxication compared to drowning and hanging cases. Lysophosphatidylcholines (LysoPCs) generally have lower values in CHD and intoxication cases compared to hanging, trauma, and drowning cases. Carnitine-related metabolites show mixed trends, but some (e.g., dodecanoylcarnitine, tetradecanoylcarnitine) appear higher in hanging, trauma, and drowning cases. L-tyrosine, phenylalanine, and nicotinamide tend to be higher in CHD and intoxication cases.

For the functional analysis in MetaboAnalyst, 2610 features were uploaded along with fold changes and p-values from four separate comparisons: drowning versus each of the four individual control groups. MetaboAnalyst identified 245 empirical compounds in the dataset. The following pathways showed a combined p-value below 0.05, based on both the Mummichog and GSEA algorithms, in at least one of the individual comparisons between drowning and a control

group: C21-steroid hormone biosynthesis and metabolism, fatty acid activation, glycolysis and gluconeogenesis, urea cycle/amino group metabolism, CoA catabolism, vitamin B5 - CoA biosynthesis from pantothenate, drug metabolism - cytochrome P450, glycerophospholipid metabolism and bioperin. Specific pathways important for each of the control groups versus drowning cases are shown in [Fig. 3](#).

4. Discussion

When a dead body is found in water, a forensic pathologist often needs to consider other possible CODs besides drowning, i.e. natural disease, intoxication, or trauma. For instance, a dead body found on a shoreline could indicate drowning, or sudden collapse from a medical event near water. Similarly, a dead body in a bathtub may be the result of some natural disease leading to a fatal collapse, a homicidal drowning, or a drowning exacerbated by factors such as drug or alcohol intoxication. The diagnosis of drowning can be difficult to make from autopsy findings alone, especially when there are findings indicating possible competing CODs [1]. Recognizing the limitations of any single marker due to variability in environmental and physiological factors, our approach seeks to identify a biomarker pattern capable of improving the accuracy of the drowning diagnosis. ([Fig. 4](#))

4.1. Selection of control groups

If a metabolomic pattern of drowning is to be used as a diagnostic tool, drowning cases need to be compared with other CODs that may come up as differential diagnoses when a dead body is found in water. Therefore, we chose three control groups with such CODs. CHD can cause sudden death due to arrhythmia or acute infarction. Arrhythmia itself can obviously not be demonstrated at autopsy, but even an acute infarction may go unnoticed since you need several hours survival time to develop macro- or microscopic infarction signs in the myocardium [9]. Such an acute cardiac event is a relevant differential diagnosis for a submerged body with signs of CHD. For example, elderly individuals found deceased in bathtubs may have suffered a fatal cardiac event leading to submersion [10]. Intoxication is another important differential diagnosis, particularly in cases of polydrug intoxications, where the cumulative or synergistic effects of substances can cause sedation severe enough to result in collapse and submersion. Ethanol, known for its sedative effects, is the most common substance found in drowning cases,

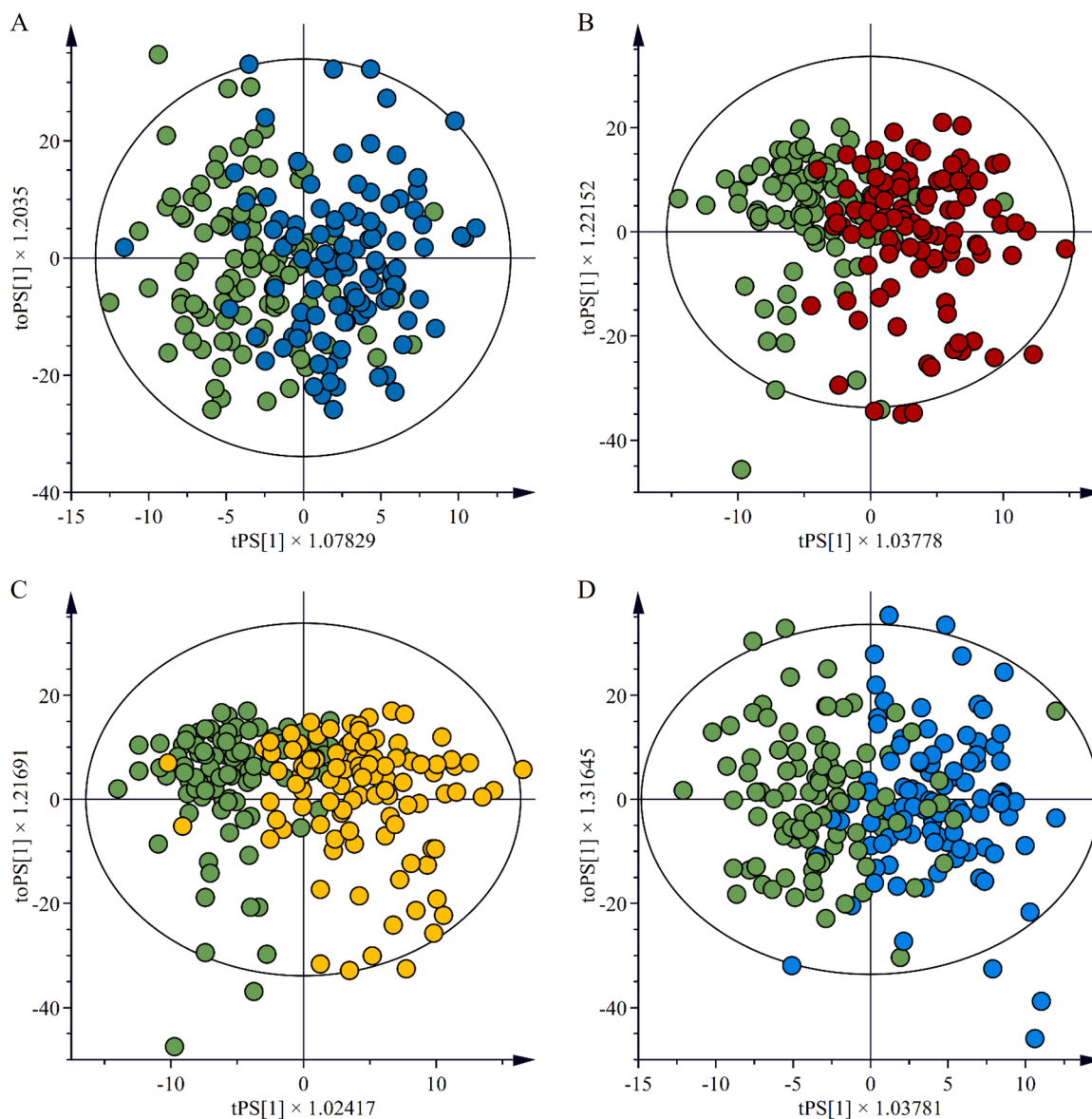


Fig. 2. Predicted score plots of the validation set in four binary OPLS-DA models: (a) drowning vs. hanging ($R^2 = 0.61$, $Q^2 = 0.40$), (b) drowning vs. chronic heart disease (CHD), ($R^2 = 0.68$, $Q^2 = 0.48$); (c) drowning vs. intoxication ($R^2 = 0.76$, $Q^2 = 0.56$), and (d) drowning vs. trauma ($R^2 = 0.70$, $Q^2 = 0.48$). Each model classifies cases based on metabolomic profiles, limited overlap and classification accuracy illustrates the potential of metabolomics in forensic cause-of-death determinations.

especially in accidental drownings [11,12]. When a body with potentially lethal injuries is found in water, the question may arise whether the person drowned or was dead before entering the water. In a suicidal jump from a high bridge, the question may be of more academic interest than relevant to the police. However, in a suspected homicide the differential diagnosis might be of great importance. Since most of the trauma cases that were included in this study had immediate or short death process, their metabolic profile should be similar to cases of sudden trauma followed by submersion. The fourth control group consisted of hangings. Hanging typically leaves distinct neck markings, making it relatively easy to differentiate from drowning, even if the body is placed in water postmortem. However, since both drowning and hanging are forms of asphyxial death, comparing their metabolic profiles may help determine whether they can still be reliably distinguished beyond physical findings. Additionally, it is reasonable to hypothesize that hanging might produce metabolic changes similar to other forms of fatal neck pressure, such as manual strangulation or arm-locks. Since these asphyxial deaths do not always leave characteristic signs, they

could pose a diagnostic challenge when a dead body is recovered from water. Investigating the metabolic similarities and differences between these types of asphyxial deaths could provide valuable forensic insights, improving the ability to distinguish drowning from other causes of death in submerged bodies.

4.2. Demographic comparison between cases and controls

The demographic comparison between drowning cases and the four control groups showed no significant differences in sex or PMI. However, some differences in age distribution and BMI were observed. The CHD and trauma groups had a statistically significant difference in age compared to the drowning cases, primarily due to differences in the lower quartiles; 53 years in CHD, 37 years in trauma, and 43 years in drowning. This could potentially influence metabolomic profiles, particularly when comparing drowning to CHD, as age-related metabolic changes may be more pronounced. The median and upper quartile differences were smaller, suggesting a limited overall impact. Similarly,

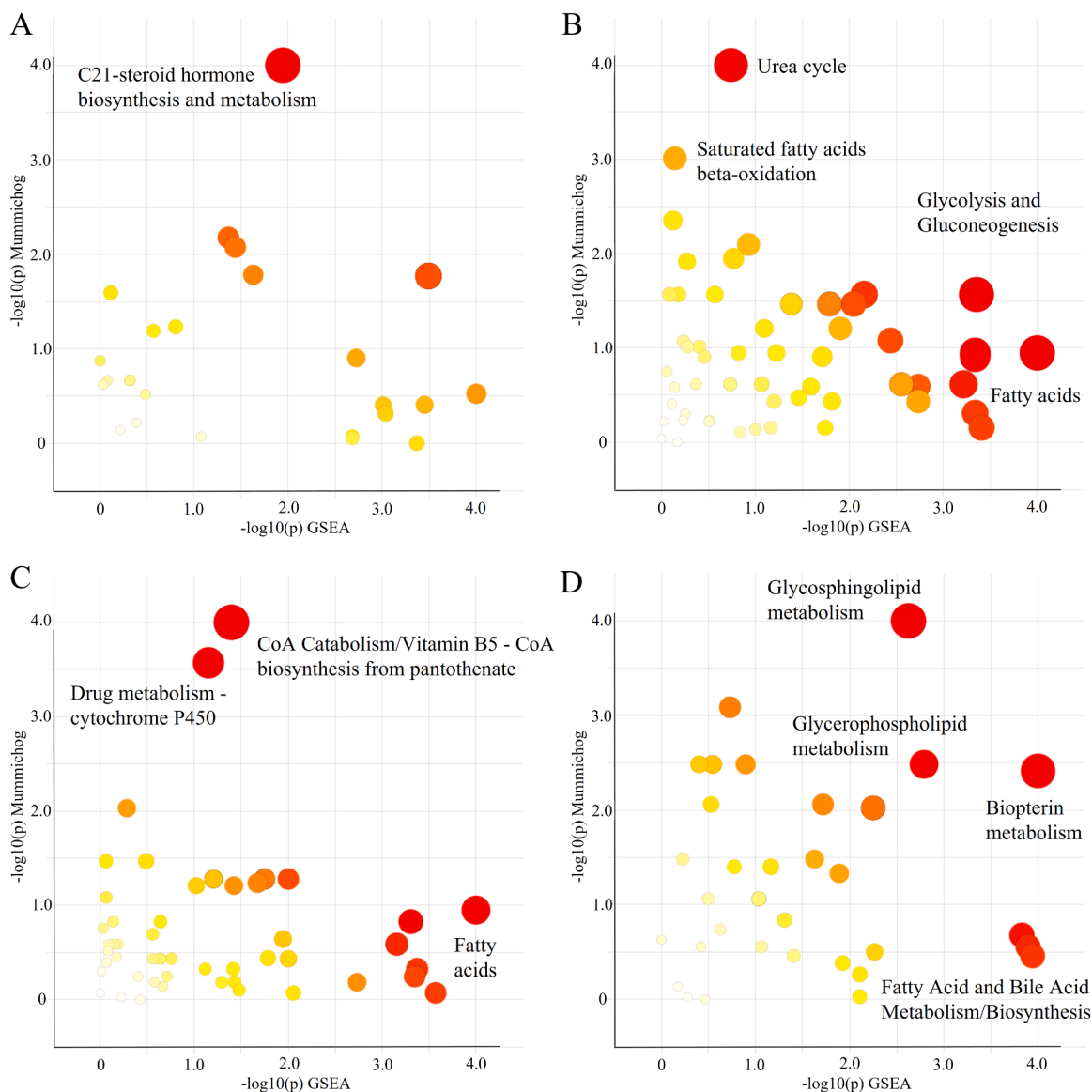


Fig. 3. Functional analysis of untargeted high-resolution mass spectrometry (HRMS) data from drowning cases compared with other causes of death, performed using MetaboAnalyst 6.0. A total of 2610 metabolite features were included, with input formatted as a complete peak list, with m/z values, with retention time (RT), p-values and fold-change. Functional analysis was conducted using both Mummichog (y-axis) and Gene Set Enrichment Analysis (x-axis) algorithms. Subfigures represent pathway enrichment results for four binary comparisons involving drowning versus other causes of death: (a) Drowning vs. Hanging, (b) Drowning vs. Chronic Heart Disease (CHD), (c) Drowning vs. Intoxication and (d) Drowning vs. Trauma. Each plot illustrates the top enriched pathways or metabolite sets that distinguish drowning from the respective comparator group.

the hanging group had a lower BMI compared to the CHD and trauma groups. While these differences were statistically significant, their absolute values were small. Given the large sample size (approximately 500 cases per group), these differences could be influenced by statistical power rather than having a meaningful impact on the metabolome. Therefore, while minor demographic differences exist between the groups, their actual effect on the metabolome remains uncertain and warrants further consideration.

4.3. Autopsy findings in drowning cases

In our study, foam in the airways was observed in 35 % of cases, which is notably lower than the 73 % reported by a previous study by Schneppe et al. [13]. In that study, foam was more seldomly noted in bodies with longer lay time in water (equivalent to our definition of postmortem submersion interval), putrefactive changes, and when resuscitation attempts had been made. Such correlations are not

surprising and might explain differences between studies. In our study 36 % of the cases had been exposed to resuscitation attempts. Another possible explanation is how well the forensic pathologists note and describe findings of foam. In our material, we noted that some pathologists explicitly described the absence of foam, indicating that they had actively looked for it. Others did not mention either presence or absence of foam, which opens up the possibility that they might have forgotten to document it.

Pulmonary edema was identified in 61 % of our cases and pleural effusion in (at least) 39 %. Thus, these findings are common in our material, consistent with previous literature, but they are unspecific findings that also occur in various other CODs. Emphysema aquosum was present in 80 % of the cases. A review on postmortem findings in drownings reported that the incidence of emphysema aquosum varies between studies, with average incidences between 65 % and 80 %, though even less than 40 % has been reported [1]. In Schneppe et al. almost all drowning cases were reported to have emphysema aquosum,

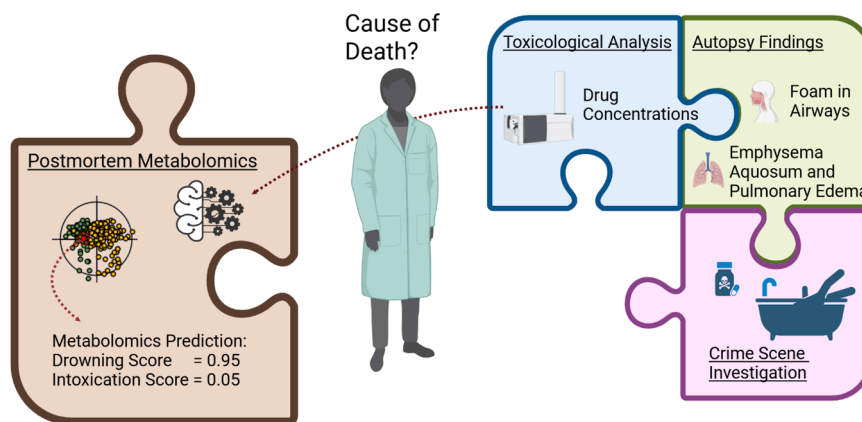


Fig. 4. Illustration of a multidisciplinary forensic investigation into the cause of death. A forensic pathologist uses multiple sources of information, such as the crime scene, autopsy findings, and toxicological results, to determine the cause of death. Postmortem metabolomics, which analyzes patterns in the metabolome, offers a novel complementary approach. By reusing data from toxicological screenings, patterns in endogenous metabolites can be modelled to predict the likely cause of death. For example, the red dot shown has a drowning score of 0.95 (where 1.00 indicates a perfect prediction), compared to a score of 0.05 for drug intoxication, suggesting drowning is the more likely cause. Based on the validation set, a threshold of 0.95 would correspond to a specificity of 98 %.

but the sign was also present in almost one in three of their non-drowning cases [13]. The variations between studies can probably, to some extent, be explained by the same factors as foam in the airways. Another plausible explanation for differing figures between studies is the subjective character of this finding, with an obvious risk of confirmation bias since the forensic pathologist expects to find emphysema aquosum when the circumstances indicate drowning.

Our results of autopsy findings, along with the findings reported in the literature, underscores the limitations of relying on individual drowning signs for forensic diagnostics as well as their partly subjective nature. One additional aspect is that many of the classical drowning signs, including emphysema aquosum and pulmonary edema, can also be present in non-drowning water deaths, reducing their specificity [13].

4.4. Modeling approaches and classification performance

In this study, two modeling approaches were used: one incorporating all CODs and another using four separate binary models. In the model including all CODs, differences were observed only at the population level, with considerable overlap between groups. As a result, the model exhibited limited predictive power, as indicated by both cross-validation and external validation. A likely reason for model overlap is the similarity in metabolomic profiles among control groups, especially between trauma and hanging, which showed strong correlations in comparison to drowning. Drowning had the highest classification accuracy (69 %) in the five-class model, while hanging (49 %) and trauma (64 %) were more often misclassified, mostly as each other or as drowning, suggesting shared metabolic features. These similarities may reflect commonalities in agonal processes rather than mechanisms of death. Despite potential diagnostic uncertainty, drowning showed the most distinct profile, likely due to specific metabolic effects from water inhalation and pre-mortem struggle.

In contrast, chronic heart disease (CHD) had the lowest classification rate (27 %) and the most dispersed misclassifications. CHD, defined here by chronic conditions, represents a heterogeneous category with varying death mechanisms—ranging from sudden arrhythmia to gradual ischemia, some of which may leave no macroscopic evidence at autopsy [9]. As a result, cases with unclear pathology, including those potentially caused by undetectable conditions such as epilepsy [14,15] or electrolyte disturbances [16], may be misclassified as CHD, contributing to its metabolomic variability.

In forensic casework, it is uncommon to consider five potential CODs simultaneously. To better reflect real-world forensic scenarios, binary

models were developed, comparing drowning against each individual alternative COD. These models demonstrated a notable increase in both sensitivity and specificity, highlighting their practical utility. The results suggest that metabolomic models could serve as valuable tools for forensic pathologists, particularly when two competing hypotheses must be evaluated and traditional autopsy findings are inconclusive. Integrating such models into forensic workflows could significantly improve the accuracy of drowning diagnoses. For example, if a body is found in water with high levels of drugs in the blood, the COD could be drowning, with intoxication as a contributory factor, or it could be an overdose, with submersion occurring postmortem. In such cases, a forensic pathologist strives to determine which scenario is more likely. A toxicological report stating the probability or the metabolome similarity of the case at hand with all previous analyzed drowning and intoxication cases could be very valuable for the forensic pathologist when determining the cause of death.

In selecting drowning cases, we used the diagnosis set by the forensic pathologist. This approach entails a risk of diagnostic misclassification bias, as some non-drowning cases may have been erroneously classified as drownings. A more conservative selection could have been to only include witnessed drownings, where one can be almost certain that drowning was at least part of the death mechanism. However, even witnessed cases are complex from a metabolomic perspective and may introduce other sources of bias, since these cases also can have parallel ongoing processes such as myocardial infarction, epileptic seizures, or other conditions that initiated the drowning event. Furthermore, a more conservative selection of cases would have created a model based on a subpopulation that is not representative of the general population of suspected drownings encountered in forensic practice. This could result in a selection bias and reduced generalizability, i.e. a model with better separation between drowning and the control groups, but a more limited use in real-world casework. Among the cases diagnosed as drownings, the majority were either witnessed or fulfilled our definition of “clear indication” of drowning. Thus, the group as a whole should be representative of true drownings. When performing a sub-group analysis with only witnessed drownings vs. hangings (Figure S2), there is some improvement in separation. This improvement could be due to a higher proportion of correctly diagnosed drownings, but it could also be explained by other properties of this subgroup since it is not matched on sex, age, BMI, and PMI.

4.5. Metabolic signatures in the context of previous studies

The MetaboAnalyst functional analysis indicates differences in stress

hormones, glycolysis, and lipid metabolism between drowning cases and the four control groups. Since no prior studies have taken this exact approach, it remains difficult to interpret the metabolic patterns observed, particularly given the complexity of multiple biological processes and death mechanisms occurring simultaneously. It is therefore important to point out that the aim of this investigation was to develop a predictive model for drowning as cause of death, rather than identify metabolites linked to specific death mechanisms. Drowning cases, for instance, have not been extensively studied in metabolomics. However, some research has focused on looking at inflammation markers, electrolytes, blood gases, pulmonary surfactant proteins, and aquaporin expression [6,7,17–20]. Research on mechanical asphyxia is also lacking, particularly in human studies. However, two animal models are of interest: one for suffocation [21] and another for drowning [5]. In Locci et al.'s pig model, succinate accumulation during the asphyxial period was observed, suggesting it may serve as a significant biomarker for mechanical asphyxia. This accumulation could be linked to cellular stress and potential damage caused by hypoxia, as succinate plays a key role in the metabolic response to oxygen deprivation [21]. In Zhang et al.'s rat model of drowning, corticosterone and lactic acid levels were elevated, likely reflecting stress and struggle during the drowning process [5]. Metabolites associated with hemolysis were also increased, which Zhang suggested may result from water entering the blood circulation through diffusion and osmosis. Although animal studies have advanced understanding of hypoxia-related mechanisms, human post-mortem metabolomics provides more directly relevant insights for forensic investigations. It captures complex, real-world biological variability and supports the reduction of animal use in research, consistent with institutional and ethical guidelines.

Our findings suggest that cortisol levels are similar in drowning, trauma, and hanging cases, while they are elevated in intoxication and CHD cases. This may be related to the degree of stress experienced and the duration of the agonal period before death. We have previously demonstrated that postmortem metabolomics can effectively distinguish drug intoxications from other causes of death with high specificity [4, 22,23]. By identifying biomarkers such as acylcarnitines for opioid toxicity and metabolic alterations associated with insulin intoxications, metabolomics provides a more precise approach to death screening. In this study, acylcarnitine levels were lower in the intoxication group compared to hanging and trauma cases, which confirms our previous results. However, the strong correlation between acylcarnitine profiles in CHD and intoxication cases may pose challenges in using it as a marker for opioid-related respiratory depression. Cardiovascular diseases have been thoroughly studied, especially for non-forensic applications. A meta-analysis by McGranaghan et al. review of 41 metabolites linked to cardiovascular disease identified consistent changes in lysophosphatidylcholines (LysoPCs) and amino acids, patterns that we also observed in our CHD population compared to the other three groups [24]. In contrast, although elevated acylcarnitine levels have been reported in some studies on cardiovascular disease, our data did not show a significant change [24]. For trauma, numerous metabolites have been identified in post mortem CSF that showed significant concentration differences in lethal traumatic brain injury samples compared with acute cardiovascular control fatalities [25].

4.6. Limitations of the model

One limitation of this study is the occurrence of false negatives, which seem to be more frequent among cases that were hospitalized before death. These cases were often misclassified as intoxication rather than drowning. A possible explanation is that medical treatment, such as the administration of drugs or resuscitation efforts, results in a protracted respiratory failure, making the metabolic profile more similar to that of an intoxication case. Our results indicate that cases with prolonged hospital stays should be excluded from such analyses to improve classification accuracy. This observation may also be relevant for

clinical metabolomics and biobank-based studies, where metabolic profiles will most probably be influenced by prior medical treatment and/or variable agonal phases. In the five misclassified drowning cases that were investigated in more depth due to their typical intoxication profile, interesting patterns were found. Four of these were found in bathtubs and they all had findings that could suggest an alternative COD-diagnosis. Hence, one could speculate that these five cases perhaps had other CODs than drowning, or that they at least had significant contributory CODs besides drowning. This could explain why they were misclassified in our model.

Another limitation of this study is that all drowning cases involved exposure to water, whereas none of the control cases did. Metabolomic changes due to merely postmortem submersion would reasonably take at least several hours to significantly affect femoral blood. Therefore, any specific metabolic changes in drowning deaths where the body was retrieved from the water within a few hours should reflect the drowning process itself, rather than postmortem submersion. Thus, by doing a subgroup analysis of these cases, it should be possible to investigate the potential confounding effect of postmortem submersion.

Decomposition progresses more slowly while a body is submerged, mainly because of the lower ambient temperature [26]. However, the decomposition has been observed to proceed rapidly once the body is removed from water [8]. To assess whether this factor introduced a bias, we constructed a PLS model incorporating submersion time and created three datasets: Cases with a short postmortem submersion interval (<24 h), cases with a short post-submersion interval (<5 days) and cases with a long post-submersion interval (>5 days). No pattern related to postmortem submersion time could be observed, and models including only short submersion times were neither significantly improved nor worsened. This suggests that time in water did not have a strong impact on the model and influence the model's classification. For post-submersion interval, a slight trend was observed ($R^2 = 0.4$) in a PLS model. However, a model trained on short post-submersion interval cases was able to predict those with long post-submersion interval and vice versa, indicating that post-submersion interval and thereby time dependent putrefaction was not the primary factor driving classification in our model. Since no drastic changes or consistent trends were observed in classification accuracy, we believe that postmortem submersion time and post-submersion interval have a minimal impact on our models.

However, whether even a short postmortem submersion interval may affect the metabolome cannot be excluded from our data. If a dead body is submersed, a passive influx of water into the lungs and stomach may occur. This water could theoretically affect the metabolome through diffusion of water into the blood. To analyze this possibility, control groups with other CODs than drowning and where the body has been put in water after death are necessary. On review of the currently available cohort, it was not possible to construct a large enough case group to build such a model. We believe that our approach comes as close as practically possible to investigate the possible influence of postmortem submersion on the metabolome. However, it would be valuable to analyze such cases with the current model to see how they are classified, and we plan to do such tests in the future.

5. Conclusion

Our findings highlight the potential of metabolomics-based models as a valuable tool for forensic investigations, particularly in cases where traditional autopsy findings are inconclusive. Drowning cases exhibited a high classification accuracy, with both high sensitivity and specificity versus all four control groups. In addition, our findings indicate that postmortem submersion time and post-submersion interval have minimal impact on metabolomic classification. However, more studies are needed to confirm the actual effect of submersion and explore the influence of other environmental factors on metabolite stability. The study also revealed that CHD cases were misclassified most frequently, likely

due to the heterogeneous nature of this group and the difficulties in determining an acute versus prolonged fatal cardiac event at autopsy. These challenges emphasize the importance of refining metabolomic models to distinguish between natural and unnatural causes of death. Furthermore, our results suggest that hospitalization prior to death may influence metabolomic profiles, potentially leading to misclassifications. Future studies within postmortem metabolomics, but also investigations using samples from the living, should further investigate the effects of hospitalization on metabolomic signatures to determine whether these cases should be analyzed separately or excluded in metabolomics investigations.

CRedit authorship contribution statement

Fredrik Tamsen: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Henrik Green:** Writing – review & editing, Funding acquisition, Conceptualization. **Fredrik C. Kugelberg:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Gustav Engvall:** Writing – review & editing, Conceptualization. **Carl Söderberg:** Writing – review & editing, Conceptualization. **Albert Elmsjö:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Liam J. Ward:** Writing – review & editing, Project administration, Conceptualization.

Declaration of Competing Interest

The authors have nothing to declare.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.forsciint.2026.112894](https://doi.org/10.1016/j.forsciint.2026.112894).

Data availability

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available, due to legal and ethical considerations.

References

- [1] A. Tyr, et al., Diagnosing fatal drownings: a review of the postmortem findings, *Forensic Sci. Int* 364 (2024) 112251.
- [2] L. Stephenson, C. Van den Heuvel, R.W. Byard, The persistent problem of drowning - a difficult diagnosis with inconclusive tests, *J. Forensic Leg. Med.* 66 (2019) 79–85.
- [3] C. Castiglioni, P. Baumann, T. Fracasso, Acute pulmonary emphysema in death by hanging: a morphometric digital study, *Int J. Leg. Med.* 130 (5) (2016) 1281–1285.
- [4] L.J. Ward, et al., Postmortem metabolomics as a high-throughput cause-of-death screening tool for human death investigations, *iScience* 27 (5) (2024) 109794.
- [5] F.Y. Zhang, et al., A preliminary study on early postmortem submersion interval (PMSI) estimation and cause-of-death discrimination based on nontargeted metabolomics and machine learning algorithms, *Int J. Leg. Med.* 136 (3) (2022) 941–954.
- [6] D. Hernandez-Romero, et al., Proteomics in deaths by drowning: diagnostic efficacy of apolipoprotein A1 and alpha-1Antitrypsin, pilot study, *Diagnostics* 10 (10) (2020).
- [7] L. Quan, et al., Postmortem serum levels of pulmonary surfactant-associated proteins A and D with regard to the cause of death in medicolegal autopsy, *Leg. Med.* 11 (1) (2009) S301–S303.
- [8] J.L. Caruso, Decomposition changes in bodies recovered from water, *Acad. Forensic Pathol.* 6 (1) (2016) 19–27.
- [9] T.D. Cummings PM, K.M. Springer, *Atlas of Forensic Histopathology*, Cambridge University Press, 2010, p. 200.
- [10] M. Guay, M. D'Amours, V. Provencher, When bathing leads to drowning in older adults, *J. Saf. Res.* 69 (2019) 69–73.
- [11] P. Lunetta, et al., Unintentional drowning in Finland 1970–2000: a population-based study, *Int. J. Epidemiol.* 33 (5) (2004) 1053–1063.
- [12] T.R. Driscoll, J.A. Harrison, M. Steenkamp, Review of the role of alcohol in drowning associated with recreational aquatic activity, *Inj. Prev.* 10 (2) (2004) 107–113.
- [13] S. Schneppe, M. Dokter, B. Bockholdt, Macromorphological findings in cases of death in water: a critical view on "drowning signs", *Int J. Leg. Med.* 135 (1) (2021) 281–291.
- [14] S. Love. *Neuropathology - A Guide for Practising Pathologists*, First ed., Springer, Berlin, Heidelberg, 2001, p. 284.
- [15] N.A. Shlobin, et al., Sudden death in epilepsy: the overlap between cardiac and neurological factors, *Brain Commun.* 6 (5) (2024) fae309.
- [16] B. Zilg, et al., Interpretation of postmortem vitreous concentrations of sodium and chloride, *Forensic Sci. Int* 263 (2016) 107–113.
- [17] T. Miyazato, et al., Molecular pathology of pulmonary surfactants and cytokines in drowning compared with other asphyxiation and fatal hypothermia, *Int J. Leg. Med.* 126 (4) (2012) 581–587.
- [18] H. Maeda, et al., Analysis of postmortem biochemical findings with regard to the lung weight in drowning, *Leg. Med (Tokyo)* 11 (1) (2009) S269–S272.
- [19] E.J. Armstrong, K.L. Erskine, Investigation of drowning deaths: a practical review, *Acad. Forensic Pathol.* 8 (1) (2018) 8–43.
- [20] M.D. Perez-Carceles, et al., Serum biochemical markers in drowning: diagnostic efficacy of Strontium and other trace elements, *Forensic Sci. Int* 214 (1–3) (2012) 159–166.
- [21] E. Locci, et al., Metabolomics improves the histopathological diagnosis of asphyxial deaths: an animal proof-of-concept model, *Sci. Rep.* 11 (1) (2021) 10102.
- [22] A. Elmsjö, et al., Postmortem metabolomics reveal acylcarnitines as potential biomarkers for fatal oxycodone-related intoxication, *Metabolites* 12 (2) (2022).
- [23] L.J. Ward, et al., Postmortem metabolomics of insulin intoxications and the potential application to find hypoglycemia-related deaths, *Metabolites* 13 (1) (2022).
- [24] P. McGranaghan, et al., Predictive value of metabolomic biomarkers for cardiovascular disease risk: a systematic review and meta-analysis, *Biomarkers* 25 (2) (2020) 101–111.
- [25] S. Bohnert, et al., Neuroforensomics: metabolites as valuable biomarkers in cerebrospinal fluid of lethal traumatic brain injuries, *Sci. Rep.* 14 (1) (2024) 13651.
- [26] P. Saukko, B. Knight. *Knight's Forensic Pathology*, Fourth ed., CRC Press, Taylor & Francis Group, 2016.