Forensic Science International: Synergy 10 (2025) 100580

Contents lists available at ScienceDirect

Forensic Science International: Synergy

journal homepage: www.sciencedirect.com/journal/forensic-science-international-synergy

Manner of death prediction: A machine learning approach to classify suicide and non-suicide using blood metabolomics

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ARTICLE INFO

Keywords:
Suicide
Machine learning
Forensic metabolome
Biomarkers
Manner of death
Nuclear magnetic resonance spectroscopy

ABSTRACT

The classification of the manner of death (MOD) is a critical step in forensic investigations. The process is based on scene investigation, autopsy, histological and toxicological findings. However, in complex suicide cases, these findings may be insufficient to clearly establish the MOD and need potential biomarkers to assist judicial determinations. This study aims to identify specific biomarkers in the blood that could distinguish suicide from the non-suicidal deaths group. Heart blood samples were collected from suicide (n = 45) and non-suicide cases (n = 45) and metabolomic profiles were analyzed using proton nuclear magnetic resonance spectroscopy. Nineteen blood metabolites were significantly different between the groups ($p < 0.05$); especially, 4-hydroxyproline, sarcosine and heparan sulfate emerged as potential biomarkers for differentiating between the groups. A logistic regression-based predictive model incorporating sarcosine and heparan sulfate achieved sensitivity and specificity values of 73 % and 72 %, respectively. The integration of machine learning with blood metabolomics holds significant potential in forensic science and may apply to the model to adopt in criminal justice.

1. Introduction

Suicide is a significant global public health issue and more than one in every 100 deaths result from suicide [1]. It is estimated that 700,000 to 750,000 people died by suicide each year [2]. After death, an autopsy is often performed to determine the cause of death and the manner of death. In most jurisdictions, the manner of death is generally classified into one of five categories including homicide, suicide, accident, natural and undetermined. Accurately determining the manner of death is an essential process for the victim's family, law enforcement, research, public health policy and insurance matters [3–6].

Over 90 % of suicide cases involve psychiatric disorders, substance abuse, anxiety disorders and schizophrenia [7]. Underestimates the true impact of suicide, as many cases are misclassified as unnatural or undetermined deaths [7]. Sometimes, relatives may alter the scene to obscure the true nature of the event due to religious or insurance-related reasons [8]. Conversely, homicides may be staged to resemble suicide, complicating forensic analysis and the determination of the manner of death [9]. The undetermined classification is used when insufficient information regarding the circumstances surrounding the death is available [3].

Signs of a struggle, suicide notes, closed-circuit television (CCTV) footage, autopsy and laboratory findings to make their determinations are circumstantial evidence for facilitating determination of the manner of death. However, suicide cases frequently occur in secluded locations where evidence is limited. Suicide notes are rare and may not reliably confirm intent [8]. This can lead to questions from relatives, law enforcement, and legal professionals, especially in cases involving individuals without a history of psychiatric illness or stress. Relatives may reject a suicide conclusion and sometimes allege homicide, which can cast suspicion on the last person to have interacted with the deceased. Therefore, an accurate manner of death determination is essential for establishing the truth and ensuring justice.

Biomarker discovery has emerged as a critical area of research, aiming to identify unique characteristics indicative of specific diseases or conditions. Recent advances in metabolomics, the study of small

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https://doi.org/10.1016/j.fsisyn.2025.100580
Received 12 November 2024; Received in revised form 16 February 2025; Accepted 19 February 2025

molecules involved in metabolic processes, allow researchers to explore biochemical changes associated with various pathological conditions to identify disease biomarkers [10]. Machine learning has developed to be a valuable tool for improving suicide prediction in psychiatric patients. Predicting suicidal behaviors is difficult because of the lack of clear psychiatric biomarkers and the low predictive power of individual risk factors [11]. In forensic science, studies have increasingly combined machine learning and metabolomics to enhance death investigations, including estimating the post-mortem interval (PMI) and determining the cause of death [12–14]. In recent years, the integration of machine learning and metabolomics has significantly advanced forensic science, particularly in determining the cause and manner of death. For instance, studies have demonstrated the potential of combining metabolomics with machine learning algorithms to estimate postmortem submersion intervals and differentiate causes of death [15]. Additionally, the application of machine learning techniques to postmortem metabolomic data has been shown to enhance the accuracy of postmortem interval predictions [14]. These approaches highlight the value of integrating advanced computational methods with biochemical analyses to improve forensic investigations.

However, no study has explored the combination of machine learning and manner of death prediction. This study aims to investigate potential machine learning models to classify suicide and non-suicide using Blood Metabolomics.

2. Materials and methods

2.1. Chemical and reagents

Acetonitrile, deuterium oxide (D_2O) and 3-(trimethylsilyl)- [2, 2, 3, 3-d4]-1-propionate sodium salt (TSP) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Subjects and study design

This study was approved by the Ethics Committee of the Faculty of Medicine, Chiang Mai University, Thailand (the study code: FOR-2566-0234). Informed consent was obtained from close relatives before specimen collection. Autopsy cases performed at the Department of Forensic Medicine, Faculty of Medicine, Chiang Mai University from June 15, 2023, to May 31, 2024, were included in this study. The individual crops aged greater than 60 years, inconclusive manner of death, decomposition and being unable to collect heart blood samples were excluded. The study groups consisted of suicide (n = 45) and non-suicide groups (n = 45). The flow chart of the sample selection process is demonstrated in Fig. 1.

2.3. Collection and preparation of heart blood specimens

Three milliliters of heart blood sample were collected in a heparin tube and stored at −80 °C before analysis. The samples were extracted with acetonitrile following the Somtua method [16]. Briefly, one mL of the blood sample was transferred into a plastic tube and extracted with 3 mL of acetonitrile. The solution was shaken using a Mix Mate (Eppendorf, Hamburg, Germany) at 2000 RPM, 25 °C, for 10 min. The supernatant was separated and lyophilized. The dried sample was re-dissolved with 0.6 mL of 0.1 mM TSP prepared in D_2O. Metabolite levels were measured using a 500 MHz NMR, employing a technique to water suppression.

2.4. Acquisition parameters

The proton NMR (^{1}H NMR) spectrum was determined on a Bruker AVANCE 500 MHz instrument (Bruker, Bremen, Germany) equipped with a Carr-Purcell-Meiboom-Gill (CPMG, RD-90°, (t-180°), n-acquire) pulse sequence for ^{1}H NMR measurements. Spectra were acquired at

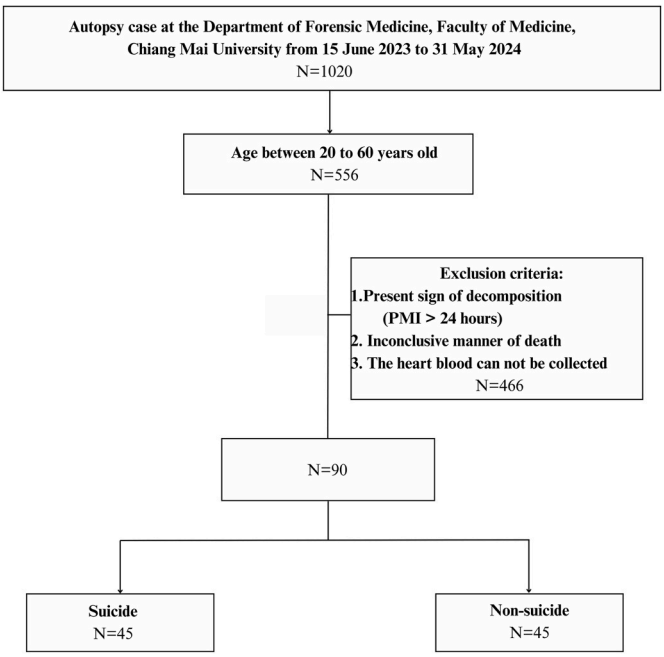

Fig. 1. The flowchart demonstrates the sample selection process based on the inclusion and exclusion criteria.

27 °C using water suppression pre-saturation, with the following parameters: 16 scans, a 1-s relaxation decay, a 3.95 s acquisition time, an 8278.146 Hz spectral window, a 0.126 Hz resolution for free induction decay (FID) and a 60.40 μs dwell time (D.W.). A 90° pulse was applied with 16 signal averages (NSAs). Baseline and phase corrections were performed using TopSpin 4.0.7 software. Spectra analysis ranged from 0.00 to 12.00 ppm, with data normalized to the total integrated area. Metabolite resonances were identified using human metabolome databases [17] and TSP was used as an internal standard to quantify 24 energy-related metabolites across all samples.

2.5. Internal standard and quality control

The TSP was selected as the internal standard which is distinctively located at 0.000 ppm and emerging from an area of higher magnetic field intensity compared to other protons. Quality control (QC) samples were prepared by combining 200 μL from each blood sample. The pooled samples were then aliquoted for QC analysis. These QC samples underwent the same processing as the primary samples, following all previously described steps. The analysis of non-targeted metabolites was conducted using the specified methods.

2.6. Peak assignment and chemical identification

Each chemical compound was identified using the Human Metabolome Database (HMDB), accessed in June 2024. Peak acquisition and *J*-coupling analysis were conducted using Bruker TopSpin software, version 4.0.7. The NMR spectra were interpreted based on chemical shift values, spin-spin coupling, signal patterns, and coupling constants. Non-targeted metabolites were matched and the chemical shift of the center peak varied to 0.01 ppm against the HMDB database.

2.7. Calculation of non-targeted metabolite concentration by quantitative NMR

The data were imported into MNOVA Software (version 12.0.0, MestreLab Research, Spain) for intensity identification. The TSP peak was adjusted and calibrated at 0.000 ppm and then, the intensity of the

209 spectra in the chromatogram were identified. After collecting the peaks, the intensity of all metabolite peaks was converted to concentrations using the equation [18] which is shown below:

$$\frac{I_A}{I_B} = \frac{H_A}{H_B} \times \frac{C_A}{C_B}$$

where I_A = the intensity value of the chemical, I_B = the intensity value of TSP, H_A = the number of hydrogen atoms in the metabolite, H_B = the number of hydrogen atoms in TSP ($H_B = 14$), C_A = the concentration of the metabolites and CB is the concentration of TSP (μM).

2.8. Analysis of specific blood metabolomic predictors

The MetaboAnalyst 6.0 free online tool, accessed on September 1, 2024, was utilized for metabolomics data analysis. Before data analysis, two classes, non-negative numbers for the compound concentrations or peak intensity values, and missing value imputations were checked. The data were normalized using quartile, log-transformed (base 10), and auto-scaled for data scaling. Partial-least square discriminant analysis (PLS-DA) was performed to understand metabolite differences between suicide and non-suicide groups. The Importance measure was analyzed by using the variable importance in projection (VIP). Wilcoxon rank-sum tests were analyzed by selecting equal group variance, non-parametric tests, raw, and a p-value threshold of 0.05 to identify significant metabolite differences between suicide and non-suicide groups. In this step, the program automatically calculated the p-value and false discovery rate (FDR). The study employed Receiver Operating Characteristic (ROC) curve analysis to evaluate the specific biomarkers to distinguish between the groups. The ROC curve is widely regarded as an effective evaluation method, as it offers a comprehensive, threshold-independent, and visually intuitive approach to assessing the discriminative power of the biomarkers. The power of the biomarkers is provided from the area under the curve (AUC) as a single, interpretable overall performance metric [19,20]. The AUC was calculated to evaluate the specific biomarkers for distinguishing between the suicide and non-suicide groups using classical univariate ROC curve analysis.

2.9. Model performance development

From the classical univariate ROC curve analysis, metabolites which had values greater than 0.7 ($p < 0.05$) were selected and matched for generating prediction models. The ROC curve-based model evaluation (Tester) was conducted to develop the suicide prediction model using a logistic regression algorithm. Then, 10-fold cross-validation, a subtype of resampling methods, was utilized to evaluate the reliability of the prediction models and prevent overfitting [21]. The values of sensitivity and specificity were evaluated for model performance. MetaboAnalyst 6.0 was used to perform all analyses for model performance development.

2.10. Metabolic pathway impact assessment

Pathway analysis was performed using the MetaboAnalyst 6.0 online platform, accessed on February 1, 2025. After data normalization, following the same protocol used in the analysis of specific blood metabolomic predictors, the KEGG pathway-based metabolite set library was selected. The parameter settings included selecting Scatter Plot as the visualization method and Mammals (Homo sapiens, KEGG) as the pathway library.

2.11. Statistical analysis

After data processing, Data are presented as quartiles. Normality was assessed using the Kolmogorov-Smirnov test. The central tendency differences between the two groups were analyzed by Wilcoxon rank-sum tests in MetaboAnalyst6.0, as described above. The Chi-square test was applied to analyze categorical variables, including sex, non-communicable diseases (NCDs), psychiatric disorders, illegal drug users, and ethanol drinkers, to identify differences in demographic data of participants between suicide and non-suicide groups. An independent samples *t*-test was used to assess differences in age and BMI between the two groups.

3. Results

3.1. Demographic data of participants

Ninety cases were separated into two groups: the suicide group (n = 45) and the non-suicide group (n = 45). In the suicide group, the causes of death included hanging (56 %), carbon monoxide poisoning (22 %), pesticide intoxication (11 %), gunshot injury (4 %), falls from height (4 %), and exsanguination (3 %). The non-suicide group consisted of homicide, natural death, and accidents. Psychiatric disorders are identified based on medical history, information provided by close relatives or the quality of psychiatric medications in the blood samples. The number of males, age, body mass index (BMI), non-communicable diseases (such as diabetes mellitus, hypertension, and dyslipidemia), exposure to illegal drugs (especially methamphetamine and THC), and alcohol use among the groups were not different between the two groups. In this study, 17 cases of psychiatric disorders were observed in the suicide group and 7 cases in the non-suicide group. The number of psychiatric disorders was significantly higher in the suicide than in the non-suicide group. The findings are presented in Table 1 and Supplementary Table 1.

3.2. Identification of specific blood biomarkers associated with suicide cases

The multivariate datasets of each group were analyzed using partial least squares discriminant analysis (PLS-DA), with results shown in Fig. 2(a). PLS-DA component 1 (8 %) is represented on the X-axis, and component 2 (11.2 %) on the Y-axis. There are some separate areas of metabolic profiles between the suicide and non-suicide groups. Thus, variable importance in projection (VIP) scores were used to investigate the specific metabolites between the groups. The metabolites which had VIP scores greater than 1.0 were considered as potential biomarkers. The top 15 VIP score value of metabolite including 4-hydroxyproline, sarcosine, heparan sulfate, pyridoxamine, biopterin, citrulline, 5-carboxycytosine, triglycerides, malic acid, cytidine, thymine, N-acetyl-D-glucosamine, epinephrine, and L-histidine are shown in Fig. 2(b). The PLSDA of cause of death are presented in Supplementary Fig. 1.

The differences in levels of metabolites between the groups were compared by a *t*-test. Significantly nineteen metabolites were presented ($p < 0.05$) and the details are shown in Table 2. From FDR (q-value), 4-hydroxyproline and sarcosine were significantly decreased in suicide cases, while heparan sulfate was significantly increased ($p < 0.001$ and

Table 1
Characteristics of suicide and non-suicide groups.

Variables	Suicide (n = 45)	Non-Suicide (n = 45)	p-value
Sex: Male (%)	32 (71 %)	36 (80 %)	0.33
Age (year-old)	41 ± 9.9	40.2 ± 12.4	0.49
Body mass index (kg/m^2)	23.08 ± 4.39	24.37 ± 4.86	0.19
Noncommunicable diseases	12 (26.7 %)	7 (15.6 %)	0.20
Psychiatric disorder	17 (37.8 %)	7 (15.6 %)	0.02*
Illegal drugs users	3 (6.7 %)	8 (17 %)	0.11
Ethanol drinkers	20 (44.4 %)	16 (35.6 %)	0.40

Continuous variables are presented as mean ± standard deviation (SD) and p-values were calculated using the independent samples *t*-test. Categorical variables are presented as numbers in each group, and p-values were calculated using the chi-square test. * Significant difference between the two groups.

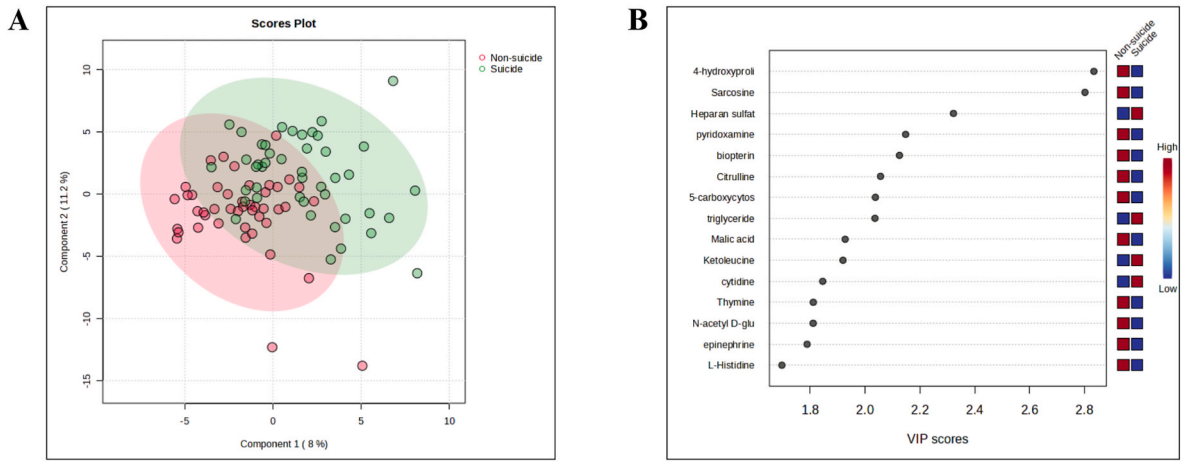

Fig. 2. Metabolomic Profiles of Suicide and Non-Suicide Cases. (a) PLS-DA analysis of suicide and non-suicide cases. (b) The top 15 metabolites with the highest VIP scores. The data were analyzed using the MetaboAnalyst 6.0 online tool.

Table 2
Nineteen metabolites showed significant differences between the suicide and non-suicide groups.

Metabolites	Suicide			Non-suicide			p-value	q-value
	Q1	Q2	Q3	Q1	Q2	Q3		
4-Hydroxyproline	30.29	64.42	273.83	43.24	145.66	325.43	<0.001	0.04*
Sarcosine	14.15	26.99	103.30	19.02	38.46	162.44	<0.001	0.04*
Heparan sulfate	6.18	16.23	61.47	6.28	16.50	59.03	<0.001	0.04*
Triglyceride	9.32	19.46	57.61	8.37	22.00	48.72	<0.001	0.19
N-acetyl glycine	31.37	82.17	331.90	41.51	104.72	395.80	0.01	0.39
Acetoacetic acid	23.24	58.11	198.12	32.74	64.66	302.48	0.01	0.39
GAL	13.18	24.83	113.24	17.62	39.83	170.55	0.01	0.44
Cholesterol	11.78	31.45	89.03	13.02	28.46	85.27	0.02	0.44
Ornithine ($\times 10^3$)	0.26	0.47	1.10	2.44	5.89	1.59	0.02	0.44
Thymine	15.16	33.45	218.52	19.98	54.05	263.57	0.03	0.44
Malic acid	70.13	152.07	522.16	54.67	170.75	497.58	0.03	0.44
Pyridoxamine	25.71	80.37	186.78	31.77	83.48	236.95	0.03	0.44
GL	6.63	21.44	77.22	8.14	18.39	69.69	0.03	0.44
Hypotaurine	27.48	57.29	227.59	30.47	63.73	173.25	0.03	0.44
Biopterin	15.80	24.78	121.33	21.05	44.12	162.01	0.03	0.44
Acetyl-CoA	3.87	24.23	62.95	4.84	12.13	57.58	0.03	0.44
L-Histidine	2.99	5.20	28.39	4.36	10.05	45.03	0.04	0.48
L-Cystathionine	24.65	68.50	177.43	26.51	66.06	191.00	0.05	0.48
5-Carboxycytosine	3.15	5.94	34.23	4.44	9.19	51.28	0.05	0.48

Data are presented as quartiles. Significant differences in central tendency between the two groups ($p < 0.05$) were analyzed using Wilcoxon rank-sum tests. * significant differences between groups after FDR adjustment assessed using MetaboAnalyst 6.0.($q < 0.05$).GAL = Gamma-aminobutyryl-lysine; GL = Sn-glycerophosphocholine.

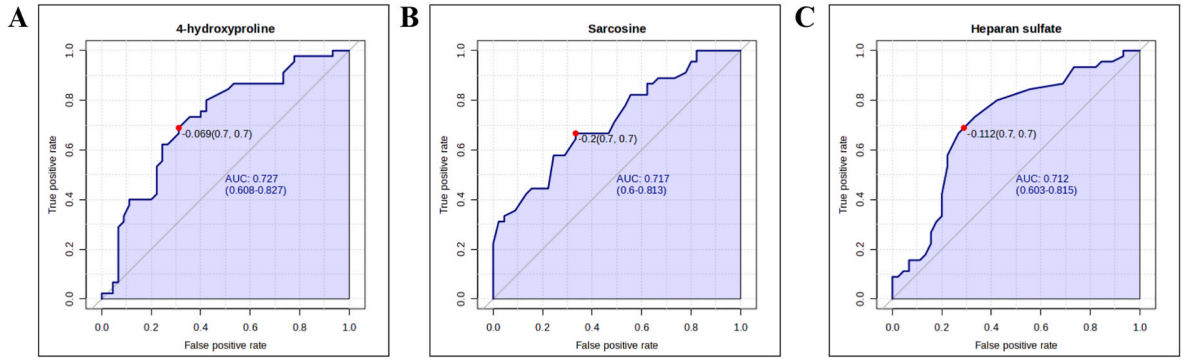

Fig. 3. ROC curves analysis for three metabolites related to suicide cases. (a) 4-Hydroxyproline: AUC = 0.73, 95 % CI = 0.61–0.83. (b) Sarcosine: AUC = 0.72, 95 % CI = 0.60–0.81. (c) Heparan sulfate: AUC = 0.71, 95 % CI = 0.60–0.82. These data were analyzed using classical univariate ROC curve analysis through the MetaboAnalyst online tool (version 6.0).

q = 0.04). The performance of biomarkers was confirmed using ROC curves and the AUC value of 4-hydroxyproline, sarcosine, and heparan sulfate presented 0.73 (95 % CI = 0.61–0.83), 0.72, (95 % CI = 0.60–0.81) and 0.71 (95 % CI = 0.60–0.82), respectively. The results are presented in Fig. 3.

3.3. Metabolic pathway impact in suicide case

Pathway analysis identified significant alterations in five metabolic pathways in suicide cases based on the criteria of $-\log_{10}(P) \geq 1.3$ and impact value ≥ 0.3. These pathways include arginine and proline metabolism, glycosaminoglycan degradation, vitamin B6 metabolism, pyrimidine metabolism, and glycine, serine and threonine metabolism. Among these, glycine, serine and threonine metabolism were identified as the most impactful pathway ($-\log_{10}(P) = 1.33$, impact value = 0.45), while arginine and proline metabolism were defined as the most statistically significant pathway ($-\log_{10}(P) = 2.52$, impact value = 0.35) in this study. The metabolic pathway impacts are presented in Fig. 4.

3.4. Machine learning for suicide prediction

We employed a ROC curve-based model evaluation (Tester) and prediction of suicide as a manner of death was conducted using a binary regression model. The results indicated that 4-hydroxyproline, sarcosine, and heparan sulfate were the most effective metabolites for generating suicide prediction models. Seven models were generated based on the three metabolites, and the models are presented in Table 3. The performance of each of the predictive models was analyzed and presented in the AUC value, sensitivity, and specificity. Subsequently, 10-fold cross-validation was performed to assess the validity of all predictive models. The results are shown in Table 4. Model 6 demonstrated the highest predictive performance, with an AUC of 0.80, sensitivity of 0.73, and specificity of 0.72. Following 10-fold cross-validation, it maintained a high AUC of 0.79, confirming its robustness and surpassing the performance of the other models.

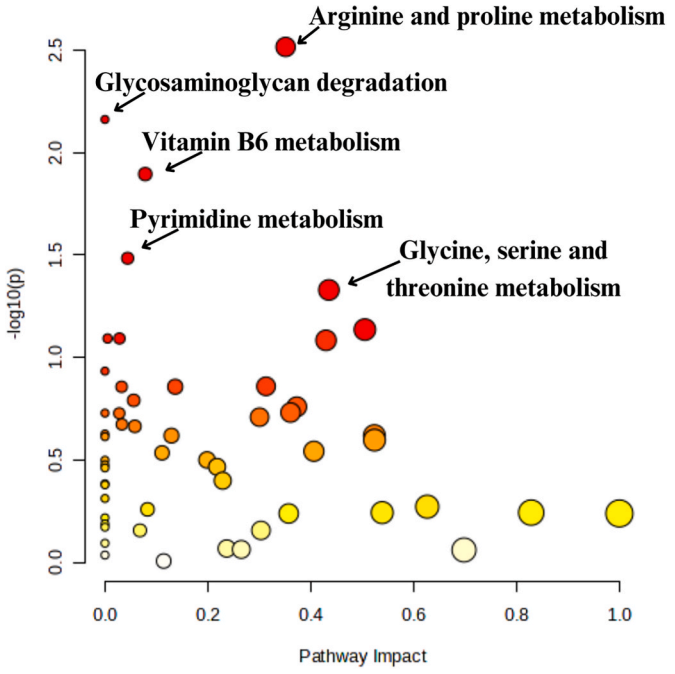

Fig. 4. Pathway analysis of metabolites in blood samples of suicide group compared to non-suicide group. The x-axis represents the pathway impact, while the y-axis highlights the most significantly altered clusters at the top. The color of each circle corresponds to the p-value, and its size reflects the pathway impact.

Table 3
The equations for the suicide predictive models, along with the optimal cutoff values, are presented.

Number	Suicide Predictive Models[a]	P Cut off value
1	logit(P) = log (P/(1 - P)) = −0.029 to 0.853 4-hydroxyproline	0.50
2	logit(P) = log (P/(1 - P)) = −0.238 to 1.742 Sarcosine	0.53
3	logit(P) = log (P/(1 - P)) = 0.1 + 1.353 Heparan sulfate	0.47
4	logit(P) = log (P/(1 - P)) = −0.221 to 0.576 4-hydroxyproline - 1.428 Sarcosine	0.47
5	logit(P) = log (P/(1 - P)) = 0.106–0.881 4-hydroxyproline + 1.462 Heparan sulfate	0.50
6	logit(P) = log (P/(1 - P)) = −0.289 to 2.376 Sarcosine + 1.805 Heparan sulfate	0.54
7	log (P/(1 - P)) = −0.226 to 0.467 4-hydroxyproline - 1.995 Sarcosine + 1.772 Heparan sulfate	0.55

[a] Predictive models were generated using ROC curve-based model evaluation (Tester) and binary regression in the MetaboAnalyst online tool (version 6.0).

4. Discussion

This is the first study in Thailand to examine the complete suicide metabolomic profiles to differentiate suicide from non-suicides in forensic cases. NMR is a favored method for clinical metabolomics studies due to its robustness, reproducibility, and ability to detect unknown metabolites in complex mixtures. It is non-destructive, non-biased, and highly quantifiable, requiring little to no sample preparation or chemical derivatization, and it facilitates the routine identification of novel compounds [22,23]. Determining the manner of death in suicide cases presents both challenges and significant importance in forensic medicine. Forensic pathologists, law enforcement, and legal professionals consider findings and evidence to determine the manner of death. Ambiguous and insufficient evidence led to the missing of the manner classification. The identification of specific biochemical markers provides valuable scientific evidence that could assess in the justification process [3,24]. 4-hydroxyproline, sarcosine and heparan sulfate had the potential to distinguish suicide from non-suicide cases. Specifically, decreased levels of 4-hydroxyproline and sarcosine, along with increased levels of heparan sulfate. The diagnostic performance of Model 6 (logit(P) = log (P/(1 - P)) = −0.289–2.376 Sarcosine + 1.805 Heparan sulfate) achieved the highest performance and maintained top performance even after 10-fold cross-validation.

Numerous factors impact forensic metabolomic analyses, including postmortem interval, sex differences, age, underlying diseases, and drug use [16,25–29]. This study, both suicide and non-suicide cases were enrolled and demographic data and medical history were recorded. Sex, job loss, low socioeconomic status, alcohol use, substance abuse, brain disease, endocrine abnormalities, and inflammatory processes have been associated with suicide [7]. These factors interrupt the interpretive process and may contribute to discrepancies between our results and previously reported data. In this study, sex, age, body mass index (BMI), non-communicable diseases (NCDs), illegal drug use, and alcohol consumption, were similar between the suicide and non-suicide groups. However, the number of psychiatric disorders was significantly higher in the suicide group compared to the non-suicide group. Psychiatric disorders, particularly mood disorders, are major risk factor for suicide [7].

All 209 metabolites were analyzed using partial least squares discriminant analysis (PLS-DA) to assess the metabolomic profile. The PLS-DA model revealed specific metabolomic profiles that can differentiate suicide cases from non-suicide cases. The AUCs for 4-hydroxyproline, sarcosine, and heparan sulfate exceeded 0.7, indicating acceptable discrimination and were the best biomarkers for determining the manner of death in cases of suicide. However, the AUCs for some metabolites were below 0.7, which may be attributed to variations in subjects' ancestry, dietary habits, living conditions, and the post-mortem interval [30–33]. Post-mortem metabolomic profiles can

Table 4
The Performance of each prediction model for categorizing suicide as the manner of death is presented.

Models	Test model				10-fold cross-validation			
	AUCs	95%CI	Sensitivity	Specificity	AUCs	95%CI	Sensitivity	Specificity
1	0.72	0.56–0.89	0.71	0.67	0.71	0.60–0.82	0.69	0.69
2	0.71	0.52–0.85	0.66	0.64	0.70	0.60–0.81	0.67	0.67
3	0.70	0.56–0.85	0.72	0.69	0.70	0.58–0.81	0.71	0.71
4	0.73	0.53–0.88	0.77	0.56	0.72	0.61–0.83	0.76	0.60
5	0.73	0.58–0.86	0.72	0.66	0.72	0.61–0.82	0.69	0.67
6	0.80	0.65–0.91	0.73	0.72	0.79	0.69–0.88	0.73	0.69
7	0.79	0.65–0.92	0.71	0.73	0.78	0.69–0.87	0.69	0.73

Data were analyzed using ROC curve-based model evaluation and logistic regression via the MetaboAnalyst online tool (version 6.0). The 95 % CI refers to the 95 % confidence interval. AUCs represent the areas under the curves provided by the ROC models.

change over time after death [33], which can affect suicide identification in forensic cases. Consequently, this study excluded deceased individuals showing signs of decomposition, typically characterized by greenish discoloration of the abdomen, indicating a postmortem interval (PMI) exceeding 24 h [3].

4-Hydroxyproline, a proline derivative, is produced through hydroxylation in collagen and serves as a major precursor for glycine, which is essential for the synthesis of glutathione, DNA, heme, and proteins and scavenges reactive oxygen species [34,35]. Recent studies in living subjects have demonstrated a correlation between urinary levels of hydroxyproline and proline with mental health issues, including stress and anxiety, suggesting a link between chronic stress and mental health [36,37]. In our study, we found that 4-hydroxyproline levels in completed suicides were lower than those in non-suicide cases. There is currently no evidence indicating decreased levels of 4-hydroxyproline in completed suicides or postmortem. In rat models, a significant decrease in hydroxyproline levels in skeletal muscles has been observed as postmortem intervals (PMI) increase, indicating collagen degradation after death [38]. The difference in tissue types may affect 4-hydroxyproline levels; therefore, the decrease in 4-hydroxyproline in postmortem human blood requires further investigation.

Our results showed that sarcosine levels decreased in suicide cases compared to non-suicide cases. In living subjects, sarcosine (N-methyl glycine) acts as a type 1 glycine transporter inhibitor enhancing glutamatergic neurotransmission [39]. Dysfunction in the glutamatergic system has been observed in cases of major depressive disorder [40]. The reduced levels of sarcosine observed in our findings may be linked to the psychological and physiological stressors that precede suicide. These findings align with prior studies in living subjects [39–42] which suggest a decrease in sarcosine levels among suicide cases, indicating that PMI may not affect these levels.

We observed an increase in heparan sulfate levels in the completed suicide cases. Heparan sulfate is a sulfated polysaccharide present on cell surfaces and in the extracellular matrix, existing in various forms of proteoglycans. It plays a crucial role in neuroinflammation and systemic inflammatory responses [43,44]. The inflammation link to the pathophysiology of suicide through several mechanisms [45]. The elevation of heparan sulfate level in our study might indicate neuroinflammatory or systemic inflammatory processes related to suicide.

The results for sarcosine and heparan sulfate are consistent across both living and postmortem subjects, whereas the levels of 4-hydroxyproline exhibit a contrasting pattern likely influenced by PMI. This discrepancy may be due to postmortem metabolic alterations of 4-hydroxyproline, for which no current human data is available. Therefore, sarcosine and heparan sulfate not only aid in suicide classification but also shed light on the underlying biochemical pathways potentially linked to suicidal behavior.

Emerging research indicates that disruptions in various metabolic pathways, including those involving arginine and proline, glycosaminoglycan degradation, vitamin B6, pyrimidine, and the metabolism of glycine, serine, and threonine, may be linked to psychiatric disorders and suicidal behavior. Alterations in arginine and proline metabolism have been associated with depressive symptoms, potentially through the mediation of inflammatory responses. Studies have observed elevated interleukin-6 (IL-6) levels in patients with major depressive disorder (MDD), suggesting that disruptions in these metabolic pathways may influence mood disorders by modulating inflammation [46]. Vitamin B6, in its active form pyridoxal 5′-phosphate (PLP), serves as a cofactor for numerous enzymes involved in neurotransmitter synthesis and amino acid metabolism. Deficiencies in PLP can lead to neurological manifestations, including epilepsy, likely due to neurotransmitter imbalances. Additionally, vitamin B6 plays roles beyond enzymatic cofactor activity, such as functioning as an antioxidant and modulating immune responses [47]. The metabolism of glycine, serine, and threonine is crucial for central nervous system function. Disruptions in these pathways can affect neurotransmitter levels and neuronal health, potentially contributing to psychiatric conditions. For instance, abnormalities in amino acid metabolism have been implicated in mood disorders, with alterations in metabolites like proline and glutamic acid observed in depressive states [48]. While direct evidence linking glycosaminoglycan degradation and pyrimidine metabolism to psychiatric disorders and suicide is limited, the interconnected nature of metabolic pathways suggests that disturbances in these areas could indirectly influence mental health. Further research is necessary to elucidate these potential connections.

This study incorporates machine learning with metabolomics to classify suicide as a manner of death. Machine learning offers significant promise in the field of forensic science, particularly in identifying distinct blood metabolites in suicide cases compared to non-suicide cases. The combinations can enhance the predictive power of machine learning models. Machine learning algorithms were used to develop predictive models based on selected biomarkers. By leveraging logistic regression and ROC curve-based model evaluation, the study constructed seven models to distinguish suicide from non-suicide cases. Model 6, which incorporated sarcosine and heparan sulfate, achieved the best predictive performance with an AUC of 0.80, sensitivity of 0.73, and specificity of 0.72. This suggests that machine learning can significantly enhance the accuracy and applicability of metabolomic findings in forensic contexts.

4.1. *Limitations and future directions*

This study demonstrates the potential of NMR-based metabolomics combined with machine learning to differentiate suicide cases from non-suicidal deaths. However, some limitations should be considered, such as the small sample size and the high inter-individual variability inherent in forensic samples. Challenges remain, including the need for larger datasets to enhance model generalizability and to account for variables like postmortem interval (PMI) and environmental factors. Expanding this research to include diverse populations and prolonged PMIs could improve the model's applicability in various forensic scenarios. Additionally, incorporating multi-omics (e.g., proteomics, genomics) with ML might strengthen biomarker-based suicide prediction, offering a more comprehensive biological profile for forensic

investigation.

5. Conclusion

4-hydroxyproline, sarcosine and heparan sulfate are key blood metabolites that significantly differ between suicide and non-suicide cases, presenting a novel approach to forensic investigation. This study also explores the potential of predictive modeling to assist forensic pathologists in determining the manner of death. The integration of machine learning with blood metabolomics demonstrates promise in forensic science, offering insights into biochemical pathways associated with psychological distress. Nonetheless, as the first study on manner-of-death biomarkers and predictive models for suicide cases in Thailand, additional research is necessary to establish the reliability and applicability of these biomarkers in forensic and public health contexts.

CRediT authorship contribution statement

Witchayawat Sunthon: Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **Thitiwat Sopananurakkul:** Methodology, Investigation, Data curation. **Giatgong Konguthaithip:** Software, Methodology. **Yutti Amornlertwatana:** Writing – review & editing. **Somlada Watcharakhom:** Writing – review & editing. **Kanicnan Intui:** Writing – review & editing. **Churdsak Jaikang:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Funding

This work was supported by the Faculty of Medicine, Chiang Mai University, grant No. 42-67.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

We would like to thank the Faculty of Medicine Chiang Mai University for supporting article publication charge and grant in this study. This work was supported by the Faculty of Medicine, Chiang Mai University, grant no 42-67. The researchers would like to express their gratitude to the deceased donors and their families for their support and permission to collect research samples. Not forgetting our gratitude to MetaboAnalyst who developed open-source metabolic analysis program and Omicsforum community who shared technique of data analysis and interpretation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsisyn.2025.100580.

References

[1] K.L. Lovero, P.F. Dos Santos, A.X. Come, M.L. Wainberg, M.A. Oquendo, Suicide in global mental health, Curr. Psychiatry Rep. 25 (6) (2023) 255–262, https://doi.org/10.1007/s11920-023-01423-x.
[2] M. Pallocci, P. Passalacqua, C. Zanovello, L. Coppeta, C. Ferrari, F. Milano, S. Gratteri, N. Gratteri, M. Treglia, Forensic characterisation of complex suicides: a literature review, Forensic Sciences 4 (3) (2024) 277–288, https://doi.org/10.3390/forensicsci4030020.
[3] V.J.M. DiMaio, D.K. Molina, Forensic Pathology, 3 ed., CRC Press, Boca Raton, FL, USA, 2022.
[4] J.C.H.I. Randy Hanzlick, Gregory J. Davis, A guide for manner of death classification, 1 ed., National Association of Medical Examiners.
[5] P. Lindqvist, L. Gustafsson, Suicide classification–clues and their use. a study of 122 cases of suicide and undetermined manner of death, Forensic Sci. Int. 128 (3) (2002) 136–140, https://doi.org/10.1016/s0379-0738(02)00188-3.
[6] A.S. Advenier, N. Guillard, J.C. Alvarez, L. Martrille, G. Lorin de la Grandmaison, Undetermined manner of death: an autopsy series, J. Forensic Sci. 61 (Suppl 1) (2016) S154–S158, https://doi.org/10.1111/1556-4029.12924.
[7] L. Sher, M.A. Oquendo, Suicide: an overview for clinicians, Med Clin North Am 107 (1) (2023) 119–130, https://doi.org/10.1016/j.mcna.2022.03.008.
[8] R.W. Byard, A. Austin, The role of forensic pathology in suicide, Forensic Sci. Med. Pathol. 7 (1) (2011) 1–2, https://doi.org/10.1007/s12024-010-9186-5.
[9] M. Irmici, M. D'Aleo, G. Pelletti, F. Pirani, A. Giorgetti, P. Fais, S. Pelotti, Homicide or suicide? A probabilistic approach for the evaluation of the manner of death in sharp force fatalities, J. Forensic Sci. 69 (1) (2024) 205–212, https://doi.org/10.1111/1556-4029.15413.
[10] M.M. Rinschen, J. Ivanisevic, M. Giera, G. Siuzdak, Identification of bioactive metabolites using activity metabolomics, Nat. Rev. Mol. Cell Biol. 20 (6) (2019) 353–367, https://doi.org/10.1038/s41580-019-0108-4.
[11] A. Pigoni, G. Delvecchio, N. Turtulici, D. Madonna, P. Pietrini, L. Cecchetti, P. Brambilla, Machine learning and the prediction of suicide in psychiatric populations: a systematic review, Transl. Psychiatry 14 (1) (2024) 140, https://doi.org/10.1038/s41398-024-02852-9.
[12] J. Cao, X. Wei, M.F. Liu, G.S. An, J. Li, Q.X. Du, J.H. Sun, Forensic identification of sudden cardiac death: a new approach combining metabolomics and machine learning, Anal. Bioanal. Chem. 415 (12) (2023) 2291–2305, https://doi.org/10.1007/s00216-023-04651-5.
[13] J. Cao, J. Li, Z. Gu, J.J. Niu, G.S. An, Q.Q. Jin, Y.Y. Wang, P. Huang, J.H. Sun, Combined metabolomics and machine learning algorithms to explore metabolic biomarkers for diagnosis of acute myocardial ischemia, Int. J. Leg. Med. 137 (1) (2023) 169–180, https://doi.org/10.1007/s00414-022-02816-y.
[14] R. Aljeaid, Application of metabolomics and machine learning for the prediction of postmortem interval, Cureus 16 (11) (2024) e74161, https://doi.org/10.7759/cureus.74161.
[15] F.Y. Zhang, L.L. Wang, W.W. Dong, M. Zhang, D. Tash, X.J. Li, S.K. Du, H.M. Yuan, R. Zhao, D.W. Guan, 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, https://doi.org/10.1007/s00414-022-02783-4.
[16] P. Somtua, C. Jaikang, G. Konguthaithip, K. Intui, S. Watcharakhom, T.E. O'Brien, Y. Amornlertwatana, Postmortem alteration of purine metabolism in coronary artery disease, Metabolites 13 (11) (2023), https://doi.org/10.3390/metabo13111135.
[17] A.C. Dona, M. Kyriakides, F. Scott, E.A. Shephard, D. Varshavi, K. Veselkov, J. R. Everett, A guide to the identification of metabolites in NMR-based metabonomics/metabolomics experiments, Comput. Struct. Biotechnol. J. 14 (2016) 135–153, https://doi.org/10.1016/j.csbj.2016.02.005.
[18] R.A. de Graaf, K.L. Behar, Quantitative 1H NMR spectroscopy of blood plasma metabolites, Anal. Chem. 75 (9) (2003) 2100–2104, https://doi.org/10.1021/ac020782+.
[19] S. Parodi, D. Verda, F. Bagnasco, M. Muselli, The clinical meaning of the area under a receiver operating characteristic curve for the evaluation of the performance of disease markers, Epidemiol Health 44 (2022) e2022088, https://doi.org/10.4178/epih.e2022088.
[20] M.W. Liemohn, A.R. Azari, N.Y. Ganushkina, L. Rastätter, The stone curve: a ROC-derived model performance assessment tool, Earth Space Sci. 7 (8) (2020) e2020EA001106, https://doi.org/10.1029/2020EA001106.
[21] R.T. Nakatsu, An evaluation of four resampling methods used in machine learning classification, IEEE Intell. Syst. 36 (3) (2021) 51–57, https://doi.org/10.1109/MIS.2020.2978066.
[22] G.A. Nagana Gowda, D. Raftery, NMR-based metabolomics, Adv. Exp. Med. Biol. 1280 (2021) 19–37, https://doi.org/10.1007/978-3-030-51652-9_2.
[23] F.A.A. Mulder, L. Tenori, C. Licari, C. Luchinat, Practical considerations for rapid and quantitative NMR-based metabolomics, J. Magn. Reson. 352 (2023) 107462, https://doi.org/10.1016/j.jmr.2023.107462.
[24] K. Tatsumi, M. Graham, Death investigation in the United States: forensic pathology, Mo. Med. 119 (5) (2022) 411–415.
[25] J. Dawidowska, M. Krzyżanowska, M.J. Markuszewski, M. Kaliszan, The application of metabolomics in forensic science with focus on forensic toxicology and time-of-death estimation, Metabolites 11 (12) (2021), https://doi.org/10.3390/metabo11120801.
[26] K. Santisukwongchote, Y. Amornlertwatana, T. Sastraruji, C. Jaikang, Possible use of blood tryptophan metabolites as biomarkers for coronary heart disease in sudden unexpected death, Metabolites 10 (1) (2019), https://doi.org/10.3390/metabo10010006.
[27] M. Costanzo, M. Caterino, G. Sotgiu, M. Ruoppolo, F. Franconi, I. Campesi, Sex differences in the human metabolome, Biol. Sex Differ. 13 (1) (2022) 30, https://doi.org/10.1186/s13293-022-00440-4.
[28] A. Mastrangelo, C. Barbas, Chronic diseases and lifestyle biomarkers identification by metabolomics, Adv. Exp. Med. Biol. 965 (2017) 235–263, https://doi.org/10.1007/978-3-319-47656-8_10.
[29] E.E. Balashova, D.L. Maslov, O.P. Trifonova, P.G. Lokhov, A.I. Archakov, Metabolome profiling in aging studies, Biology 11 (11) (2022), https://doi.org/10.3390/biology11111570.
[30] S.L. Navarro, G.A. Nagana Gowda, L.F. Bettcher, R. Pepin, N. Nguyen, M. Ellenberger, C. Zheng, L.F. Tinker, R.L. Prentice, Y. Huang, T. Yang, F.

K. Tabung, Q. Chan, R.L. Loo, S. Liu, J. Wactawski-Wende, J.W. Lampe, M. L. Neuhouser, D. Raftery, Demographic, health and lifestyle factors associated with the metabolome in older women, Metabolites 13 (4) (2023), https://doi.org/10.3390/metabo13040514.

[31] K.M. Reynolds, A. Horimoto, B.M. Lin, Y. Zhang, N. Kurniansyah, B. Yu, E. Boerwinkle, Q. Qi, R. Kaplan, M. Daviglus, L. Hou, L.Y. Zhou, J. Cai, S.R. Shaikh, T. Sofer, S.R. Browning, N. Franceschini, Ancestry-driven metabolite variation provides insights into disease states in admixed populations, Genome Med. 15 (1) (2023) 52, https://doi.org/10.1186/s13073-023-01209-z.

[32] M.C. Playdon, S.C. Moore, A. Derkach, J. Reedy, A.F. Subar, J.N. Sampson, D. Albanes, F. Gu, J. Kontto, C. Lassale, L.M. Liao, S. Männistö, A.M. Mondul, S. J. Weinstein, M.L. Irwin, S.T. Mayne, R. Stolzenberg-Solomon, Identifying biomarkers of dietary patterns by using metabolomics, Am. J. Clin. Nutr. 105 (2) (2017) 450–465, https://doi.org/10.3945/ajcn.116.144501.

[33] E.A. Zelentsova, L.V. Yanshole, A.D. Melnikov, I.S. Kudryavtsev, V.P. Novoselov, Y. P. Tsentalovich, Post-mortem changes in metabolomic profiles of human serum, aqueous humor and vitreous humor, Metabolomics 16 (7) (2020) 80, https://doi.org/10.1007/s11306-020-01700-3.

[34] Z. Wu, Y. Hou, Z. Dai, C.A. Hu, G. Wu, Metabolism, nutrition, and redox signaling of hydroxyproline, Antioxid Redox Signal 30 (4) (2019) 674–682, https://doi.org/10.1089/ars.2017.7338.

[35] S. Hu, W. He, G. Wu, Hydroxyproline in animal metabolism, nutrition, and cell signaling, Amino Acids 54 (4) (2022) 513–528, https://doi.org/10.1007/s00726-021-03056-x.

[36] K.W. Lee, S.J. Kim, J.B. Park, K.J. Lee, Relationship between depression anxiety stress scale (DASS) and urinary hydroxyproline and proline concentrations in hospital workers, J Prev Med Public Health 44 (1) (2011) 9–13, https://doi.org/10.3961/jpmph.2011.44.1.9.

[37] H. Chen, J. Wang, S. Chen, X. Chen, J. Liu, H. Tang, J. Zhou, Y. Tian, X. Wang, X. Cao, J. Zhou, Abnormal energy metabolism, oxidative stress, and polyunsaturated fatty acid metabolism in depressed adolescents associated with childhood maltreatment: a targeted metabolite analysis, Psychiatry Res. 335 (2024) 115795, https://doi.org/10.1016/j.psychres.2024.115795.

[38] T.M. Saber, B.H.F. Omran, M.M. El Deib, N.I. El-Sharkawy, M.M.M. Metwally, Y. M. Abd-Elhakim, Early postmortem biochemical, histological, and immunohistochemical alterations in skeletal muscles of rats exposed to boldenone undecylenate: forensic implication, J Forensic Leg Med 83 (2021) 102248, https://doi.org/10.1016/j.jflm.2021.102248.

[39] D. Curtis, A possible role for sarcosine in the management of schizophrenia, Br. J. Psychiatry 215 (6) (2019) 697–698, https://doi.org/10.1192/bjp.2019.194.

[40] I.D. Henter, R.T. de Sousa, C.A. Zarate Jr., Glutamatergic modulators in depression, Harv Rev Psychiatry 26 (6) (2018) 307–319, https://doi.org/10.1097/hrp.0000000000000183.

[41] C.C. Huang, I.H. Wei, C.L. Huang, K.T. Chen, M.H. Tsai, P. Tsai, R. Tun, K. H. Huang, Y.C. Chang, H.Y. Lane, G.E. Tsai, Inhibition of glycine transporter-I as a novel mechanism for the treatment of depression, Biol. Psychiatry 74 (10) (2013) 734–741, https://doi.org/10.1016/j.biopsych.2013.02.020.

[42] C.H. Chang, C.H. Lin, C.Y. Liu, S.J. Chen, H.Y. Lane, Efficacy and cognitive effect of sarcosine (N-methylglycine) in patients with schizophrenia: a systematic review and meta-analysis of double-blind randomised controlled trials, J. Psychopharmacol. 34 (5) (2020) 495–505, https://doi.org/10.1177/0269881120908016.

[43] X. Zhang, B. Wang, J.P. Li, Implications of heparan sulfate and heparanase in neuroinflammation, Matrix Biol. 35 (2014) 174–181, https://doi.org/10.1016/j.matbio.2013.12.009.

[44] B.L. Farrugia, M.S. Lord, J. Melrose, J.M. Whitelock, The role of heparan sulfate in inflammation, and the development of biomimetics as anti-inflammatory strategies, J. Histochem. Cytochem. 66 (4) (2018) 321–336, https://doi.org/10.1369/0022155417740881.

[45] L. Brundin, E.Y. Bryleva, K. Thirtamara Rajamani, Role of inflammation in suicide: from mechanisms to treatment, Neuropsychopharmacology 42 (1) (2017) 271–283, https://doi.org/10.1038/npp.2016.116.

[46] Y. Du, J. Wei, Z. Zhang, X. Yang, M. Wang, Y. Wang, X. Qi, L. Zhao, Y. Tian, W. Guo, Q. Wang, W. Deng, M. Li, D. Lin, T. Li, X. Ma, Plasma metabolomics profiling of metabolic pathways affected by major depressive disorder, Front Psychiatry 12 (2021) 644555, https://doi.org/10.3389/fpsyt.2021.644555.

[47] M.P. Wilson, B. Plecko, P.B. Mills, P.T. Clayton, Disorders affecting vitamin B(6) metabolism, J. Inherit. Metab. Dis. 42 (4) (2019) 629–646, https://doi.org/10.1002/jimd.12060.

[48] X. Bian, N. Zhou, Y. Zhao, Y. Fang, N. Li, X. Zhang, X. Wang, Y. Li, J.L. Wu, T. Zhou, Identification of proline, 1-pyrroline-5-carboxylate and glutamic acid as biomarkers of depression reflecting brain metabolism using carboxylomics, a new metabolomics method, Psychiatry Clin Neurosci 77 (4) (2023) 196–204, https://doi.org/10.1111/pcn.13517.