Predicting Critically Ill Patients' Outcome in the ICU using UHPLC-HRMS Data

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***Abstract*—**The available scores to predict patients' outcomes in specific settings generally present low sensitivities and specificities when applied to intensive care units' (ICUs) populations. Advancements in analytical techniques, notably Ultra-High Performance Liquid Chromatography- Mass Spectrometry (UHPLC-HRMS) transformed biomarker identification, enabling a comprehensive profiling of biofluids, including serum. In the current work, untargeted metabolomics, utilizing UHPLC-HRMS serum analysis, was performed on 16 ICU patients, categorized as either discharged (n=8), or deceased (n=8) in average seven days post sample collection. Linear discriminant analysis (LDA) or principal component analysis (PCA)-LDA models involving different metabolite sets were developed, enabling to predict patients' outcomes in the ICU with 92% accuracy and 83% sensitivity on validation datasets. These results highlight the advantages of UHPLC-HRMS as a platform capable of providing a set of clinically significant biomarkers to predict patients' outcome.

***Keywords*—** Biomarkers, Intensive care unit, Predictive models, Metabolomics, Mass Spectrometry

I. Introduction

Precision medicine has emerged as a novel approach in healthcare, aiming to tailor medical treatment to the characteristics of each patient. No other setting demonstrates the potential of precision medicine as the intensive care unit (ICU) where critically ill patients require complex and often time-sensitive interventions. Effective ICU patient management relies on the ability to predict outcomes accurately, guiding clinicians in making timely and informed decisions to improve patient care and outcomes [1]. Biomarker discovery plays a pivotal role in this endeavor by identifying molecular indicators that can predict patients' outcomes. These biomarkers can provide valuable insights into the underlying mechanisms of disease progression, response to treatment, and prognosis, thereby facilitating personalized therapeutic strategies in the ICU setting [2]. In recent years, advances in analytical techniques, particularly Ultra-High Performance Liquid Chromatography Mass Spectrometry (UHPLC-HRMS), have revolutionized biomarker identification and characterization. UHPLC-HRMS offers unparalleled sensitivity, resolution, and throughput, enabling comprehensive profiling of complex biological samples with exceptional accuracy and precision [3]. Thus, precision medicine can be easily and seamlessly integrated as a new technique for patient management. For instance, routine blood draws can be leveraged for liquid biopsy [4], quickly generating a patient's metabolomic profile [5]. Analyzing this data with predictive algorithms against a comprehensive metabolomic database can optimize therapeutic interventions in real-time, leading to an improved and more efficient management of patients and medical resources [6]. Thus, the present work aims to identify serum metabolomic biomarkers using UHPLC-HRMS, with the goal of predicting mortality in critically ill patients.

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II. Methodology

A. Study Design

Blood samples from 16 male ICU patients at *Hospital São José* (Lisbon, Portugal), who tested positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), through positive real-time polymerase chain reaction testing were collected and analyzed according to legal and ethical requirements (Ethics Commission project approval: 1043/2021, 20/05/2020). Clinical data was obtained from the hospital's medical records. The cohort was categorized by ICU outcome, discharged or deceased.

B. Serum Metabolome

Serum was obtained from blood, by centrifugation at 3000 rpm for 10 min, and kept at -80 °C until analysis. The metabolome acquired from serum was analyzed with an UHPLC-HRMS platform as outlined by Fonseca *et al.* [7]. Briefly, samples were submitted to two chromatographic modes, reverse phase (RP) and hydrophilic interaction liquid chromatography (HILIC). For RP, a constant temperature of 40 °C, and a gradient elution was used at a flow rate of 250

µL/min as follows (mobile phase A, 0.1% formic acid in water; mobile phase B, 0.1% formic acid in acetonitrile): 0.0−0.5 min 0% B, 0.5−1.5 min 0−20% B, 1.5−4.0 min 20−60% B, 4.0−6.0 min 60−100% B, 6.0−9.0 min 100% B, 9.0−10.0 min 100−0% B, and 10.0−15.0 min 0% B. For HILIC, a constant temperature of 40 °C was used with a flow rate of 250 µL/min, a gradient elution of 10 mM ammonium acetate in water containing 0.1% acetic acid (A) and 10 mM ammonium acetate in acetonitrile containing 2% water and 0.1% acetic acid (B) was applied as follows: 0−2 min 90% B, 2−6 min 90−70% B, 6−9 min 70−30% B, 9−13 min 30% B, 13−18 min 30−90% B, 18−22 min 90% B. MS acquisition parameters were set as follows: capillary voltage of 3 kV (ESI−) or 4.5 kV (ESI+), end plate offset of 500 V, nebulizer of 4.0 bar, dry gas flow of 8.0 L/min, and dry heater temperature of 200 °C. Spectral acquisition was performed with an absolute threshold of 25 counts per 1000, with a m/z range from 50 to 1300 and a 3 Hz spectra rate. Three full scans and one auto MS/MS scan were performed for each sample and for each mode. Quality control samples were analyzed every 6h for consistent chromatographic resolution and spectrometer detection over time. MS data was pre-processed with Data Analysis (versions 4.1, 4.4, and 4.5, Bruker Daltonics), converted to mzXML using ProteoWizard MSConvert [8], and uploaded to the XCMS server [9], where data processing, pairwise sample comparison, multimodal analysis (independent of separation and acquisition modes), and global meta-analysis were performed.

C. Multivariate Data Analysis

Principal component analysis (PCA), linear discriminant analysis (LDA), and PCA-LDA models based on metabolites identified by UPLC-HRMS were developed using The Unscrambler X, version 10.4 (CAMO software AS, Oslo, Norway). Samples were divided into 75% train (calibration) and 25% test (validation) sets. This was repeated four times using a rotation scheme to ensure no overlap between samples. To assess the models' performance, indicators such as accuracy, sensitivity, specificity, and precision were provided.

D. Other Statistical Analysis

Continuous variables were presented as mean ± standard deviation (S.D.), and categorical data as absolute frequencies and percentages, using IBM SPSS Statistics software, version 26 (IBM Corp., New York, United States). For spectral bands and MS intensities, comparisons between groups were performed using Welch's t-test, in the XCMS platform. Statistical significance was set for two-sided p-values of less than 0.05.

III. Results and Discussion

Serum samples were obtained from 8 discharged and 8 deceased patients in an average of five days after ICU admission. Discharged patients did not require invasive mechanical ventilation (IMV), relying on high-flow oxygenation (HFO), whereas deceased patients who died on average seven days after ICU admission, were under IMV at the time of sample collection (**Table I**).

TABLE I
OVERVIEW OF PATIENTS' CHARACTERISTICS IN BOTH GROUPS, STRATIFIED BASED ON THEIR ICU OUTCOME.

Clinical Variables	***Patients' Outcome***	
	Discharged (n=8)	***Deceased (n=8)***
Age, years (mean ± S.D.)	52.25 ± 11.42	63.25 ± 5.73
Body Mass Index, kg/m^2 (mean ± S.D.)	30.29 ± 6.63	29.36 ± 3.59
Arterial hypertension; n (%)	2 (25.0)	3 (37.5)
Obesity; n (%)	2 (25.0)	2 (25.0)
Diabetes; n (%)	2 (25.0)	3 (37.5)
Dyslipidemia; n (%)	2 (25.0)	2 (25.0)
Chronic respiratory disease; n (%)	2 (25.0)	3 (37.5)
HFO; n (%)	8 (100.0)	2 (25.0)
Days with IMV (mean ± S.D.)	-	10.63 ± 6.32
Days in the ICU (mean ± S.D.)	7.38 ± 2.77	12.50 ± 4.84

The employed UHPLC-HRMS analysis involved two column types, namely RP and HILIC, to enhance metabolite coverage. HILIC and RP chromatography display a complementary capacity to resolve metabolites based on their polarity. Their combined use allows a clearer separation of metabolites across a wider polarity and hydrophilicity range, resulting in a more comprehensive analysis [10]. Thus, applying both methods yielded approximately 23,000 m/z peaks per group, with roughly half resulting from each column type, underscoring the complementary nature of the two chromatographic techniques. Pairwise comparisons made between samples from discharged and deceased patients revealed a total of 52 features (*i.e.*, m/z peaks), corresponding to 24 metabolites significantly different between the two groups (p-value <0.0001) (**Table II)**.

TABLE II
DIFFERENTLY EXPRESSED METABOLITES DETECTED BY UHPLC-HRMS (P<0.0001) BETWEEN DISCHARGED AND DECEASED PATIENTS.

Metabolites	
Up Regulated	***Fold change*** [a]
Thiamin	7.76
α-L-iduronate	5.22
Octanoate	4.23
α,α-trehalose	3.84
3'-dephospho-CoA	3.83
6-methoxy-3-methyl-2-all-trans-decaprenyl-1,4-benzoquinol	3.61
4-acetamidobutanoate	3.52
Flavin mononucleotide	3.20
4-hydroxyphenylpyruvate	2.68
Ribose-1-arsenate	2.66
S-adenosyl-L-homocysteine	2.48
Homovanillate	2.11
Xylitol	1.89
N-acetyl-β-neuraminate	1.76

Pyruvate	1.62
α-D-glucose	1.61
(R)-lactate	1.38
Down Regulated	***Fold change*** [a]
Bilirubin	2.44
Fumarate	2.39
(R)-mevalonate	1.84
4-methyl-2-oxopentanoate	1.80
6-phospho-D-glucono-1,5-lactone	1.68
3-ureido-isobutyrate	1.49
(3R,5S)-1-pyrroline-3-hydroxy-5-carboxylate	1.47

a. Change trend of group C energy intensities.

Seven molecules were downregulated in the samples from deceased patients, in comparison to those from discharged ones. These included organic acids and organic carbonic acids (*i.e.*, *(3R,5S)*-1-pyrroline-3-hydroxy-5-carboxylate and 3-ureido-isobutyrate), carbohydrate 6-phospho-D-glucono-1,5-lactone, the hydroxy fatty acid *(R)*-mevalonate, keto acid 4-methyl-2-oxopentanoate, fumarate and bilirubin. On the other hand, deceased patients had higher results in molecular families of carbohydrates (α-D-glucose; α,α-trehalose; ribose-1-arsenate; xylitol; N-acetyl-β-neuraminate), lipid molecules (S-adenosyl-L-homocysteine; octanoate; 6-methoxy-3-methyl-2-all-trans-decaprenyl-1,4-benzoquinol), benzenoids (4-hydroxyphenylpyruvate; homovanillate), the carboxylic acids 4-acetamidobutanoate, keto acid pyruvate, hydroxy acid *(R)*-lactate, nucleotide flavin mononucleotide and 3'-dephospho-CoA, constituent of glycosaminoglycans α-L-iduronate and vitamin thiamine.

Employing PCA to the 24 identified metabolites retrieved good distinction between deceased and discharged patients emerged in the PC1 versus PC2 score-plot (**Fig. 1a**). This highlights the potential of these metabolites to predict the patients' outcome. Furthermore, 13 metabolites were highlighted by the PCA' loadings (**Fig. 1b**). On the negative side of PC1, (R)-mevalonate, 4-methyl-2-oxopentanoate and (3R,5S)-1-pyrroline-3-hydroxy-5-carboxylate were identified, while on the positive side of PC1, α-D-glucose and (R)-lactate were detected. Furthermore, on the positive side of both PC1 and PC2, the following metabolites were identified: pyruvate, N-acetyl-β-neuraminate, S-adenosyl-L-homocysteine, 4-hydroxyphenylpyruvate, xylitol, α-L-iduronate, homovanillate, and 4-acetamidobutanote.

In line with the current study, other authors have reported similar findings concerning the 13 metabolites highlighted by the PCA' loadings. Tang *et al.* [11], for example, reported a down-regulation of *(3R,5S)-1-pyrroline-3-hydroxy-5-carboxylate*, a metabolite involved in the arginine and proline metabolism, among severe coronavirus disease 2019 (COVID-19) patients in comparison to healthy controls. *(R)-mevalonate* down-regulation in deceased patients can be indicative of induced muscle weakness cause by prolonged hospitalization [12]. This molecule can have a dual mechanism, as it can be recruited by viral infected mechanism to support viral replication and at the same time plays an immunoregulatory role [13]. Concerning *4-methyl-2-oxopentanoate*, an intermediate in the leucine degradation pathway, its down-regulation in deceased patients aligns with previous findings demonstrating its negative association with COVID-19 severity [14]. When it comes to the metabolites that were up-regulated in deceased patients, *S-adenosyl-L-homocysteine* has emerged as a biomarker for severe sepsis and systemic inflammatory response syndrome in the ICU. Elevated levels of this biomarker, linked to tissue hypoxia, predict disease severity and outcome [15]. *(R)-lactate* and *pyruvate* are associated with unfavorable clinical outcomes and an increased risk of ICU mortality, particularly when present in high concentrations. In ICU settings, elevated blood lactate and lactate-to-pyruvate ratio are commonly observed, stemming from metabolic dysregulations, including hypoxia from ischemic events in septic patients and other phenomena such as the Warburg Effect [16]. In the context of disease severity-driven bioenergetic changes, *α-D-glucose* correlates positively with increased morbidity, and unfavorable clinical outcomes [17]. Stress-induced hyperglycemia is a prevalent occurrence in the ICU environment, attributed to a range of factors encompassing inflammatory responses and neuroendocrine disturbances, contributing for instance to insulin resistance [18]. *N-acetyl-β-neuraminate* levels have been associated with chronic kidney disease severity, end-stage kidney disease, increased heart failure risk, and adverse outcomes in heart failure patients [19], [20]. Considering homovanillate, while reduced levels have been reported in patients with traumatic brain injury [21], other studies have described elevated levels of this metabolite in non-survivor patients. Examples include critically ill children experiencing complicated severe malnutrition [22], as well as acute critical ill patients diagnosed with nosocomial pneumonia [23] and septic shock [24]. In a serum multiomics study, *4-hydroxyphenylpyruvate* was identified as part of a predictive signature for COVID-19 severity [25]. The buildup of 4-hydroxyphenylpyruvate may be related to vitamin C depletion, as it serves as a cofactor in the breakdown of 4-hydroxyphenylpyruvate into homogentisate within the tyrosine metabolic pathway. This can occur in conditions such as hypovitaminosis or when vitamin C is redirected to other biochemical reactions, involving processes related to the activity of reactive oxygen species and the various inflammatory responses observed in conditions like COVID-19 and septic shock [26], [27]. *Xylitol*, a common component in parenteral nutrition, has been associated with COVID-19 severity, showing increased levels in critical cases, along with e.g., other sugar alcohols, arabinose, ribose and trehalose [28].

LDA or PCA-LDA models were developed using different sets of metabolites to predict the patients' outcome. Succinctly, sets employing all identified metabolites (n=24), metabolites from the PCA's influence plot (**Fig. 1b**), metabolites with the lowest p-values, and the highest fold change between the two groups of patients (**Table II**), were considered. In total, seven metabolite sets and corresponding

LDA/PCA-LDA models were developed (**Table III)**. Overall, good predictive models were developed, with the best performances achieving 92% accuracy and 83% sensitivity on the validation dataset. This model incorporated seven metabolites with the highest fold-change, all of which were up-regulated in deceased patients.

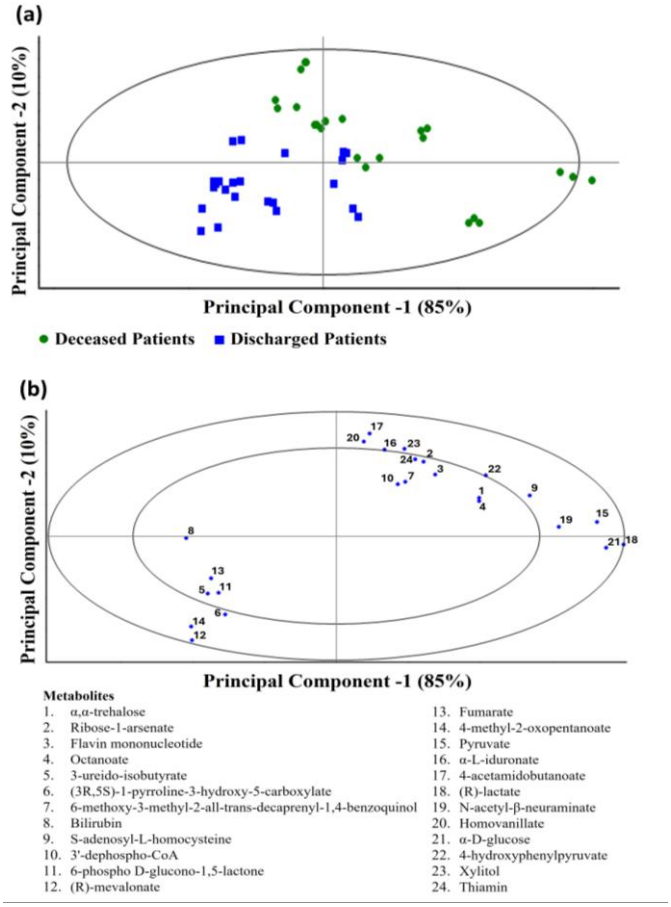

Fig. 1 PCA from the 24 metabolites differently expressed between discharged and deceased patients (a); Correlation loadings from both PC1 and PC2, highlighting the most influential metabolites. The plot contains two ellipses that indicate how much variance is considered. The outer ellipse is the unit-circle and indicates 100% explained variance. The inner ellipse indicates 50% of explained variance (b).

Thiamine, or vitamin B1, which is a cofactor in the energy metabolism, exhibited the highest fold-change. Deficiency or low levels of this metabolite have been associated with increased disease severity and poorer outcomes [29]. The up-regulation of thiamine among deceased patients is most likely attributed to the administration of thiamine-rich diets or direct supplementation in the ICU. *α-L-iduronate*, the metabolite with the second-highest fold-change, which was also highlighted in the PCA analysis, has been associated with neurological complications in septic events [30]. Elevated iduronate levels might be linked to the degradation of glycosaminoglycan dermatan sulfate, a process associated with SARS-CoV-2 infection, both through the virus's direct impact on tissues and indirectly through inflammatory mechanisms [31]. *Octanoate*, also included in the model, acts as the main endogenous substrate for lipoic acid in lipoate biosynthesis, having a protective role in ischemic events, coagulopathy and COVID-19-induced endothelial disorders [32], [33]. Thus, higher octanoate serum levels could be indicative of lipoate synthesis blockade, leading to worse outcomes. Furthermore, this metabolite is considered a potential biomarker for the severity of acute respiratory distress syndrome [34]. *α,α-Trehalose* is a disaccharide, hydrolyzed into D-glucose in the small intestine. It is a common ingredient in some aliments and pharmaceutical products. As previously observed, high sugar serum levels exhibit a positive correlation with increased morbidity, prolonged hospitalization, and unfavorable clinical outcomes [35]. *3'-Dephospho-CoA* serves as a precursor to coenzyme A during the pantothenate biosynthesis process that takes place in the cell's cytosol. Elevated serum levels of this metabolite may suggest cell leakage resulting from cellular damage, a characteristic observed in patients with greater SARS-COV-2 severity [36]. The sixth metabolite that was included as a predictive variable in the model, is *6-methoxy-3-methyl-2-all-trans-decaprenyl-1,4-benzoquinol*. This hydroquinone participates in ubiquinol biosynthesis and is a precursor to ubiquinol-10 (CoQ-10), which is an essential cofactor in the electron transport chain of the phosphorylative oxidation system, exhibiting anti-inflammatory properties [37]. The last metabolite incorporated in the model, which was also highlighted in the PCA analysis, was *4-acetamidobutanoate*. Elevated levels are associated with hepatorenal dysfunction and increased mortality in cirrhosis patients [38].

IV. Conclusion

Serum metabolomics can represent an essential platform for identifying biomarkers for disease diagnosis and, in this instance, outcome, especially within the highly diverse critical care population in ICUs. The discovery of new biomarkers through metabolomics will permit the development of pathophysiological profiles enabling the creation of new prognostic/diagnostic tools base on innovative approaches with higher precision and efficacy. Although this research presents a reduced sample size, we point out the possibility to develop classification models for patient mortality with an average sample collection of seven days until patients' death. This achievement highlights the sensibility of patients' metabolome analysis. In the future we aim to reproduce this analysis with a larger sample size, as this work will serve as a guiding point towards what metabolites we should be looking for. By validating these results, it will be possible to translate these findings into a clinical context.

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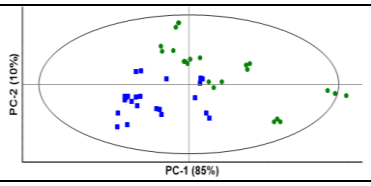
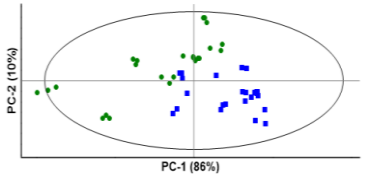
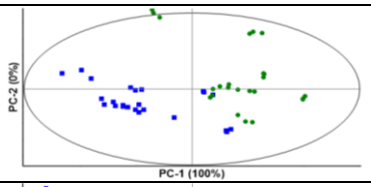
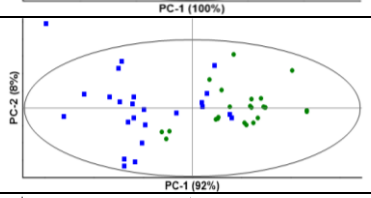
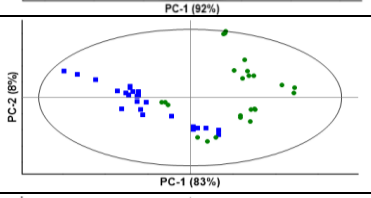
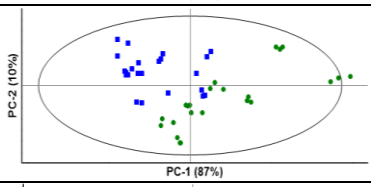
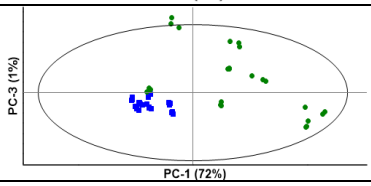
TABLE III
DIFFERENTLY EXPRESSED METABOLITES DETECTED BY UHPLC-HRMS (P<0.0001) BETWEEN DISCHARGED AND DECEASED PATIENTS.

	Prediction Models			**Principal Component Analysis**
	Performance	***Calibration***	***Validation***	
Set 1: All metabolites (n = 24)	Accuracy	97%	88%	
	Sensitivity	94%	88%	
	Specificity	100%	88%	
	Precision	100%	92%	
Set 2: According to PCA loading (n = 13) *1-pyrroline-3-hydroxy-5-carboxylate; S-adenosyl-L-homocysteine; Pyruvate; (R)-mevalonate; 4-methyl-2-oxopentanoate; Xylitol; 4-hydroxyphenylpyruvate; α-L-iduronate; (R)-lactate; Homovanillate; N-acetyl-β-neuraminate; α-D-glucose; 4-acetamidobutanoate*	Accuracy	100%	71%	
	Sensitivity	100%	54%	
	Specificity	100%	88%	
	Precision	100%	-	
Set 3: Lowest *p-values* (n = 2) *(R)-mevalonate; Octanoate*	Accuracy	93%	70%	
	Sensitivity	97%	70%	
	Specificity	89%	70%	
	Precision	90%	73%	
Set 4: Lowest *p-values* (n = 6) *Set 3; Fumarate; Xylitol; 4-hydroxyphenylpyruvate; 4-methyl-2-oxopentanoate*	Accuracy	99%	90%	
	Sensitivity	97%	83%	
	Specificity	100%	96%	
	Precision	100%	94%	
Set 5: Lowest *p-values* (n = 13) *Set 4; Thiamine; Pyruvate; α-L-iduronate; 3-ureido-isobutyrate; α,α-trehalose; 4-acetamidobutanoate; (3R,5S)-1-pyrroline-3-hydroxy-5-carboxylate*	Accuracy	100%	73%	
	Sensitivity	100%	58%	
	Specificity	100%	88%	
	Precision	100%	88%	
Set 6: Lowest *p-values* (n = 17) *Set 5; (R)-lactate; 6-methoxy-3-methyl-2-all-trans-decaprenyl-1,4-benzoquinol; Ribose-1-arsenate; Flavin Mononucleotide*	Accuracy	100%	81%	
	Sensitivity	100%	63%	
	Specificity	100%	100%	
	Precision	100%	100%	
Set 7: Highest fold-change (n=7) *Thiamin; α-L-iduronate; Octanoate; α,α-trehalose; 3'-dephospho-CoA; 6-methoxy-3-methyl-2-all-trans-decaprenyl-1,4-benzoquinol; 4-acetamidobutanoate*	Accuracy	**100%**	**92%**	
	Sensitivity	**100%**	**83%**	
	Specificity	**100%**	**100%**	
	Precision	**100%**	**100%**	

Best calibration and validation results are highlighted in **bold**. PCAs include discharged (blue squares) and deceased (green circles) patients. Regarding LDA, deceased patients were considered true positives.

REFERENCES

[1] R. Méndez Hernández and F. Ramasco Rueda, "Biomarkers as Prognostic Predictors and Therapeutic Guide in Critically Ill Patients: Clinical Evidence," *J Pers Med*, vol. 13, no. 2, p. 333, Feb. 2023. https://doi.org/10.3390/jpm13020333

[2] R. Araújo, L. F. N. Bento, T. A. H. Fonseca, C. P. Von Rekowski, B. R. da Cunha, and C. R. C. Calado, "Infection Biomarkers Based on Metabolomics," *Metabolites*, vol. 12, no. 2, p. 92, Feb. 2022. https://doi.org/10.3390/metabo12020092

[3] L. Cui, H. Lu, and Y. H. Lee, "Challenges and emergent solutions for LC-MS/MS based untargeted metabolomics in diseases," *Mass Spectrom Rev*, vol. 37, no. 6, pp. 772–792, Nov. 2018. https://doi.org/10.1002/mas.21562

[4] K. K. Adhit, A. Wanjari, S. Menon, and S. K, "Liquid Biopsy: An Evolving Paradigm for Non-invasive Disease Diagnosis and Monitoring in Medicine," *Cureus*, vol. 15, no. 12, p. e50176, Dec. 2023. https://doi.org/10.7759/cureus.50176

[5] J. Peng, C. Qiu, J. Zhang, and X. Xiao, "Serum metabolite profiling reveals metabolic characteristics of sepsis patients using LC/MS-based metabolic profiles: a cross-sectional study," *BMC Med Genomics*, vol. 16, no. 1, p. 224, Dec. 2023. https://doi.org/10.1186/s12920-023-01666-w

[6] D. Wang et al., "A Machine Learning Model for Accurate Prediction of Sepsis in ICU Patients," *Front Public Health*, vol. 9, p. 754348, Oct. 2021. https://doi.org/10.3389/fpubh.2021.754348

[7] T. A. H. Fonseca et al., "The Impact of the Serum Extraction Protocol on Metabolomic Profiling Using UPLC-MS/MS and FTIR Spectroscopy," *ACS Omega*, vol. 8, no. 23, pp. 20755–20766, Jun. 2023. https://doi.org/10.1021/acsomega.3c01370

[8] M. C. Chambers et al., "A cross-platform toolkit for mass spectrometry and proteomics.," *Nat. Biotechnol.*, vol. 30, no. 10, pp. 918–20, Oct. 2012. https://doi.org/10.1038/nbt.2377

[9] H. Gowda et al., "Interactive XCMS Online: Simplifying Advanced Metabolomic Data Processing and Subsequent Statistical Analyses," *Anal. Chem.*, vol. 86, no. 14, pp. 6931–6939, Jul. 2014. https://doi.org/10.1021/ac500734c

[10] C. Pommié, S. Levadoux, R. Sabatier, G. Lefranc, and M.-P. Lefranc, "IMGT standardized criteria for statistical analysis of immunoglobulin V-REGION amino acid properties," *J. Mol. Recognit.*, vol. 17, no. 1, pp. 17–32, Jan. 2004. https://doi.org/10.1002/jmr.647

[11] Tang, Y. Liu, Y. Ruan, L. Ge, and Q. Zhang, "Reconstructed Genome-Scale Metabolic Model Characterizes Adaptive Metabolic Flux Changes in Peripheral Blood Mononuclear Cells in Severe COVID-19 Patients," Int. J. Mol. Sci., vol. 23, no. 20, p. 12400, Oct. 2022. https://doi.org/10.3390/ijms232012400

[12] C. Goossens et al., "Altered cholesterol homeostasis in critical illness-induced muscle weakness: effect of exogenous 3-hydroxybutyrate," *Crit Care*, vol. 25, no. 1, pp. 1–12, Dec. 2021. https://doi.org/10.1186/s13054-021-03688-1

[13] M. C. Proto et al., "Lipid homeostasis and mevalonate pathway in COVID-19: Basic concepts and potential therapeutic targets," *Prog Lipid Res*, vol. 82, p. 101099, Apr. 2021. https://doi.org/10.1016/j.plipres.2021.101099

[14] H. A. S. Chatelaine et al., "Nucleotide, Phospholipid, and Kynurenine Metabolites Are Robustly Associated with COVID-19 Severity and Time of Plasma Sample Collection in a Prospective Cohort Study," *Int J Mol Sci*, vol. 25, no. 1, p. 346, Dec. 2023. https://doi.org/10.3390/ijms25010346

[15] F.-S. Centner et al., "S-Adenosylhomocysteine Is a Useful Metabolic Factor in the Early Prediction of Septic Disease Progression and Death in Critically Ill Patients: A Prospective Cohort Study," *Int. J. Mol. Sci.*, vol. 24, no. 16, p. 12600, Aug. 2023. https://doi.org/10.3390/ijms241612600

[16] D. Bar-Or, M. Carrick, A. Tanner, M. J. Lieser, L. T. Rael, and E. Brody, "Overcoming the Warburg Effect: Is it the key to survival in sepsis?," *J. Crit. Care*, vol. 43, pp. 197–201, Feb. 2018. https://doi.org/10.1016/j.jcrc.2017.09.012

[17] D. Sotello, S. Yang, and K. Nugent, "Glucose and Lactate Levels at Admission as Predictors of In-hospital Mortality," Cureus, vol. 11, no. 10, Oct. 2019. https://doi.org/10.7759/cureus.6027

[18] Godinjak et al., "Hyperglycemia in Critically Ill Patients: Management and Prognosis," *Med. Arch.*, vol. 69, no. 3, p. 157, 2015. https://doi.org/10.5455/medarh.2015.69.157-160

[19] T. Kimura et al., "Identification of biomarkers for development of end-stage kidney disease in chronic kidney disease by metabolomic profiling," *Sci. Rep.*, vol. 6, no. 1, p. 26138, May 2016. https://doi.org/10.1038/srep26138

[20] C. Li et al., "Prognostic Value of Elevated Levels of Plasma N-Acetylneuraminic Acid in Patients With Heart Failure," *Circ. Hear. Fail.*, vol. 14, no. 11, p. E008459, Nov. 2021. https://doi.org/10.1161/CIRCHEARTFAILURE.121.008459

[21] M. Kharouba, D. D. Patel, R. H. Jaber, and S. H. Mahmoud, "Metabolomic Analysis in Neurocritical Care Patients," *Metabolites*, vol. 13, no. 6, p. 745, Jun. 2023. https://doi.org/10.3390/metabo13060745

[22] B. Wen et al., "Systemic inflammation and metabolic disturbances underlie inpatient mortality among ill children with severe malnutrition," *Sci. Adv.*, vol. 8, no. 7, Feb. 2022. https://doi.org/10.1126/sciadv.abj6779

[23] E. Chernevskaya et al., "Serum and fecal profiles of aromatic microbial metabolites reflect gut microbiota disruption in critically ill patients: a prospective observational pilot study," *Crit. Care*, vol. 24, no. 1, p. 312, Dec. 2020. https://doi.org/10.1186/s13054-020-03031-0

[24] N. V. Beloborodova, Y. N. Sarshor, A. Y. Bedova, E. A. Chernevskaya, and A. K. Pautova, "Involvement of Aromatic Metabolites in the Pathogenesis of Septic Shock," *Shock*, vol. 50, no. 3, pp. 273–279, Sep. 2018. https://doi.org/10.1097/SHK.0000000000001064

[25] S. K. Byeon et al., "Development of a multiomics model for identification of predictive biomarkers for COVID-19 severity: a retrospective cohort study," *Lancet Digit. Heal.*, vol. 4, no. 9, pp. e632–e645, Sep. 2022. https://doi.org/10.1016/S2589-7500(22)00112-1

[26] U. Shahbaz et al., "Role of vitamin C in preventing of COVID-19 infection, progression and severity," *AIMS Microbiol.*, vol. 8, no. 1, pp. 108–124, 2022. https://doi.org/10.3934/microbiol.2022010

[27] L. P. Mciteka, "A Synthesis Review of Vitamins Involved in the Fight against Covid-19," *ChemBioEng Rev.*, vol. 10, no. 3, pp. 236–257, Jun. 2023. https://doi.org/10.1002/cben.202200032

[28] F. X. Danlos et al., "Metabolomic analyses of COVID-19 patients unravel stage-dependent and prognostic biomarkers," *Cell Death Dis*, vol. 12, no. 3, p. 258, Mar. 2021. https://doi.org/10.1038/s41419-021-03540-y

[29] P. Attaluri, A. Castillo, H. Edriss, and K. Nugent, "Thiamine Deficiency: An Important Consideration in Critically Ill Patients," *Am. J. Med. Sci.*, vol. 356, no. 4, pp. 382–390, Oct. 2018. https://doi.org/10.1016/j.amjms.2018.06.015

[30] J. A. Hippensteel et al., "Circulating heparan sulfate fragments mediate septic cognitive dysfunction," *J. Clin. Invest.*, vol. 129, no. 4, pp. 1779–1784, Mar. 2019. https://doi.org/10.1172/JCI124485

[31] J. Y. Wang, M. W. Roehrl, V. B. Roehrl, and M. H. Roehrl, "A master autoantigen-ome links alternative splicing, female predilection, and COVID-19 to autoimmune diseases," *J. Transl. Autoimmun.*, vol. 5, no. 1, pp. 100147, Jan. 2022. https://doi.org/10.1016/j.jtauto.2022.100147

[32] Y. Ding, Y. Zhang, W. Zhang, J. Shang, Z. Xie, and C. Chen, "Effects of Lipoic Acid on Ischemia-Reperfusion Injury," Oxid. Med. Cell. Longev., vol. 2021, pp. 1–15, Oct. 2021. https://doi.org/10.1155/2021/5093216

[33] L. Rochette and S. Ghibu, "Mechanics Insights of Alpha-Lipoic Acid against Cardiovascular Diseases during COVID-19 Infection," Int. J. Mol. Sci., vol. 22, no. 15, p. 7979, Jul. 2021. https://doi.org/10.3390/ijms22157979

[34] S. Lin et al., "Explore potential plasma biomarkers of acute respiratory distress syndrome (ARDS) using GC–MS metabolomics analysis," *Clin Biochem*, vol. 66, pp. 49–56, Apr. 2019. https://doi.org/10.1016/j.clinbiochem.2019.02.009

[35] D. Martinon, V. F. Borges, A. C. Gomez, and K. Shimada, "Potential Fast COVID-19 Containment With Trehalose," *Front Immunol*, vol. 11, p. 1623, Jul. 2020, doi: 10.3389/FIMMU.2020.01623. https://doi.org/10.3389/fimmu.2020.01623

[36] S. Montazersaheb et al., "COVID-19 infection: an overview on cytokine storm and related interventions," *Virol. J.*, vol. 19, no. 1, p. 92, May 2022. https://doi.org/10.1186/s12985-022-01814-1

[37] M. Fakhrolmobasheri et al., "Coenzyme Q10 and Its Therapeutic Potencies Against COVID-19 and Other Similar Infections: A Molecular Review," *Adv. Pharm. Bull.*, vol. 13, no. 2, pp. 233–243, Mar. 2023. https://doi.org/10.34172/apb.2023.026

[38] E. L. Tsalik et al., "Renal systems biology of patients with systemic inflammatory response syndrome," *Kidney Int.*, vol. 88, no. 4, pp. 804–814, Oct. 2015. https://doi.org/10.1038/ki.2015.150