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Metabolomic profiling using 1H NMR spectroscopy in emergency department patients with sepsis: association with SOFA severity scores and in-hospital mortality. A prospective observational study

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Key words: sepsis; septic shock; SOFA score; metabolomics; 1H NMR; emergency department; branched-chain amino acids; mortality.

Abstract

Metabolomics offers mechanistic insights into the pathophysiology of sepsis and may complement conventional severity scoring. This prospective study evaluated the serum metabolomic profile of patients with sepsis presenting to the Emergency Department (ED) and its relationship with Sequential Organ Failure Assessment (SOFA) scores and in-hospital mortality. One hundred and fifty-five adults (≥18 years) meeting Sepsis-3 criteria were enrolled

consecutively from a tertiary care ED between July 2023 and July 2024. Serum samples were collected at two standardised time points: admission (T1, 0 h) and 72 hours (T2). Samples were analysed using 1H NMR spectroscopy on an 800 MHz Bruker Avance III spectrometer. SOFA scores were recorded at T1 and T2. The primary outcome was in-hospital mortality. Associations between metabolites, SOFA scores, and mortality were examined using univariable non-parametric tests, Spearman correlation, and a parsimonious multivariable logistic regression model. All metabolite p-values are exploratory given the absence of formal multiplicity correction. Of 155 patients, 98 had sepsis and 57 had septic shock; 38 (24.5%) died in hospital. Baseline characteristics did not differ significantly between survivors and non-survivors. At T2, non-survivors had significantly lower levels of methanol (10.6 vs 18.1 μM, p=0.02), mannose (52.6 vs 72.1 μM, p=0.044), isoleucine (p=0.015), leucine (p=0.027), and valine (p=0.02), and higher pyruvate (56.2 vs 27.4 μM, p=0.011). No significant metabolite differences were observed at T1. SOFA score was associated with in-hospital mortality (OR 1.36, 95% CI 1.06–1.74, p=0.017; AUC 0.70, 95% CI 0.55–0.84). Distinct metabolic alterations at 72 hours — but not at ED admission — particularly in branched-chain amino acids, mannose, methanol, and pyruvate, were associated with in-hospital mortality in sepsis patients. These findings suggest that early metabolic trajectories may complement ongoing risk stratification after initial ED resuscitation, but require validation in larger, multicentre cohorts before clinical translation.

Introduction

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection and remains a leading cause of in-hospital mortality worldwide.[1] Despite advances in critical care, early recognition and risk stratification remain challenging, particularly in emergency and resource-limited settings where rapid triage decisions must be made with incomplete information. Conventional severity scores, such as the Sequential Organ Failure Assessment (SOFA) score, provide important prognostic information but capture physiological and laboratory parameters rather than underlying biochemical heterogeneity.[2,3]

Metabolomics — the systematic analysis of small-molecule metabolites in biological fluids — has emerged as a powerful approach to characterise the biochemical alterations accompanying systemic inflammation and organ dysfunction. Prior studies have demonstrated that sepsis induces profound disruptions in amino acid, lipid, and energy metabolism, and that these perturbations associate with disease severity and outcomes.[4,5] Large-scale profiling in ICU cohorts has differentiated survivors from non-survivors and identified metabolic clusters associated with organ dysfunction.[6,7] Mosevoll *et al.* demonstrated that a substantial proportion of metabolites distinguished Sepsis-3 patients and correlated significantly with SOFA scores.[8]

However, the majority of metabolomic investigations have been conducted in Western Intensive Care Unit (ICU) populations. Data from emergency departments in Low- and Middle-Income Countries (LMICs) are scarce, despite the fact that distinct comorbidity profiles, resource constraints, and delayed presentations may shape both metabolic responses and outcomes in these settings.[9] Whether the metabolic signatures identified in ICU-based studies are reproducible at the time of ED presentation — before ICU-level interventions — remains underexplored.

Against this background, we conducted a prospective 1H Nuclear Magnetic Resonance (NMR)-based metabolomics study in ED patients with sepsis. The primary objective was to explore associations between the serum metabolomic profile at admission and at 72 hours with SOFA severity scores and in-hospital mortality, in a real-world, resource-limited tertiary care ED in India.

Materials and Methods

Study design and ethical approval

This was a prospective observational study conducted in the Emergency Department of a tertiary care academic medical institute in India between July 2023 and July 2024. The study protocol was approved by the Institutional Ethics Committee (IEC No: 2022-182-MD-129, *SGPGIMS, Lucknow*). Written informed consent was obtained from all patients or their legally authorized

representatives prior to enrolment. Patient care was not altered by study procedures and adhered to Surviving Sepsis Campaign 2021 (SSC-2021) guideline recommendations throughout.[10]

Patient population

Consecutive adult patients (≥18 years) presenting to the ED with a clinical diagnosis of sepsis according to Sepsis-3 criteria were eligible.[1] Sepsis was defined as suspected or confirmed infection with an acute increase in SOFA score ≥2 points. Septic shock was defined as sepsis with vasopressor requirement to maintain a mean arterial pressure (MAP) ≥65 mmHg and serum lactate >2 mmol/L despite adequate fluid resuscitation. Patients with known metabolic disorders, haematological malignancies, pregnancy, or refusal to consent were excluded.

Sample size

Sample size was estimated using Power Analysis and Sample Size (PASS) software version 16. Based on published metabolomics data in sepsis populations[4,6] and assuming a moderate correlation coefficient of 0.65 between SOFA scores and key metabolite changes — with a two-sided alpha of 0.05 and 80% power — the calculated required sample size was 156. A total of 155 patients were enrolled; the marginal shortfall is unlikely to have materially affected study power. Given the exploratory nature of the metabolomics analyses, the study was not formally powered for multiple metabolite comparisons, and all metabolite findings should be regarded as hypothesis-generating.

Clinical data collection

Demographic data, comorbidities, source of infection, clinical parameters, and standard laboratory results were captured using a structured case record form. SOFA scores were calculated at T1 (admission, 0 h) and T2 (72 ± 4 hours after admission). In-hospital mortality was ascertained at discharge or death.

Sample collection and processing

Venous blood samples were collected at two standardised time points: T1 (within 1 hour of ED presentation, 0 h) and T2 (72 ± 4 hours after admission). Samples were collected in sterile vacutainers, allowed to clot at room temperature for 30 minutes, and centrifuged at 2000 × g for 10 minutes. Serum aliquots (≥500 µL) were stored at −80°C until batch analysis. Reference samples from age- and sex-matched healthy fasting volunteers (minimum 8-hour fast) were collected to confirm instrument performance and quantification accuracy; these were not included in comparative clinical analyses.

NMR sample preparation

For 1H NMR spectroscopy, 300 µL of serum was mixed with 300 µL of phosphate-buffered D2O (0.9% NaCl, 50 mM sodium phosphate, 0.2 mM 3-Trimethylsilylpropanoic acid [TSP] as an internal chemical shift and concentration reference). The mixture was centrifuged at 1500 × g for 5 minutes and 550 µL of supernatant was transferred into 5 mm NMR tubes for analysis.

NMR spectroscopy

Spectra were acquired on an 800 MHz Bruker Avance III spectrometer (Bruker BioSpin, Rheinstetten, Germany) using a standard one-dimensional 1H Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence to attenuate macromolecular and lipid signals. Data were processed in TopSpin v3.6 with Fourier transformation, manual phase correction, and automated baseline correction. Metabolites were identified and quantified using the CHENOMX NMR Suite (v9.0, CHENOMX Inc., Edmonton, Canada) referenced against the 800 MHz metabolite spectral library.

Statistical analysis

Statistical analyses were performed using SPSS (v25.0, IBM Corp.), GraphPad Prism (v9.0), and MetaboAnalyst (v5.0). All analyses were performed on complete-case data; patients with missing T2 samples were noted and their exclusion from T2 analyses was documented.

Descriptive statistics: Continuous variables were assessed for normality using the Shapiro–Wilk test and visual inspection of distribution plots, and are presented as median (interquartile range, IQR) or mean (Standard Deviation, SD) as appropriate. Categorical variables are presented as frequency (percentage).

Group comparisons: comparisons between survivors and non-survivors, and between sepsis and septic shock groups, were performed using the Mann–Whitney U test for non-normally distributed continuous variables, the independent-samples t-test for normally distributed variables, and the chi-square or Fisher's exact test for categorical variables.

Metabolite–SOFA correlations: spearman's rank correlation coefficients (r) with 95% confidence intervals were used to quantify associations between individual metabolite concentrations and SOFA scores at T1 and T2. Correlation magnitude was interpreted as: negligible ($|r| < 0.1$), weak (0.1–0.3), mild (0.3–0.5), moderate (0.5–0.7), or strong (>0.7).

Multivariable logistic regression: to explore the independent association of SOFA score with in-hospital mortality, a pre-specified parsimonious logistic regression model was constructed including SOFA score at T2 and sepsis category (sepsis vs septic shock) as covariates. With 38 in-hospital deaths, the Events-Per-Variable (EPV) constraint of ≥10 events per predictor limited the model to ≤3 degrees of freedom; accordingly, no metabolite variable was entered as a formal covariate. Odds ratios (ORs) with 95% CIs are reported. Model discrimination was assessed by the area under the receiver operating characteristic curve (AUC-ROC) with 95% CI.

Multiple comparisons: this study tests multiple metabolites across two time points and multiple comparison groups. No formal correction for multiplicity was applied, given the exploratory intent. All p-values are two-sided; $p<0.05$ is used as the reporting threshold, and all metabolite associations are interpreted as hypothesis-generating signals requiring independent validation.

Multivariate metabolomics (MetaboAnalyst v5.0): unsupervised Principal Component Analysis (PCA) and supervised Partial Least Squares Discriminant Analysis (PLS-DA) were performed

after log-transformation and Pareto scaling. PLS-DA model quality was assessed by R^2 (goodness of fit) and Q^2 (predictive ability) from 7-fold cross-validation, and statistical robustness was confirmed by 1000-permutation testing. Metabolites contributing most to group separation were identified using Variable Importance in Projection (VIP >1.0) scores.

In the present cohort, PCA showed partial overlap between groups, while PLS-DA demonstrated modest separation for survivors versus non-survivors at T2 and poorer separation at T1, consistent with the univariate findings. Cross-validated multivariate performance was therefore interpreted cautiously and used primarily for signal exploration rather than definitive classification. To avoid overstatement, only metabolites showing concordant directionality in univariable analyses and biologically plausible patterns are emphasised in the main text.

Results

Baseline characteristics

A total of 155 adult patients with sepsis were enrolled: 98 (63.2%) with sepsis and 57 (36.8%) with septic shock. The median age was 54 years (IQR 39–67) with a male predominance (101/155, 65.2%). Common comorbidities included diabetes mellitus or hypertension (70%), chronic liver disease (35%), coronary artery disease (20%), and COPD (10%). In-hospital mortality was 24.5% (38/155). Mortality was significantly higher in the septic shock group (28/57, 49.1%) than in the sepsis group (10/98, 10.2%; $p<0.001$). Baseline demographics and comorbidities did not differ significantly between survivors and non-survivors, or between sepsis and septic shock groups. SOFA scores were significantly higher in non-survivors at both T1 (median 8 [IQR 4–13] vs 5 [IQR 4–7], $p<0.01$) and T2 (9 [IQR 5–12] vs 5 [IQR 4–7], $p=0.009$).

Metabolite differences: survivors vs non-survivors

No statistically significant differences in metabolite concentrations were observed at T1 between survivors and non-survivors, indicating that the serum metabolome at ED presentation did not

discriminate outcomes in this cohort. At T2 (72 hours), non-survivors showed significantly lower concentrations of selected metabolites across lipid, carbohydrate, and protein classes.

Lipids: methanol was significantly lower in non-survivors at T2 (10.6 [IQR 7.6–18.0] vs 18.1 [IQR 10.0–26.3] μM, p=0.02).

Carbohydrates: mannose was significantly lower (52.6 [IQR 23.1–82.0] vs 72.1 [IQR 45.6–119.6] μM, p=0.044). Pyruvate was significantly higher in non-survivors (56.2 [IQR 21.1–69.3] vs 27.4 [IQR 14.7–36.8] μM, p=0.011).

Branched-Chain Amino Acids (BCAAs): isoleucine (p=0.015), leucine (p=0.027), and valine (p=0.020) were all significantly lower in non-survivors at T2.

These differences were absent at T1 for all listed metabolites, emphasising that prognostically relevant metabolic divergence manifests at 72 hours rather than at initial presentation. All p-values are exploratory and should be interpreted in the context of multiple comparisons without formal correction.

Minimal multivariate analysis supported these observations. PCA did not show clear unsupervised separation between survivors and non-survivors at T1, whereas PLS-DA at T2 showed modest group discrimination, in keeping with the emergence of significant metabolite differences at 72 hours rather than at presentation. Given the sample size and exploratory design, these multivariate findings were used to support pattern recognition and were not interpreted as standalone predictive models.

Values: median (IQR), μM. Mann–Whitney U test. *p<0.05 (exploratory; no multiplicity correction applied). T1 = admission (0 h); T2 = 72 hours. Abbreviations: BCAA = branched-chain amino acid; TSP = 3-trimethylsilylpropanoic acid (internal reference).

Metabolite differences: sepsis vs septic shock

At T1, septic shock patients exhibited lower concentrations of acetone, methanol, and glucose compared with sepsis patients, but paradoxically higher concentrations of isoleucine, leucine, and valine. This cross-sectional pattern likely reflects acute catecholamine-driven proteolysis in

haemodynamically decompensated patients at presentation. The T2 BCAA depletion in non-survivors represents a distinct longitudinal trajectory — reflecting sustained hypercatabolism and failure of metabolic recovery — and is not contradicted by the T1 septic shock pattern. These two comparisons describe different biological processes at different time points.

SOFA score and mortality

SOFA scores were significantly higher in the septic shock group compared with sepsis at both T1 (median 9 [IQR 7–12] vs 4 [IQR 3–6], $p<0.001$) and T2, and in non-survivors compared with survivors at both time points. In logistic regression adjusted for sepsis category, each one-unit increase in SOFA score at T2 was associated with increased odds of in-hospital mortality (OR 1.36, 95% CI 1.06–1.74, $p=0.017$). ROC analysis for the model-predicted probability yielded an AUC of 0.70 (95% CI 0.55–0.84, $p=0.013$). At a model probability threshold of 0.50, sensitivity was 61.1% and specificity was 75.0% for in-hospital death. This represents moderate discriminative performance, consistent with the established but imperfect prognostic utility of SOFA alone in heterogeneous ED populations.

Discussion

Sepsis induces profound metabolic reprogramming that begins early and evolves dynamically over the course of illness. This prospective 1H NMR metabolomics study of ED patients with sepsis identified significant associations between serum metabolite concentrations at 72 hours — particularly in BCAAs, mannose, methanol, and pyruvate — and in-hospital mortality. Critically, no significant metabolite differences were observed at admission, suggesting that the prognostic metabolic signal in this cohort emerges during early disease evolution rather than at initial presentation.

These findings extend the metabolomics literature into a previously understudied setting: the emergency department of an LMIC tertiary care centre. Most prior metabolomic investigations have been conducted in ICU populations at Western institutions.[4–7] By demonstrating comparable metabolic signatures at the time of ED triage in an Indian cohort, this study supports

the potential generalisability of metabolomics approaches across diverse healthcare settings.[9] The absence of significant metabolite differences at T1 is itself informative — it suggests that in this population, a single admission metabolic measurement may be insufficient for prognostication without serial sampling.

The reduction in BCAAs (isoleucine, leucine, valine) in non-survivors at 72 hours is consistent with enhanced proteolysis and skeletal muscle catabolism driven by sustained systemic inflammation.4 Under prolonged septic physiology, BCAAs are consumed as peripheral energy substrates and for hepatic gluconeogenesis, leading to progressive depletion in those failing to mount metabolic recovery. Langley *et al.* first demonstrated that integrated clinico-metabolomic models incorporating amino acid alterations improved mortality prediction in sepsis beyond conventional clinical variables.[4] Our findings replicate this BCAA depletion signal in an ED-based LMIC setting using NMR spectroscopy.

The elevation of pyruvate in non-survivors at 72 hours warrants attention. Under normal conditions, pyruvate is channelled into the Tricarboxylic Acid (TCA) cycle for oxidative phosphorylation. In critical illness with mitochondrial dysfunction, impaired pyruvate dehydrogenase activity results in pyruvate accumulation and shunting toward lactate and alanine.[5] Elevated pyruvate at 72 hours in our non-survivors may therefore reflect persistent mitochondrial failure rather than active aerobic metabolism, and merits investigation as a dynamic biomarker of energy metabolism failure in sepsis.

The observation that BCAAs were paradoxically higher in septic shock vs sepsis at T1 — while lower in non-survivors *vs.* survivors at T2 — deserves explicit explanation. These comparisons are cross-sectional (phenotype comparison at one time point) versus longitudinal (survival trajectory over 72 hours). Initial BCAA elevation in shock likely reflects acute catecholamine-stimulated proteolysis in haemodynamically compromised patients, whereas T2 BCAA depletion in eventual non-survivors reflects exhaustion of protein reserves and failure of anabolic recovery. These patterns are biologically distinct and not contradictory.

The SOFA score demonstrated moderate discrimination for in-hospital mortality (AUC 0.70, 95% CI 0.55–0.84), consistent with its established but imperfect prognostic performance in heterogeneous ED cohorts.[3] While SOFA was the primary prognostic variable in this study, the

metabolite alterations at 72 hours may represent complementary biochemical information not captured by SOFA's physiological parameters. Formal evaluation of whether metabolite biomarkers improve discrimination (delta-AUC) or reclassification (NRI/IDI) when added to SOFA requires larger, adequately powered cohorts and was not possible within the EPV constraints of this study.

From an emergency care perspective, the clinical value of this study lies less in immediate triage at the front door and more in defining an early metabolic trajectory during the first 72 hours of sepsis care. In many EDs and acute medical units, disposition decisions, escalation to higher-dependency care, and reassessment after initial resuscitation occur within this same window. Accordingly, a metabolite signature that evolves after admission may still be operationally relevant for early inpatient escalation, ICU referral refinement, and identification of patients failing metabolic recovery after standard sepsis management. Future work should evaluate whether earlier serial sampling at 6–24 hours, or derivation of a smaller targeted metabolite panel from these discovery data, can translate this signal into a practical ED-facing prognostic tool.

Limitations

Several limitations must be acknowledged. First, this was a single-centre observational study with a modest sample size (n=155; 38 deaths), limiting statistical power for multivariable metabolite modelling and generalisability. Second, the prognostically relevant metabolite differences emerged only at 72 hours, not at admission, which restricts the immediate clinical utility of these findings for initial ED triage. Third, the absence of a non-septic critically ill control group limits assessment of the specificity of these metabolic changes for sepsis-related organ dysfunction. Fourth, metabolite concentrations may have been influenced by confounders including dietary intake, comorbid metabolic disease, antibiotic therapy, vasopressor exposure, and fluid administration, none of which were formally adjusted for. Fifth, 1H NMR spectroscopy, while robust and reproducible, has lower sensitivity than mass spectrometry-based platforms (LC-MS/MS, GC-MS) and captures a narrower range of metabolites.[11-13] Sixth, the study was not designed to formally evaluate incremental prognostic value of metabolomics over

SOFA. Seventh, all metabolite p-values are exploratory and no correction for multiple comparisons was applied; type I error inflation must be considered when interpreting individual metabolite findings. Finally, multivariate PCA/PLS-DA results were interpreted only as supportive pattern-recognition tools and were not presented as definitive predictive classifiers.

Conclusions

In this prospective 1H NMR metabolomics study of patients with sepsis presenting to an Indian emergency department, distinct and biologically plausible metabolic alterations — including depletion of branched-chain amino acids, mannose, and methanol, and elevation of pyruvate — were identified at 72 hours in patients who did not survive to hospital discharge. These alterations were not present at ED admission. SOFA score demonstrated moderate discrimination for in-hospital mortality (AUC 0.70). These findings are hypothesis-generating and require validation in multicentre cohorts with larger sample sizes, complementary metabolomics platforms, and formal evaluation of incremental prognostic value beyond established clinical scoring systems.

References

1. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016;315:801–10.

2. Ghatak T, Sajjad SA, Das A, et al. The diagnostic accuracy of serum procalcitonin for sepsis in adult emergency department patients: a systematic review and meta-analysis. CHEST Critical Care 2025;3:100179.

3. Shankar-Hari M, Phillips GS, Levy ML, et al. Developing a new definition and assessing new clinical criteria for septic shock. JAMA 2016;315:775–87.

4. Langley RJ, Tsalik EL, van Velkinburgh JC, et al. An integrated clinico-metabolomic model improves prediction of death in sepsis. Sci Transl Med 2013;5:195ra95.

5. Langley-Evans SC, Gardner DS, Jackson AA, et al. Metabolic profiling identifies key biochemical pathways altered in human sepsis. Crit Care Med 2015;43:2534–42.

6. Seymour CW, Yende S, Scott MJ, et al. Metabolomics in pneumonia and sepsis: an analysis of the GenIMS cohort study. Intensive Care Med 2013;39:1423–34.

7. Rogers AJ, McGeachie M, Baron RM, et al. Plasma metabolites in early sepsis identify distinct clusters of patients with differential outcomes. Crit Care Explor 2021;3:e0480.

8. Mosevoll KA, Gundersen T, Skrede S, et al. Metabolic profiling of Sepsis-3 patients compared to SIRS and Sepsis-2 patients. Biomolecules 2023;13:223.

9. Pandey N, Kumar S, Singh R, et al. Metabolomics in sepsis: a comprehensive review of clinical and experimental studies. Clin Exp Emerg Med 2025;12:4–15.

10. Evans L, Rhodes A, Alhazzani W, et al. Surviving Sepsis Campaign: international guidelines for management of sepsis and septic shock 2021. Intensive Care Med 2021;47:1181–247.

11. Chen Q, Liang X, Wu T, et al. Integrative analysis of metabolomics and proteomics reveals amino acid metabolism disorder in sepsis. J Transl Med 2022;20:123.

12. Peng J, Qiu C, Zhang J, Xiao X. Serum metabolite profiling reveals metabolic characteristics of sepsis patients using LC/MS-based metabolic profiles. BMC Med Genomics 2023;16:224.

13. Feng S, Cui N, Zhao W, et al. Prognostic biomarkers for sepsis mortality based on the literature and LC-MS-based metabolomics. Am J Transl Res 2023;15:5757–68.

Table 1. Baseline characteristics and SOFA scores by survival outcome (N=155).

Variable	Total (N=155)	Non-Survivors (n=38)	Survivors (n=117)	p-value
Age, years, median (IQR)	54 (39, 67)	55 (44, 68)	54 (36, 65)	0.391
Male sex, n (%)	101 (65.2)	23 (60.5)	78 (66.7)	0.490
Septic Shock, n (%)	57 (36.8)	28 (73.7)	29 (24.8)	<0.001*
Diabetes/Hypertension, n (%)	109 (70.3)	26 (68.4)	83 (70.9)	0.760
Chronic Liver Disease, n (%)	54 (34.8)	14 (36.8)	40 (34.2)	0.760
CAD, n (%)	31 (20.0)	8 (21.1)	23 (19.7)	0.857
COPD, n (%)	16 (10.3)	4 (10.5)	12 (10.3)	0.965
SOFA T1, median (IQR)	6 (4, 8)	8 (4, 13)	5 (4, 7)	<0.01*
SOFA T2, median (IQR)	6 (4, 8)	9 (5, 12)	5 (4, 7)	0.009*

T1, admission (0 h); T2, 72 hours. *p<0.05. CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease.

Table 2. Serum metabolite concentrations (µM) at T1 and T2 by survival outcome.

Metabolite	Time	Total (N=155)	Non-Survivors (n=38)	Survivors (n=117)	p-value
Methanol	T1	15.0 (9.5–24.5)	13.9 (10.1–20.6)	15.2 (9.0–28.7)	0.533
Methanol	T2	15.7 (9.2–24.2)	10.6 (7.6–18.0)	18.1 (10.0–26.3)	0.02*

Mannose	T1	72.7 (44.2–121.7)	49.9 (29.0–120.8)	76.0 (51.0–125.9)	0.078
Mannose	T2	67.2 (38.5–113.2)	52.6 (23.1–82.0)	72.1 (45.6–119.6)	0.044*
Pyruvate	T1	38.6 (22.4–60.4)	40.3 (23.6–72.2)	36.5 (22.2–59.6)	0.599
Pyruvate	T2	28.0 (18.9–44.5)	56.2 (21.1–69.3)	27.4 (14.7–36.8)	0.011*
Isoleucine	T1	37.8 (20.2–67.8)	30.2 (14.0–48.5)	39.3 (20.8–71.1)	0.128
Isoleucine	T2	33.8 (17.5–51.6)	19.4 (12.5–37.4)	35.1 (18.4–59.4)	0.015*
Leucine	T1	55.7 (29.8–86.7)	48.5 (25.4–72.8)	57.5 (34.4–86.7)	0.388
Leucine	T2	46.1 (22.3–75.7)	34.8 (10.9–46.8)	51.1 (23.2–77.7)	0.027*
Valine	T1	112.9 (60.6–174.9)	97.9 (50.3–141.7)	118.1 (65.3–181.6)	0.115
Valine	T2	93.4 (53.1–141.0)	64.2 (41.8–114.3)	101.0 (58.2–180.0)	0.02*

Values are median (IQR), µM. Mann–Whitney U test. *$p<0.05$ (exploratory; no multiplicity correction applied). T1, admission (0 h); T2, 72 hours. Abbreviations: BCAA, branched-chain amino acid; TSP, 3-trimethylsilylpropanoic acid.

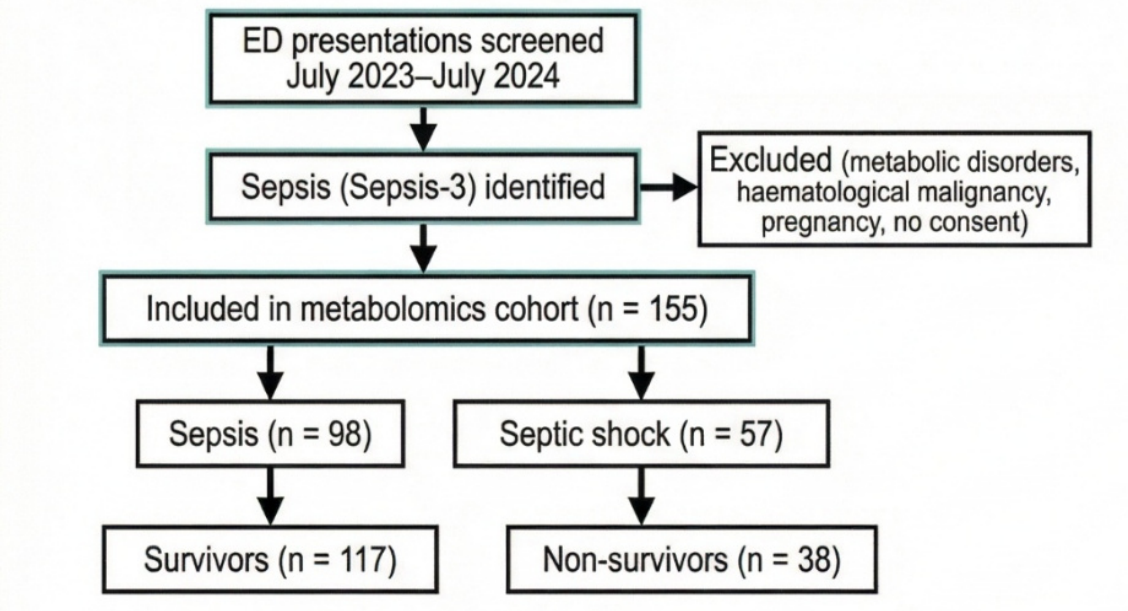

Figure 1. Study flow of the emergency department sepsis cohort.

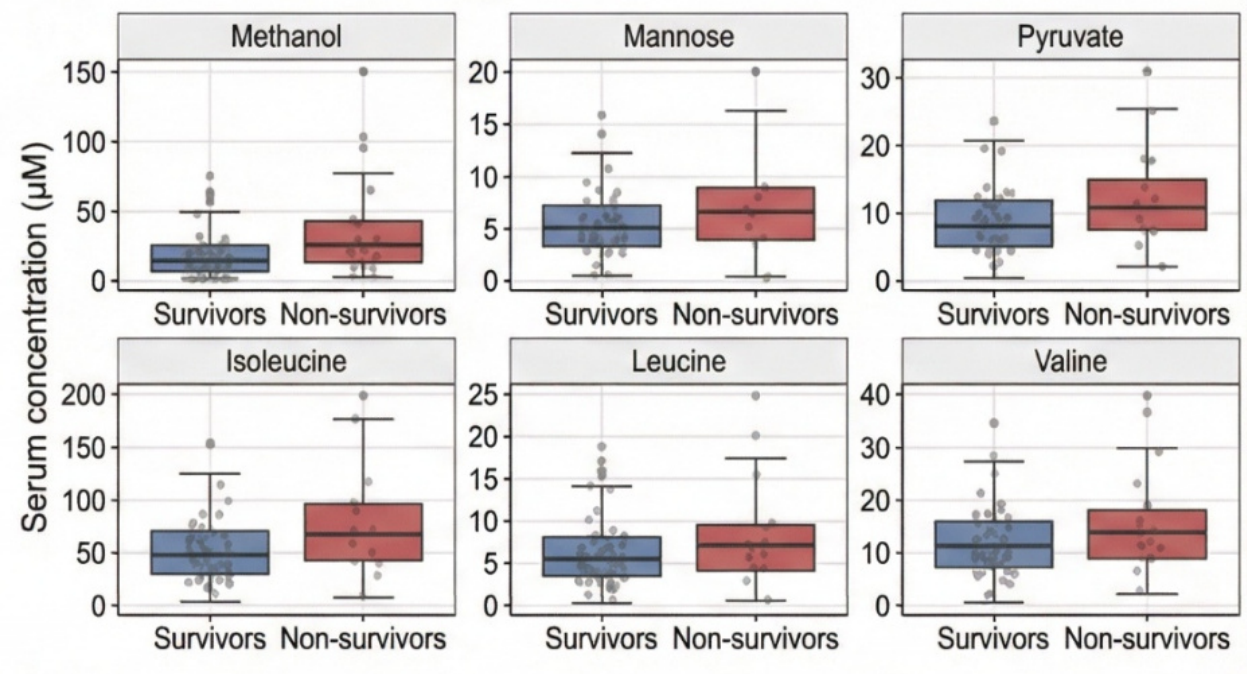

Figure 2. Boxplots comparing circulatory levels of key metabolites at 72 hours (T2) by survival outcome (survivors versus non-survivors. No corresponding differences were observed at admission (T1).

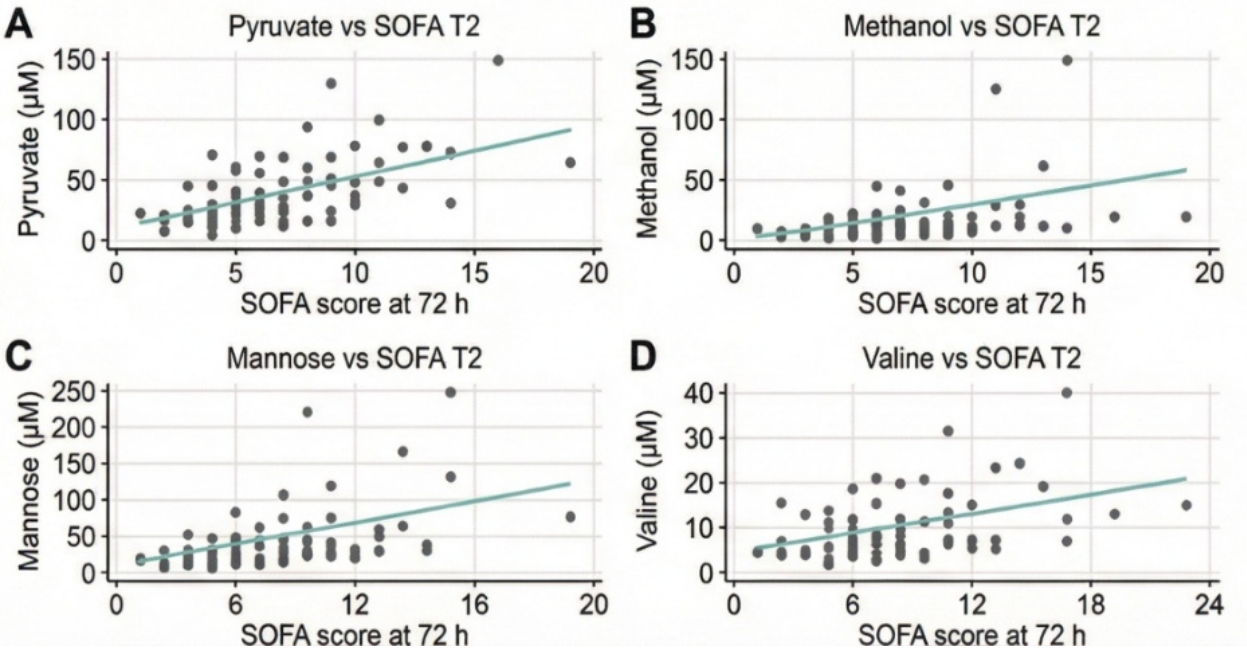

Figure 3. Correlation between 72-hour (T2) serum metabolites and SOFA score. Scatter plots with fitted trend lines show the relationship between SOFA score at 72 hours and (A) pyruvate, (B) methanol, (C) mannose, and (D) valine. Associations were assessed using Spearman's rank correlation.

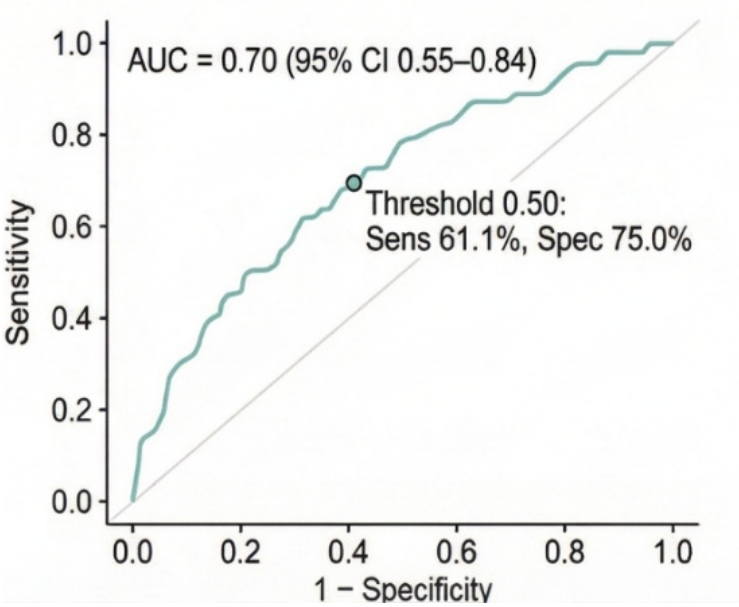

Figure 4. Discriminative performance of the 72-hour SOFA-based logistic regression model for in-hospital mortality. Receiver operating characteristic (ROC) curve for the model-predicted probability of death (SOFA score at 72 hours, adjusted for sepsis category); AUC = 0.70 (95% CI 0.55–0.84). The marked point indicates the sensitivity (61.1%) and specificity (75.0%) at a predicted-probability threshold of 0.50.

Contributions: Ratender Kumar Singh, Tanmoy Ghatak, and Puja Gautam contributed to study conception and design, ethical approval, and patient evaluation, screening, and sample collection.

Puja Gautam additionally compiled the clinical and demographic data for all patients from the institutional digital records. Gurvinder Singh and Pragati Gupta prepared samples for NMR analysis and acquired, processed, and analyzed the NMR spectral data. Dinesh Kumar performed the multivariate statistical analysis. Prabhakar Mishra performed the univariate statistical analysis. All authors reviewed and approved the final manuscript.

Conflict of interest: the authors declare no potential conflict of interest, and all authors confirm accuracy.

Ethics approval: the Ethics Committee of Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh approved this study (*IEC No: 2022-182-MD-129*). The study is conformed with the Helsinki Declaration of 1964, as revised in 2013, concerning human and animal rights.

Informed consent: all patients participating in this study signed a written informed consent form for participating in this study.

Patient consent for publication: written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

Availability of data and materials: all data generated or analyzed during this study are included in this published article.

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