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Identification of Distinct Metabolic Phenotypes in Aneurysmal Subarachnoid Hemorrhage: A Hypothesis- Generating Pilot Study using Hierarchical Clustering

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Research Article

Keywords: Aneurysmal subarachnoid hemorrhage, Cerebrospinal fluid, Uric acid, Biomarkers, Prognosis, Metabolic profiling

Posted Date: June 15th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9784716/v1

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Additional Declarations: No competing interests reported.

Abstract

Background/Objective:

Aneurysmal subarachnoid hemorrhage (aSAH) is associated with high mortality and heterogeneous clinical outcomes that are not fully explained by conventional prognostic models. Emerging evidence suggests that dynamic metabolic alterations may contribute to disease progression. This exploratory pilot study aimed to investigate dynamic cerebrospinal fluid (CSF) metabolic changes and explore clinically accessible metabolic markers associated with disease trajectory in patients with aSAH.

Methods

In this prospective, single-center cohort study, 20 patients with aSAH were enrolled and followed in the intensive care unit. Clinical and biochemical parameters were recorded at admission (Day 1) and follow-up (Day 5). CSF uric acid (UA) and protein levels were measured, and derived indices including the CSF UA/protein ratio were analyzed. Temporal changes, correlation analyses, ROC analysis, principal component analysis, and exploratory multivariate Cox regression analyses were performed.

Results

CSF protein levels significantly decreased over time ($p = 0.019$), whereas CSF UA showed a non-significant downward trend. The CSF UA/protein ratio showed an exploratory association with altered survival dynamics in multivariate Cox regression analysis (HR: 0.204, $p = 0.048$). However, its discriminative performance was limited (AUC: 0.558, 95% CI: 0.287–0.829). No correlation was observed between CSF UA and systemic renal parameters, while a strong inverse correlation was identified between baseline CSF UA levels and their temporal change (rho = − 0.781, $p < 0.001$). PCA suggested that neurological and physiological severity were the major contributors to patient stratification, with additional influence from metabolic parameters.

Conclusion

Dynamic CSF biomarker changes may reflect heterogeneous metabolic responses following aSAH. The CSF UA/protein ratio appeared to be associated with prolonged disease trajectory rather than improved clinical outcome. These exploratory findings suggest that metabolic biomarkers may provide complementary biological information alongside clinical assessment; however, their prognostic utility remains limited and should be interpreted cautiously given the small sample size and pilot nature of the study.

Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) continues to be associated with substantial early mortality and long-term neurological disability despite advances in treatment strategies.[1] Clinical outcomes after aSAH are highly heterogeneous, even among patients with comparable neurological grade and radiological severity at presentation, suggesting that conventional prognostic frameworks incompletely capture the biological complexity of the disease.[2]

Beyond structural brain injury, aSAH induces a profound systemic and central metabolic response that evolves dynamically during the early phase after hemorrhage. Metabolomic studies in plasma and cerebrospinal fluid have consistently demonstrated a broad range of metabolic alterations affecting lipid turnover, amino acid pathways, and cellular energy utilization.[3,4] Several metabolites—such as sphingolipids, taurine, kynurenine, and intermediates of glycolysis and the tricarboxylic acid cycle—have been linked to delayed cerebral ischemia and functional outcome, underscoring the clinical relevance of metabolic dysregulation after aSAH.[5]

Importantly, these metabolic alterations are not uniform across patients. Recent data-driven approaches using unsupervised methods, including hierarchical clustering, suggest that aSAH patients can be stratified into biologically distinct metabolic phenotypes characterized by coordinated changes across lipid, amino acid, and energy-metabolism pathways, with meaningful associations to complications and outcome.[1,6,7] However, most prior studies have relied on high-dimensional metabolomics platforms that are not readily available in routine clinical practice. Whether clinically accessible metabolic and physiological variables, tracked dynamically during early neurocritical care, can define comparable metabolic phenotypes remains largely unexplored.

In this hypothesis-generating pilot study, we aimed to explore whether routinely measured metabolic and clinical parameters could reveal heterogeneous metabolic patterns in patients with aSAH using hierarchical clustering analysis. We further investigated the relationship between these exploratory metabolic profiles, early dynamic metabolic deterioration, and in-hospital mortality, with the goal of providing a pragmatic and clinically accessible framework for future biologically informed risk assessment in aSAH.

Methods

Study Design and Patient Selection

This was a single-center prospective observational cohort study conducted following approval from the Clinical Research Ethics Committee of Ondokuz Mayıs University Faculty of Medicine (Approval No: 2023/267; September 2023). The study was supported by the Scientific Research Projects Coordination Unit of Ondokuz Mayıs University (Project No: BAP01-2024-4955) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients or their legal representatives prior to inclusion.

Between March 2024 and January 2025, 20 patients diagnosed with aneurysmal subarachnoid hemorrhage (aSAH) and admitted to the intensive care unit were prospectively enrolled.

CSF samples were obtained through external ventricular drainage catheters placed for standard clinical indications and were collected at predefined time points (Day 1 and Day 5) according to the study protocol. Demographic characteristics, clinical variables, and routine laboratory parameters were obtained from the hospital electronic medical record system.

Patients with traumatic subarachnoid hemorrhage, hemorrhage secondary to arteriovenous malformations, or incomplete clinical/laboratory follow-up data were excluded.

CSF Sampling and Biochemical Analysis

CSF samples were centrifuged at 3,500 × g for 5 minutes. The supernatant was collected, aliquoted, and stored at − 80°C until analysis. Uric acid concentrations were measured using a commercially available Uric Acid Colorimetric Assay Kit (Elabscience, Houston, TX, USA, Cat. No: E-BC-K016-M), in accordance with the manufacturer's protocol. CSF protein concentrations were measured using standard biochemical methods in the hospital central laboratory.

Absorbance values were measured at a wavelength of 690 nm using a spectrophotometric method. The assay had a detection range of 1.30–80 mg/L and a sensitivity of 1.30 mg/L. The intra-assay coefficient of variation (CV) ranged from 1.8% to 2.3%, while the inter-assay CV ranged from 0.7% to 4.4%. Uric acid concentrations were expressed in mg/L. All samples were processed using a standardized protocol to minimize preanalytical variability.

Data Collection

Demographic characteristics (age, sex), comorbidities (hypertension, diabetes mellitus, coronary artery disease), lifestyle characteristics (e.g., smoking status, alcohol use), and radiological findings were obtained from the hospital electronic medical record system. Radiological evaluation was based on computed tomography scans, and the presence of subarachnoid hemorrhage, intraventricular hemorrhage, and intraparenchymal hemorrhage was recorded.

Disease severity was assessed using the following scoring systems:

- **Clinical**: Glasgow Coma Scale (GCS), World Federation of Neurological Surgeons (WFNS) score[1]
- **Radiological**: Modified Fisher score, Rotterdam score[1,8,9]
- **Physiological**: Acute Physiology and Chronic Health Evaluation II (APACHE II)[10]

Laboratory parameters, including complete blood count, biochemical tests, arterial blood gas analysis, and CSF analysis, were recorded separately for day 1 (acute phase) and day 5 (subacute/vasospasm risk

phase) of intensive care unit admission.

Statistical Analysis

All statistical analyses were performed using R software (version 4.5.1) and the RStudio integrated development environment. Data manipulation and graphical visualization were conducted using the tidyverse ecosystem (dplyr, tidyr, ggplot2).

The following R packages were used for specialized analyses:

- pROC: exploratory receiver operating characteristic (ROC) curve analysis and estimation of optimal cut-off values
- Hmisc and corrplot: evaluation and visualization of correlations between variables
- pheatmap: exploratory hierarchical clustering and heatmap visualization
- gsignif: graphical annotation of statistical significance

Normality of continuous variables was assessed using the Shapiro–Wilk test. Continuous variables were expressed as mean ± standard deviation for normally distributed data and median (interquartile range) for non-normally distributed data. Categorical variables were presented as number and percentage (n, %).

- Between-group comparisons: Independent samples t-test was used for normally distributed continuous variables, while the Mann–Whitney U test was applied for non-normally distributed variables. Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate.
- Within-group temporal comparisons: Differences between Day 1 and Day 5 measurements (Δ values) were analyzed using the paired t-test or Wilcoxon signed-rank test.
- Advanced analyses: Associations between variables were evaluated using Spearman correlation analysis. ROC analysis was performed as an exploratory assessment of the discriminatory performance of selected parameters for in-hospital mortality. Exploratory hierarchical clustering analysis was conducted using Euclidean distance and complete linkage methods to evaluate heterogeneous metabolic and clinical patterns among patients. The optimal number of clusters was determined based on visual inspection of the dendrogram and clinical interpretability.

Given the limited sample size and high event rate, all multivariable, ROC, and clustering analyses were considered exploratory and hypothesis-generating.

A two-sided p value < 0.05 was considered statistically significant for all analyses; however, findings were interpreted cautiously given the exploratory nature of the study.

Only patients with complete clinical and laboratory data at both time points (Day 1 and Day 5) were included in the analysis. No imputation methods were applied.

Results

Baseline Characteristics and Temporal Changes

Baseline demographic, clinical, radiological, and laboratory characteristics are summarized in Table 1.

Table 1
Baseline demographic, clinical, radiological, and laboratory characteristics of the study cohort (N = 20)

Variable Group	Variable	Total (N = 20)
Demographics & Follow-up	Age (Years) (Mean ± SD)	64.35 ± 13.21
	Age Group: < 65 Years (Middle Age) (n, %)	10 (50.0%)
	Age Group: ≥ 65 Years (Elderly) (n, %)	10 (50.0%)
	Follow-up Duration (Days) (Mean ± SD)	85.90 ± 132.37
	Smoking History (n, %)	2 (10.0%)
Clinical Scores & Findings	GCS - Day 1 (Mean ± SD)	10.05 ± 3.80
	GCS - Day 5 (Mean ± SD)	6.70 ± 3.10
	APACHE II Score - Day 1 (Mean ± SD)	16.40 ± 5.07
	APACHE II Score - Day 5 (Mean ± SD)	20.90 ± 6.46
	WFNS Score (Mean ± SD)	3.70 ± 1.13
	WFNS Grade: 1–3 (Low Risk) (n, %)	7 (35.0%)
	WFNS Grade: 4–5 (High Risk) (n, %)	13 (65.0%)
	Fisher Score (Mean ± SD)	3.85 ± 0.67
	Fisher Grade: 3–4 (High Risk) (n, %)	19 (95.0%)
	Rotterdam Score (Mean ± SD)	1.85 ± 0.67
	GOS Score (Mean ± SD)	1.50 ± 1.05
	GOS Group: Poor Outcome (1–3) (n, %)	18 (90.0%)
Radiological Findings (CT[f])	Isolated SAH (n, %)	7 (35.0%)
	SAH + Intraventricular Hemorrhage (n, %)	8 (40.0%)
	Intraventricular Hemorrhage: Present (n, %)	12 (60.0%)
Comorbidities & Interventions	Comorbidity: Present (n, %)	16 (80.0%)
	Hypertension (n, %)	10 (50.0%)
	Mechanical Ventilation Requirement (n, %)	16 (80.0%)
	Tracheostomy (n, %)	5 (25.0%)

Abbreviations: SD, standard deviation; **GCS**, Glasgow Coma Scale; **APACHE II**, Acute Physiology and Chronic Health Evaluation II; **WFNS**, World Federation of Neurological Surgeons; **GOS**, Glasgow Outcome Scale; **CT**, Computed Tomography; **SAH**, subarachnoid hemorrhage; **CSF**, cerebrospinal fluid.

Variable Group	Variable	Total (N = 20)
Day 1 Laboratory Findings	Serum uric acid (mg/dL) (Mean ± SD)	4.21 ± 2.29
	CSF uric acid (mg/dL) (Mean ± SD)	2.06 ± 1.44
	Serum Glucose (mg/dL) (Mean ± SD)	157.40 ± 30.60
	CSF Protein (g/L) (Mean ± SD)	1.98 ± 2.23
Day 5 Laboratory Findings	Serum uric acid (mg/dL) (Mean ± SD)	3.75 ± 2.23
	CSF uric acid (mg/dL) (Mean ± SD)	1.43 ± 0.87
	Serum Glucose (mg/dL) (Mean ± SD)	175.55 ± 60.91
	CSF Protein (g/L) (Mean ± SD)	0.69 ± 0.37
Clinical Outcomes	Mortality (In-hospital): Deceased (n, %)	16 (80.0%)

Abbreviations: SD, standard deviation; **GCS**, Glasgow Coma Scale; **APACHE II**, Acute Physiology and Chronic Health Evaluation II; **WFNS**, World Federation of Neurological Surgeons; **GOS**, Glasgow Outcome Scale; **CT**, Computed Tomography; **SAH**, subarachnoid hemorrhage; **CSF**, cerebrospinal fluid.

While the study population exhibits a symmetrical distribution in terms of age (with middle-aged and elderly groups equally represented), the baseline characteristics of the patients generally indicate a high-severity clinical profile. At least one comorbidity was present in 80% of the sample, with hypertension alone reported in half of the patients (50.0%). When examining the Fisher Grade, it is observed that 95% of the patients were categorized as high-risk (Grade 3–4), and 80% required mechanical ventilation support. The in-hospital mortality rate was 80%.

Figure 1 shows changes in APACHE II and GCS scores between Day 1 and Day 5, as well as the distribution of clinical outcomes according to age group.

A comparative analysis of baseline (Day 1) clinical and metabolic variables was performed to identify differences between the middle-aged and elderly subgroups. As shown in Table 2, several parameters differed significantly between the groups. Serum glucose levels were higher in the elderly group (≥ 65 years) compared to the middle-aged group (170.50 ± 21.88 mg/dL vs. 144.30 ± 33.42 mg/dL; p = 0.011). Baseline serum uric acid levels were also higher in elderly patients (5.60 ± 2.11 mg/dL vs. 2.81 ± 1.52 mg/dL; p = 0.003).

Table 2
Comparative Analysis of Baseline Clinical and Metabolic Variables by Age Group

Feature (Unit)	< 65 Years (n = 10)	≥ 65 Years (n = 10)	Test Statistic	p-value
Age (Years)	53.50 ± 7.37	75.20 ± 7.24	U = 0	< 0.001
Serum Glucose (mg/dL)	144.30 ± 33.42	170.50 ± 21.88	U = 16	0.011
Serum Uric Acid (mg/dL)	2.81 ± 1.52	5.60 ± 2.11	T = − 3.38	0.003

Temporal changes in CSF biomarkers between Day 1 and Day 5 are presented in Table 3.

Table 3
Temporal Evolution of CSF Biomarkers from Admission (Day 1) to Follow-up (Day 5)

CSF Variable (Unit)	Day 1 (Baseline)	Day 5 (Follow-up)	p-value
CSF Uric Acid (mg/dL)	2.06 ± 1.44	1.43 ± 0.87	0.104
CSF Protein (g/L)	1.98 ± 2.23	0.69 ± 0.37	0.019
CSF Glucose (mg/dL)	70.99 ± 40.28	74.01 ± 50.44	0.835
Abbreviation: CSF, cerebrospinal fluid.			

CSF protein levels decreased significantly between Day 1 and Day 5 (− 1.27 g/L, p = 0.019), whereas CSF uric acid showed a non-significant downward trend.

Intragroup comparisons of clinical and metabolic parameters between Day 1 and Day 5 are presented in Table 4.

Table 4
Intragroup Comparison of Clinical and Metabolic Trajectories from Day 1 to Day 5 Stratified by Age

Age Group	Variable	Day 1	Day 5	Test Statistic	p-value
< 65 years	Serum Sodium (mmol/L)	140(138 – 141)	147(138.5 – 149.5)	W = 3.50	0.049
	Serum Magnesium (mg/dL)	2.42(1.95 – 2.53)	1.94(1.46 – 2.20)	W = 33.50	0.035
	White Blood Cell (103/ µL)	14.90 ± 5.74	11.37 ± 2.95	t (9) = 2.28	0.048
≥ 65 years	GCS	11.20 ± 2.97	6.20 ± 2.57	t (9) = 5.75	< 0.001
	APACHE II Score	16.00 ± 3.33	22.50 ± 6.82	t (9) = – 3.70	0.005
	Serum Uric Acid (mg/dL)	5.60 ± 2.11	4.33 ± 2.18	t (9) = 2.30	0.046
	Blood pH	7.38 ± 0.06	7.41 ± 0.08	t (9) = – 2.33	0.045
	Bicarbonate (mmol/L)	22.50 ± 3.06	28.02 ± 6.81	t (9) = – 3.10	0.012

Abbreviations: GCS, Glasgow Coma Scale; **APACHE II**, Acute Physiology and Chronic Health Evaluation II.

In the elderly group (≥ 65 years), GCS scores decreased from 11.20 ± 2.97 to 6.20 ± 2.57 ($p < 0.001$), while APACHE II scores increased from 16.00 ± 3.33 to 22.50 ± 6.82 ($p = 0.005$). Serum uric acid levels decreased from 5.60 ± 2.11 mg/dL to 4.33 ± 2.18 mg/dL ($p = 0.046$). Blood pH increased from 7.38 ± 0.06 to 7.41 ± 0.08 ($p = 0.045$), and bicarbonate levels increased from 22.50 ± 3.06 mmol/L to 28.02 ± 6.81 mmol/L ($p = 0.012$).

In the middle-aged group (< 65 years), serum sodium levels increased from 140 (138–141) to 147 (138.5–149.5) mmol/L ($p = 0.049$), while serum magnesium levels decreased from 2.42 (1.95–2.53) to 1.94 (1.46–2.20) mg/dL ($p = 0.035$). White blood cell counts decreased from 14.90 ± 5.74 to 11.37 ± 2.95 ($p = 0.048$).

Inter-group comparisons of clinical and metabolic parameters according to hemorrhage pattern are summarized in Table 5. A significant difference was observed in serum chloride levels at baseline ($p = 0.007$), while GCS scores did not differ significantly between groups at either time point.

Table 5
Inter-group Comparison of Clinical and Metabolic Parameters According to Hemorrhage Pattern

Variable	Isolated SAH (n = 7)	SAH + IVH (n = 8)	SAH + Other* (n = 5)	Test Statistic	p-value
Baseline (Day 1)					
GCS	10.29 ± 3.55	7.88 ± 3.87	13.20 ± 1.30	H = 5.88	0.053
Serum Chloride (mmol/L)	108.57 ± 3.10	112.12 ± 12.56	97.60 ± 4.45	H = 9.70	0.007[a]
Follow-up (Day 5)					
GCS	7.29 ± 3.77	4.88 ± 2.10	8.80 ± 1.92	H = 5.85	0.054

* SAH + Other includes patients with concomitant parenchymal or subdural hemorrhages.

[a] Inter-group comparison (Kruskal–Wallis test).

Abbreviations: GCS, Glasgow Coma Scale; **SAH**, subarachnoid hemorrhage; **IVH**, intraventricular hemorrhage.

A trend toward lower GCS scores was observed in the aSAH + intraventricular hemorrhage (IVH) group at Day 5 (H = 5.85, p = 0.054).

Within the aSAH + IVH group, intragroup analysis demonstrated significant increases in APACHE II score, serum glucose, and blood pH between Day 1 and Day 5 (all $p < 0.05$).

Longitudinal changes in CSF uric acid levels according to comorbidity status are presented in Table 6. No difference was observed at baseline, whereas a significant difference emerged at Day 5, with higher levels in patients with comorbidities.

Table 6
Longitudinal Analysis of CSF Uric Acid Dynamics Based on Comorbidity Status

Variable: CSF Uric Acid (mg/dL)	Total Group (n = 20)	Comorbidity Absent (n = 4)	Comorbidity Present (n = 16)	p-value
Day 1 (Admission)	2.06 ± 1.44	2.23 ± 1.26	2.02 ± 1.52	0.786
Day 5 (Follow-up)	1.43 ± 0.87	0.63 ± 0.20	1.63 ± 0.85	< 0.001

Abbreviations: **CSF**, cerebrospinal fluid.

Correlation analysis using Spearman's rank test showed no significant relationship between changes in CSF protein (ΔProtein) and CSF uric acid (ΔUA) (rho = − 0.058, p = 0.826; Fig. 2).

Changes in CSF erythrocyte and leukocyte counts were also evaluated (Fig. 3). Neither erythrocyte counts (p = 0.34) nor leukocyte counts (p = 0.28) showed significant temporal variation. No significant

association was observed between cellular changes and the delta (Δ) values of CSF protein or uric acid.

Although a mean reduction in CSF protein levels of − 1.27 g/L was observed by Day 5, this change was not significantly associated with variations in CSF uric acid levels.

Outcome-based differences in CSF uric acid dynamics and derived indices are summarized in **Supplementary Table S1**. Due to the limited number of patients with good outcomes (n = 2), findings are presented descriptively.

A reduction in both CSF uric acid and protein levels was observed in both outcome groups by Day 5. The mean decrease in CSF uric acid was − 1.14 ± 1.14 mg/dL in the good outcome group and − 0.57 ± 1.73 mg/dL in the poor outcome group. CSF protein levels also decreased in both groups.

At baseline (Day 1), the CSF UA/Protein ratio was higher in the poor outcome group (840.00%) compared to the good outcome group (204.00%).

The CSF/Serum uric acid index decreased in the good outcome group (Δ = −0.135), whereas an increase was observed in the poor outcome group (Δ = +0.089).

Given the limited number of patients in the good outcome group (n = 2), these findings are presented descriptively.

In the multivariate Cox model, age and the CSF UA/protein ratio (> 128.64) were independently associated with survival (HR: 0.204, p = 0.048). Correlation analyses demonstrated no relationship between serum and CSF parameters; however, a strong negative correlation was observed between baseline CSF uric acid levels and their change over time (Table 7).

Table 7
Multivariate Risk Factors and Biochemical Correlation Analysis

Analysis Domain	Variable	Statistical Summary
Cox Survival Analysis	Age	HR: 1.112 (p = 0.005)
	WFNS Score	HR: 1.827 (p = 0.094)
	CSF UA/Protein Ratio (ROC Cut-off > 128.64)	HR: 0.204 (p = 0.048)
Correlation Analysis	Renal vs. CSF	rho: 0.004 (p = 0.987)
	Serum UA vs. CSF UA	rho: 0.088 (p = 0.712)
	Baseline vs. Δ Change	rho: -0.781 (p < 0.001)

Abbreviations: CSF, cerebrospinal fluid; **UA**, uric acid; **WFNS**, World Federation of Neurosurgical Societies; **ROC**, receiver operating characteristic; **HR**, hazard ratio; **rho**, Spearman's correlation coefficient; **Δ**, change.

Kaplan–Meier survival analysis was performed using the ROC-derived cut-off value (128.64) for the change in the CSF UA/Protein ratio (Δ). Patients were categorized into low (≤ 128.64) and high (> 128.64) groups. No statistically significant difference in survival distribution was observed between the groups (log-rank p = 0.90; Fig. 4).

ROC analysis demonstrated limited discriminative ability of the CSF UA/protein ratio for predicting mortality, with an area under the curve (AUC) of 0.558 (95% CI: 0.287–0.829). The optimal cut-off value of 128.64 yielded a sensitivity of 46.2%, indicating poor predictive performance when used as a standalone marker.

Principal component analysis (PCA) demonstrated that neurological severity (GCS Day 5) and systemic physiological status (APACHE II Day 5) were the main contributors to variance. The variable contribution plot is presented in Fig. 5, while patient clustering according to survival status is shown in Fig. 6.

Discussion

In this prospective pilot study, we investigated dynamic metabolic alterations following aneurysmal subarachnoid hemorrhage (aSAH) using routinely available biochemical and physiological parameters, with a particular focus on cerebrospinal fluid (CSF) biomarkers. Our findings revealed a significant temporal reduction in CSF protein levels during the early critical care period, whereas CSF uric acid (UA) showed a consistent, albeit non-significant, decline. These observations are consistent with previous studies demonstrating dynamic changes in CSF composition and the progressive clearance of blood-derived components following aSAH.[11]

The non-significant decrease in CSF UA suggests that uric acid may follow a distinct kinetic profile compared to total protein. As the terminal product of purine degradation, UA plays a dual role in acute brain injury, contributing to both antioxidant defense and pro-inflammatory signaling. Beyond this, UA reflects a dynamic balance between oxidative stress, cellular injury, and metabolic turnover. Following aSAH, erythrocyte breakdown and neuronal damage increase purine metabolism, while blood–brain barrier disruption alters CSF composition. The observed dissociation between CSF UA and protein dynamics in our cohort reinforces the notion that UA kinetics are not merely a reflection of passive clearance but likely represent intrinsic intracranial metabolic regulation and ongoing oxidative stress.[3,12,13]

A central finding of our study is the emergence of the CSF UA/protein ratio as a potential prognostic indicator. In our multivariate Cox regression model, this ratio was significantly associated with survival duration, suggesting that the balance between oxidative metabolites and protein clearance carries more clinical relevance than isolated parameters.[14] Importantly, composite indices such as the CSF UA/protein ratio may better capture the complex interplay between metabolic activity and clearance mechanisms, integrating two biologically distinct processes into a single clinically interpretable marker. Interestingly, no significant correlation was observed between changes in Glasgow Coma Scale (GCS)

scores and dynamic alterations in the CSF UA/protein ratio. This lack of association suggests that metabolic processes within the central nervous system may evolve independently of overt clinical neurological changes. From a pathophysiological perspective, this finding implies that ongoing oxidative stress and metabolic dysregulation may persist even in the absence of detectable changes in bedside neurological assessment. Clinically, this dissociation highlights a critical limitation of relying solely on macroscopic neurological scales, as cellular-level injury and metabolic deterioration may continue despite apparently stable or even improving clinical status.

Importantly, the observed association between the CSF UA/protein ratio and survival should be interpreted with caution. Although the Cox regression model suggested a protective effect (HR < 1), this finding is more likely to reflect delayed mortality rather than true survival benefit. Violation of the proportional hazards assumption further indicates that the effect of this variable is not constant over time. Clinically, patients with higher CSF UA/protein ratios were not characterized by recovery, but rather by prolonged survival under intensive supportive care, often requiring tracheostomy and long-term mechanical ventilation, followed by delayed mortality.

This pattern likely reflects a form of survivorship bias or a “chronic critical illness” trajectory, in which early mortality is deferred without meaningful neurological recovery. Therefore, the CSF UA/protein ratio may be more accurately interpreted as a marker of prolonged disease course rather than improved survival. Interestingly, while the regression model showed significance, Kaplan–Meier analysis did not demonstrate a significant difference in survival between groups. This discrepancy—commonly observed in small, high-risk neurocritical care cohorts such as ours—likely reflects the fact that Cox models can adjust for multiple covariates (such as GCS and APACHE II), whereas Kaplan–Meier analysis remains a univariate approach limited by sample size and event distribution. Importantly, the limited discriminative performance observed in ROC analysis further supports this inconsistency, suggesting that the CSF UA/protein ratio has restricted ability to accurately classify outcomes when used as a standalone predictor. Taken together, these findings indicate that the observed association is likely driven by complex interactions within a multivariable context rather than reflecting robust independent predictive performance. This methodological divergence is well recognized in neurocritical care research and highlights the importance of multivariate modeling in small heterogeneous cohorts.[2,5,6,15]

Beyond descriptive changes, our study proposes a clinically accessible framework for metabolic phenotyping. While prior metabolomic investigations have consistently reported widespread metabolic reprogramming following aSAH—including shifts toward glycolysis, lipid mobilization, and altered amino acid metabolism—these approaches remain largely dependent on high-dimensional platforms.[1,3–6,14] In contrast, we demonstrate that simple, routinely measured variables—such as CSF UA and protein—can capture clinically meaningful biological variation. This translational approach may bridge the gap between experimental metabolomics and routine clinical decision-making and may facilitate bedside implementation.

Furthermore, we observed that comorbidity status significantly influences CSF UA dynamics, with comorbid patients exhibiting higher UA levels at Day 5. This suggests that systemic comorbidity burden may modulate neuroinflammatory responses and impair metabolic clearance trajectories following acute brain injury (2, 11). This interpretation is further supported by the absence of correlations between CSF UA and systemic renal parameters, confirming that CSF-derived biomarkers directly capture central pathophysiological changes rather than reflecting systemic metabolism.[2,12]

Importantly, our findings also suggest that radiological hemorrhage patterns—particularly the presence of intraventricular hemorrhage (IVH)—may play a central role in shaping systemic physiological deterioration and clinical outcomes. Although statistical significance was not consistently achieved, IVH was associated with a markedly increased risk of mortality, suggesting a clinically meaningful effect size that warrants further investigation in larger cohorts.

IVH was consistently linked to markers of systemic metabolic decompensation, including elevated APACHE II scores, secondary hyperglycemia, and electrolyte disturbances. From a pathophysiological perspective, IVH may reflect a more extensive hemorrhagic burden with greater disruption of cerebrospinal fluid dynamics, increased intracranial pressure, and impaired neurovascular regulation. These changes can trigger a cascade of autonomic and systemic responses, contributing to progressive physiological instability.

In this context, the observed metabolic alterations should not be interpreted in isolation but rather as components of a broader, multidimensional phenotype characterized by the interaction between central neurological injury and systemic physiological failure. This integrated framework better captures the complexity of disease progression in aSAH than single-parameter models.

Notably, the strong inverse correlation between baseline CSF UA levels and the magnitude of change further supports the presence of a regulated metabolic response rather than a passive diffusion process. This may reflect a ceiling effect or feedback-regulated clearance mechanism within the intracranial compartment.

From a systems-level perspective, our PCA findings further support the multidimensional nature of disease progression. Neurological severity (GCS Day 5) and systemic physiological burden (APACHE II Day 5) were identified as primary contributors to variance, while metabolic parameters such as CSF UA also contributed to patient stratification. These findings are consistent with emerging literature demonstrating that clustering and multivariate approaches can identify distinct metabolic and physiological states associated with outcomes after acute brain injury.[3,6,16]

From a clinical perspective, the dynamic deterioration in neurological (GCS) and physiological (APACHE II) scores observed in our cohort reinforces the progressive nature of early brain injury after aSAH.[7,9,10] Integrating these clinical trajectories with biochemical markers provides a more comprehensive understanding of disease evolution.

Several limitations should be acknowledged. First, the small sample size and single-center design limit the generalizability of our findings. Second, the high mortality rate reflects a highly selected, high-risk cohort, which may introduce selection bias. Third, the imbalance between outcome groups, particularly the very limited number of patients with favorable outcomes, restricts the ability to draw definitive conclusions regarding prognostic discrimination. Fourth, although we employed advanced analytical techniques such as hierarchical clustering and principal component analysis, these findings remain exploratory and hypothesis-generating in the absence of external validation.[3,6] Finally, the lack of long-term functional outcome measures beyond in-hospital mortality restricts the broader clinical applicability of our results.

Despite these limitations, our study provides novel and clinically relevant insights into the dynamic metabolic landscape of aSAH using a pragmatic and accessible approach. The identification of differential CSF biomarker trajectories and the potential prognostic role of the CSF UA/protein ratio highlight the value of integrating biochemical and clinical data for risk stratification. Future studies with larger, multi-center cohorts are warranted to refine these metabolically informed prognostic models and enhance individualized management strategies in patients with aSAH.[2,14]

Conclusions

Dynamic changes in CSF biomarkers following aSAH reveal distinct patterns of metabolic regulation that reflect underlying pathophysiological heterogeneity. The CSF UA/protein ratio, as a readily available and clinically interpretable parameter, emerges as a clinically accessible marker associated with altered survival dynamics and prolonged disease trajectory, rather than improved clinical outcome. These findings support the integration of simple biochemical markers with clinical parameters to better characterize disease progression in aSAH. Larger prospective studies are needed to validate these exploratory observations and determine their clinical applicability.

Declarations

The authors confirm that this manuscript complies with all Instructions for Authors of Neurocritical Care.

Author Contributions

M.İ., Ö.Y.Ç., and F.Ü. contributed to the concept and design of the study. M.İ., E.Ç., and T.S.A. collected the data. M.İ., Ö.Y.Ç., N.Ü.A., and M.D. performed data analysis and interpretation. Statistical analysis was conducted by Ö.Y.Ç. and M.İ. Ö.Y.Ç. and M.İ. drafted the manuscript. F.Ü., M.D., B.B., and N.Ü.A. critically revised the manuscript. All authors reviewed, approved the final version of the manuscript, and agree to be accountable for all aspects of the work.

This manuscript has not been published previously and is not under consideration for publication elsewhere.

Ethics Approval and Informed Consent

This study was approved by the Clinical Research Ethics Committee of Ondokuz Mayıs University Faculty of Medicine (Approval No: 2023/267; Date: September 2023) and conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all patients or their legal representatives prior to inclusion.

Clinical trial number: Not applicable.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Reporting Guideline

This study was prepared in accordance with the STROBE reporting guideline for observational cohort studies.

Funding

This study was supported by the Scientific Research Projects Coordination Unit of Ondokuz Mayıs University (Project No: BAP01-2024-4955). The grant recipient was Prof. Dr. Melda Dilek.

Artificial Intelligence (AI)–Assisted Technology

The authors used ChatGPT (OpenAI) for language editing, grammatical refinement, and organizational support during manuscript preparation. All scientific content, interpretations, and final revisions were critically reviewed and approved by the authors.

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Figures

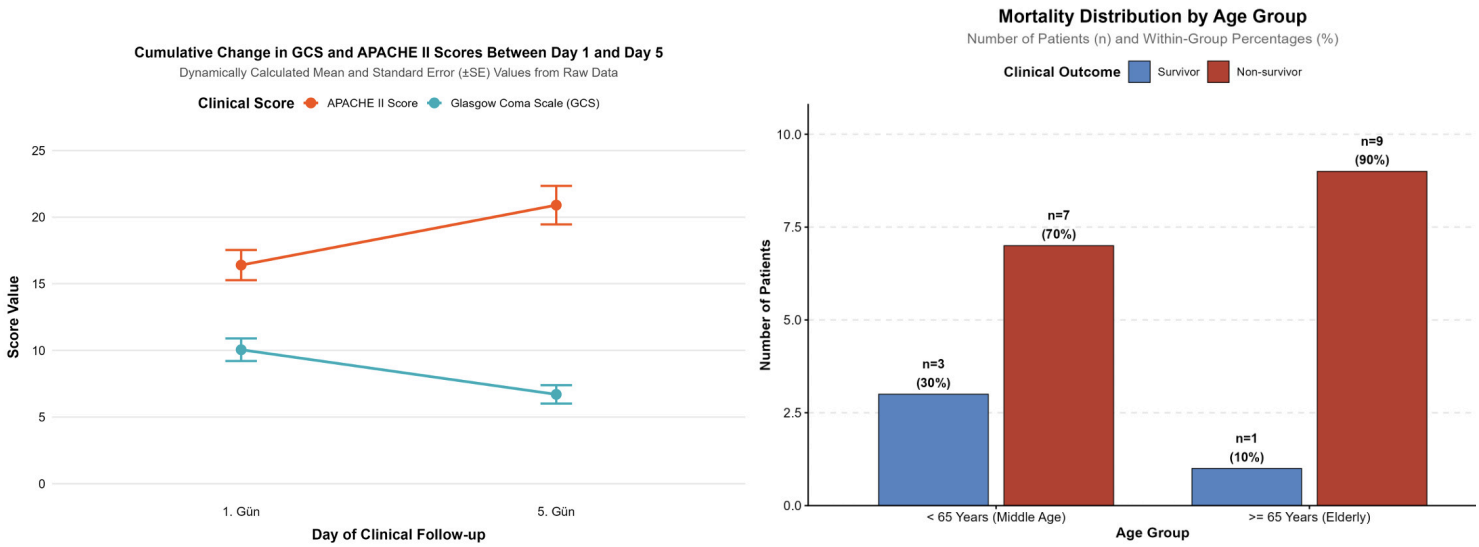


Figure 1

Temporal Changes in Neurological and Disease Severity Scores and Their Association with Mortality Across Age Groups. **(Left panel)** Changes in APACHE II and Glasgow Coma Scale (GCS) scores between Day 1 and Day 5. **(Right panel)** Distribution of clinical outcomes according to age groups.
Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; **GCS,** Glasgow Coma Scale; **SE,** standard error.

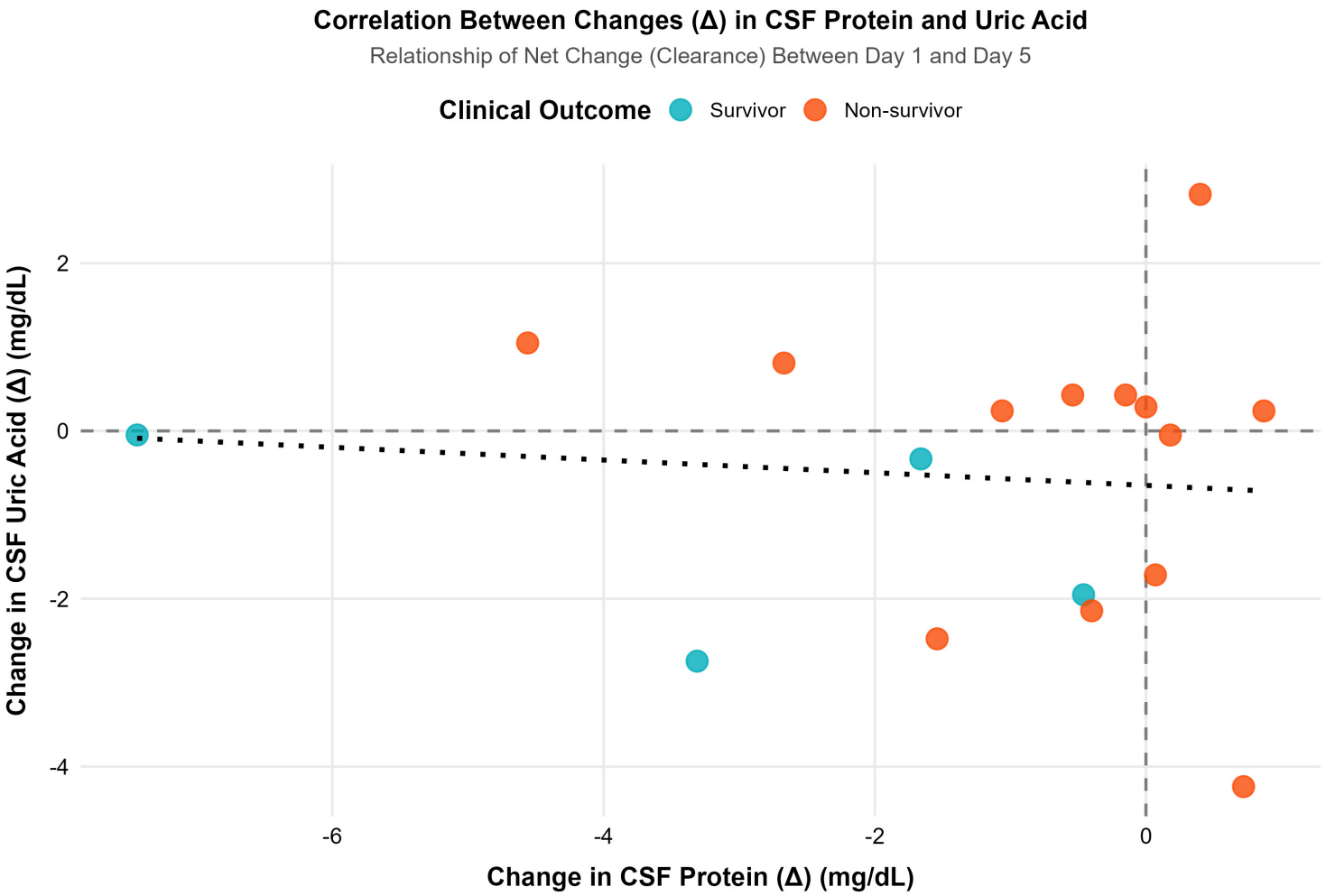


Figure 2

Correlation Between Changes in CSF Protein and Uric Acid. Scatter plot showing the relationship between ΔCSF protein and ΔCSF uric acid between Day 1 and Day 5.
Abbreviations: CSF, cerebrospinal fluid; **Δ,** change.

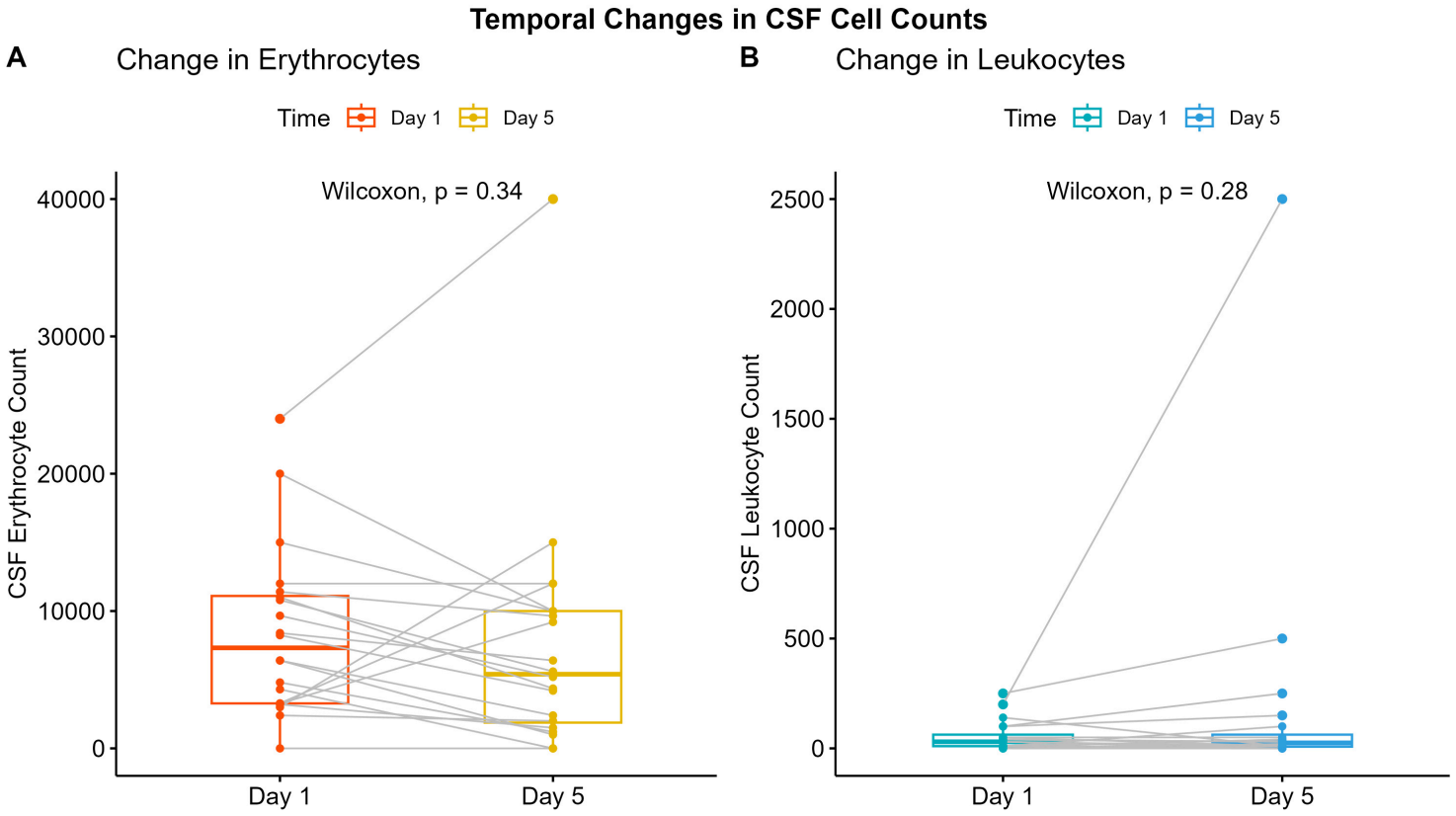


Figure 3

Temporal Changes in CSF Cellular Components. **(A)** CSF erythrocyte counts and **(B)** CSF leukocyte counts between Day 1 and Day 5.

Abbreviations: CSF, cerebrospinal fluid.

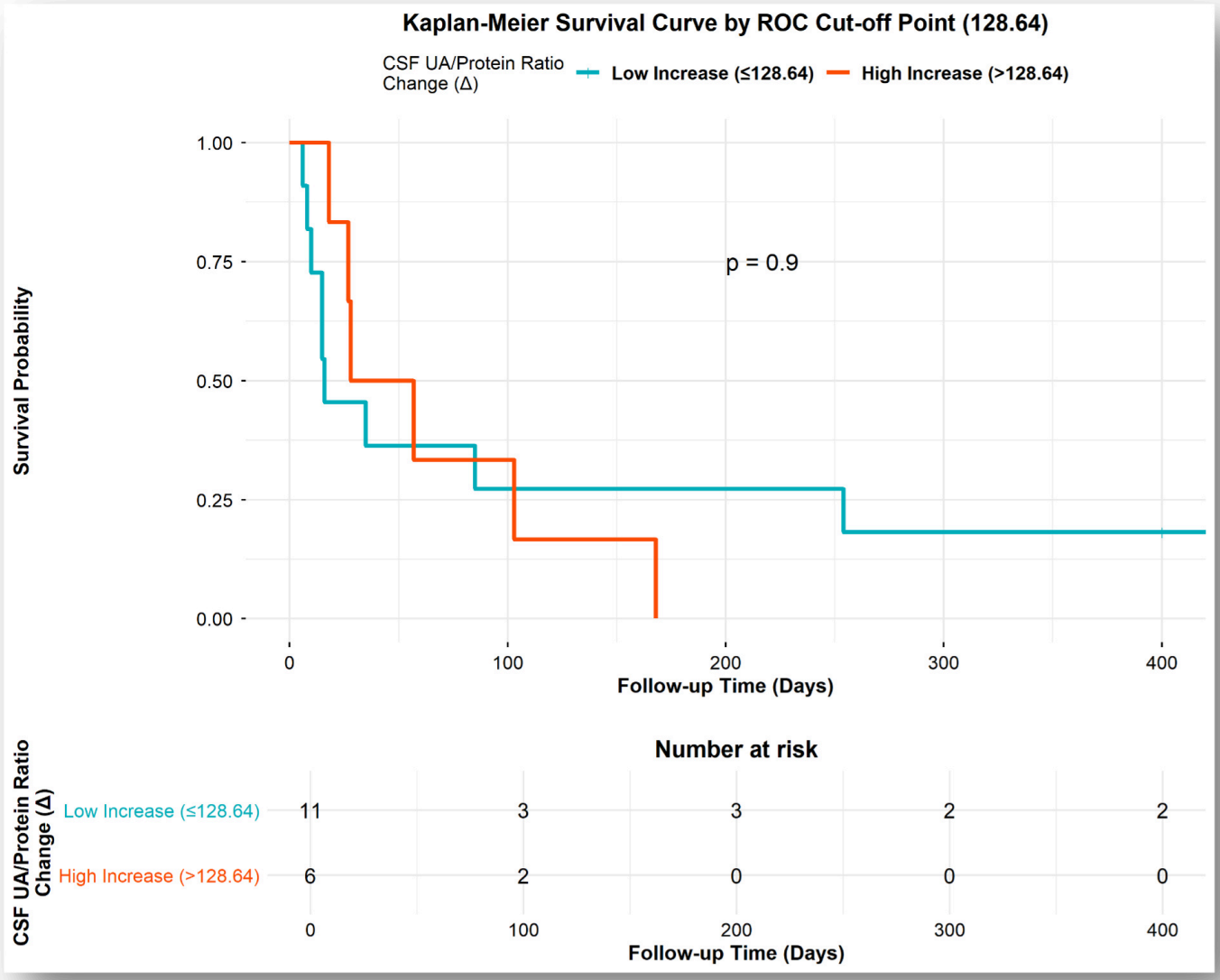


Figure 4

Kaplan–Meier Survival Curves According to ROC-Derived Cut-off of CSF UA/Protein Ratio Change (Δ).
Abbreviations: CSF, cerebrospinal fluid; **UA,** uric acid; **ROC,** receiver operating characteristic; **Δ,** change.

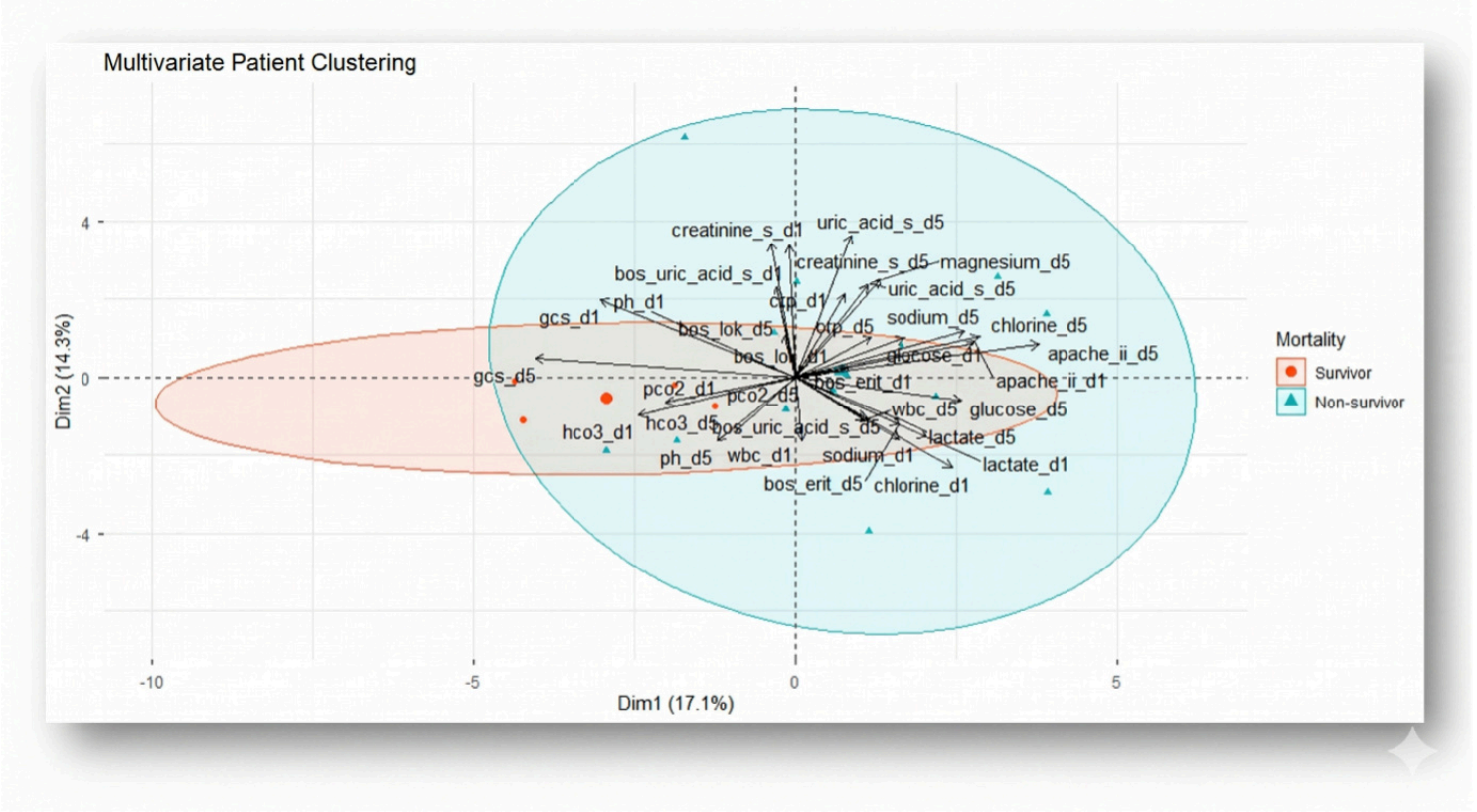


Figure 5

Principal Component Analysis (PCA) Biplot of Clinical and Biochemical Variables According to Mortality Status.

Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; **GCS,** Glasgow Coma Scale; **PCA,** principal component analysis.

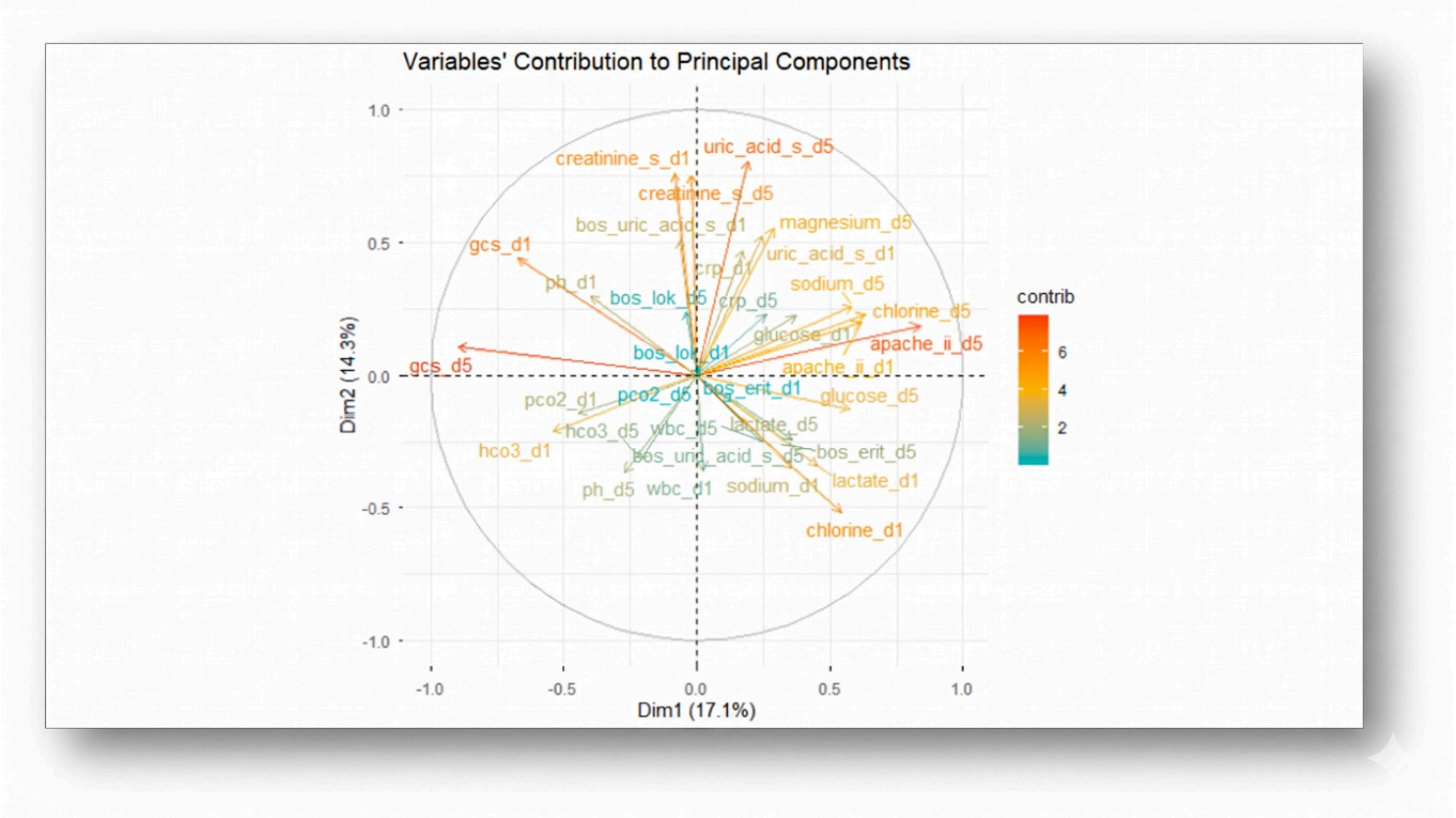


Figure 6

Variable Contributions to Principal Components Based on Principal Component Analysis (PCA). **Abbreviations: APACHE II,** Acute Physiology and Chronic Health Evaluation II; **GCS,** Glasgow Coma Scale; **PCA,** principal component analysis.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTableS1.docx