Subphenotyping Aneurysmal Subarachnoid Hemorrhage Using Clinical and Biological Data Clustering

Manel Santafé[1,2], Manuel Quintana[4,5], Anna Sánchez[3], Rosa-Maria Gràcia[3], Daniel Campos-Fernandez[4,5], Laura Abraira[4,5,], Estevo Santamarina[4,5]

[1] Intensive Care Department, Hospital Universitari Parc Taulí, I3PT, Universitat Autònoma de Barcelona, Barcelona, Spain

[2] Departament de Medicina, Universitat Autònoma de Barcelona

[3] Intensive Care Department, Hospital Universitari Vall d'Hebron, Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona, Barcelona, Spain

[4] Epilepsy Unit, Neurology Department, Hospital Universitari Vall d'Hebron, Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona, Barcelona, Spain

[5]Research Group on Status Epilepticus and Acute Seizures, Vall d'Hebron Research Institute (VHIR), Vall d'Hebron University Hospital, Barcelona, Spain

Word Count: *2554*

Figures: *4*

Tables: *2*

References*: 29*

MeSH terms: *Subarachnoid Hemorrhage, Cerebral Ischemia, Biomarkers, Proteomics, Neuroinflammation, Intensive Care Units*

Abstract

Background:

Aneurysmal subarachnoid hemorrhage (aSAH) is an heterogeneous disease with variable outcomes, even among patients with similar clinical and radiological severity. Additional research is needed to better stratify aSAH patients.

Objectives:

To identify distinct clinical subphenotypes of aSAH, we applied clustering analysis using clinical, radiological, and laboratory data.

Methods:

We conducted a retrospective cohort study of adult patients with aSAH admitted to the ICU between 2010 and 2021. K-means clustering was applied to standardized demographic, clinical, and laboratory variables collected at admission. Principal component analysis was used for dimensionality reduction and visualization. Additionally, we analyzed whether these clusters were associated with serum biomarkers (S100B, HMGB1, and TLR4) in a subset of patients.

Results:

The study included 511 patients with aSAH. Two distinct subphenotypes were identified: a High-Risk Cluster (n=301, 58.9%) characterized by severe systemic complications, and higher mortality, and a Low-Risk Cluster (n=210, 41.1%) with less severe symptoms and better outcomes. Serum S100B levels were significantly elevated in the High-Risk Cluster (0.077 [0.056–0.179] vs. 0.055

[0.040–0.079] µg/L, p=0.008) and showed moderate discriminatory power (AUC=0.72).

Conclusions:

Clustering analysis revealed two aSAH subphenotypes associated with DCI, mortality and functional. Integrating early clinical and biomarker data could enhance patient stratification.

INTRODUCTION

Spontaneous Subarachnoid Hemorrhage is a neurological emergency characterized by nontraumatic bleeding into the subarachnoid space. In approximately 80% of cases, SAH is caused by the rupture of an arterial aneurysm (aSAH) (1). Although the incidence of aSAH is relatively low—around 6 cases per 100,000 person-years—it carries a high pre-hospital mortality rate ranging from 12% to 26%, and a substantial burden of morbidity among survivors.

Up to 46% of patients who survive the initial hemorrhagic event suffer long-term cognitive impairment, significantly affecting functional independence and quality of life (1-3). The clinical course of aSAH is shaped by a combination of early brain injury, systemic complications (e.g., neurogenic cardiomyopathy, acute pulmonary edema), and late secondary neurological events such as delayed cerebral ischemia (DCI)(4-8). DCI has multiple contributory factors including vasospasm, microthrombosis, cortical spreading depolarizations, and neuroinflammation (9-13). These different pathways contribute to the marked heterogeneity of the disease as a result, patients with comparable radiological findings may experience a very different prognosis(14-17).

In addition to traditional clinical and radiological markers, recent studies have identified serum biomarkers related to inflammation and blood-brain barrier disruption as potential contributors to neurological outcomes after aSAH(18, 19). The investigation of these biomarkers in aSAH may provide additional biological characterization of clinical subphenotypes and contribute to understanding the pathophysiology of DCI.

Given the substantial heterogeneity among patients with SAH and the wide variability in clinical outcomes—even among individuals with similar radiological severity—we conducted a clustering analysis to identify distinct subphenotypes of aSAH, using data-driven clustering, and to explore their association with clinical outcomes and serum biomarkers of brain injury and inflammation.

METHODS

Patients

We conducted a retrospective, longitudinal study of adults with a confirmed diagnosis of aSAH admitted to the ICU between January 2010 and December 2021.

For the k-means clustering analysis, the variables collected included; demographics and comorbidities: age, hypertension, diabetes, dyslipidemia, and migraine history; clinical presentation and severity: presence of headache at onset, seizure at onset, cranial nerve palsy, pupillary abnormalities (anisocoria, mydriasis), and cardiac arrest at ictus; radiological severity: modified Fisher scale; vital signs (heart rate, systolic blood pressure, respiratory rate, SpO2, body temperature); cardiac involvement: electrocardiographic changes (ST-segment, T-wave abnormalities), angina; laboratory parameters: hemoglobin, leukocyte count, neutrophil count, blood glucose, and sodium levels; initial hemorrhage characteristics: presence of intracerebral, subdural, or intraventricular hemorrhage on initial imaging and treatment strategy (surgical clipping or endovascular treatment).

These variables were chosen to comprehensively characterize the initial clinical profile, encompassing the spectrum of neurological compromise, systemic

physiological derangement, and bleeding patterns that define early disease heterogeneity in aSAH. Variables not available at admission (e.g., delayed complications) or with >20% missing data were excluded from the clustering analysis.

For subsequent outcome analysis and cohort characterization, we also collected data on treatment strategy, SAH-related complications (e.g., rebleeding, vasospasm, delayed cerebral ischemia [DCI]), the occurrence of seizures during hospitalization, and functional status assessed using the modified Rankin Scale (mRS).

All adult patients (≥18 years) with a diagnosis of subarachnoid hemorrhage (20) and a confirmed aneurysmal etiology were included. The aneurysmal etiology was established by contrast-enhanced brain CT, MRI, or conventional angiography. We excluded patients who did not undergo vascular evaluation because of futility of care (e.g. signs of brain death) or a follow-up <3 days (death, transfer to other facility, etc).

DCI was defined as the occurrence of a new focal neurological deficit or a decrease of ≥2 points in the Glasgow Coma Scale (GCS) lasting at least one hour, after carefully excluding other causes such as rebleeding, hydrocephalus, seizures, fever, or metabolic disturbances. In comatose patients, DCI was defined as the appearance of a new cerebral infarction on follow-up neuroimaging not attributable to the initial hemorrhage or confirmed at autopsy (21). Given the retrospective design of the study, the entire cohort was systematically reviewed post hoc to verify the accuracy of DCI diagnoses and to identify possible cases that might have been misclassified or overlooked at the time of initial assessment.

All clinical scores were obtained from the medical records; if not explicitly documented, they were inferred from the neurological examination at admission.

Cerebral vasospasm was defined as a narrowing of the cerebral arteries identified by digital subtraction angiography or CT angiography (CTA).

Cardiac involvement was defined as elevated troponin T, the presence of new or reversible segmental wall motion abnormalities, or new global dysfunction on echocardiography.

Biomarker Analysis

For this study, serum samples were available from a subset of 80 patients from the main cohort. These samples had been prospectively collected at ICU admission as part of a previous research project conducted by our group, aimed at investigating inflammatory and neuroinjury biomarkers after aneurysmal subarachnoid hemorrhage. The biospecimens were stored under standardized conditions at −80°C and were subsequently used for the present analysis.

We selected high mobility group box 1 (HMGB1), toll-like receptor 4 (TLR-4), and S100 calcium-binding protein B (S100B) for the analysis because they represent complementary pathways involved in neuroinflammation, blood–brain barrier disruption, and glial injury, all of which are central mechanisms in early brain injury and delayed cerebral ischemia (DCI) after aSAH (22-24). Together, these markers capture complementary aspects of the neuroinflammatory axis that links early brain injury with subsequent ischemic complications. Their combined evaluation thus provides a biologically coherent panel for exploring pathophysiological subphenotypes of aSAH.

Biomarker levels were obtained from blood samples collected within the first 24 hours of admission and analyzed using commercially available ELISA kits according to manufacturer instructions.

Baseline demographic and clinical characteristics of this biomarker subset were comparable to those of the overall cohort, minimizing selection bias.

Statistical analysis

Statistical analysis was conducted using R software (version 4.4.1) and the software IBM SPSS Statistics version 27.0.

Cluster analysis by K-means was used including demographic, clinical variables and others factors and scales collected in ICU admission. For this purpose, quantitative and categorical variables were normalized according to James D. McCaffrey's approach (25).

For the cluster analysis, we evaluated first the optimal number of clusters with the use of the function fviz_nbclust from "factoextra" R package, checking two methods of clusters selection: the average silhouette width and the elbow method (based in the total within sum of square). Thus, clustering with kmeans function was performed and principal component analysis was done to reduce the dimensionality of data, in order to show a plot of the resulting clusters with individuals and variables in a same figure (fviz_pca_biplot function).

After cluster attribution, patients were compared for clinical characteristics and outcomes. Continuous variables were expressed as mean (standard deviation) and compared with Student's t test, clinical scales were reported as median (inter-quartile range) and compared with Wilcoxon signed rank test, and categorical variables as frequency (percentage) and compared with Pearson's chi square.

Stacked box-plots were performed to show some associations between clusters and outcomes.

For the biomarker subanalysis, the relationship between the identified clusters and biomarker levels was assessed using the Mann-Whitney U test. A receiver operating characteristic (26) curve was performed to evaluate the discriminatory power of the most representative biomarker (S100B) in classifying patients. The optimal cutoff point for patient stratification into high- or low-risk groups was determined using the Youden index.

A p-value < 0.05 was considered as statistically significant.

Ethics Approval

This study was approved by the Institutional Review Board of our institution (reference PR(AG)72/2022). For the blood-analysis sub-study, separate approval was granted under reference PR(AG)212/2017. Informed consent was waived for the retrospective component, whereas written informed consent was obtained from all patients or their legal representatives participating in the prospective biomarker study prior to enrollment. The study was conducted in accordance with the Declaration of Helsinki and applicable local regulations.

RESULTS

Demographics and Clinical Characteristics

From an initial database of 743 SAH patients, 511 were included in the final analysis after applying the predefined exclusion criteria. The mean age was 56.9 years (SD ± 14.0), and 65.8% were male. Baseline characteristics are summarized in **Table 1.**

Cluster analysis

The optimal number of clusters was determined using two methods: average silhouette width and the elbow method, both supporting the selection of two clusters.

A principal component analysis was performed to represent the results in two dimensions. The clustering revealed two groups (**Figure 1**): Cluster 1, comprising 310 patients (58,9%), and Cluster 2, comprising 210 patients (41,1%).

Two distinct clinical profiles emerged from the clustering analysis (see **Table 2**). The High-Risk Cluster was defined by greater initial severity, higher rates of systemic and neurological complications, and more pronounced laboratory abnormalities. In contrast, the Low-Risk Cluster presented with milder neurological impairment, more frequent headache at onset, and a higher prevalence of prior migraine. These divergent profiles translated into clear prognostic differences, with the High-Risk Cluster showing a higher incidence of DCI, worse functional outcomes and higher mortality at both discharge and 3 months.

Figure 2 illustrates the distribution of functional outcomes at 3 months according to cluster. Patients in the High-Risk Cluster showed markedly poorer prognosis, with nearly 70% presenting a mRS >2, compared with only about 20% of patients in the Low-Risk Cluster.

Even though cerebral vasospasm was not associated with the high-risk cluster (vasospasm: 20.5% vs. 29.6%, $p = 0.021$), delayed cerebral ischemia (DCI) was significantly associated with the high-risk cluster (DCI: 17.1% vs. 28.9%, $p = 0.002$).

Severity scales were not included in the clustering model; however, post-hoc comparisons showed that the High-Risk cluster corresponded to patients with lower GCS scores (12 [6–14] vs. 15 [15–15], $p < 0.001$) and higher WFNS (4 [2–5] vs. 1 [1–2], $p < 0.001$) and Hunt–Hess grades (4 [4–4] vs. 2 [1–2], $p < 0.001$) compared with the Low-Risk cluster.

Biomarker subanalysis

In the subgroup of 80 patients (47 in the High-Risk Cluster and 33 in the Low-Risk Cluster), we analyzed three candidate biomarkers associated with brain injury and neuroinflammation. HMGB1 and TLR-4 showed no significant differences between clusters [HMGB1: 1041 (781–1516) vs. 1045 (829–1349), p=0.868; TLR-4: 0.64 (0.59–1.22) vs. 0.88 (0.57–1.27), p=0.56, respectively]. However, S100B levels were significantly elevated in the High-Risk Cluster [0.077 (0.056–0.179) vs. 0.055 (0.040–0.079), p=0.008] (**Figure 3**).

ROC analysis of S100B (**Figure 4**) yielded an area under the curve (AUC) of 72.4% (95% CI: 60.6%–84.2%) for distinguishing High-Risk from Low-Risk clusters. The optimal cutoff value was 0.063 µg/L, achieving a negative predictive value (NPV) of 63.6% and a positive predictive value (PPV) of 78.6% for Low-Risk and High-Risk cluster assignment, respectively.

DISCUSSION

In this study, we applied an automated clustering approach to patients with aSAH to identify distinct clinical phenotypes based on demographic, clinical, radiological, and biochemical characteristics at admission. Our findings suggest that early phenotypic stratification can effectively distinguish patients with markedly different clinical courses, complication profiles, and outcomes.

We did not include neurological severity scales (GCS, WFNS, or Hunt–Hess) in the clustering analysis. This approach aimed to capture multidimensional physiological patterns without the potential circularity introduced by predefined severity grading. Instead, we included variables that comprehensively describe the initial clinical presentation, systemic physiological derangement, and bleeding characteristics that define early disease heterogeneity in aSAH.

The High-Risk Cluster showed marked systemic derangement and the poorest outcomes, with higher rates of delayed cerebral ischemia, cardiac involvement, seizures, and intracerebral or intraventricular hemorrhage. Laboratory findings reflected metabolic and inflammatory stress (hyperglycemia, leukocytosis, and neutrophilia). Although severity scales were not included in the model, post-hoc comparisons confirmed that this cluster corresponded to patients with lower GCS and higher WFNS and Hunt–Hess grades, validating its clinical coherenceAs vasospasm is a delayed complication, it was not included among the clustering variables; nonetheless, future studies could apply this subphenotyping framework to assess whether early profiles predict its occurrence or outcomes, as recently explored in endovascular series (27).

These findings are consistent with previous literature that connects early systemic inflammatory responses and neurocardiogenic injury to worse neurological outcomes in aSAH patients (4-7).

While this cluster overlaps with established severity patterns, our data-driven approach objectively integrates neurological, systemic, and inflammatory dimensions into a unified high-risk phenotype. The strong association of the High-Risk phenotype with delayed cerebral ischemia supports the idea that these clusters reflect early biological vulnerability rather than only initial neurological

impairment. Furthermore, functional outcomes and mortality were significantly worse in this group, both at discharge and at 3 months. This supports the notion that clinical and systemic severity at admission is a strong predictor of long-term prognosis, as shown in earlier studies (6, 16, 28).

On the other hand, the Low-Risk Cluster was characterized by milder presentation, preserved consciousness, and better systemic parameters, along with a higher prevalence of prodromal symptoms such as headache or migraine history. These features may indicate earlier recognition of symptoms or a less aggressive initial hemorrhagic event. However, patients in the High-Risk group may have been unable to report headache because of reduced consciousness, potentially inflating this difference. Even though this limitation must be acknowledged, previous studies have described a similar association between headache and more favorable outcomes (29), suggesting that headache at onset may identify a subgroup of patients with inherently better prognosis.

Levels of S100B were significantly higher in the High-Risk Cluster and showed good discriminatory capacity (AUC 72.4%), reinforcing the biological divergence between these phenotypes. Previous studies in acute stroke and aSAH have shown that S100B correlates with final infarct volume and functional outcome, but its delayed kinetics mean that a single measurement may not capture the full evolution of brain injury (30, 31). A limitation of our study is that S100B was measured only within the first 24 hours; Serial sampling could improve its prognostic precision. While not routinely used in aSAH management, our results support its potential for early risk stratification, pending external validation.

Emerging studies suggest that circulating and cerebrospinal microRNAs may serve as prognostic biomarkers in aneurysmal aSAH (32). While these results

are still preliminary, future multi-omic approaches incorporating microRNA profiling could further refine data-driven subphenotyping by capturing molecular dimensions of inflammation and vascular injury.

Our study has some limitations. It was conducted at a single center, and some laboratory values were incomplete. Advanced neuroimaging data were not available, which could further enhance clustering resolution, and follow-up beyond three months might better characterize recovery trajectories. Nevertheless, our findings establish a reproducible and clinically relevant foundation for phenotyping patients with aSAH.

Clustering techniques enabled us to uncover patterns not evident through conventional univariate or multivariate analyses, highlighting the heterogeneity of aSAH and supporting a move toward individualized risk stratification.

Future studies with external validation cohorts and prospective designs are needed to confirm the reproducibility of these clusters. In addition to incorporating dynamic information such as serial biomarkers and continuous physiological monitoring, further work could integrate complementary omic layers—including transcriptomic, microRNA, and genetic profiles—to capture the biological pathways underlying early brain injury and delayed complications(33-35), Advanced imaging modalities, such as perfusion CT and vessel-wall MRI, may also refine phenotyping by quantifying microvascular dysfunction and secondary ischemia(36, 37), while adding more accurate imaging and biomarker profiles could better distinguish the different subphenotypes.

Beyond cluster identification, developing predictive models through logistic regression or simplified scoring systems could allow bedside estimation of

subphenotype probability at admission, similar to approaches recently applied in other inflammatory critical illnesses such as ARDS and sepsis (26, 38). These tools would facilitate early recognition of high-risk profiles and support the design of phenotype-guided interventions aimed at improving outcomes. Finally, prospective interventional studies could determine whether early identification of high-risk phenotypes enables personalized management and improved outcomes.

In conclusion, our study presents a novel, data-driven classification of aSAH patients using early-phase clinical and biochemical data. The High-Risk Cluster was consistently associated with worse outcomes and higher S100B levels, suggesting both clinical and biological coherence. This work provides a framework for personalized care and paves the way for biomarker-integrated prognostic models in acute aSAH.

BIBLIOGRAPHY

1. Hoh BL, Ko NU, Amin-Hanjani S, Chou S-Y, Cruz-Flores S, Dangayach NS, et al. 2023 Guideline for the Management of Patients With Aneurysmal Subarachnoid Hemorrhage: A Guideline From the American Heart Association/American Stroke Association. Stroke. 2023;54(7):e314-e70.

2. Treggiari MM, Rabinstein AA, Busl KM, Caylor MM, Citerio G, Deem S, et al. Guidelines for the Neurocritical Care Management of Aneurysmal Subarachnoid Hemorrhage. Neurocritical care. 2023;39(1):1-28.

3. Mayer SA, Kreiter KT, Copeland D, Bernardini GL, Bates JE, Peery S, et al. Global and domain-specific cognitive impairment and outcome after subarachnoid hemorrhage. Neurology. 2002;59(11):1750-8.

4. Zaroff JG, Leong J, Kim H, Young WL, Cullen SP, Rao VA, et al. Cardiovascular predictors of long-term outcomes after non-traumatic subarachnoid hemorrhage. Neurocritical care. 2012;17(3):374-81.

5. van der Bilt I, Hasan D, van den Brink R, Cramer MJ, van der Jagt M, van Kooten F, et al. Cardiac dysfunction after aneurysmal subarachnoid hemorrhage: relationship with outcome. Neurology. 2014;82(4):351-8.

6. Richard C. Stress-related cardiomyopathies. Ann Intensive Care. 2011;1(1):39.

7. Lee VH, Oh JK, Mulvagh SL, Wijdicks EF. Mechanisms in neurogenic stress cardiomyopathy after aneurysmal subarachnoid hemorrhage. Neurocritical care. 2006;5(3):243-9.

8. Friedman JA, Pichelmann MA, Piepgras DG, McIver JI, Toussaint LG, 3rd, McClelland RL, et al. Pulmonary complications of aneurysmal subarachnoid hemorrhage. Neurosurgery. 2003;52(5):1025-31; discussion 31-2.

9. Wenneberg SB, Block L, Sörbo A, Naredi S, Oras J, Hendén PL, et al. Long-term outcomes after aneurysmal subarachnoid hemorrhage: A prospective observational cohort study. Acta neurologica Scandinavica. 2022;146(5):525-36.

10. Stienen MN, Smoll NR, Weisshaupt R, Fandino J, Hildebrandt G, Studerus-Germann A, et al. Delayed cerebral ischemia predicts neurocognitive impairment following aneurysmal subarachnoid hemorrhage. World neurosurgery. 2014;82(5):e599-605.

11. Duan W, Pan Y, Wang C, Wang Y, Zhao X, Wang Y, et al. Risk Factors and Clinical Impact of Delayed Cerebral Ischemia after Aneurysmal Subarachnoid Hemorrhage: Analysis from the China National Stroke Registry. Neuroepidemiology. 2018;50(3-4):128-36.

12. Brown RJ, Kumar A, Dhar R, Sampson TR, Diringer MN. The relationship between delayed infarcts and angiographic vasospasm after aneurysmal subarachnoid hemorrhage. Neurosurgery. 2013;72(5):702-7; discussion 7-8.

13. Vergouwen MD. Vasospasm versus delayed cerebral ischemia as an outcome event in clinical trials and observational studies. Neurocritical care. 2011;15(2):308-11.

14. Dreier JP, Major S, Manning A, Woitzik J, Drenckhahn C, Steinbrink J, et al. Cortical spreading ischaemia is a novel process involved in ischaemic damage in patients with aneurysmal subarachnoid haemorrhage. Brain : a journal of neurology. 2009;132(Pt 7):1866-81.

15. Macdonald RL, Schweizer TA. Spontaneous subarachnoid haemorrhage. Lancet (London, England). 2017;389(10069):655-66.

16. Budohoski KP, Guilfoyle M, Helmy A, Huuskonen T, Czosnyka M, Kirollos R, et al. The pathophysiology and treatment of delayed cerebral ischaemia following subarachnoid haemorrhage. Journal of neurology, neurosurgery, and psychiatry. 2014;85(12):1343-53.

17. Rabinstein AA, Weigand S, Atkinson JL, Wijdicks EF. Patterns of cerebral infarction in aneurysmal subarachnoid hemorrhage. Stroke. 2005;36(5):992-7.

18. Kedziora J, Burzynska M, Gozdzik W, Kübler A, Uryga A, Kasprowicz M, et al. Brain-Specific Biomarkers as Mortality Predictors after Aneurysmal Subarachnoid Haemorrhage. Journal of clinical medicine. 2020;9(12).

19. Miller BA, Turan N, Chau M, Pradilla G. Inflammation, vasospasm, and brain injury after subarachnoid hemorrhage. BioMed research international. 2014;2014:384342.

20. Chesnut R, Aguilera S, Buki A, Bulger E, Citerio G, Cooper DJ, et al. A management algorithm for adult patients with both brain oxygen and intracranial pressure monitoring: the Seattle International Severe Traumatic Brain Injury Consensus Conference (SIBICC). Intensive care medicine. 2020;46(5):919-29.

21. Hijdra A, Van Gijn J, Stefanko S, Van Dongen KJ, Vermeulen M, Van Crevel H. Delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage: clinicoanatomic correlations. Neurology. 1986;36(3):329-33.

22. Uryga A, Czyż M, Adamik B, Tabakow P, Kasprowicz M, Burzyńska M. Serum biomarkers and cerebral autoregulation as early warnings of delayed cerebral ischemia risk in patients after aneurysmal subarachnoid haemorrhage. Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia. 2021;87:35-43.

23. Paudel YN, Angelopoulou E, Piperi C, Othman I, Shaikh MF. HMGB1-Mediated Neuroinflammatory Responses in Brain Injuries: Potential Mechanisms and Therapeutic Opportunities. International journal of molecular sciences. 2020;21(13).

24. Jabbarli R, Pierscianek D, Darkwah Oppong M, Sato T, Dammann P, Wrede KH, et al. Laboratory biomarkers of delayed cerebral ischemia after subarachnoid hemorrhage: a systematic review. Neurosurgical review. 2020;43(3):825-33.

25. McCaffrey JD. Clustering Mixed Categorical and Numeric Data Using k-Means with C#. Visual Studio Magazine. 2024 May 2024.

26. Blot PL, Chousterman BG, Santafè M, Cartailler J, Pacheco A, Magret M, et al. Subphenotypes in patients with acute respiratory distress syndrome treated with high-flow oxygen. Critical care (London, England). 2023;27(1):419.

27. McGrath K, Hey G, MacNeil A, Wahbeh T, Lucke-Wold B, Chowdhury MAB, et al. Functional outcomes following endovascular treatment of vasospasm secondary to aneurysmal subarachnoid Hemorrhage: A Single center retrospective analysis. Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia. 2025;135:111205.

28. Foreman B. The Pathophysiology of Delayed Cerebral Ischemia. Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society. 2016;33(3):174-82.

29. Lin CM, Wang AY, Chen CC, Wu YM, Liu CH, Tsay PK, et al. Warning headache correlates survival rate in aneurysmal subarachnoid hemorrhage. Biomedical journal. 2019;42(5):352-7.

30. Kapapa T, Pfnür A, Halbgebauer R, Broer P, Halbgebauer S, Tumani H, et al. Biomarkers in Aneurysmatic and Spontaneous Subarachnoid Haemorrhage:

A Clinical Prospective Multicentre Biomarker Panel Study of S100B, Claudin-5, Interleukin-10, TREM-1, TREM-2 and Neurofilament Light Chain As Well As Immunoglobulin G and M. Molecular neurobiology. 2025;62(9):11499-516.

31. Dassan P, Keir G, Brown MM. Criteria for a clinically informative serum biomarker in acute ischaemic stroke: a review of S100B. Cerebrovascular diseases (Basel, Switzerland). 2009;27(3):295-302.

32. Segherlou ZH, Saldarriaga L, Azizi E, Vo KA, Reddy R, Siyanaki MRH, et al. MicroRNAs' Role in Diagnosis and Treatment of Subarachnoid Hemorrhage. Diseases (Basel, Switzerland). 2023;11(2).

33. Khurana VG, Sohni YR, Mangrum WI, McClelland RL, O'Kane DJ, Meyer FB, et al. Endothelial nitric oxide synthase gene polymorphisms predict susceptibility to aneurysmal subarachnoid hemorrhage and cerebral vasospasm. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2004;24(3):291-7.

34. Solodovnikova Y, Ivaniuk A, Marusich T, Son A. Meta-analysis of associations of genetic polymorphisms with cerebral vasospasm and delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. Acta neurologica Belgica. 2022;122(6):1547-56.

35. Ahn SH, Savarraj JPJ, Parsha K, Hergenroeder GW, Chang TR, Kim DH, et al. Inflammation in delayed ischemia and functional outcomes after subarachnoid hemorrhage. Journal of neuroinflammation. 2019;16(1):213.

36. Cremers CH, van der Schaaf IC, Wensink E, Greving JP, Rinkel GJ, Velthuis BK, et al. CT perfusion and delayed cerebral ischemia in aneurysmal subarachnoid hemorrhage: a systematic review and meta-analysis. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2014;34(2):200-7.

37. Aksakal M, Chang D, Kharaji M, Watt C, Levitt MR, Hippe DS, et al. Predicting Vasospasm and Delayed Cerebral Ischemia in Aneurysmal Subarachnoid Hemorrhage: The Role of Vessel Wall MRI. AJNR American journal of neuroradiology. 2025.

38. Seymour CW, Kennedy JN, Wang S, Chang CH, Elliott CF, Xu Z, et al. Derivation, Validation, and Potential Treatment Implications of Novel Clinical Phenotypes for Sepsis. Jama. 2019;321(20):2003-17.

Figure Legends

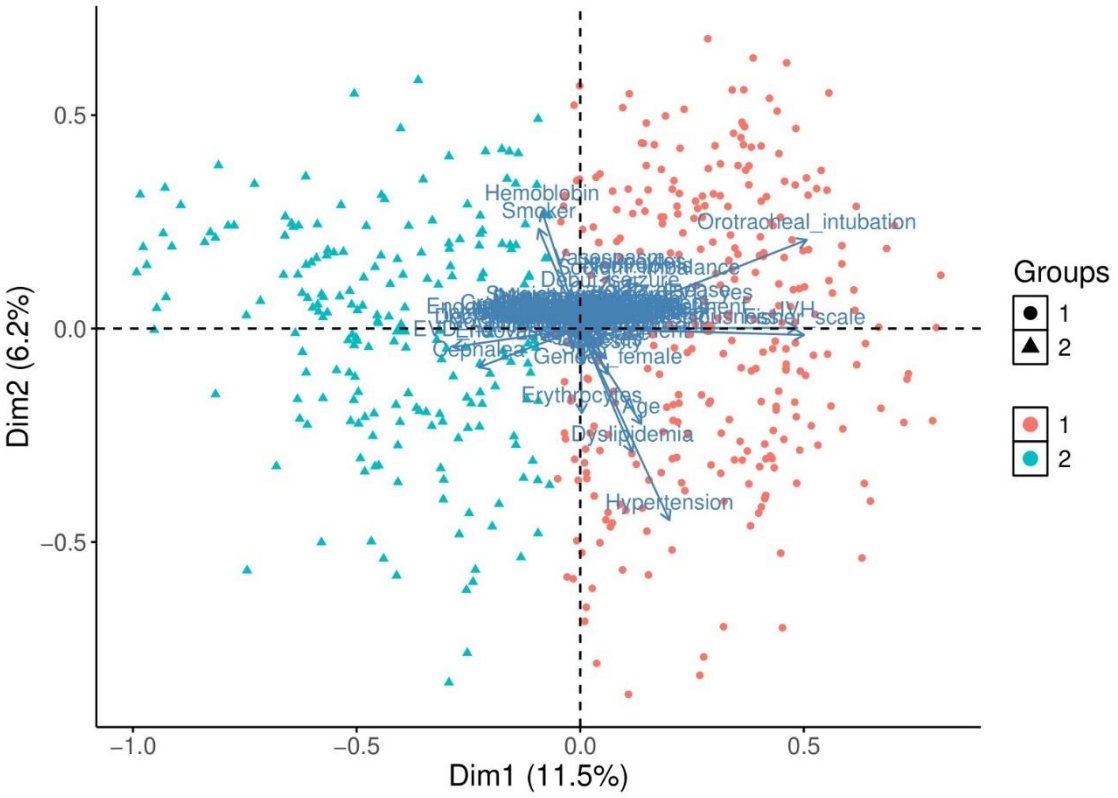

Figure 1. Clustering analysis. The plot shows the distribution of 511 patients with spontaneous subarachnoid hemorrhage according to k-means clustering based on clinical, radiological, and laboratory variables. Principal component analysis (PCA) was used to reduce dimensionality and visualize the clustering results. Cluster 1 (red dots, circles) and Cluster 2 (blue triangles) represent distinct clinical subphenotypes. The blue arrows (loadings) indicate the direction and strength of the contribution of the original clinical variables to the principal components.

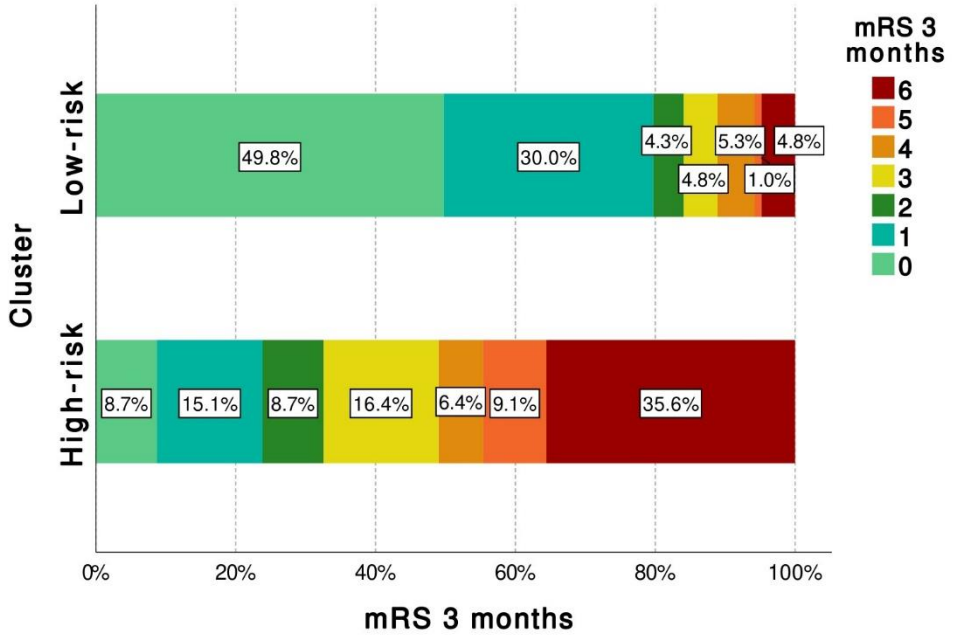

Figure 2. Modified Rankin Scale (mRS) low-Risk and high-Risk Cluster.

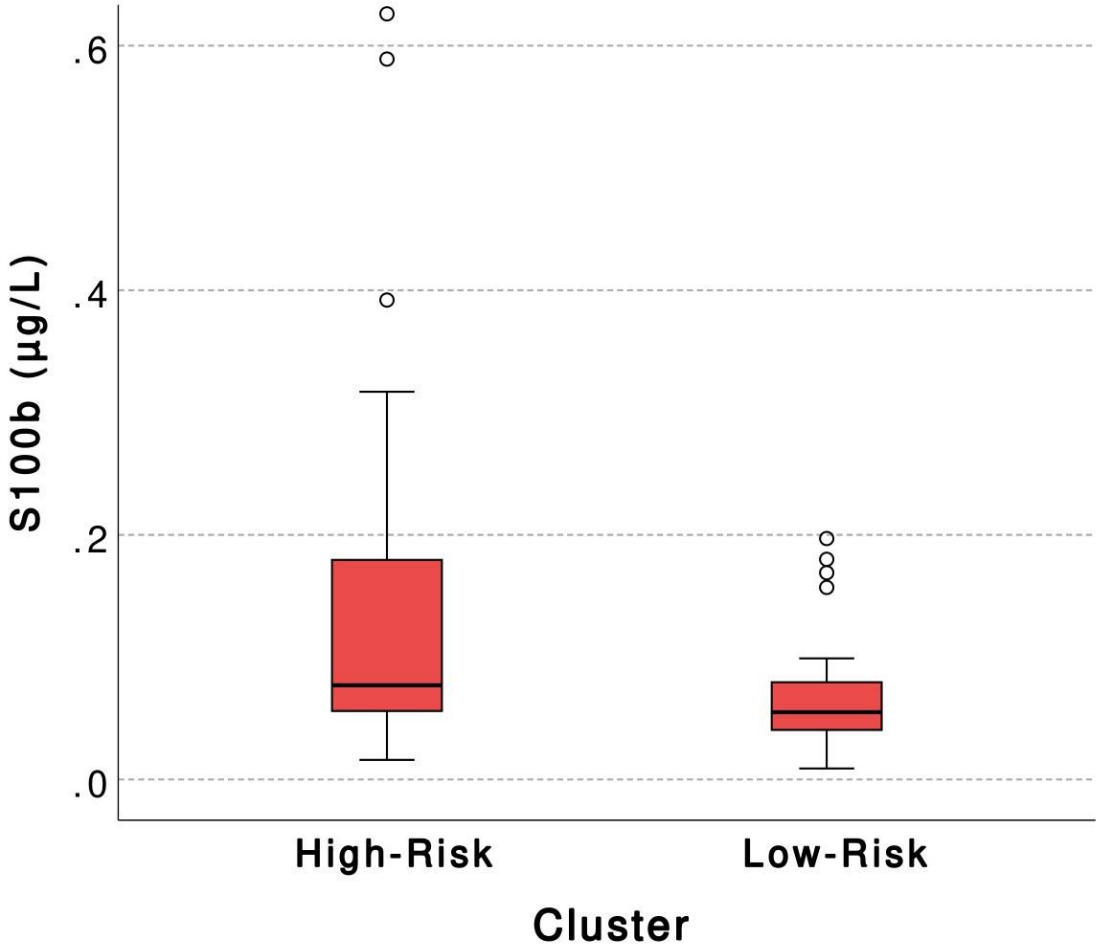

Figure 3. *Comparison of S100B levels between clusters.*

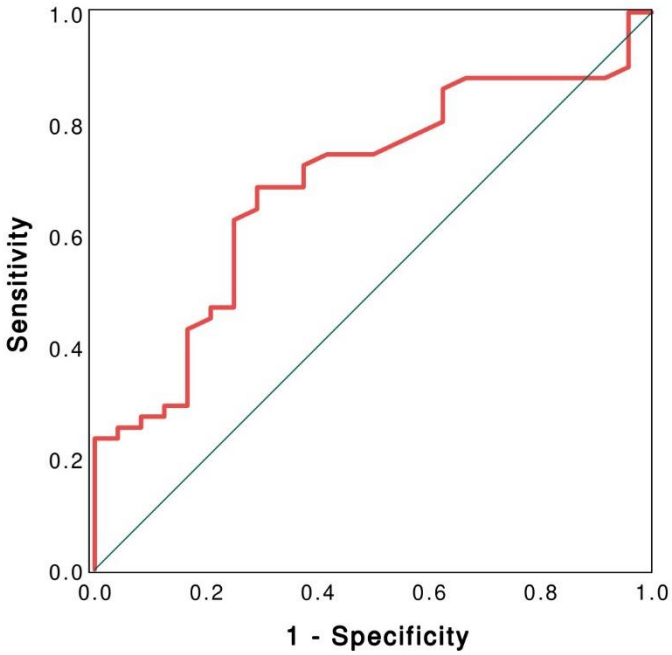

Figure 4. *ROC curve of S100B for identifying High-Risk cluster*

Table Legends

	Value
Sex, male (%)	65.8%
Age, years ± sd	56.9 ± 14.0
mRS, mean ± sd	0.33 ± 0.67
WFNS, mean ± sd	2.71 ± 1.60
mFisher scale, mean ± sd	3.59 ± 0.74
Hunt & Hess, mean ± sd	2.77 ± 1.48
GCS at ICU admission, mean ± sd	10.9 ± 4.9
Vasograde (%)	Red 36.8%
	Yellow 55.2%
	Green 8%
Hypertension (%)	43.8%

Diabetes mellitus (%)	6.7%
Dyslipidemia (%)	25.0%
Chronic pulmonary disease (%)	9.0%

Table 1. Demographics and clinical characteristics. mRS: modified Rankin Scale; WFNS: World Federation of Neurosurgical Societies score; mFisher scale: modified fisher scale; GCS: Glasgow Coma Scale; ICU: Intensive Care Unit.

	Low-Risk Cluster (n=210)	**High-Risk Cluster (n=301)**	**p-value**
Clinical characteristics			
Gender (male)	127 (60.5%)	209 (69.4%)	0.036
Age (years)	53.4±13.7	59.4±13.6	<0.001
Diabetes mellitus	8 (3.8%)	26 (8.6%)	0.031
Dyslipidemia	36 (17.1%)	92 (30.6%)	<0.001
BMI >30	11 (5.2%)	48 (15.9%)	<0.001
CKD	2 (1.0%)	16 (5.3%)	0.008
Heart rate (bpm)	74.2±14.9	80.2±19.4	<0.001
Invasive ventilation	23 (11.0%)	243 (80.7%)	<0.001
Headache at onset	184 (87.6%)	202 (67.1%)	<0.001
Seizure at onset	18 (8.6%)	59 (19.6%)	<0.001
Migraine headache	37 (7.6%)	20 (6.6%)	<0.001
Cardiac arrest	1 (0.5%)	12 (4.0%)	0.013
Anormal ECG	8 (3.8%)	49 (16.3%)	<0.001
Cardiac involvement	18 (8.6%)	75 (24.9%)	<0.001
Intracerebral hemorrhage	19 (9.0%)	96 (31.9%)	<0.001
Intraventricular hemorrhage	51 (24.3%)	258 (85.7%)	<0.001
Ventricular drainage	13 (6.2%)	192 (63.8%)	<0.001

Vasospasm	43 (20.5%)	89 (29.6%)	0.021
Acute symptomatic seizure	21 (10.0%)	74 (24.6%)	<0.001
Laboratory values			
Hemoglobin (g/dL)	12.6±2.9	11.8±3.2	0.005
Leukocytes (10^9/L)	12.3±4.2	14.4±5.9	<0.001
Neutrophils (10^9/L)	9.8±4.2	12.2±5.5	<0.001
Creatinine (mg/dL)	0.69±0.18	0.76±0.41	0.011
Glucose (mg/dL)	126.8±43.9	159.5±51.8	<0.001
Outcomes			
DCI	36 (17.1%)	87 (28.9%)	0.002
Death at discharge	10 (4.8%)	97 (32.2%)	<0.001
mRS at discharge	1 (0-2)	4 (2-6)	<0.001
GOS at discharge	5 (5-5)	3 (1-4)	<0.001
Death at 3 months	10 (4.8%)	106 (35.6%)	<0.001
mRS at 3 months	1 (0-1)	4 (2-6)	<0.001
GOS at 3 months	5 (5-5)	3 (1-5)	<0.001

Table 2. Comparison of clinical variables between clusters. DCI: Delayed Cerebral Ischemia; mFisher scale: modified Fisher scale; ECG: electrocardiogram; mRS: modified Rankin Scale; GOS: Glasgow Outcome Scale