



Original Research

Circulating inflammasome biomarkers provide independent prognostic value beyond clinical scores in aneurysmal subarachnoid hemorrhage

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ABSTRACT

Background: Aneurysmal subarachnoid hemorrhage (aSAH) is associated with high mortality and poor functional outcome, driven in part by neuroinflammatory mechanisms. Inflammasome activation has emerged as a key contributor to early brain injury and delayed cerebral ischemia (DCI), yet the prognostic value of circulating inflammasome-related biomarkers remains incompletely defined. This study aimed to evaluate the temporal dynamics and clinical relevance of serum interleukin-18 (IL-18), caspase-1 p20, and gasdermin D N-terminal (GSDMN) in aSAH, and to determine their incremental prognostic value beyond established clinical severity scores.

Methods: In this single-center prospective observational cohort study, 183 patients with aSAH were enrolled. Serum levels of IL-18, caspase-1 p20, and GSDMN were measured on days 1, 5, and 9 after ictus using ELISA. Clinical severity was assessed using WFNS and modified Fisher scores. The primary outcome was 3-month functional status (modified Rankin Scale). Associations were analyzed using mixed-effects models and multi-variable logistic regression adjusted for clinical covariates. Model performance was evaluated using ROC analysis.

Results: Caspase-1 p20 levels were significantly elevated in patients and consistently higher in those with unfavorable outcome across all time points. In multivariable analyses, caspase-1 p20 (day 1 and day 5) independently predicted poor outcome and DCI, improving model performance beyond clinical variables (AUC increase from 0.880 to 0.919). IL-18 showed time-dependent changes but lacked independent prognostic significance, while GSDMN levels did not differentiate outcome groups. Longitudinal analyses revealed distinct biomarker trajectories, with significant time–outcome interactions for caspase-1 p20 only.

Conclusion: Circulating caspase-1 p20 is a robust and independent biomarker of inflammasome activation with significant prognostic value in aSAH. Its integration into clinical models enhances outcome prediction, supporting its potential role in risk stratification and biomarker-guided therapeutic strategies.

1. Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) remains a severe form of stroke associated with high mortality and long-term disability despite advances in neurocritical care [1–3]. Early clinical severity scales, such as the World Federation of Neurosurgical Societies (WFNS) score and the modified Fisher (mFisher) scale, are widely used to predict outcome; however, these measures incompletely reflect the complex

biological processes underlying secondary brain injury and may lack sufficient granularity and reliability in predicting individual patient outcomes [4–6]. aSAH is increasingly recognized as a condition in which early brain injury and delayed complications, particularly delayed cerebral ischemia (DCI), jointly contribute to outcome variability beyond the initial clinical presentation [7–9]. Neuroinflammation plays a central role in the pathophysiology of aSAH, contributing to blood–brain barrier disruption, neuronal injury, and DCI [10,11]. Among

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inflammatory pathways, activation of the inflammasome has emerged as a key mechanism linking sterile inflammation to neuronal damage. The NLRP3 inflammasome promotes the activation of caspase-1, which in turn mediates the maturation of pro-inflammatory cytokines such as interleukin-1 β and interleukin-18 (IL-18), and induces pyroptotic cell death via gasdermin D N-terminal (GSDMN) [12–14]. Experimental studies have demonstrated that inflammasome activation contributes to early brain injury after aSAH and intracerebral hemorrhage (ICH) and may represent a therapeutic target, as inhibition of the NLRP3 inflammasome attenuates neuroinflammation, neuronal injury, and blood–brain barrier disruption in experimental models [15–18]. Circulating biomarkers reflecting inflammasome activation, including IL-18, caspase-1, and GSDMN, have gained increasing attention in neurological disorders [19–21]. Elevated levels of inflammasome-related cytokines, including interleukin-1 β and interleukin-18, have been detected in the cerebrospinal fluid of patients with aneurysmal subarachnoid hemorrhage and are associated with early brain injury and clinical outcome [22]. More recently, inflammasome-associated proteins have been proposed as reliable biomarkers of the inflammatory response in aSAH, further supporting their potential role in disease monitoring and prognostication [23]. Recent studies have highlighted the clinical relevance of caspase-1 as a key mediator of inflammasome activation in aneurysmal subarachnoid hemorrhage. Elevated levels of caspase-1 and related inflammasome proteins have been detected in cerebrospinal fluid and peripheral blood of aSAH patients, and are associated with disease severity and unfavorable neurological outcome. Moreover, increased caspase-1 activity has been linked to secondary brain injury and the development of delayed complications, supporting its role as both a biomarker and a potential therapeutic target [24,25]. GSDMN has emerged as a key executor of pyroptotic cell death downstream of inflammasome activation and plays a critical role in neuroinflammatory injury. Experimental studies have demonstrated that activation of the NLRP3–caspase-1–GSDMN pathway contributes to neuronal damage and early brain injury following subarachnoid hemorrhage, while its inhibition attenuates neuroinflammation and improves neurological outcomes [26,27]. Similarly, in ischemic stroke models, targeting GSDMN has been shown to reduce brain injury and limit neutrophil-mediated damage, highlighting its therapeutic potential across acute brain injuries [28,29]. Caspase-1 p20 represents the cleaved, active form of caspase-1 generated following inflammasome activation and is directly involved in the maturation of pro-inflammatory cytokines and induction of pyroptosis. Unlike total caspase-1 levels, the p20 subunit reflects functional inflammasome activity, providing a more specific marker of pathway activation [26]. Measurement of caspase-1 p20 may offer improved sensitivity for detecting inflammasome-mediated neuroinflammation [30,31]. Therefore, the present study aimed to investigate the temporal profile and clinical relevance of inflammasome-related biomarkers (IL-18, caspase-1 p20 and GSDMN) following aSAH. We hypothesized that these biomarkers exhibit dynamic changes over time and could offer independent prognostic value beyond established clinical severity scores. The primary objective was to assess the association between inflammasome-related biomarkers and 3-month functional outcome. Secondary objectives included (i) characterization of longitudinal biomarker trajectories, (ii) evaluation of associations with DCI, (iii) assessment of incremental predictive value beyond clinical models, and (iv) exploration of interrelationships between biomarkers.

2. Materials and methods

2.1. Participants

This single-center prospective observational cohort study enrolled consecutive patients with aSAH treated at our institution between December 2022 and December 2024. A total of 183 patients were included in the final analysis. Inclusion criteria were: (1) age $\geq$ 18 years;

(2) diagnosis of aSAH confirmed by non-contrast head computed tomography (CT) and aneurysm verification by computed tomography angiography (CTA) or digital subtraction angiography (DSA); and (3) admission within 24 h of symptom onset. Exclusion criteria comprised traumatic subarachnoid hemorrhage, pregnancy, delayed admission (>24 h after ictus), absence of aneurysm treatment, hemorrhage due to arteriovenous malformation, missing informed consent, aneurysm rerupture, or clinical deterioration during treatment. Patients with significant systemic comorbidities (including malignancy, severe hepatic or renal insufficiency, chronic pulmonary disease, inflammatory bowel disease or other chronic gastrointestinal disorders), chronic infections, or evidence of acute infection at admission were also excluded to avoid confounding inflammatory biomarker levels. The control group consisted of age- and sex-matched healthy healthcare personnel without known neurological disease. In these participants, blood sampling was performed after eligibility confirmation and exclusion of ongoing infection based on laboratory parameters. Clinical data were prospectively collected, including demographic characteristics (age, sex), vascular risk factors (hypertension, diabetes mellitus, smoking status), admission laboratory values, and clinical severity scores using the World Federation of Neurosurgical Societies (WFNS) grading system [32] and the modified Fisher (mFisher) scale [33]. Data on intensive care management (e.g., mechanical ventilation, external ventricular or lumbar drainage), occurrence of systemic or central nervous system infections, and delayed complications such as DCI were also recorded. Functional outcome was assessed at 3 months using the modified Rankin Scale (mRS) via structured telephone interview or in-person evaluation. Favorable outcome was defined as mRS 0–3, and unfavorable outcome as mRS 4–6, given the severity of aSAH and the clinical relevance of moderate disability (mRS 3), as this threshold has been widely used in neurocritical care studies to define a clinically meaningful favorable outcome [34]. Outcome assessments were performed by trained investigators blinded to biomarker data and not involved in patient care. Outcome assessors and laboratory personnel were blinded to clinical data and outcomes.

2.2. Study design and protocol

The study was designed to investigate the temporal dynamics and clinical relevance of circulating inflammasome-related biomarkers following aSAH. Serial blood samples were collected at predefined time points: day 1 (D1), day 5 (D5), and day 9 (D9) after ictus (defined as time of symptom onset; in cases of uncertainty, time of admission was used as a proxy). Sampling time points were selected to reflect distinct phases of post-hemorrhagic inflammatory responses, including early brain injury (D1), the typical temporal window of delayed cerebral ischemia development (D5), and later-stage neurocritical care inflammation (D9). Serum levels of interleukin-18 (IL-18), caspase-1 p20, and GSDMN were quantified using enzyme-linked immunosorbent assay (ELISA) techniques as described later in Methods. All patients received standard-of-care treatment according to institutional protocols, including prophylactic administration of nimodipine (60 mg orally every 4 h starting from day 1) to prevent vasospasm. Additional neurocritical care interventions were applied as clinically indicated. DCI was defined according to established consensus criteria (new neurological deficit and/or new infarction not attributable to other causes) [35]. Systemic and central nervous system infections were diagnosed based on a combination of clinical signs (e.g., fever $> 38^{\circ}\text{C}$), elevated inflammatory markers (e.g., C-reactive protein or procalcitonin > 0.5 ng/mL), and confirmatory diagnostic testing (e.g., chest imaging, microbiological cultures, cerebrospinal fluid analysis, or urine testing).

The primary objective of the study was to evaluate the association between inflammasome-related biomarkers and 3-month functional outcome assessed by mRS. Secondary objectives included the assessment of biomarker trajectories over time, their relationship with delayed cerebral ischemia, and their potential to provide additional prognostic

information beyond established clinical severity scores. The study protocol was approved by the institutional review board (IV/8468–1/2021/EKU and BM/4629–1/2024), and written informed consent was obtained from all participants or their legal representatives prior to inclusion.

2.3. Detection of IL-18, Caspase-1 p20 and GSDMN by ELISA

These samples were centrifuged within 30 min, and the serum supernatant was stored at -80°C until analysis. To remove debris, serum samples were centrifuged at $10,000 \times g$ for 5 min at 4°C before running the assay. All samples were handled according to a standardized protocol, and repeated freeze–thaw cycles were avoided. Serum levels of IL-18, Caspase-1 p20 and GSDMN were determined using commercially available sandwich ELISA kits (Enzyme Linked Immunosorbent Assay, EH0011, EH5100 and EH5523; Wuhan Fine Biotech Co., Ltd., China). All samples were measured in duplicate, and mean values were used for analysis. Serum samples were diluted 1:2 in the sample diluent provided in the kit and all measurements were carried out according to the manufacturer's protocol. Briefly, 100 μl of standards and samples were added to wells pre-coated with a specific monoclonal antibody and incubated at 37°C for 90 min. Following two washing steps to remove unbound material, 100 μl of biotin-labeled detection antibody was added and incubated at 37°C for 60 min. After further 3 washing steps, streptavidin-HRP conjugate was added and incubated for 30 min at 37°C . Following five more washes, color development was achieved using a TMB substrate, and the reaction was stopped with the STOP solution of the kit. Absorbance was measured at 450 nm using a microplate reader, with additional measurement at 570 nm for background correction. The corrected absorbance values were calculated by subtracting the 570 nm reading from the 450 nm reading. The concentration of IL-18, Caspase-1 p20 and GSDMN was calculated from a standard curve generated using standards.

2.4. Statistical analysis

Statistical analyses were performed using GraphPad Prism (version 10, GraphPad Software, San Diego, CA, USA). Additional analyses and visualizations were performed using Python (version 3.11) with the use of standard scientific libraries (pandas, NumPy, Matplotlib, SciPy). Continuous variables are presented as mean $\pm$ standard deviation (SD) or median with interquartile range (IQR), depending on data distribution. Normality was assessed using the Shapiro–Wilk test. For comparisons involving more than two groups, non-normally distributed data were analyzed using the Kruskal–Wallis test followed by Dunn's test with Dunn's correction to account for multiple testing and control the type I error rate. For two-group comparisons, the Mann–Whitney U test or Student's t -test was applied, as appropriate. A small proportion of biomarker measurements was missing (D1: $n = 3$; D5: $n = 5$; D9: $n = 5$). Given the low frequency of missing data relative to the total sample size, no imputation was performed. Analyses were conducted using all available data, assuming missing at random, and sensitivity analyses were not performed due to the low proportion of missing data. Analyses were conducted using all available data, assuming missing at random. The number of available samples per time point was as follows: D1 ($n = 180$), D5 ($n = 178$), and D9 ($n = 178$). To evaluate longitudinal changes in biomarker levels and their association with clinical outcome, linear mixed-effects models were applied using restricted maximum likelihood estimation. Time (day 1, day 5, and day 9) and clinical outcome (favorable vs. unfavorable) were included as fixed effects, along with their interaction term (time $\times$ outcome). The reference categories were day 1 (D1) for time and favorable outcome for group comparisons. Subject-specific variability was accounted for by including patient ID as a random intercept. An unstructured covariance matrix was used to model within-subject correlations. Separate models were fitted for each biomarker (interleukin-18, caspase-1 p20, and GSDMN). Model

estimates are reported as β coefficients with 95% confidence intervals (CI). Estimated marginal means were calculated, and pairwise comparisons between time points were adjusted using Bonferroni correction. Model assumptions were evaluated by inspection of residuals. In case of non-normal distribution, logarithmic transformation of biomarker levels was applied.

For regression analyses, biomarker levels were entered as continuous variables and scaled per 10 pg/mL increase to facilitate interpretability of odds ratios. Binary logistic regression analyses were performed to identify predictors of 3-month outcome and DCI, adjusted for clinically relevant covariates (age, WFNS, and mFisher score). Results are reported as odds ratios (OR) with 95% confidence intervals. Model performance was assessed using Nagelkerke R^2 to estimate explained variance and the Hosmer–Lemeshow test to evaluate goodness-of-fit. Discrimination ability was further assessed using the area under the receiver operating characteristic curve (AUC). Confidence intervals for AUC were estimated using bootstrap resampling (1,000 iterations). Spearman's rank correlation coefficients were calculated to assess pairwise associations between biomarkers. Correlation matrices were visualized using hierarchical clustering-based heatmaps, with clustering performed using correlation distance ($1 - \rho$). All statistical tests were two-tailed, and a p -value < 0.05 was considered statistically significant.

3. Results

3.1. Patients characteristics

A total of 183 patients were included, of whom 99 (54.1%) had a favorable and 84 (45.9%) an unfavorable outcome. The overall mean age of the cohort was 57.8 years, with no significant difference between groups ($p = 0.453$). In total, 53 patients (29.0%) were female, without a significant sex difference between outcome groups ($p = 0.157$). DCI occurred in 41 patients (22.4%) overall and was significantly more frequent in the unfavorable group (42.9% vs. 5.1%, $p < 0.001$). The mean time to DCI was 9 ± 4 days (median 8, IQR 7–9 days). Patients with unfavorable outcome had significantly higher rates of hypertension (68.7% vs. 51.5%, $p = 0.019$), diabetes (18.1% vs. 6.1%, $p = 0.012$), and seizure at onset (15.9% vs. 4.0%, $p = 0.007$). They also presented with significantly worse clinical severity, reflected by higher WFNS and mFisher scores (both $p < 0.001$). Laboratory parameters showed significantly elevated glucose ($p = 0.001$), CRP ($p = 0.020$), WBC ($p = 0.013$), and neutrophil-to-lymphocyte ratio ($p < 0.001$), along with lower platelet counts ($p = 0.009$) in the unfavorable group. Furthermore, unfavorable outcome was associated with a markedly higher need for intensive interventions, including external ventricular drainage (79.8% vs. 10.1%, $p < 0.001$), decompressive craniotomy (15.5% vs. 0%, $p < 0.001$), and mechanical ventilation (81.0% vs. 14.1%, $p < 0.001$), as well as a significantly higher rate of infection (67.9% vs. 15.2%, $p < 0.001$). No significant differences were observed in smoking, alcohol consumption, lumbar drainage, or aneurysm location distribution between groups. A control group of 44 individuals was included for comparison. The mean age of the control group was 57.5 ± 10.9 years, and 31 participants (70.5%) were female. There were no significant differences in age or sex distribution between the control group and the overall patient population. A small proportion of biomarker measurements was missing across time points (D1: $n = 3$; D5: $n = 5$; D9: $n = 5$). The number of available samples per time point was as follows: D1 ($n = 180$), D5 ($n = 178$), and D9 ($n = 178$). Considering the low frequency of missing data relative to the total sample size, the potential impact on the results is expected to be minimal, and no systematic bias is assumed. The detailed patient characteristics of the study population are presented in Table 1.

Data are presented as mean $\pm$ standard deviation (SD) for normally distributed continuous variables, median with interquartile range [IQR] for non-normally distributed variables, and number (percentage) for categorical variables. P -values were calculated using Student's t -test or

Table 1
Patient characteristics by outcome.

Variable	Favorable (n = 99)	Unfavorable (n = 84)	P-value
Age (mean ± SD)	56.96 ± 11.00	58.83 ± 11.98	0.453
Female, N (%)	66 (66.7%)	64 (76.2%)	0.157
Hypertension, N (%)	51 (51.5%)	57 (68.7%)	0.019
Diabetes, N (%)	6 (6.1%)	15 (18.1%)	0.012
Smoking, N (%)	35 (35.4%)	31 (37.3%)	0.780
Alcohol consumption, N (%)	7 (7.1%)	6 (7.2%)	0.967
Seizure at onset, N (%)	4 (4.0%)	13 (15.9%)	0.007
Loss of consciousness, N (%)	34 (34.3%)	39 (47.6%)	0.071
Aneurysm location, N (%)			
ICA	7 (7.1%)	6 (7.8%)	
MCA	23 (23.2%)	24 (31.2%)	
ACom	31 (31.3%)	22 (28.6%)	
PCom	13 (13.1%)	8 (10.4%)	
ACA	4 (4.0%)	6 (7.8%)	
VB	9 (9.1%)	10 (13.0%)	
PCA	0 (0%)	1 (1.3%)	
WFNS, median [IQR]	1 [1,2]	4 [2–5]	<0.001
mFisher, median [IQR]	2 [1–3]	4 [3,4]	<0.001
Glucose*, median [IQR]	7.4 [5.9–8.6]	9.0 [8.0–10.9]	0.001
Creatinine*, median [IQR]	61 [50–70]	66 [52–80]	0.090
CRP*, median [IQR]	7.1 [2.7–16.1]	18.0 [2.6–70.8]	0.020
WBC*, median [IQR]	10.4 [8.5–12.0]	12.1 [9.4–15.3]	0.013
Neutrophil-lymphocyte ratio*, median [IQR]	4.2 [2.8–6.9]	6.7 [4–11.9]	<0.001
Platelet*, median [IQR]	245 [197–292]	214 [170–261]	0.009
Delayed cerebral ischemia, N (%)	5 (5.1%)	36 (42.9%)	<0.001
Extraventricular drain, N (%)	10 (10.1%)	67 (79.8%)	<0.001
Lumbar drain, N (%)	50 (50.5%)	46 (54.8%)	0.508
Decompressive craniotomy, N (%)	0 (0%)	13 (15.5%)	<0.001
Mechanical ventilation, N (%)	14 (14.1%)	68 (81.0%)	<0.001
Infection, N (%)	15 (15.2%)	57 (67.9%)	<0.001

Mann–Whitney *U* test for continuous variables, and χ^2 test or Fisher’s exact test for categorical variables, as appropriate. Favorable outcome was defined as: modified Rankin Scale (mRS) 0–3 at 3-month follow-up, while unfavorable outcome was defined as mRS 4–6 at 3-month follow up. ICA, internal carotid artery; MCA, middle cerebral artery; Acom, anterior communicating artery; Pcom, posterior communicating artery; ACA, anterior cerebral artery; VB, vertebrobasilar system; PCA, posterior cerebral artery; WFNS, World Federation of Neurosurgical Societies score; mFisher, modified Fisher scale; CRP, C-reactive protein; WBC, white blood cell count; NLR, neutrophil-to-lymphocyte ratio; DCI, delayed cerebral ischemia; EVD, external ventricular drainage.

3.2. Temporal distribution of serum inflammasome-related biomarkers according to clinical outcome

Serum caspase-1 p20 levels differed between groups at all examined time points (Fig. 1A–C). At D1 (Fig. 1A), both favorable and unfavorable groups showed significantly higher levels compared with controls ($p < 0.001$ and $p < 0.0001$, respectively; $\Delta = +410$ pg/mL for unfavorable vs. control), with an additional difference between favorable and unfavorable patients ($p < 0.01$). At D5 (Fig. 1B) and D9 (Fig. 1C), caspase-1 p20 levels remained significantly elevated in both patient groups compared with controls (all $p < 0.05$), with further differences observed between favorable and unfavorable groups at each time point. For IL-18 (Fig. 1D–F), at D1 (Fig. 1D), a significant difference was observed between controls and the favorable group ($p < 0.01$), while no significant differences were detected between controls and the unfavorable group or between outcome groups. At D5 (Fig. 1E), both favorable and unfavorable groups differed significantly from controls ($p < 0.0001$ and $p < 0.001$, respectively; $\Delta = -56$ pg/mL for favorable vs. control), while no difference was observed between the outcome groups. At D9 (Fig. 1F), both patient groups showed significant differences compared with

controls ($p < 0.0001$ and $p < 0.01$, respectively; $\Delta = -57$ pg/mL for favorable vs. control), with an additional difference between favorable and unfavorable groups ($p < 0.05$). GSDMN levels (Fig. 1G–I) were significantly lower in both favorable and unfavorable groups compared with controls at all time points, including D1 (Fig. 1G), D5 (Fig. 1H), and D9 (Fig. 1I) (all $p \leq 0.001$; Δ ranging from -90 to -97 pg/mL vs. controls). No significant differences were observed between favorable and unfavorable groups at any time point. All pairwise comparisons between groups at each time point were adjusted for multiple testing using Dunn’s post hoc correction following Kruskal–Wallis analysis. Reported *p*-values reflect these adjusted comparisons. All median differences are provided in Table S1.

Linear mixed-effects models with random intercepts for subjects showed a significant decrease in IL-18 levels over time in the favorable outcome group, with lower levels at D9 compared with D1 ($\beta = -31.73$, 95% CI -50.40 to -13.05 , $p = 0.001$), while no overall effect of outcome was observed ($\beta = 0.23$, 95% CI -60.26 to 60.72 , $p = 0.994$). For caspase-1 p20, baseline levels were significantly higher in the unfavorable group ($\beta = 438.81$, 95% CI 276.30 to 601.31 , $p < 0.001$), and significant time-by-outcome interactions were detected at both D5 ($\beta = -212.88$, 95% CI -383.30 to -42.45 , $p = 0.014$) and D9 ($\beta = -203.64$, 95% CI -376.72 to -30.56 , $p = 0.021$), indicating a different temporal trajectory between outcome groups. In contrast, GSDMN showed no significant main effects of time or outcome, and no significant interaction terms.

Fig. 2 shows serum IL-18 and caspase-1 p20 levels measured on days 1, 5, and 9 (D1,D5,D9) following aSAH in patients with and without DCI. A total of 41 patients developed DCI, while 142 did not. IL-18 levels differed significantly between groups at day 5 ($**p < 0.01$), whereas no significant differences were observed at days 1 and 9. Serum caspase-1 p20 levels were significantly higher in the DCI group at day 1 ($***p < 0.001$), with no significant differences at days 5 and 9. The largest difference between DCI and no DCI groups was observed for caspase-1 p20 at day 1 ($\Delta = 314$ pg/mL), whereas IL-18 showed moderate differences (Δ up to 59 pg/mL), and GSDMN levels demonstrated minimal separation between groups ($\Delta \leq 6$ pg/mL), Table S1.

Linear mixed-effects models with random intercepts for subjects showed no significant main effect of DCI status on IL-18 levels ($\beta = 28.41$, 95% CI -12.33 to 69.15 , $p = 0.170$), and no significant time-by-DCI interaction, indicating similar temporal trajectories between groups. For caspase-1 p20, baseline levels were significantly higher in patients who developed DCI ($\beta = 302.77$, 95% CI 168.41 to 437.12 , $p < 0.001$), while no significant interaction between time and DCI status was observed, suggesting comparable longitudinal changes in both groups. GSDMN levels showed no significant main effects of time or DCI status, and no interaction, indicating no association with DCI occurrence.

Binary logistic regression analyses were performed to assess predictors of 3-month outcome and DCI, Table 2. For the 3-month outcome, univariate analysis showed significant associations for age (OR 1.05, 95% CI 1.02–1.07, $p = 0.001$), WFNS (OR 2.5, 95% CI 1.94–3.23, $p < 0.001$), mFisher score (OR 4.07, 95% CI 2.27–7.31, $p < 0.001$), and caspase-1 p20 levels at D1, D5, and D9. In multivariate analysis, age (OR 1.05, 95% CI 1.00–1.11, $p = 0.045$), WFNS (OR 2.15, 95% CI 1.4–3.32, $p = 0.001$), caspase-1 p20 D1 (OR 1.32, 95% CI 1.09–1.60, $p = 0.005$), and caspase-1 p20 D5 (OR 1.26, 95% CI 1.04–1.52, $p = 0.018$) remained significant, while mFisher score and caspase-1 p20 D9 did not. Model performance improved with the inclusion of caspase-1 p20 D1 (Nagelkerke R^2 0.67, Hosmer–Lemeshow $p = 0.43$).

For DCI, univariate analysis identified WFNS, mFisher score, infection, and caspase-1 p20 D1 as significant variables. In multivariate analysis, mFisher score (OR 2.5, 95% CI 1.1–5.87, $p = 0.035$), infection (OR 0.28, 95% CI 0.10–0.89, $p = 0.032$), and caspase-1 p20 D1 (OR 1.24, 95% CI 1.06–1.45, $p = 0.008$) remained significant, while WFNS did not. The association between infection and DCI should be interpreted cautiously, as it may reflect complex interactions between systemic inflammation, treatment intensity, and survival bias. The addition

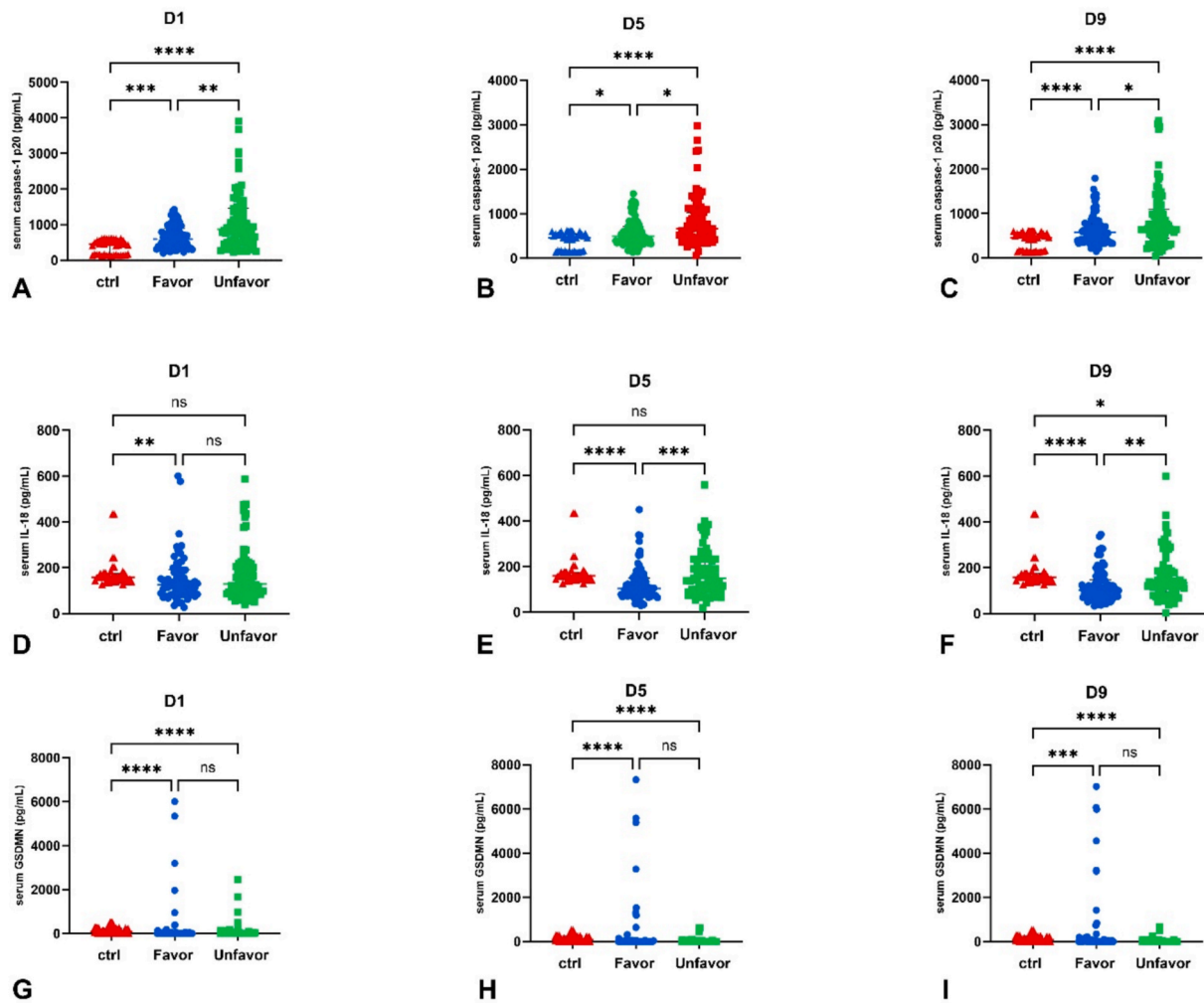


Fig. 1. Temporal changes in serum inflammasome-related biomarkers in aneurysmal subarachnoid hemorrhage patients stratified by outcome. Serum levels of inflammasome-related biomarkers measured on day 1 (D1), day 5 (D5), and day 9 (D9) following aneurysmal subarachnoid hemorrhage (aSAH). Patients were stratified into control (ctrl), N = 44, favorable outcome (mRS 0–2), N = 99, and unfavorable outcome (mRS 3–6), N = 84 groups. (A–C) Caspase-1 p20 serum levels at D1 (A), D5 (B), and D9 (C). (D–F) IL-18 serum levels at D1 (D), D5 (E), and D9 (F). (G–I) Gasdermin D N-terminal (GSDMN) serum levels at D1 (G), D5 (H), and D9 (I). Data are presented as individual values. Statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.00001$; ns, not significant. aSAH, aneurysmal subarachnoid hemorrhage; mRS, modified Rankin Scale; D1/D5/D9, day 1/5/9 after ictus.

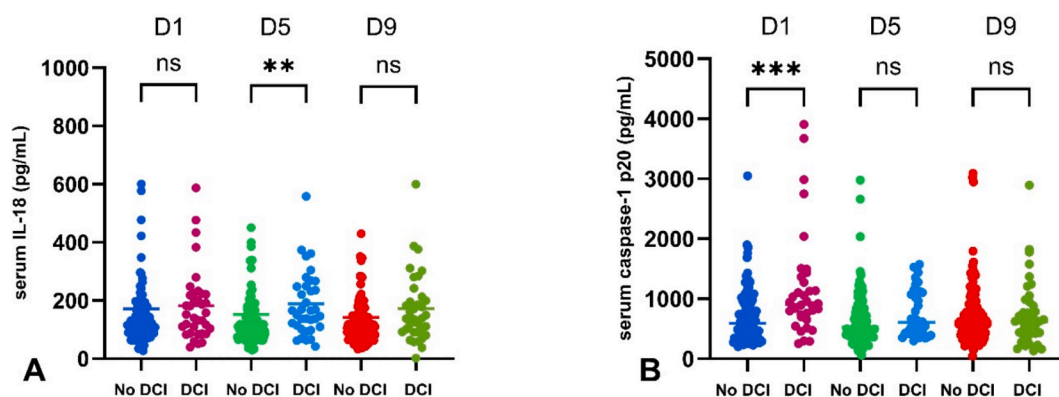


Fig. 2. Serum IL-18 and caspase-1 p20 levels according to delayed cerebral ischemia status at different time points. Serum levels of IL-18 and caspase-1 p20 measured on day 1 (D1), day 5 (D5), and day 9 (D9) following aneurysmal subarachnoid hemorrhage (aSAH), stratified by the occurrence of delayed cerebral ischemia (DCI vs. no DCI). A total of 41 patients developed DCI, while 142 patients did not. (A) Serum IL-18 levels at D1, D5, and D9. (B) Serum caspase-1 p20 levels at D1, D5, and D9. Data are presented as individual values. Statistical comparisons between groups are indicated for each time point. Significance levels are denoted as follows: ** $p < 0.01$; *** $p < 0.001$; ns, not significant. DCI, delayed cerebral ischemia; D1/D5/D9, day 1/5/9 after ictus.

of caspase-1 p20 D1 increased the Nagelkerke R^2 from 0.29 to 0.47, with a Hosmer–Lemeshow p -value of 0.59. Although IL-18 showed significant

Table 2
Combined logistic regression results and model performance for 3-month outcome and delayed cerebral ischemia (DCI).

Outcome	Variable	Univariate OR (95% CI)	P-value	Multivariate OR (95% CI)	P-value	Model	Nagelkerke R ²	Hosmer–Lemeshow p
3-month outcome	Age	1.05 (1.02–1.07)	0.001	1.05 (1.00–1.11)	0.045	Clinical model	0.55	0.65
	WFNS	2.5 (1.94–3.23)	<0.001	2.15 (1.4–3.32)	0.001	+ Caspase-1 p20 D1	0.67	0.43
	mFisher score	4.07 (2.27–7.31)	<0.001	1.98 (0.95–4.11)	0.067	+ Caspase-1 p20 D5	0.61	0.10
	Caspase-1 p20 D1	1.17 (1.08–1.27)	<0.001	1.32 (1.09–1.60)	0.005	+ Caspase-1 p20 D9	0.55	0.40
	Caspase-1 p20 D5	1.14 (1.05–1.24)	0.002	1.26 (1.04–1.52)	0.018			
	Caspase-1 p20 D9	1.10 (1.03–1.18)	0.006	1.12 (0.98–1.28)	0.09			
DCI	WFNS	1.36 (1.1–1.69)	0.005	0.9 (0.58–1.41)	0.651	Clinical model	0.29	0.75
	mFisher score	2.89 (1.56–5.38)	0.001	2.5 (1.1–5.87)	0.035	+ Caspase-1 p20 D1	0.47	0.59
	Infection	0.17 (0.08–0.37)	<0.001	0.28 (0.10–0.89)	0.032			
	Caspase-1 p20 D1	1.13 (1.05–1.21)	0.001	1.24 (1.06–1.45)	0.008			

Binary logistic regression analyses were performed to evaluate predictors of 3-month functional outcome and delayed cerebral ischemia (DCI) following aneurysmal subarachnoid hemorrhage. Both univariate and multivariate models are presented as odds ratios (ORs) with 95% confidence intervals (CIs). For the 3-month outcome, the clinical model included age, WFNS, and mFisher score, and was extended by adding Caspase-1 p20 levels measured at different time points (Day 1, Day 5, and Day 9). For DCI, the clinical model included WFNS, mFisher score, and infection, with additional analysis including Caspase-1 p20 Day 1. Model performance is summarized using Nagelkerke R² to indicate explained variance and the Hosmer–Lemeshow test to assess goodness-of-fit. Abbreviations: OR, odds ratio; CI, confidence interval; WFNS, World Federation of Neurological Societies score; mFisher, modified Fisher score; DCI, delayed cerebral ischemia; D1/D5/D9, Day 1/5/9; aSAH, aneurysmal subarachnoid hemorrhage.

differences between outcome groups in univariate analysis, this association did not remain significant in multivariable logistic regression, suggesting that its effect is not independent of established clinical predictors.

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the discriminative performance of the clinical model and its extensions including caspase-1 p20 measurements at different

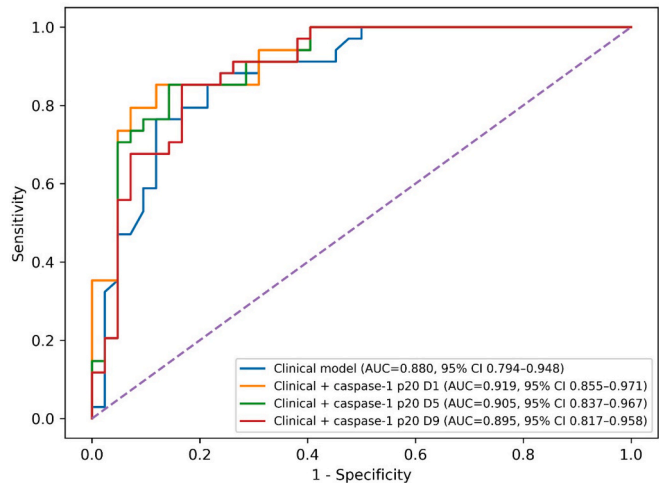


Fig. 3. Receiver operating characteristic (ROC) curves for prediction of 3-month outcome. Receiver operating characteristic (ROC) curves showing the performance of the clinical model and biomarker-augmented models for predicting 3-month functional outcome following aSAH. The clinical model included age, World Federation of Neurosurgical Societies (WFNS) score, and modified Fisher (mFisher) score. Extended models additionally incorporated serum caspase-1 p20 levels measured at day 1 (D1), day 5 (D5), and day 9 (D9). Area under the curve (AUC) values with corresponding 95% confidence intervals (CI) are displayed in the legend for each model. The diagonal dashed line represents random classification (AUC = 0.5). Abbreviations: ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval; WFNS, World Federation of Neurosurgical Societies score; mFisher, modified Fisher scale; D1/D5/D9, day 1/5/9 after ictus.

time points (Fig. 3). The clinical model, incorporating age, WFNS, and mFisher score, demonstrated good discrimination for predicting 3-month outcome, with an area under the curve (AUC) of 0.880 (95% CI 0.803–0.957, $p < 0.001$). The addition of caspase-1 p20 at day 1 (D1) further improved model performance, yielding the highest discriminative ability among all models (AUC 0.919, 95% CI 0.859–0.980, $p < 0.001$). Models incorporating caspase-1 p20 at day 5 (D5) and day 9 (D9) also showed improved discrimination compared with the clinical model alone, with AUCs of 0.905 (95% CI 0.837–0.974, $p < 0.001$) and 0.895 (95% CI 0.824–0.966, $p < 0.001$), respectively. Among the evaluated models, the inclusion of caspase-1 p20 at D1 resulted in the largest increase in AUC ($\Delta\text{AUC} = +0.039$), whereas the improvements associated with D5 ($\Delta\text{AUC} = +0.025$) and D9 ($\Delta\text{AUC} = +0.015$) were smaller, Fig. 3.

Spearman correlation analysis was performed to assess pairwise associations between serum levels of IL-18, caspase-1 p20, and GSDMN measured at different time points (D1, D5, and D9). The resulting correlation matrix is presented as a hierarchical clustering-based heatmap (Fig. 4.), with correlation coefficients displayed for each variable pair. The strongest correlations were observed within the same biomarker across different time points, particularly for caspase-1 p20 and IL-18 measurements, indicating temporal consistency. In contrast, correlations between different biomarkers were generally weaker, especially for GSDMN, which showed limited association with IL-18 and caspase-1 p20, Fig. 4.

4. Discussion

In this prospective cohort of patients with aSAH, circulating inflammasome-related biomarkers exhibited distinct temporal dynamics and differential clinical relevance. Among the investigated markers, caspase-1 p20 showed the strongest and most consistent association with unfavorable functional outcome and delayed cerebral ischemia, providing independent prognostic value beyond established clinical severity scores. In contrast, IL-18 demonstrated time-dependent but less robust associations, while gasdermin D N-terminal did not discriminate between outcome groups. These findings suggest that upstream inflammasome activation is more reliably captured in peripheral blood

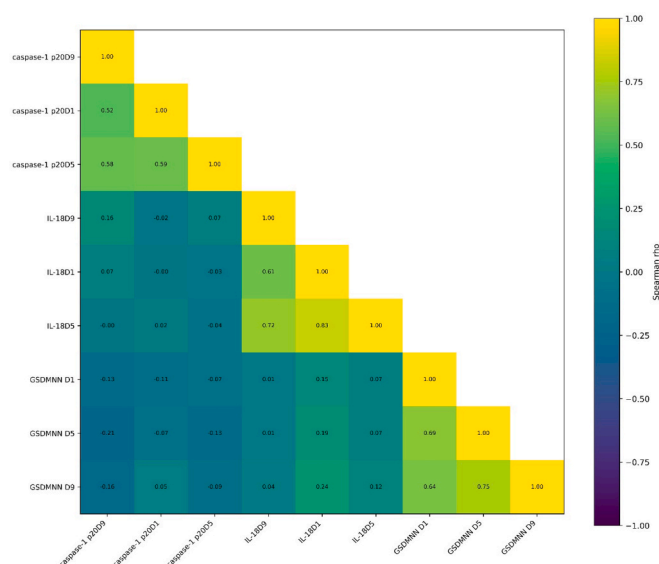


Fig. 4. Spearman correlation heatmap of inflammasome-related biomarkers across time points. A hierarchical clustering-based triangular heatmap illustrates pairwise Spearman correlation coefficients (ρ) between serum levels of IL-18, caspase-1 p20, and gasdermin D N-terminal (GSDMN) measured at day 1 (D1), day 5 (D5), and day 9 (D9) following aneurysmal subarachnoid hemorrhage. Correlation coefficients are displayed within each cell. Only the lower triangle of the symmetric correlation matrix is shown. Variables are ordered according to hierarchical clustering using correlation distance ($1 - \rho$). The color scale represents the strength and direction of correlations.

than downstream effector signaling following aSAH. Caspase-1 is a central effector of inflammasome activation, generated through proteolytic cleavage of pro-caspase-1 within multiprotein complexes, and mediates the maturation of key pro-inflammatory cytokines such as IL-1 β and IL-18, while also inducing pyroptotic cell death via gasdermin D cleavage [18,20]. Through these mechanisms, caspase-1 contributes to blood–brain barrier disruption, cerebral edema, neuronal injury, and amplification of neuroinflammation following aSAH, and has been consistently linked to disease severity and poor functional outcomes [20,24]. Consistent with these mechanistic insights, elevated circulating caspase-1 p20 levels in our cohort support the presence of early and clinically relevant inflammasome activation, particularly in patients with unfavorable outcomes and DCI [23–25]. While previous studies—especially those based on CSF—have demonstrated strong associations between inflammasome activation, cytokine release, and pyroptosis, we observed weaker or inconsistent changes in IL-18 and gasdermin D [23–25,36]. This discrepancy likely reflects compartmentalization between central and systemic inflammatory responses, as CSF biomarkers more directly capture intracerebral processes, whereas circulating markers may be diluted, regulated (e.g., by binding proteins), or temporally dissociated from local pyroptotic activity [36]. Although longitudinal mixed-effects modeling did not reveal significant group-level differences, logistic regression analyses showed that caspase-1 levels at specific time points independently predicted unfavorable outcome, suggesting that individual variability drives prognostic value. This apparent discrepancy indicates that prognostic value arises from inter-individual variability rather than consistent group-level differences, highlighting that biomarkers may carry predictive information even in the absence of clear separation at the population level. Several inflammatory, endothelial, metabolic, and neurovascular biomarkers have previously been proposed for prediction of delayed cerebral ischemia and outcome after aSAH, including inflammatory cytokines, endothelial dysfunction markers, metabolic stress indicators, and markers of neurovascular injury [37–41]. However, despite promising results, few biomarkers have demonstrated sufficient specificity or

reproducibility for routine clinical implementation. In this context, circulating caspase-1 p20 may provide additional translational value by more directly reflecting inflammasome-associated inflammatory activation, a pathway increasingly implicated in secondary brain injury after aSAH. Furthermore, serum-based measurement of caspase-1 p20 may represent a clinically feasible approach for longitudinal biomarker assessment during neurocritical care monitoring. Collectively, these findings identify caspase-1 as a sensitive upstream marker of inflammasome activation in aSAH and support its potential as an accessible surrogate marker of early brain injury. Importantly, although systemic inflammation may influence circulating biomarker levels, the independent association of caspase-1 p20 with outcome after adjustment for clinical severity supports its specific relevance to inflammasome-driven pathophysiology rather than reflecting nonspecific inflammatory activation.

The observation of lower circulating IL-18 levels in patients compared with controls, although counterintuitive, is biologically plausible and may reflect compartmentalized inflammasome signaling rather than absence of pathway activation. Inflammatory responses after aSAH differ substantially between central and systemic compartments, and serum IL-18 does not necessarily parallel cerebrospinal fluid inflammasome activity [42]. In this context, circulating IL-18 may not directly reflect intracerebral cytokine production. This interpretation is further supported by elevated caspase-1 p20 levels, indicating upstream inflammasome activation, while lower circulating GSDMN may suggest limited systemic pore-mediated cytokine release [43]. In addition, total IL-18 levels may be influenced by IL-18-binding protein, which can reduce detectable free IL-18 during systemic inflammation, potentially leading to underestimation of biologically active cytokine levels in the acute post-hemorrhagic setting [44]. The selected longitudinal sampling design enabled assessment of both early and delayed inflammasome-associated inflammatory responses following aSAH. Persistent elevations of IL-18 and caspase-1 p20 may reflect sustained inflammasome activation beyond the immediate early brain injury phase, whereas later alterations may additionally be influenced by secondary neuro-inflammatory processes, systemic complications, and delayed cerebral ischemia.

Circulating GSDMN has been detected in both serum and plasma in several human studies, including metabolic, infectious, and oncological conditions, where it has been proposed as a biomarker of pyroptosis-related inflammation [45–48]. In our cohort, serum GSDMN levels differed significantly between patients with aSAH and healthy controls, indicating systemic inflammasome activation. However, no differences were observed between favorable and unfavorable outcome groups, suggesting limited prognostic utility. This may reflect the predominantly intracellular and tissue-specific role of GSDMN in mediating pyroptotic cell death. Unlike caspase-1, which can be detected in circulation as an indicator of inflammasome activation, GSDMN fragments may remain localized within affected tissues or be rapidly cleared from the bloodstream [43]. Consequently, circulating GSDMN may not adequately capture the extent of pyroptotic activity in the injured brain.

Taken together, our findings support a hierarchical model of inflammasome signaling in aSAH, in which upstream activation markers such as caspase-1 are more reliably reflected in peripheral circulation, whereas downstream mediators are subject to compartmentalization and regulatory mechanisms that limit their detectability. This likely explains the superior prognostic performance of caspase-1 p20 compared with IL-18 and GSDMN and underscores the importance of selecting biomarkers that best capture the systemic footprint of neuroinflammation. Several limitations should be acknowledged. First, this was a single-center study, which may limit generalizability. Second, inflammasome-associated biomarkers were measured exclusively in peripheral serum samples, and no paired cerebrospinal fluid analyses were available to directly distinguish intracerebral from systemic inflammasome activation. Furthermore, severe systemic illness, infection, mechanical ventilation, EVD placement, and differences in

neurocritical care intensity may have contributed to circulating caspase-1 p20 levels. Therefore, the observed biomarker elevations should be interpreted as reflecting systemic inflammasome-associated inflammatory responses following aSAH rather than direct evidence of intracerebral inflammasome activation. Third, although longitudinal sampling was performed, the selected time points may not fully capture peak inflammasome dynamics. Patients who died before the typical temporal window of DCI onset were not excluded, and early mortality may therefore have influenced DCI-related analyses. Similarly, the inverse association observed between infection and DCI in multivariable analysis should be interpreted cautiously and likely reflects complex temporal relationships, limited sample size, collinearity with disease severity variables, and potential survival or treatment-related biases rather than a biologically protective effect. Finally, patients with unruptured intracranial aneurysms were not included, and therefore it remains unclear whether inflammasome activation is specifically associated with aneurysm rupture or may already be present in unruptured aneurysm disease. Future comparative studies including both ruptured and unruptured aneurysm cohorts are warranted. Furthermore, severe systemic illness, infection, mechanical ventilation, EVD placement, and differences in neurocritical care intensity may have contributed to circulating caspase-1 p20 levels. Therefore, the observed biomarker elevations should be interpreted as reflecting systemic inflammasome-associated inflammatory responses following aSAH rather than direct evidence of intracerebral inflammasome activation. Despite these limitations, the prospective design and relatively large cohort strengthen the robustness and internal validity of our findings.

5. Conclusion

In conclusion, circulating caspase-1 p20 represents a promising and clinically accessible biomarker of inflammasome activation that provides independent prognostic value in aSAH, supporting its potential role in risk stratification and future biomarker-guided therapeutic approaches. Future studies integrating both serum and cerebrospinal fluid biomarkers may further clarify compartment-specific inflammasome dynamics and improve the biological interpretation of systemic inflammatory signatures in aSAH.

CRedit authorship contribution statement

Emoke Hegedus: Investigation, Data curation, Conceptualization. **Blanka Nagybyanyai-Nagy:** Writing – original draft, Visualization, Investigation, Data curation. **Patricia Neuperger:** Visualization, Software, Methodology, Data curation. **Fanni Balogh:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Beáta Kari:** Resources, Methodology, Data curation. **Gábor J. Szebeni:** Writing – review & editing, Writing – original draft, Conceptualization. **Tihamer Molnar:** Writing – review & editing, Validation, Supervision, Project administration. **Peter Csecsei:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jocn.2026.112165>.

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