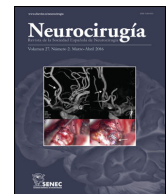


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## Clinical Research

## Research of the effects of myelin basic protein (MBP) and ischemia modified albumin (IMA) levels in blood and cerebrospinal fluid on vasospasm, clinical progress and outcome in patients with non-traumatic subarachnoid hemorrhage

*Estudio de los niveles de proteína básica de mielina (MBP) y albúmina modificada por isquemia (IMA) en sangre y líquido cefalorraquídeo, y su relación con el vasoespasmo, la evolución clínica y el pronóstico en pacientes con hemorragia subaracnoidea no traumática*

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## ABSTRACT

**Background:** Non-traumatic subarachnoid hemorrhage (SAH) is associated with high morbidity and mortality, mainly due to secondary brain injury mechanisms such as cerebral vasospasm and delayed cerebral ischemia. Reliable biochemical biomarkers for monitoring disease progression and early outcome assessment remain limited.

**Objective:** To investigate temporal changes in serum and cerebrospinal fluid (CSF) levels of myelin basic protein (MBP) and ischemia-modified albumin (IMA) in patients with non-traumatic SAH and to evaluate their associations with clinical severity and functional outcome.

**Methods:** In this prospective single-center study, 20 patients with non-traumatic SAH were included. Serum and CSF MBP and IMA levels were measured on days 1, 5, and 10 after admission. Clinical severity was assessed using the Modified Fisher scale, Glasgow Coma Scale (GCS), Hunt–Hess scale (HHS), and World Federation of Neurosurgical Societies (WFNS) scale. Functional outcome was evaluated at discharge using the Glasgow Outcome Scale (GOS). Temporal changes and correlations between biomarkers and clinical parameters were analyzed.

**Results:** Serum and CSF MBP levels increased significantly over time ( $p < 0.001$ ), reflecting progressive tissue injury, but showed no consistent association with clinical scores or functional outcome. Serum IMA levels remained elevated without significant temporal variation. In contrast, CSF IMA levels demonstrated a late-phase increase and were significantly associated with clinical deterioration. On day 10, CSF IMA levels correlated negatively with GCS and GOS scores and positively with WFNS scores.

**Conclusion:** MBP reflects cumulative brain injury following SAH, whereas CSF IMA is more closely associated with late-phase neurological deterioration and early functional outcome. CSF IMA may represent a complementary biomarker for prognostic assessment in non-traumatic SAH.

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## RESUMEN

**Palabras clave:**

Hemorragia subaracnoidea  
Myelin basic protein (MBP)  
Ischaemia modified albumin (IMA)  
Vasospasmo

**Introducción:** La hemorragia subaracnoidea (HSA) no traumática se asocia con una elevada morbimortalidad, principalmente debido a mecanismos de lesión cerebral secundaria como el vasoespasmo y la isquemia cerebral tardía. Existen pocos biomarcadores fiables para el seguimiento evolutivo y la evaluación temprana del pronóstico.

**Objetivo:** Analizar los cambios temporales en los niveles séricos y en líquido cefalorraquídeo (LCR) de la proteína básica de mielina (MBP) y la albúmina modificada por isquemia (IMA) en pacientes con HSA no traumática, y evaluar su relación con la gravedad clínica y el resultado funcional.

**Métodos:** Estudio prospectivo unicéntrico que incluyó a 20 pacientes con HSA no traumática. Los niveles de MBP e IMA en suero y LCR se midieron mediante ELISA los días 1, 5 y 10 tras el ingreso. La gravedad clínica se evaluó mediante la escala de Fisher modificada, la Escala de Coma de Glasgow (GCS), la escala de Hunt-Hess y la escala WFNS. El resultado funcional se valoró al alta mediante la Escala de Resultado de Glasgow (GOS). Se analizaron los cambios temporales y las correlaciones entre biomarcadores y parámetros clínicos.

**Resultados:** Los niveles séricos y en LCR de MBP aumentaron significativamente con el tiempo ( $p < 0.001$ ), sin asociación consistente con las escalas clínicas ni con el pronóstico funcional. Los niveles séricos de IMA permanecieron elevados sin cambios significativos. En contraste, la IMA en LCR mostró una asociación significativa con el deterioro clínico tardío. En el día 10, los niveles de IMA en LCR se correlacionaron negativamente con GCS y GOS, y positivamente con WFNS.

**Conclusión:** La MBP refleja la lesión cerebral acumulativa tras la HSA, mientras que la IMA en LCR se asocia con el deterioro neurológico tardío y el resultado funcional temprano. La IMA en LCR podría constituir un biomarcador complementario en la evaluación pronóstica de la HSA no traumática.

**Introduction**

Subarachnoid hemorrhage (SAH) is a high-risk cerebrovascular condition associated with substantial morbidity and mortality, caused by arterial or venous bleeding into the subarachnoid space due to trauma, aneurysm rupture, vascular malformations, bleeding disorders, brain tumors, or anticoagulant-related complications.<sup>1,2</sup> The incidence is estimated to be 2–22 per 100,000 people, and 60% of the patients is between the age of 40 and 60.<sup>3</sup> The most common cause of subarachnoid hemorrhage (SAH) is trauma, whereas ruptured cerebral aneurysms represents the leading cause of non-traumatic SAH. In this context, cerebral vasospasm constitutes the principal determinant of morbidity and mortality following aneurysmal SAH, despite the fact that its underlying pathophysiology and optimal therapeutic strategies remain incompletely understood.<sup>4</sup> Although cerebral vasospasm is detected radiologically in up to 70% of patients, only 20–30% develop clinically significant vasospasm. Accordingly, prognosis in non-traumatic SAH is influenced by multiple interrelated factors, including the location and volume of hemorrhage, rebleeding, vasospasm, acute hydrocephalus, and the overall clinical course.<sup>5,6</sup> Cerebral vasospasm starts on the 3rd day after SAH, peaks on the 6th and 8th days and lasts for 2–3 weeks.<sup>7</sup> It is a clinical diagnosis, and digital subtraction angiography (DSA) remains the gold standard for its detection.<sup>8</sup> Despite advances in clinical and radiological monitoring, the role of biochemical biomarkers in the follow-up of subarachnoid hemorrhage remains controversial, and there is still a need for reliable biomarkers.

Myelin basic protein (MBP) is the second most abundant protein in central nervous system (CNS) myelin after proteolipid protein and plays a crucial role in myelin sheath formation by oligodendrocytes.<sup>9</sup> MBP is widely accepted as a biomarker of brain tissue injury in both traumatic and non-traumatic neurological conditions. Serum MBP levels increase in traumatic brain injury<sup>10</sup> and have been shown to be elevated in cerebrospinal fluid in multiple sclerosis, benign and malignant intracranial tumors, central nervous system infections, and cerebrovascular disorders.<sup>11–13</sup> In addition, serum MBP concentrations may rise significantly in cerebral injuries such as cerebrovascular accident, intracranial tumors, and head trauma.<sup>14</sup> Importantly, elevated peripheral blood MBP levels after aneurysmal subarachnoid hemorrhage reflect the severity of brain parenchymal damage and have been associated with treatment outcomes.<sup>9</sup>

Ischaemia-modified albumin (IMA) is generated by a reduction in the metal-binding capacity of albumin under conditions of oxidative stress

and tissue ischaemia and was initially described in acute ischaemic stroke.<sup>15</sup> It is considered a non-specific biochemical marker reflecting ischaemia and oxidative stress. IMA has been widely used in the diagnosis and clinical assessment of various ischaemia-related conditions, including myocardial infarction, brain and spinal cord injuries, diabetes mellitus, pregnancy-related complications, gynaecological disorders, and other ischaemic pathologies.<sup>16</sup> Elevated serum IMA levels have been observed in a wide range of ischaemia-related conditions, such as cardiovascular and cerebrovascular diseases, metabolic disorders, malignancies, infections, and peripheral vascular diseases.<sup>17–22</sup>

In our study, we aimed to obtain an insight on the correlation between MBP and IMA values in serum and CSF with radiological evaluation, neurological examination, vasospasm and clinical courses and outcome in patients with non-traumatic SAH.

**Materials and methods**

This single-centre, prospective methodological study was conducted on twenty patients admitted to Karadeniz Technical University (KTU) Faculty of Medicine, Farabi Hospital, with a diagnosis of non-traumatic SAH. Patients with a history of traumatic SAH were excluded.

This study was approved by the Karadeniz Technical University Faculty of Medicine Scientific Research Ethics Committee (Institutional Review Board approval number 2022/174, dated 29/07/2022), and all procedures involving human participants were conducted in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all individual participants included in the study.

M. Fisher score was recorded on day 1, and GCS, WFNS, and Hunt-Hess scores were assessed on days 1, 5, and 10. Serum and CSF levels of MBP and IMA were measured using ELISA (enzyme-linked immunosorbent assay) on the 1st, 5th and 10th days at the KTU Biochemistry Laboratory. Correlations were examined between biomarker levels, demographic data, hypertension, radiological and clinical scores, vasospasm, and GOS score at discharge.

**Collection and biochemical analysis of serum and CSF samples**

Blood samples were collected into serum-separating tubes and allowed to clot at room temperature for 20 min, followed by centrifugation at 1800×g for 10 min. The supernatant serum was aliquoted into 1.5 mL polypropylene tubes. Concurrent cerebrospinal fluid (CSF) samples were

obtained and processed under standard sterile conditions. All specimens were stored at  $-80^{\circ}\text{C}$  until analysis.

Quantification of myelin basic protein (MBP) and ischemia-modified albumin (IMA) levels in serum and CSF samples was performed using enzyme-linked immunosorbent assay (ELISA) kits in accordance with the manufacturers' instructions (MBP: Elabscience, Cat No: E-EL-H0161, Lot: ZD40FN4R8949, Wuhan, China; IMA: Bioassay Technology Laboratory, Cat No: E1172Hu, Lot: 202304011, Shanghai, China). Prior to analysis, frozen samples were thawed at room temperature. Standard curves were generated, and appropriate sample volumes were loaded into microplate wells, followed by incubation with specific antibodies and streptavidin–HRP conjugates. After substrate reaction and stop solution addition, absorbance was measured at 450 nm using a VERSA microplate reader (Molecular Devices, CA, USA).

MBP results were reported in  $\mu\text{g/mL}$  and IMA results in  $\text{ng/mL}$ . The intra-assay coefficients of variation (%CV) were 3.78% (serum) and 2.49% (CSF) for MBP, and 4.46% (serum) and 5.20% (CSF) for IMA.

### Statistical evaluation

Statistical analysis was performed by SPSS 29.0 and R-Studio 2026.01.0 softwares. Descriptive statistics of continuous variables are given with mean and standard deviation or with median, minimum and maximum values; qualitative variables are given with frequency and percent. Shapiro–Wilk test was used for test of normality. Friedman and repeated measures Anova test used for  $k$  related sample comparison. Bonferroni corrected Wilcoxon test used for post-hoc analysis. Spearman correlation coefficient used to evaluate the relationship between quantitative variables. For all statistical comparisons with a  $p$  value below 0.05 was assumed as statistically significant.

## Results

### Patient characteristics and baseline clinical data

A total of 20 patients with non-traumatic SAH were included in the study. The mean age of the cohort was  $56.6 \pm 13.6$  years (range: 31–91 years). Thirteen patients (65%) were male and seven (35%) were female. Fourteen patients (60%) had a history of hypertension, while six (40%) did not.

The mean M. Fisher score was  $2.05 \pm 1.36$ . On day 1, the mean Glasgow Coma Scale (GCS) score was  $10.40 \pm 4.91$ , the mean HHS score was  $2.80 \pm 1.40$ , and the mean WFNS score was  $2.95 \pm 1.67$ . No statistically significant changes were observed in GCS, HHS, or WFNS scores across days 1, 5, and 10 ( $p > 0.05$  for all).

### Temporal changes in serum and CSF MBP and IMA levels

Serum MBP levels increased progressively over time, with mean values of  $71.46 \pm 2.30$   $\text{ng/mL}$  on day 1,  $90.20 \pm 18.64$   $\text{ng/mL}$  on day 5, and  $103.37 \pm 23.94$   $\text{ng/mL}$  on day 10. Friedman test analysis demonstrated a statistically significant overall change in serum MBP levels across time points ( $p < 0.001$ ).

CSF MBP levels also showed a marked and continuous increase, with mean values of  $104.43 \pm 21.32$   $\text{ng/mL}$  on day 1,  $143.20 \pm 19.43$   $\text{ng/mL}$  on day 5, and  $182.62 \pm 45.59$   $\text{ng/mL}$  on day 10. The overall temporal change in CSF MBP levels was statistically significant ( $p < 0.001$ ).

Serum IMA levels demonstrated elevated mean values at all time points (day 1:  $112.29 \pm 77.21$   $\text{ng/mL}$ ; day 5:  $126.07 \pm 89.36$   $\text{ng/mL}$ ; day 10:  $126.55 \pm 72.46$   $\text{ng/mL}$ ); however, no statistically significant change was observed over time ( $p = 0.157$ ). Similarly, CSF IMA levels showed a gradual increase from day 1 ( $165.96 \pm 46.11$   $\text{ng/mL}$ ) to day 10 ( $193.83 \pm 34.81$   $\text{ng/mL}$ ), but this change did not reach statistical significance ( $p = 0.059$ ).

Temporal changes in serum and CSF MBP and IMA levels are shown in Fig. 1.

### Longitudinal comparisons of biomarkers and clinical scores

The results of longitudinal comparisons for biomarkers and clinical scores are summarized in Table 1.

Post-hoc pairwise analyses revealed that the increase in serum MBP levels was statistically significant between day 1 and day 5 ( $p = 0.005$ ) and between day 1 and day 10 ( $p < 0.001$ ), whereas the difference between day 5 and day 10 was not statistically significant ( $p = 0.246$ ).

For CSF MBP, pairwise comparisons demonstrated statistically significant increases between day 1 and day 5 ( $p = 0.003$ ), day 1 and day 10 ( $p < 0.001$ ), and day 5 and day 10 ( $p = 0.013$ ).

No statistically significant pairwise differences were identified for serum IMA or CSF IMA levels. Additionally, no significant temporal changes were observed in GCS, HHS, or WFNS scores across the three time points.

### Correlation analyses

#### Day 1 correlations

Correlation analyses among day 1 parameters are shown in Table 2. Age was not significantly correlated with any biochemical or clinical parameter ( $p > 0.05$ ).

The M. Fisher score demonstrated a strong negative correlation with day 1 GCS scores ( $r = -0.701$ ,  $p = 0.001$ ) and a strong positive correlation with day 1 HHS scores ( $r = 0.782$ ,  $p < 0.001$ ). A moderate positive correlation was observed between the M. Fisher score and day 1 WFNS scores ( $r = 0.693$ ,  $p = 0.001$ ).

A moderate positive correlation was identified between day 1 serum MBP levels and day 1 HHS scores ( $r = 0.502$ ,  $p = 0.024$ ). Day 1 CSF IMA levels showed a moderate positive correlation with day 1 HHS scores ( $r = 0.471$ ,  $p = 0.036$ ).

Day 1 GCS scores were strongly and negatively correlated with both day 1 HHS scores ( $r = -0.795$ ,  $p < 0.001$ ) and day 1 WFNS scores ( $r = -0.978$ ,  $p < 0.001$ ).

#### Day 5 correlations

Correlation analyses for day 5 variables are presented in Table 3.

The M. Fisher score demonstrated a strong negative correlation with day 5 GCS scores ( $r = -0.736$ ,  $p < 0.001$ ) and strong positive correlations with day 5 HHS scores ( $r = 0.797$ ,  $p < 0.001$ ) and WFNS scores ( $r = 0.729$ ,  $p < 0.001$ ).

A moderate negative correlation was observed between day 5 serum IMA and day 5 serum MBP levels ( $r = -0.492$ ,  $p = 0.028$ ).

Day 5 GCS scores showed strong negative correlations with day 5 HHS scores ( $r = -0.908$ ,  $p < 0.001$ ) and WFNS scores ( $r = -0.986$ ,  $p < 0.001$ ). A strong positive correlation was found between day 5 HHS and WFNS scores ( $r = 0.881$ ,  $p < 0.001$ ).

#### Day 10 correlations

Day 10 correlation analyses are summarized in Table 4.

The M. Fisher score demonstrated a strong negative correlation with day 10 GCS scores ( $r = -0.743$ ,  $p < 0.001$ ) and strong positive correlations with day 10 HHS scores ( $r = 0.756$ ,  $p < 0.001$ ) and WFNS scores ( $r = 0.769$ ,  $p < 0.001$ ).

A moderate negative correlation was identified between day 10 CSF IMA levels and day 10 GCS scores ( $r = -0.507$ ,  $p = 0.022$ ), while a moderate positive correlation was observed between day 10 CSF IMA levels and day 10 WFNS scores ( $r = 0.457$ ,  $p = 0.043$ ).

Day 10 GCS scores were strongly and negatively correlated with both day 10 HHS scores ( $r = -0.977$ ,  $p < 0.001$ ) and WFNS scores ( $r = -0.980$ ,  $p < 0.001$ ). A strong positive correlation was observed between day 10 HHS and WFNS scores ( $r = 0.969$ ,  $p < 0.001$ ).

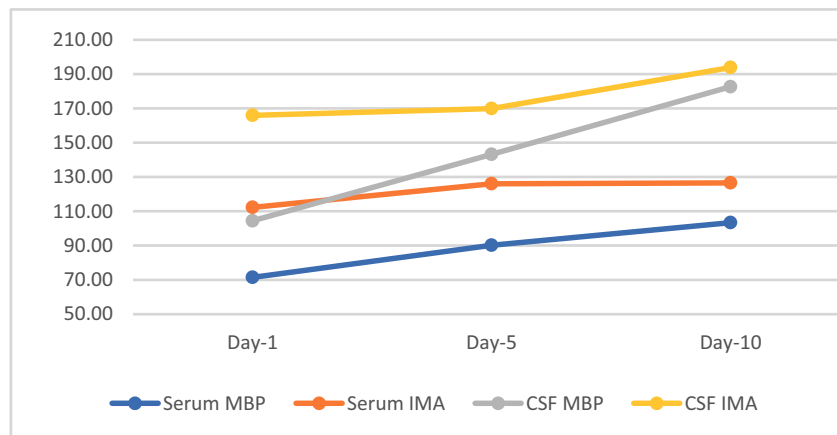


Fig. 1. Temporal changes in serum and CSF MBP and IMA levels.

Table 1

Longitudinal comparisons for biomarkers and clinical scores.

	Day 1	Day 5	Day 10	p
Serum MBP	71.46 ± 2.30	90.20 ± 18.64	103.37 ± 23.94	< 0.001 <sup>a</sup>
Serum IMA	112.29 ± 77.21	126.07 ± 89.36	126.55 ± 72.46	0.157 <sup>d</sup>
CSF MBP	104.43 ± 21.32	143.20 ± 19.43	182.62 ± 45.59	< 0.001 <sup>a</sup>
CSF IMA*	165.96 ± 46.11	169.89 ± 40.27	193.83 ± 34.81	0.059 <sup>b</sup>
GCS	10.40 ± 4.91	10.35 ± 4.90	9.75 ± 5.31	0.832 <sup>d</sup>
Hunt-Hess	2.80 ± 1.40	2.60 ± 1.54	2.85 ± 1.76	0.283 <sup>d</sup>
WFNS	2.95 ± 1.67	2.95 ± 1.76	3.00 ± 1.89	0.529 <sup>d</sup>

<sup>a</sup> Friedman test.

<sup>b</sup> Repeated measures ANOVA.

<sup>c</sup> Bonferonni corrected Wilcoxon test.

Table 2

Correlations between day 1 biomarker levels and clinical scores.

	Age	M. Fisher	Serum MBP	Serum IMA	CSF MBP	CSF IMA	GCS	Hunt-Hess
M. Fisher	$r = 0.051$ $p = 0.832$	–						
Serum MBP	$r = -0.189$ $p = 0.426$	$r = 0.182$ $p = 0.441$	–					
Serum IMA	$r = -0.075$ $p = 0.752$	$r = -0.034$ $p = 0.886$	$r = 0.300$ $p = 0.199$	–				
CSF MBP	$r = 0.005$ $p = 0.982$	$r = 0.044$ $p = 0.855$	$r = -0.188$ $p = 0.428$	$r = -0.323$ $p = 0.164$	–			
CSF IMA	$r = -0.157$ $p = 0.510$	$r = 0.352$ $p = 0.128$	$r = 0.213$ $p = 0.368$	$r = 0.104$ $p = 0.663$	$r = -0.048$ $p = 0.840$	–		
GCS	$r = 0.086$ $p = 0.720$	$r = -0.701$ $p = 0.001a$	$r = -0.226$ $p = 0.338$	$r = 0.020$ $p = 0.934$	$r = 0.356$ $p = 0.123$	$r = -0.256$ $p = 0.275$	–	
Hunt-Hess	$r = -0.099$ $p = 0.678$	$r = 0.782$ $p < 0.001a$	$r = 0.502$ $p = 0.024b$	$r = 0.096$ $p = 0.688$	$r = -0.255$ $p = 0.277$	$r = 0.471$ $p = 0.036b$	$r = -0.795$ $p < 0.001a$	–
WFNS	$r = -0.020$ $p = 0.935$	$r = 0.693$ $p = 0.001b$	$r = 0.216$ $p = 0.360$	$r = -0.028$ $p = 0.906$	$r = -0.282$ $p = 0.229$	$r = 0.232$ $p = 0.326$	$r = -0.978$ $p < 0.001a$	$r = 0.798$ $p < 0.001a$

Spearman correlation analysis.

<sup>a</sup> Strong correlation.

<sup>b</sup> Moderate correlation.

#### Correlation between GOS and biomarker levels

Spearman correlation analysis was performed to evaluate the relationship between Glasgow Outcome Scale (GOS) scores and serum and cerebrospinal fluid (CSF) biomarker levels measured on days 1, 5, and 10. No statistically significant correlations were observed between GOS scores and serum MBP levels on day 1 ( $r = -0.253$ ,  $p = 0.281$ ), day 5 ( $r = 0.258$ ,  $p = 0.272$ ), or day 10 ( $r = 0.217$ ,  $p = 0.358$ ). Similarly,

GOS scores were not significantly correlated with CSF MBP levels on day 1 ( $r = 0.352$ ,  $p = 0.128$ ), day 5 ( $r = 0.205$ ,  $p = 0.387$ ), or day 10 ( $r = 0.120$ ,  $p = 0.615$ ).

With respect to ischemia-modified albumin (IMA), no significant correlations were identified between GOS scores and serum IMA levels on day 1 ( $r = 0.111$ ,  $p = 0.641$ ), day 5 ( $r = 0.334$ ,  $p = 0.150$ ), or day 10 ( $r = 0.226$ ,  $p = 0.338$ ). In contrast, a statistically significant moderate negative correlation was observed between GOS scores and CSF IMA

**Table 3**  
Correlations between day 5 biomarker levels and clinical scores.

	Age	M. Fisher	Serum MBP	Serum IMA	CSF MBP	CSF IMA	GCS	Hunt-Hess
M. Fisher	$r = 0.051$ $p = 0.832$							
Serum MBP	$r = -0.072$ $p = 0.762$	$r = -0.179$ $p = 0.449$	–					
Serum IMA	$r = -0.020$ $p = 0.932$	$r = -0.290$ $p = 0.215$	$r = -0.492$ $p = 0.028^b$	–				
CSF MBP	$r = -0.110$ $p = 0.645$	$r = 0.125$ $p = 0.600$	$r = 0.169$ $p = 0.476$	$r = -0.029$ $p = 0.902$	–			
CSF IMA	$r = -0.214$ $p = 0.365$	$r = 0.318$ $p = 0.172$	$r = 0.104$ $p = 0.663$	$r = -0.083$ $p = 0.729$	$r = 0.367$ $p = 0.111$	–		
GCS	$r = 0.003$ $p = 0.991$	$r = -0.736$ $p < 0.001^a$	$r = 0.188$ $p = 0.428$	$r = 0.286$ $p = 0.221$	$r = 0.233$ $p = 0.323$	$r = -0.055$ $p = 0.818$	–	
Hunt-Hess	$r = -0.007$ $p = 0.978$	$r = 0.797$ $p < 0.001^a$	$r = -0.279$ $p = 0.234$	$r = -0.217$ $p = 0.358$	$r = -0.105$ $p = 0.660$	$r = 0.120$ $p = 0.614$	$r = -0.908$ $p < 0.001^a$	–
WFNS	$r = 0.074$ $p = 0.757$	$r = 0.729$ $p < 0.001^a$	$r = -0.171$ $p = 0.472$	$r = -0.290$ $p = 0.215$	$r = -0.281$ $p = 0.230$	$r = 0.027$ $p = 0.911$	$r = -0.986$ $p < 0.001^a$	$r = 0.881$ $p < 0.001^a$

Spearman correlation analysis.

<sup>a</sup> Strong correlation.

<sup>b</sup> Moderate correlation.

**Table 4**  
Correlations between day 10 biomarker levels and clinical scores.

	Age	M. Fisher	Serum MBP	Serum IMA	CSF MBP	CSF IMA	GCS	Hunt-Hess
M. Fisher	$r = 0.051$ $p = 0.832$							
Serum MBP	$r = 0.263$ $p = 0.262$	$r = -0.284$ $p = 0.225$	–					
Serum IMA	$r = -0.050$ $p = 0.835$	$r = -0.085$ $p = 0.722$	$r = -0.283$ $p = 0.227$	–				
CSF MBP	$r = 0.081$ $p = 0.736$	$r = 0.145$ $p = 0.542$	$r = 0.123$ $p = 0.604$	$r = -0.120$ $p = 0.613$	–			
CSF IMA	$r = 0.249$ $p = 0.289$	$r = 0.319$ $p = 0.171$	$r = -0.146$ $p = 0.539$	$r = -0.441$ $p = 0.052$	$r = 0.092$ $p = 0.701$	–		
GCS	$r = -0.015$ $p = 0.950$	$r = -0.743$ $p < 0.001^a$	$r = 0.272$ $p = 0.246$	$r = 0.159$ $p = 0.503$	$r = 0.126$ $p = 0.598$	$r = -0.507$ $p = 0.022^b$	–	
Hunt-Hess	$r = 0.014$ $p = 0.953$	$r = 0.756$ $p < 0.001^a$	$r = -0.265$ $p = 0.258$	$r = -0.094$ $p = 0.694$	$r = -0.162$ $p = 0.495$	$r = 0.407$ $p = 0.075$	$r = -0.977$ $p < 0.001^a$	–
WFNS	$r = 0.047$ $p = 0.844$	$r = 0.769$ $p < 0.001^a$	$r = -0.264$ $p = 0.260$	$r = -0.105$ $p = 0.661$	$r = -0.078$ $p = 0.744$	$r = 0.457$ $p = 0.043^b$	$r = -0.980$ $p < 0.001^a$	$r = 0.969$ $p < 0.001^a$

Spearman correlation analysis.

<sup>a</sup> Strong correlation.

<sup>b</sup> Moderate correlation.

levels measured on day 10 ( $r = -0.531$ ,  $p = 0.016$ ). No significant correlations were found between GOS scores and CSF IMA levels on day 1 ( $r = -0.227$ ,  $p = 0.336$ ) or day 5 ( $r = 0.106$ ,  $p = 0.656$ ).

## Discussion

Spontaneous SAH is a complex clinical condition associated with high early mortality and morbidity, with a clinical course that is particularly characterized by the development of vasospasm and delayed cerebral ischemia within the first 10 days. Secondary brain injury occurring during this period progresses through mechanisms such as neuroinflammation, microcirculatory dysfunction, oxidative stress, and disruption of blood–brain barrier integrity.

Biochemical biomarkers are widely used in the diagnosis, prognostic assessment, and outcome prediction of various neuropathological conditions.<sup>23,24</sup> However, no specific biochemical biomarker is currently available for the monitoring of non-traumatic SAH patients or SAH-associated vasospasm. Therefore, monitoring early-phase biomarkers

following SAH has gained increasing importance for predicting clinical deterioration and determining prognosis.

In the present study, the temporal changes in serum and CSF levels of MBP and IMA on days 1, 5, and 10 were evaluated, and the associations of these biomarkers with clinical scoring systems were investigated. Our findings indicate that MBP levels increased significantly in both serum and CSF from the early phase onward, whereas IMA levels did not show a significant change in serum but may be associated with clinical deterioration in CSF, particularly in the late phase. In addition, correlations between biomarker levels and functional outcome assessed by the GOS were examined, and a significant association was identified between CSF IMA levels measured on day 10 and GOS scores.

Serum MBP levels demonstrated a statistically significant increase from day 1 to day 10 ( $p < 0.001$ ). The observation that this increase was significant particularly between days 1–5 ( $p = 0.005$ ) and days 1–10 ( $p < 0.001$ ), while the change between days 5–10 did not reach statistical significance ( $p = 0.246$ ), suggests that serum MBP rises predominantly in the early period following SAH and

that the rate of increase may subsequently enter a relative plateau phase.

Wasik et al. reported that peripheral blood MBP concentrations following aneurysmal SAH reflect the severity of brain parenchymal injury and are associated with treatment outcomes.<sup>9</sup> Similarly, in a study by Thomas et al., comparisons of serum MBP levels in patients with intracranial pathologies versus those with spinal or peripheral nerve disorders demonstrated elevated serum MBP levels in intracranial pathologies, whereas no such increase was observed in patients with spinal or peripheral nerve involvement.<sup>14</sup>

In another study conducted by Hoyle et al., patients followed for spontaneous SAH exhibited progressively increasing preoperative serum MBP levels, which reached their peak on postoperative day 10.<sup>25</sup> Furthermore, Zheng et al. reported higher serum MBP levels in spontaneous SAH patients who developed symptomatic vasospasm. Elevated serum MBP levels were also found to be associated with vasospasm and poor 6-month outcomes and were correlated with WFNS scores.<sup>26</sup>

Consistent with these previous studies, our findings demonstrate a gradual increase in serum MBP levels (overall temporal change:  $p < 0.001$ ). Importantly, our results suggest that serum MBP may serve as a valuable biomarker for detecting early-phase tissue injury following spontaneous SAH.

CSF MBP levels demonstrated a more pronounced and continuous increasing pattern. The change in CSF MBP levels between days 1, 5, and 10 was statistically significant ( $p < 0.001$ ), and all pairwise comparisons (days 1–5, 1–10, and 5–10) also showed statistical significance (day 1–5:  $p = 0.003$ ; day 1–10:  $p < 0.001$ ; day 5–10:  $p = 0.013$ ). These findings support the notion that MBP may act as a more direct and cumulative marker of injury within the CSF compartment.

In a study published by Vries et al., CSF MBP levels were evaluated in neurosurgical patients. CSF MBP levels were found to be significantly elevated in patients with intracranial hemorrhage secondary to aneurysm or arteriovenous malformation, whereas no significant elevation was observed in patients with benign intracranial pathologies.<sup>11</sup> Similarly, Hirasma et al. investigated CSF MBP levels together with SAH extent, degree of cerebral infarction, and discharge outcomes, and suggested that CSF MBP may serve as a marker reflecting the severity of vasospasm-related brain injury.<sup>27</sup>

In our study, consistent with the literature, both serum and CSF MBP levels showed an increasing trend. However, when evaluated together with clinical scoring systems, no association was observed between CSF or serum MBP levels and clinical scores, except for a moderate positive correlation between day 1 serum MBP levels and the HHS scores ( $r = 0.502$ ,  $p = 0.024$ ). This may be explained by the lack of significant changes in GCS, HHS, and WFNS scores between days 1, 5, and 10 (all  $p > 0.05$ ), and the absence of a clear group-level clinical trend. Nevertheless, the significant increase in MBP levels in both serum and CSF suggests that clinical scoring systems may not always fully capture the dynamics of biological injury. In addition, factors such as sedation, mechanical ventilation, hemodynamic optimization, and other supportive intensive care interventions may reduce the variability of clinical scores.

Since MBP is one of the principal structural components of central nervous system myelin, increases in serum and CSF MBP levels may be interpreted as a biochemical reflection of myelin sheath and axonal involvement during secondary injury processes following SAH. From this perspective, MBP elevation may represent not only the primary effect of hemorrhage but also the cumulative burden of tissue injury resulting from secondary mechanisms, including blood–brain barrier disruption, inflammatory responses, and hypoperfusion.

IMA is a biomarker associated with ischemia and oxidative stress and reflects structural modifications of albumin. In intracranial pathologies, Gunduz et al. reported that serum IMA levels measured within the first 24 h were higher in patients with acute cerebrovascular disease compared with healthy controls.<sup>24</sup> Similarly, Han et al. demonstrated a

marked elevation in serum IMA levels as early as 3 h after the onset of acute cerebrovascular pathology.<sup>28</sup>

In a study by Elshony et al., serum fibulin-5 and IMA levels measured within the first hours after hospital admission were significantly higher in patients with ischemic stroke, intracerebral hemorrhage, and SAH compared with controls. Additionally, fibulin-5 and IMA levels showed positive correlations with ischemic lesion volume and hemorrhage/SAH severity.<sup>29</sup>

In our study, serum IMA levels did not show a statistically significant change between days 1, 5, and 10 ( $p = 0.157$ ); however, the mean values at all time points remained elevated. The lack of significant temporal variation in serum IMA levels may be related to concomitant clinical conditions that could influence the ischemic oxidative stress response, as well as intensive care-related factors such as sedation, mechanical ventilation, and the use of vasopressor agents for hemodynamic optimization.

Although IMA has been proposed as an early indicator of ischemia and oxidative stress, it has also been reported to increase in various systemic conditions other than cardiac ischemia, including sepsis, metabolic syndrome, and diabetes.<sup>16,17,19–22</sup> Therefore, serum IMA levels should not be interpreted independently of the clinical context and should preferably be evaluated together with more compartment-specific measurements or other clinical parameters.

In our study, serum IMA was measured using an ELISA method and expressed in ng/mL. Therefore, direct comparisons with studies using the Albumin Cobalt Binding test, which typically reports results in U/mL, may not be appropriate. However, in the literature, Aydogan et al. measured serum IMA levels using ELISA in ng/mL in patients with acute ischemic stroke and found significantly higher median serum IMA levels compared with healthy controls. They also defined cut-off values of  $> 58$  ng/mL for acute ischemic stroke diagnosis,  $> 76.7$  ng/mL for stroke severity, and  $> 65.4$  ng/mL for poor 3-month outcomes.<sup>30</sup>

Despite the absence of a significant temporal increase, the median serum IMA values measured on days 1, 5, and 10 ( $\sim 98$ – $109$  ng/mL) are consistent with previous literature demonstrating elevated IMA levels in acute and ischemia-related processes such as SAH.

To the best of our knowledge, no previous studies have evaluated CSF IMA levels in patients with non-traumatic SAH. Since multiple systemic and clinical factors can influence serum IMA levels and affect the clinical oxidative stress response, we hypothesized that measuring IMA in CSF, as a more central nervous system-specific compartment, may better reflect the clinical course.

In our study, CSF IMA levels demonstrated an increasing trend; however, this change remained at borderline statistical significance ( $p = 0.059$ ). Despite the borderline temporal change, it is noteworthy that CSF IMA showed significant correlations with clinical scores, particularly on day 10. A moderate negative correlation was observed between day 10 CSF IMA levels and day 10 GCS scores ( $r = -0.507$ ,  $p = 0.022$ ), whereas a moderate positive correlation was found between day 10 CSF IMA levels and day 10 WFNS scores ( $r = 0.457$ ,  $p = 0.043$ ). These findings suggest that CSF IMA may be more closely associated with late-phase clinical deterioration following SAH.

This observation is consistent with the hypothesis that ischemic and oxidative stress burden within the CSF compartment may increase during the period when vasospasm and delayed ischemic injury risk become more prominent in spontaneous SAH patients (approximately days 7–10).

An important strength of our study is that correlation analyses were evaluated separately for each time point. The identification of a moderate positive correlation between day 1 serum MBP levels and day 1 HHS scores ( $r = 0.502$ ,  $p = 0.024$ ) suggests that MBP may be associated with clinical severity in the early phase. Similarly, the moderate positive correlation observed between day 1 CSF IMA levels and day 1 HHS scores ( $r = 0.471$ ,  $p = 0.036$ ) indicates that early oxidative stress/ischemic responses may be associated with clinical severity.

On the other hand, a moderate negative correlation was observed between day 5 serum IMA and day 5 serum MBP levels ( $r = -0.492$ ,  $p = 0.028$ ), which may appear contradictory. However, this finding may indicate that these biomarkers do not necessarily represent the same pathophysiological axis in all patients. In some cases, markers of myelin/axonal injury may predominate, whereas in others, oxidative stress responses may be more prominent. Considering the heterogeneous clinical course of SAH, this finding supports the possibility that different biological processes may contribute to disease progression with varying relative impact across individuals.

When studies evaluating the relationship between GOS and biomarkers are considered, heterogeneous associations between myelin basic protein (MBP) and functional outcome have been reported. Previous studies by Zheng et al. and Wasik et al. identified associations between elevated serum MBP levels and unfavorable long-term outcomes assessed at 3 and 6 months.<sup>9,26</sup> In our study, no significant correlations were observed between GOS and serum MBP or serum IMA at any time point (all  $p > 0.05$ ). Functional outcome in our study was evaluated at the time of discharge or death, which may partly account for the lack of comparable MBP-GOS associations. Notably, unlike prior studies focusing primarily on serum biomarkers, the present analysis included cerebrospinal fluid measurements and identified a significant association between day 10 CSF IMA levels and GOS scores ( $r = -0.531$ ,  $p = 0.016$ ). This finding underscores the potential relevance of CSF-based biomarkers in outcome assessment and highlights CSF IMA as a marker warranting further investigation in larger cohorts.

The presence of strong correlations among clinical grading scales supports the internal consistency of the cohort, including strong negative correlations between M. Fisher and GCS scores (day 1:  $r = -0.701$ ,  $p = 0.001$ ; day 5:  $r = -0.736$ ,  $p < 0.001$ ; day 10:  $r = -0.743$ ,  $p < 0.001$ ) and strong positive correlations between M. Fisher and Hunt-Hess/WFNS scores across all time points (all  $p < 0.001$ ).

## Conclusion

The significant early increase of MBP levels in both serum and CSF suggests that MBP may represent a promising biomarker candidate for monitoring secondary brain injury following SAH. Although no significant temporal change was observed in serum IMA levels, the finding that CSF IMA demonstrated significant correlations with clinical deterioration, particularly on day 10, suggests that CSF IMA may have prognostic relevance during the later phase of the disease course. Notably, the significant association observed between day 10 CSF IMA levels and GOS scores further supports the potential value of CSF IMA in relation to early functional outcome assessment. Future studies with larger sample sizes, stratification according to the presence of vasospasm, and support from multivariate analyses will be necessary to clarify the clinical utility and potential translational applicability of these biomarkers.

## Limitations

The main limitations of this study are the relatively small sample size ( $n = 20$ ) and the absence of long-term follow-up data. Although the limited cohort size may restrict statistical power and generalizability, the consistency of biomarker trends and their correlations with established clinical grading scales support the internal validity of the findings. In addition, functional outcome was assessed at discharge, precluding evaluation of long-term prognostic implications; therefore, larger prospective studies with extended follow-up are warranted to further clarify the clinical relevance of these biomarkers.

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## Ethical considerations

This study was approved by the Karadeniz Technical University Faculty of Medicine Scientific Research Ethics Committee (Institutional Review Board approval number 2022/174, dated 29/07/2022), and all procedures involving human participants were conducted in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

## Informed consent

Written informed consent was obtained from all individual participants included in the study. The confidentiality of all participants was protected.

## Declaration of generative AI and AI-assisted technologies in the writing process

None declared.

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## Conflict of interests

The authors declare no conflicts of interest.

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