

Serum Sestrin2 Emerges as a Prognostic Biomarker of Human Aneurysmal Subarachnoid Hemorrhage: A Prospective Observational Cohort Single-Center Study

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Background: Sestrin2 functions as a neuroprotective factor. Herein, serum sestrin2 was investigated with respect to its associations with severity, delayed cerebral ischemia (DCI) and prognosis of aneurysmal subarachnoid hemorrhage (aSAH).

Methods: In this prospective, observational, cohort, single-center study, serum sestrin2 levels were measured at entry into the study in 45 healthy controls and at admission in 135 aSAH patients. Also, they were gauged in other time points (namely, at days 1, 2, 3, 5 and 7) among 45 patients. Unfavorable prognosis was defined as extended Glasgow Outcome Scale (GOSE) scores of 1–4 at six months after aSAH.

Results: Serum sestrin2 levels were immediately raised at admission in patients, increased thereafter, peaked at day 2, declined afterwards till day 7, and were significantly higher than those in controls (all $P < 0.001$). Serum sestrin2 levels had independent correlation with Hunt-Hess scores (beta, 1.715; 95% confidence interval (CI), 0.595–2.835; $P = 0.003$) and modified Fisher scores (beta, 2.505; 95% CI, 1.102–3.907; $P = 0.001$). Alternatively, serum sestrin2 levels, which were independently correlated with 6-month GOSE scores (beta, -0.050 ; 95% CI, -0.099 – 0.001 ; $P = 0.044$), were independently associated with DCI (odds ratio, 1.079; 95% CI, 1.008–1.156; $P = 0.029$) and unfavorable prognosis (odds ratio, 1.093; 95% CI, 1.020–1.172; $P = 0.012$). DCI and prognosis prediction models, which were composed of serum sestrin2, Hunt-Hess scores and modified Fisher scores, were comparatively stable and clinically beneficial under calibration curve and decision curve. Prognosis prediction model showed significantly higher area under receiver operating characteristic curve than serum sestrin2, Hunt-Hess scores and modified Fisher scores alone (all $P < 0.05$).

Conclusion: A significant enhancement of serum sestrin2 levels after aSAH is independently related to severity, DCI and poor prognosis following aSAH. The models incorporating serum sestrin2 perform well in predicting the DCI and prognosis of aSAH patients. Presumably, determination of serum sestrin2 may be of clinical significance in aSAH.

Keywords: aneurysm, subarachnoid hemorrhage, Sestrin2, delayed cerebral ischemia, prognosis, severity

Introduction

Aneurysmal subarachnoid hemorrhage (aSAH), which is characterized as a hemorrhagic stroke resulting from aneurysmal rupture, is the principal cause of death and disability among survivors around the world.¹ When bleedings accumulate inside the subarachnoid space, some subsequent phenomena, such as early brain injury (EBI) and delayed cerebral ischemia (DCI), occur.^{2,3} Both EBI and DCI are the two key pathophysiological processes pertaining to acute brain injury following aSAH.⁴ Oxidative stress and inflammation have been acknowledged as the two main molecular mechanisms contributing to acute brain injury after aSAH.^{5,6} The excessive generation of reactive oxygen species and cytokines results in cellular toxicity, thereby inducing neuronal death, brain edema and blood–brain barrier disruption; and such brain damage elicited neurologic dysfunction and even death of patients with aSAH.⁷ Clinically, several severity assessment scales, such as the Hunt-Hess scale and modified Fisher scales, have been universally adopted as the prognostic predictors of aSAH.⁸ Noteworthy,

biochemical markers in peripheral blood, such as glial fibrillary astrocyte protein, myelin basic protein, S100B, C-reactive protein and interleukin-6, are ubiquitously studied and exploration of apposite prognostic biomarkers has attracted widespread attentions in aSAH-related neurologic science during recent decades.^{9,10}

Sestrin2 is recognized as hypoxia-inducible gene 95, which is a very important member of the sestrin family.¹¹ Its production can be induced by various insults, such as hypoxia, oxidative stress, inflammation and ischemia.¹² Accumulating evidence has pointed to a consensus concept that sestrin2 may be a protective shield because of its anti-oxidant and anti-inflammatory properties in numerous diseases, such as chronic obstructive pulmonary disease, coronary artery disease and type 2 diabetes mellitus.^{13–15} Also, there has been a common view that high blood sestrin2 levels in those patients may reflect a compensatory response to enhanced insults.¹⁶ In central nervous system, sestrin2 may be mainly expressed by neurons and microglia.^{17–19} It is believably demonstrated that sestrin2 may be neuroprotective based on experimental studies concerning ischemic stroke,¹⁷ traumatic brain injury¹⁸ and subarachnoid hemorrhage.¹⁹ Intriguingly, serum sestrin2 levels had been found to be were significantly elevated in patients with Parkinson's disease,²⁰ Alzheimer's disease or mild cognitive impairment.²¹ It is referred that serum sestrin2 may be a biomarker for reflecting acute brain injury. Herein, we measured serum sestrin2 levels and further explored its significance as a prognostic biochemical marker of aSAH.

Methods

Study Design

This was a prospective, observational, cohort study, which was implemented at an academic medical center in Ningbo, Zhejiang Province, China from May 2018 to October 2021. This study was assigned into the two parts. In the first part, we intended to observe dynamic change of serum sestrin2 levels after aSAH and thus peripheral blood samples were collected at entry into the study from controls and at admission and days 1, 2, 3, 5 and 7 from all patients, who agreed with blood-drawings at multiple time-points. In the second part, we attempted to investigate the prognostic role of serum sestrin2 in aSAH and therefore peripheral blood was acquired at admission from all patients, who gave consent to blood-obtainment at admission. The 2008 Revision of the Declaration of Helsinki and its later amends were obeyed when the current study went on. The study protocol was endorsed by the Institutional Review Committee at Ningbo Medical Center Lihuli Hospital (No. LHH23209). Prior to blood-collection, informed consent was signed by patients' legal representatives or controls themselves.

Participant Enrollment

The consecutively recruited subjects in the current study included patients and healthy controls. Inclusion criteria for patients were depicted as follows: (1) Consent to participation in this study; (2) Hospital admission with first-ever stroke; (3) A computerized tomography (CT) confirmation of SAH; (4) An age of 18 years or older; (5) A CT angiography or digital subtraction angiography diagnosis of intracranial aneurysm; (6) Rupture of single aneurysm; (7) Hospitalization of 24 hours after symptom onset; and (8) Aneurysm clipping or interventional treatment of 48 hours following hospital admission. Exclusion criteria for patients were shown below: (1) Rerupture of the aneurysm; (2) Pseudoaneurysm; (3) Previous or present other neurological diseases, such as cerebrovascular disorders, brain degenerative diseases and intracranial tumors; (4) Previous or present certain diseases, such as malignancies, immune system diseases and severe dysfunction of other organs; and (5) Other specific conditions, such as loss to follow-up, incomplete information and hemolysis. Controls were chosen from healthy volunteers. They did not have chronic comorbidities, such as hypertension, diabetes mellitus and coronary heart disease, there were normal data in some routine blood tests, such as blood sugar detection, blood cell analyses and blood electrolyte measurements, and some specific auxiliary examinations, such as chest x-ray check and routine electrocardiogram, did not show obvious abnormal results.

Data Collection

We collected some conventional information, such as demographical data, adverse lifestyle habits, chronic comorbidities, medication histories and vital signs. The Hunt-Hess scale, varying from 1 to 5, was adopted to evaluate neurologic status and the modified Fisher grading scale, varying from 0 to 4, was applied as a radiological severity indicator. We recorded

conformation, location and size of aneurysms. For the sake of securing aneurysms, neurosurgical clipping or endovascular intervention was employed. Head CT scans were implemented on the first day, the first week, the second week and the first month postoperatively, and diagnosis of DCI was verified in accordance with the previous criteria.²² Intraventricular hemorrhage and acute hydrocephalus were experienced in some patients. As necessary, external ventricular drainage was performed. The recorded complications included pneumonia and seizure. Extended Glasgow Outcome Scale (GOSE) scores varied from 1 to 8 and GOSE scores of 1–4 at six months after the onset of symptom was deemed as an unfavorable prognosis.²³

Immune Analysis

Venous blood was drawn, once controls were permitted into the study. Among a portion of patients, venous blood samples were obtained at admission. And among another portion of patients, venous blood samples were collected at days 0 (at admission) 1, 2, 3, 5 and 7 after aSAH. All blood samples were immediately placed in 5 mL gel-containing biochemistry tubes. Blood was centrifugated and thereby serum was separated. Afterwards, an aliquot of extracted serum sample was stored at a -80°C refrigerator for later measurements. Every at most three months, a batch of serum samples were melted and serum sestrin2 levels were gauged. All measurements were in duplicate done by the same skilled technician, who was inaccessible to the clinical materials. The enzyme-linked immunosorbent assay kits (catalog number, E3437Hu) were bought from Bioassay Technology Laboratory (Shanghai, China). The detection sensitivity was 0.01 ng/mL, detection range was from 0.05 to 15 ng/mL, intra-assay coefficients of variation were $<8\%$ and inter-assay coefficients of variation were $<10\%$.

Statistical Analysis

As for data analysis, we used the three statistical software packages as follows: SPSS (version 22.0; SPSS Inc., Chicago, IL, USA), MedCalc 20.1 (MedCalc Software, Mariakerke, Belgium) and R software (version 3.5.1; <https://www.r-project.org>). Qualitative variables, which are shown in the form of the counts (percentages), were compared between the group using the chi-square test or Fisher's exact test as appropriate. As regard to quantitative variables, the Shapiro–Wilk test was done to evaluate data distributions. Subsequently, they were presented as the mean (standard deviation, SD) or the median (25th–75th percentiles) as appropriate. Also, the Mann–Whitney *U*-test or the independent *t*-test was adopted for intergroup comparisons. The Kruskal–Wallis test or Friedman test was employed for multiple-group comparisons of serum sestrin2 levels. The Spearman correlation coefficient test and the multivariable linear regression analysis was sequentially used for bivariate correlation analyses. The binary logistic regression models were constructed, in which DCI and 6-month poor prognosis were regarded as dependent variables. The independent predictors were incorporated into nomograms, and then calibration curves, decision curves and restricted cubic splines were drawn to assess the prediction model. Area under receiver operating characteristic (ROC) curve (AUC) was estimated to assess discriminative efficiency. The Z-test was carried out for comparing AUCs. The cutoff criterion was selected via the Youden method. When two-tailed P-value was less than 0.05, the difference was deemed as statistical significance.

Results

Participant Recruitments

Based on the specified inclusion criteria, an aggregate of 186 patients, who were inflicted with aSAH, underwent an initial observational assessment. Afterwards, fifty-one patients were removed from the current study owing to the reasons displayed in Figure 1. Eventually, one hundred and thirty-five patients were chosen for the data analysis. Among them, there were 48 cases with the development of six-month unfavorable prognosis, 35 cases presenting with DCI and 45 cases who accepted blood-sample collections at admission and at days 1, 2, 3, 5 and 7 after aSAH. Additionally, forty-five controls were selected.

Participants' Basic Characteristics

Controls were aged from 33 to 77 years (mean, 47.4 years; SD, 9.8 years), comprised 23 females and 22 males, and consisted of 15 alcohol drinkers and 14 cigarette smokers, as well as their body mass index ranged from 19.3 to 30.2 kg/

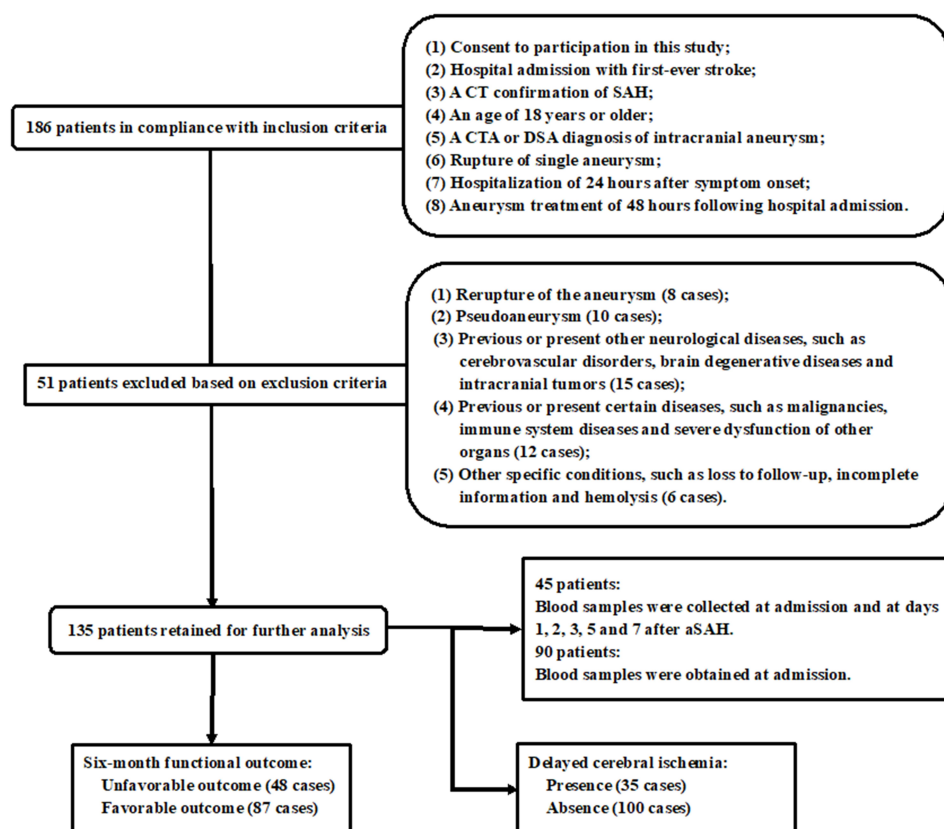


Figure 1 Flowing diagram for enrolling eligible patients with aneurysmal subarachnoid hemorrhage. A total of 186 patients obtained an initial evaluation based on the presented inclusion criteria; 51 patients were removed according to the prespecified exclusion criteria; and ultimately 135 patients were retained for statistical analysis. **Abbreviations:** aSAH, aneurysmal subarachnoid hemorrhage; CT, computed tomography; CTA, computed tomography angiography; DSA, digital subtraction angiography.

m^2 (mean, 24.1 kg/m^2 ; SD, 2.8 kg/m^2). Among this special group of 45 patients, there were 28 females and 17 males, the age ranged from 32–73 years (mean, 49.9 years; SD, 10.5 years), 13 alcohol drinkers and 13 cigarette smokers were revealed, and body mass index ranged from 19.6 to 28.7 kg/m^2 (mean, 23.3 kg/m^2 ; SD, 2.4 kg/m^2). As for the differences of mean age and body mass index, as well as gender, alcohol drinker and cigarette smoker percentages between controls and that special group of 45 aSAH patients, statistical significances were not confirmed (all $P > 0.05$).

This cohort of 135 aSAH patients (76 females and 59 males) were aged from 28 to 73 years (mean, 52.1 years; SD, 10.7 years). Their body mass index ranged from 19.0 to 30.1 kg/m^2 (mean, 23.6 kg/m^2 ; SD, 2.5 kg/m^2). There were 41 cigarette smoker, 36 alcohol drinkers, 25 hypertensive individuals, 14 diabetic subjects, 30 dyslipidemic suffers and 7 patients sustaining coronary heart disease. In total, 20, 7, 13 and 21 patients orally took statins, anticoagulants, antiplatelet and antihypertensive agents, respectively. And eleven patients were orally administrated with hypoglycemic drugs or were subcutaneously injected with insulin. Systolic arterial blood pressure, diastolic arterial blood pressure and mean arterial blood pressure ranged from 84 to 192 mmHg (mean, 131.1 mmHg; SD, 21.3 mmHg), from 48 to 110 mmHg (mean, 81.9 mmHg; SD, 13.3 mmHg) and from 66 to 151 mmHg (mean, 106.5 mmHg; SD, 16.2 mmHg), respectively. The Hunt-Hess scores varied from 1 to 5, with a median value of 3 (lower-upper quartiles, 2–4) and the modified Fisher scores varied from 1 to 4, with a median value of 2 (lower-upper quartiles, 2–3). Intracranial aneurysms in 104 patients were located in anterior circulation and those in the remainders were in posterior circulation; 113 patients occupied cystic aneurysms and other shapes were displayed in aneurysms of the remaining patients. Aneurysms with diameter $< 10 \text{ mm}$ and $\geq 10 \text{ mm}$ were revealed in 74 and 61 patients, respectively. Surgical clipping was done for securing aneurysms among 53 patients and 82 patients underwent endovascular intervention for embolizing aneurysms. Acute hydrocephalus, intraventricular hemorrhage, pneumonia and seizure were observed in 24, 22, 26 and 17 patients, respectively. External ventricular drainage was implemented for 22 patients. Admission time varied from 0.4 to 24.0 hours (median,

8.6 hours; 25th–75th percentiles, 4.5–13.8 hours). Blood-sample collection time varied from 0.6 to 26.5 hours (median, 9.4 hours; lower-upper quartiles, 5.1–14.6 hours).

Dynamic Change of Serum Sestrin2 Levels After aSAH

In the current study, 45 patients gave permission to blood-collection at the multiple time points. As plotted in Figure 2, serum sestrin2 levels were immediately raised at admission, afterwards were gradually increased at day 1, peaked at day 2, and were declined at day 3 until day 7; and serum sestrin2 levels were substantially higher in patients at admission and at days 1, 2, 3, 5 and 7 than controls (all $P<0.001$).

Relationship Between Serum Sestrin2 Levels and Illness Severity After aSAH

As displayed in Table 1, there was a tight correlation between serum sestrin2 levels and Hunt-Hess scores ($P<0.001$), between serum sestrin2 levels and modified Fisher scores ($P<0.001$), between serum sestrin2 levels and external ventricular drainage ($P<0.05$), between serum sestrin2 levels and intraventricular hemorrhage ($P<0.01$), between serum sestrin2 and acute hydrocephalus ($P<0.05$), as well as between serum sestrin2 levels and blood glucose levels ($P<0.01$). In the Multivariate linear regression model incorporating the above-mentioned six variables of significant correlation, serum sestrin2 levels had independent correlation with Hunt-Hess scores (beta, 1.715; 95% CI, 0.595–2.835; VIF, 1.749; $t=3.029$; $P=0.003$) and modified Fisher scores (beta, 2.505; 95% CI, 1.102–3.907; VIF, 1.722; $t=3.534$; $P=0.001$). In total, 24, 24, 51, 31 and 5 patients had Hunt-Hess scores 1, 2, 3, 4 and 5, respectively. In aggregate, modified Fisher scores 1, 2, 3 and 4 were found in 24, 51, 48 and 12 patients, respectively. As depicted in Figure 3, as compared to patients with Hunt-Hess score 1, serum sestrin2 levels were substantially elevated in those with Hunt-Hess score 2, followed by those with Hunt-Hess scores 3 and 4, and were significantly highest in those with Hunt-Hess score 5 ($P<0.001$); and its levels were markedly raised in the order of modified Fisher scores from 1 to 4 ($P<0.001$; Figure 4).

Relation of Serum Sestrin2 Levels to Six-Month GOSE Scores After aSAH

At six months following aSAH, GOSE scores varied from 1 to 8, its median value was 5, as well as its lower and upper quartiles were 4 and 7 respectively. Totally, 8, 11, 12, 17, 25, 18, 19 and 25 patients experienced GOSE scores 1, 2, 3, 4, 5, 6, 7 and 8, respectively. In Figure 5, serum sestrin2 levels were significantly declined in the order of GOSE scores from 1 to 8 ($P<0.001$). As

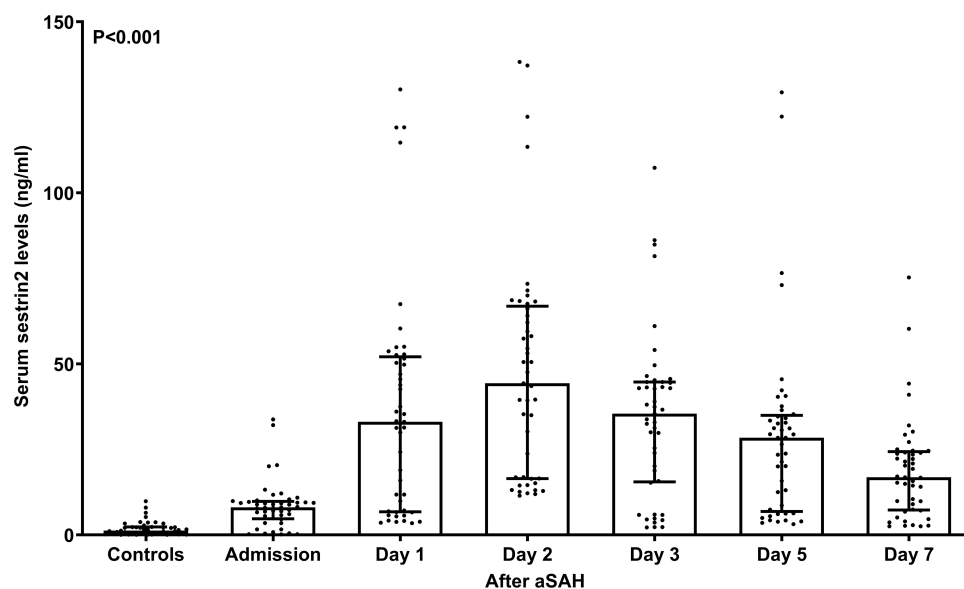


Figure 2 Temporal change of serum sestrin2 levels after aneurysmal subarachnoid hemorrhage. Serum sestrin2 levels were substantially increased at admission in patients, reached a highest level at day 2, and afterwards diminished gradually until day 7. Serum sestrin2 levels were substantially higher in patients during seven days after stroke than in controls ($P<0.001$).

Abbreviation: aSAH, aneurysmal subarachnoid hemorrhage.

Table 1 Relationship Between Serum Sestrin2 Levels and Other Variables in Patients with Aneurysmal Subarachnoid Hemorrhage

	ρ	P value
Demographic data		
Gender (male/female)	0.020	0.820
Age (years)	0.114	0.189
Body mass index (kg/m ²)	−0.056	0.515
Adverse lifestyle habits		
Cigarette smoking	−0.026	0.768
Alcohol consumption	−0.045	0.606
Chronic diseases		
Hypertension	0.008	0.924
Diabetes mellitus	0.099	0.254
Dyslipidemia	0.089	0.306
Coronary heart disease	0.141	0.102
Medication history		
Statin use	−0.012	0.895
Anticoagulant use	−0.149	0.085
Antiplatelet use	−0.005	0.956
Antihypertensive use	−0.043	0.620
Hypoglycemic or insulin use	0.108	0.212
Vital signs		
Systolic arterial blood pressure (mmHg)	−0.001	0.987
Diastolic arterial blood pressure (mmHg)	0.045	0.601
Mean arterial blood pressure (mmHg)	0.044	0.612
Severity scales		
Hunt-Hess scores	0.613	<0.001
Modified Fisher scores	0.567	<0.001
Aneurysm-related parameters		
Position (posterior/anterior circulation)	0.077	0.376
Shape (cystic/others)	−0.157	0.069
Diameter (<10 mm/≥10 mm)	0.016	0.852
Treatments (clipping/endovascular)	0.036	0.680
External ventricular drain	0.187	0.030
Complications		
Acute hydrocephalus	0.192	0.024
Intraventricular bleeding	0.245	0.004
Pneumonia	0.083	0.339
Seizure	0.047	0.591
Biochemical indices		
Blood glucose levels (mmol/l)	0.268	0.002
Blood leukocyte count ($\times 10^9/l$)	0.073	0.397
Others		
Admission time (h)	0.072	0.405
Blood-collection time (h)	0.078	0.370

Note: Bivariate correlations were evaluated by Spearman's rank correlation test.

listed in Table 2, GOSE scores were intimately correlated with serum sestrin2 levels ($P<0.001$), Hunt-Hess scores ($P<0.001$), modified Fisher scores ($P<0.001$), external ventricular drainage ($P<0.05$), intraventricular hemorrhage ($P<0.01$), acute hydrocephalus ($P<0.01$) and blood glucose levels ($P<0.05$). Using the multivariate linear regression analysis, the factors, which were retained as the independent correlated variables, were serum sestrin2 levels (beta, -0.050 ; 95% CI, $-0.099-0.001$; VIF, 1.583; $t=-2.037$; $P=0.044$), Hunt-Hess scores (beta, -0.670 ; 95% CI, $-0.993-0.347$; VIF, 1.874; $t=-4.108$; $P<0.001$) and modified Fisher scores (beta, -0.665 ; 95% CI, $-1.074-0.256$; VIF, 1.890; $t=-3.214$; $P=0.002$).

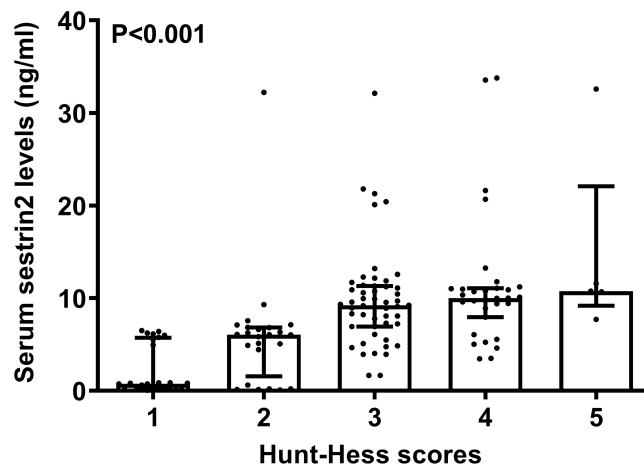


Figure 3 Serum sestrin2 levels across Hunt-Hess scores after aneurysmal subarachnoid hemorrhage. Using the Kruskal–Wallis test, serum sestrin2 levels were dramatically increased in the order of Hunt-Hess scores ($P<0.001$).

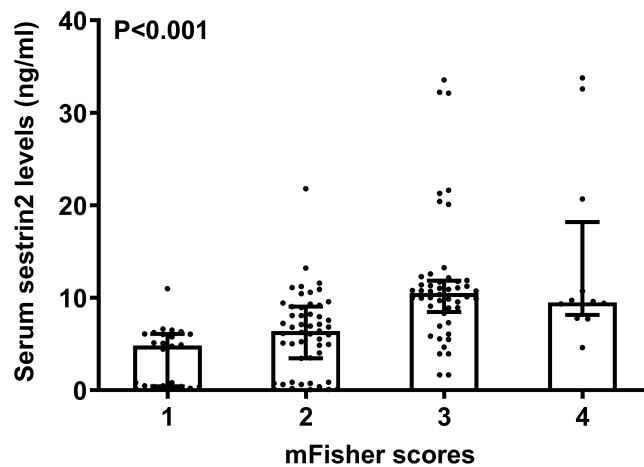


Figure 4 Serum sestrin2 levels across modified Fisher scores after aneurysmal subarachnoid hemorrhage. Using the Kruskal–Wallis test, serum sestrin2 levels had a significant enhancement in the order of modified Fisher scores ($P<0.001$).

Abbreviation: mFisher, modified Fisher.

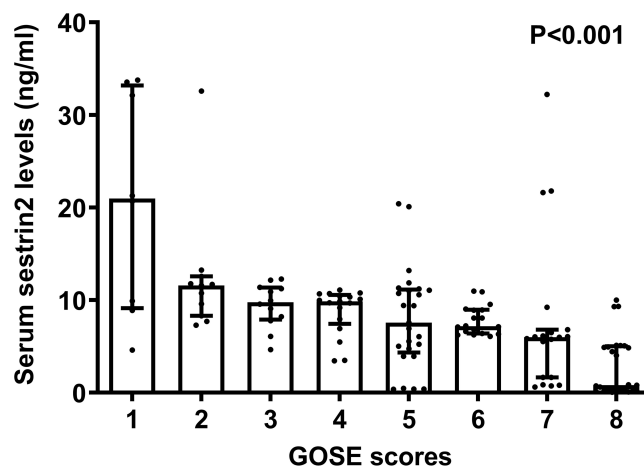


Figure 5 Serum sestrin2 levels across six-month Glasgow outcome scale scores after aneurysmal subarachnoid hemorrhage. Using the Kruskal–Wallis test, there was a substantial decline of serum sestrin2 levels in the order of Glasgow outcome scale scores ($P<0.001$).

Abbreviation: GOSE, Glasgow outcome scale scores.

Table 2 Relationship Between Postinjury 6-Month Extended Glasgow Outcome Scale Scores and Other Variables in Patients with Aneurysmal Subarachnoid Hemorrhage

	ρ	P value
Demographic data		
Gender (male/female)	−0.047	0.585
Age (years)	−0.141	0.103
Body mass index (kg/m ²)	0.061	0.482
Adverse lifestyle habits		
Cigarette smoking	−0.050	0.564
Alcohol consumption	0.001	0.989
Chronic diseases		
Hypertension	−0.100	0.248
Diabetes mellitus	−0.018	0.834
Dyslipidemia	−0.055	0.526
Coronary heart disease	−0.005	0.957
Medication history		
Statin use	0.023	0.795
Anticoagulant use	−0.052	0.549
Antiplatelet use	−0.005	0.956
Antihypertensive use	−0.114	0.189
Hypoglycemic or insulin use	−0.029	0.737
Vital signs		
Systolic arterial blood pressure (mmHg)	−0.085	0.325
Diastolic arterial blood pressure (mmHg)	−0.114	0.188
Mean arterial blood pressure (mmHg)	−0.108	0.212
Severity scales		
Hunt-Hess scores	−0.639	<0.001
Modified Fisher scores	−0.590	<0.001
Aneurysm-related parameters		
Position (posterior/anterior circulation)	−0.073	0.402
Shape (cystic/others)	0.124	0.153
Diameter (<10 mm/≥10 mm)	0.058	0.502
Treatments (clipping/endovascular)	−0.013	0.881
External ventricular drain	−0.193	0.025
Complications		
Acute hydrocephalus	−0.226	0.009
Intraventricular bleeding	−0.240	0.005
Pneumonia	−0.100	0.248
Seizure	−0.098	0.256
Biochemical indices		
Blood glucose levels (mmol/l)	−0.207	0.016
Blood leukocyte count ($\times 10^9/l$)	−0.121	0.160
Serum sestrin2 levels (ng/mL)	−0.601	<0.001
Others		
Admission time (h)	−0.011	0.899
Blood-collection time (h)	−0.017	0.841

Note: Bivariate correlations were evaluated by Spearman's rank correlation test.

Association of Serum Sestrin2 Levels with Poor Outcome at Postinjury Six Months

A total of 48 patients experienced six-month poor outcome (GOSE scores 1–4) following aSAH. Using the restricted cubic spline (Figure 6), serum sestrin2 levels were linearly related to the risk of poor outcome ($P=0.439$). Under the ROC curve (Figure 7),

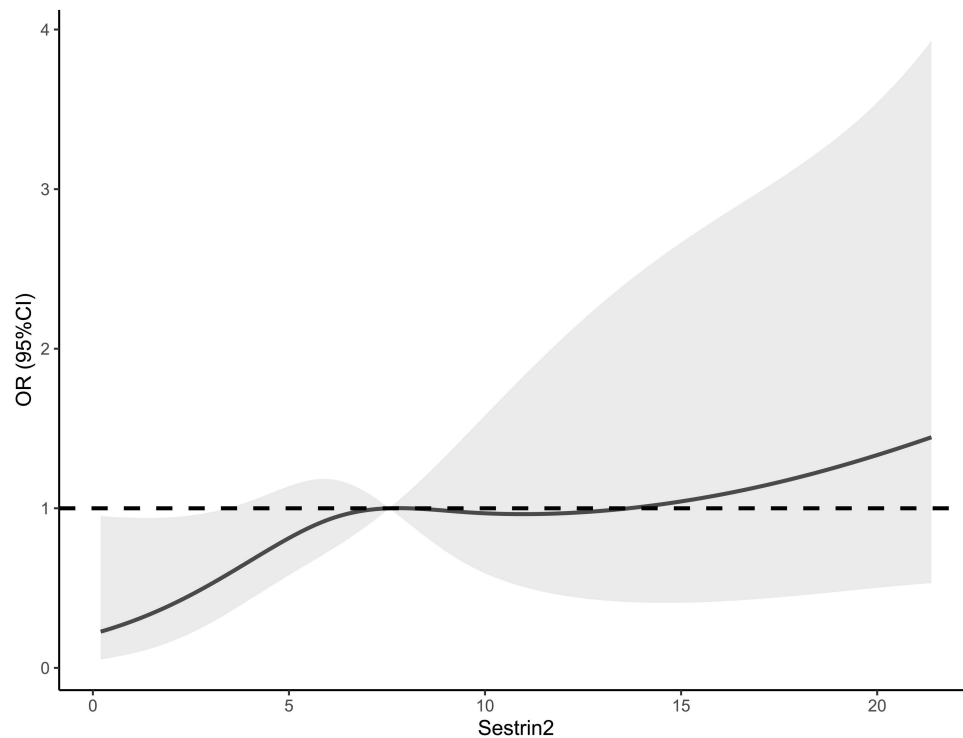


Figure 6 Restricted cubic spline describing a linear correlation between serum sestrin2 levels and risk of 6-month poor prognosis after aneurysmal subarachnoid hemorrhage. Serum sestrin2 levels were linearly related to risk of poor prognosis at six-months.

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.

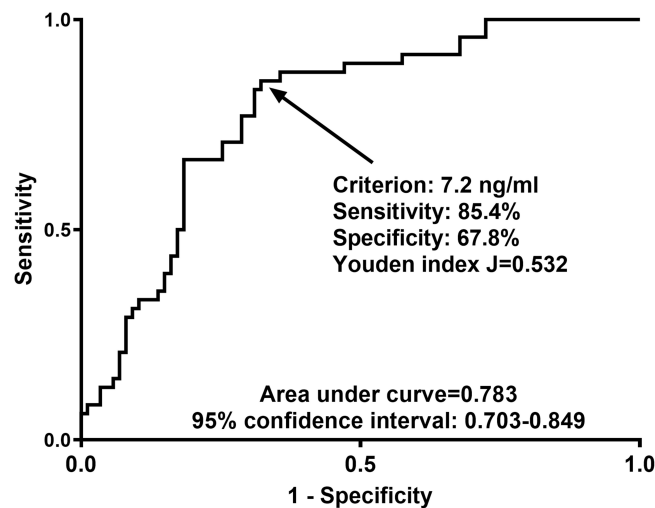


Figure 7 Receiver operating characteristic curve with respect to serum sestrin2 levels for predicting 6-month poor prognosis after aneurysmal subarachnoid hemorrhage. Serum sestrin2 levels significantly discriminated risk of poor prognosis and an optimal value was identified using the Youden method.

serum sestrin2 levels significantly discriminated patients at risk of poor outcome and sestrin2 levels above 7.2 ng/mL predicted poor outcome with the maximum Youden index.

Table 3 shows that, patients with the development of poor outcome, in comparison to those presenting with good outcome, had substantially elevated Hunt-Hess scores ($P<0.001$), modified Fisher scores ($P<0.001$), serum sestrin2 levels ($P<0.001$) and blood glucose levels ($P<0.05$), as well as displayed significantly increased proportions of intraventricular bleeding ($P<0.05$), acute hydrocephalus ($P<0.05$) and external ventricular drainage ($P<0.05$). When the binary logistic

Table 3 Factors Associated with 6-Month Functional Outcome After Aneurysmal Subarachnoid Hemorrhage

	Poor Prognosis	Good Prognosis	P value
Demographic data			
Gender (male/female)	19/29	40/47	0.473
Age (years)	53.7±11.0	51.3±10.5	0.224
Body mass index (kg/m ²)	23.1±2.4	23.8±2.6	0.146
Adverse lifestyle habits			
Cigarette smoking	17 (35.4%)	24 (27.6%)	0.344
Alcohol consumption	16 (33.3%)	21 (24.1%)	0.252
Chronic diseases			
Hypertension	11 (22.9%)	14 (16.1%)	0.328
Diabetes mellitus	6 (12.5%)	8 (9.2%)	0.547
Dyslipidemia	11 (22.9%)	19 (21.8%)	0.885
Coronary heart disease	2 (4.2%)	5 (5.7%)	0.692
Medication history			
Statin use	6 (12.5%)	14 (16.1%)	0.574
Anticoagulant use	3 (6.3%)	4 (4.6%)	0.699
Antiplatelet use	5 (10.4%)	8 (9.2%)	0.818
Antihypertensive use	8 (16.7%)	13 (14.9%)	0.791
Hypoglycemic or insulin use	4 (8.3%)	7 (8.1%)	0.953
Vital signs			
Systolic arterial blood pressure (mmHg)	130.3±21.9	131.5±21.1	0.754
Diastolic arterial blood pressure (mmHg)	81.8±12.7	82.0±13.6	0.935
Mean arterial blood pressure (mmHg)	106.0±16.6	106.7±16.2	0.812
Severity scales			
Hunt-Hess scores	3 (3–4)	2 (1–3)	<0.001
Modified Fisher scores	3 (3–3)	2 (1–2.5)	<0.001
Aneurysm-related parameters			
Position (posterior/anterior circulation)	13/35	18/69	0.398
Shape (cystic/others)	38/10	75/12	0.289
Diameter (<10 mm/≥10 mm)	25/23	49/38	0.636
Treatments (clipping/endovascular)	18/30	35/52	0.756
External ventricular drain	12 (25.0%)	10 (11.5%)	0.042
Complications			
Acute hydrocephalus	13 (27.1%)	11 (12.6%)	0.036
Intraventricular bleeding	12 (25.0%)	10 (11.5%)	0.042
Pneumonia	12 (25.0%)	14 (16.1%)	0.209
Seizure	8 (16.7%)	9 (10.3%)	0.289
Biochemical indices			
Blood glucose levels (mmol/l)	10.1 (7.3–16.5)	8.7 (7.1–11.1)	0.025
Blood leukocyte count (×10 ⁹ /l)	9.4 (6.8–11.5)	7.1 (4.8–12.1)	0.051
Serum sestrin2 levels (ng/mL)	10.0 (8.3–11.6)	6.1 (1.3–9.1)	<0.001
Others			
Admission time (h)	8.8 (4.6–13.5)	8.5 (4.4–13.9)	0.744
Blood-collection time (h)	9.5 (5.3–14.6)	9.3 (5.1–14.6)	0.749

Notes: Variables were shown as number (percentage), mean + standard deviation or median (percentiles 25th–75th) as appropriate and statistical methods included the student t-test, Mann–Whitney test, Fisher's exact test and χ^2 test.

regression model, in which the preceding seven significant variables were forced, was configured, Hunt-Hess scores (OR, 3.439; 95% CI, 1.649–7.171; $P=0.001$), modified Fisher scores (OR, 3.325; 95% CI, 1.526–7.244; $P=0.003$) and serum sestrin2 levels (OR, 1.093; 95% CI, 1.020–1.172; $P=0.012$) were verified to be the three independent predictors of poor six-month outcome following aSAH.

The Hunt-Hess scores, modified Fisher scores and serum sestrin2 levels were integrated to form a prediction model using the combined logistic regression method. A nomogram (Figure 8), in which the prediction model was visually displayed, was configured to describe the risk of poor outcome. Using the Hosmer–Lemeshow goodness-of-fit test, the model was relatively stable ($P=0.688$). Under the calibration curve (Figure 9), the model was valid. Using the decision

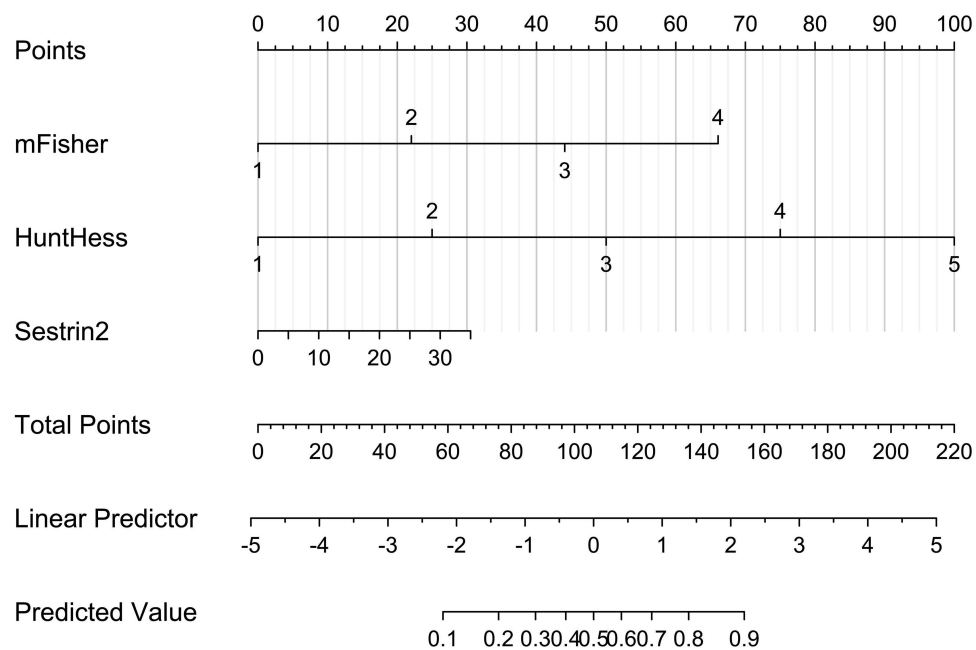


Figure 8 Nomogram displaying prognostic prediction model after aneurysmal subarachnoid hemorrhage. The model was composed of Hunt-Hess scores, modified Fisher scores and serum sestrin2 levels. Base on nomogram, risk of poor prognosis could be estimated.

Abbreviation: mFisher, modified Fisher.

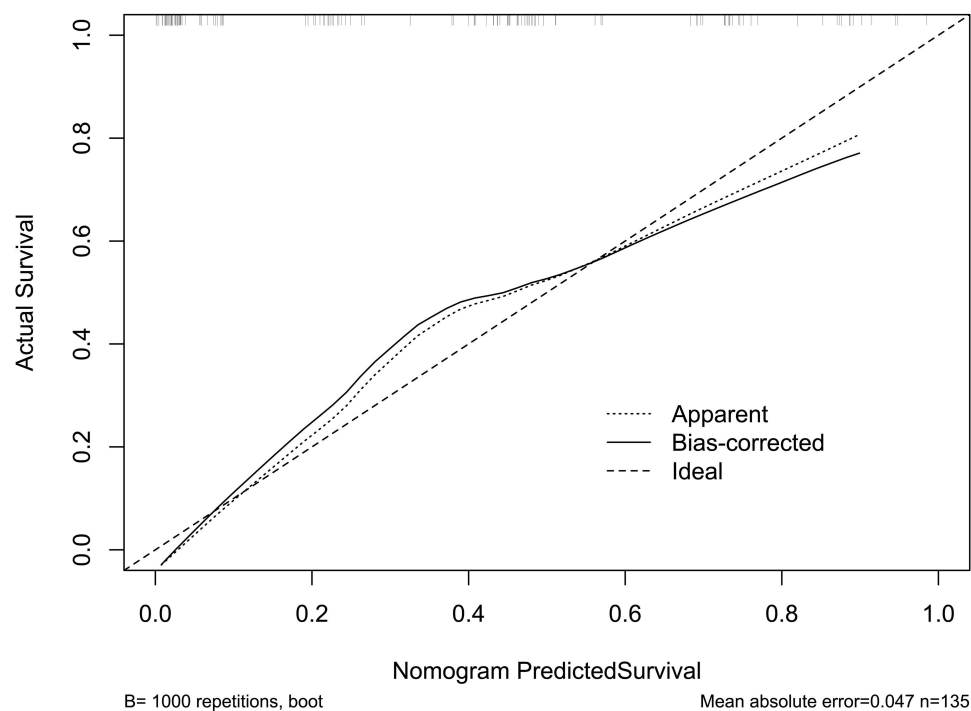


Figure 9 Calibration curve assessing prognostic prediction model after aneurysmal subarachnoid hemorrhage. The model, which consisted of Hunt-Hess scores, modified Fisher scores and serum sestrin2 levels, had rather stability.

curve analysis (Figure 10), the model was of clinical benefit. Under the ROC curve (Figure 11), the predictive model had substantially higher discriminatory efficiency for poor prognosis than the Hunt-Hess scores, modified Fisher scores and serum sestrin2 levels alone (all $P < 0.05$).

Relation of Serum Sestrin2 Levels to DCI Following aSAH

A total of 35 patients suffered from DCI after aSAH. Using the restricted cubic spline (Figure 12), there was a linear relationship between serum sestrin2 levels and the DCI risk ($P = 0.397$). As for the discriminatory efficiency under the ROC curve (Figure 13), serum sestrin2 levels substantially distinguished the DCI risk and sestrin2 levels more than 9.6 ng/mL discriminated patients at risk of DCI with the maximum Youden index.

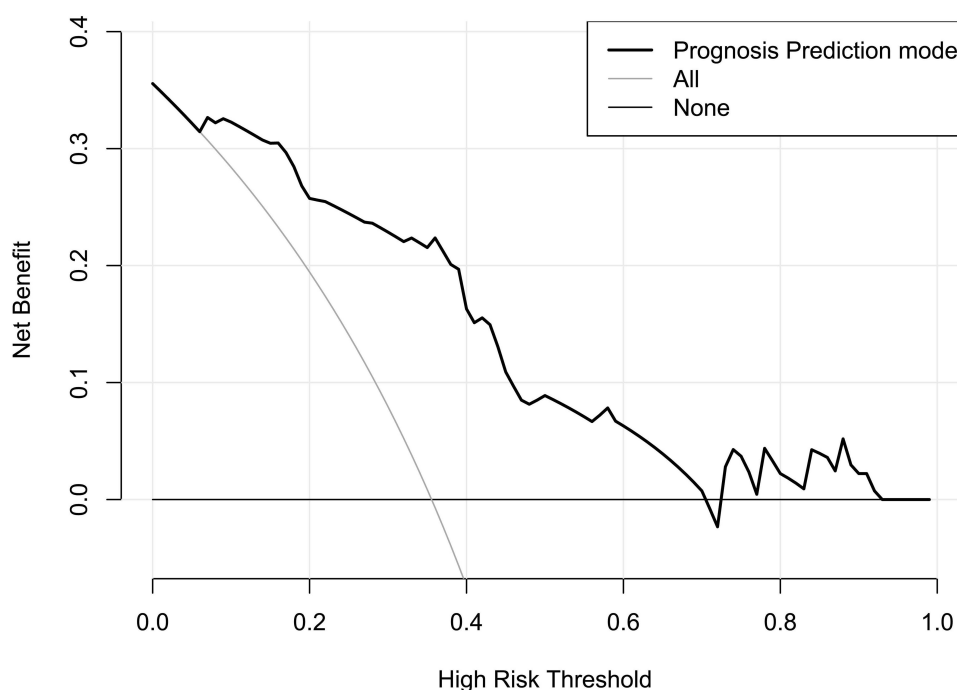


Figure 10 Decision curve evaluating prognostic prediction model following aneurysmal subarachnoid hemorrhage. The model, in which Hunt-Hess scores, modified Fisher scores and serum sestrin2 levels were integrated, was of clinical benefit.

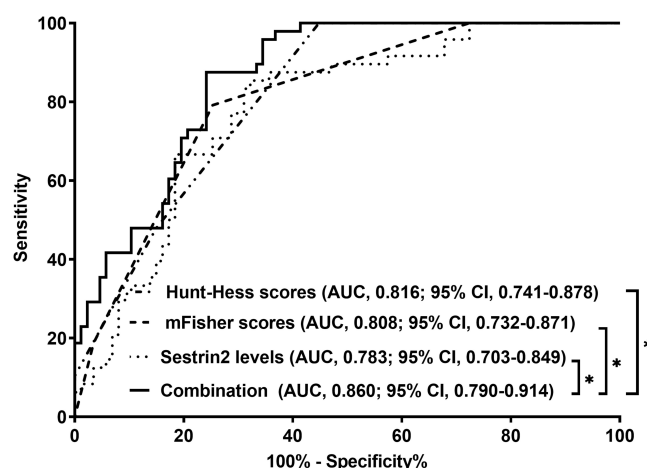


Figure 11 Predictive value of prognostic prediction model after aneurysmal subarachnoid hemorrhage. The model, in which Hunt-Hess scores, modified Fisher scores and serum sestrin2 levels were forced, displayed markedly higher prognostic predictive performance than Hunt-Hess scores, modified Fisher scores and serum sestrin2 levels alone (all $P < 0.05$). Asterisk indicates significant difference ($P < 0.05$).

Abbreviations: AUC, area under curve; 95% CI, 95% confidence interval.

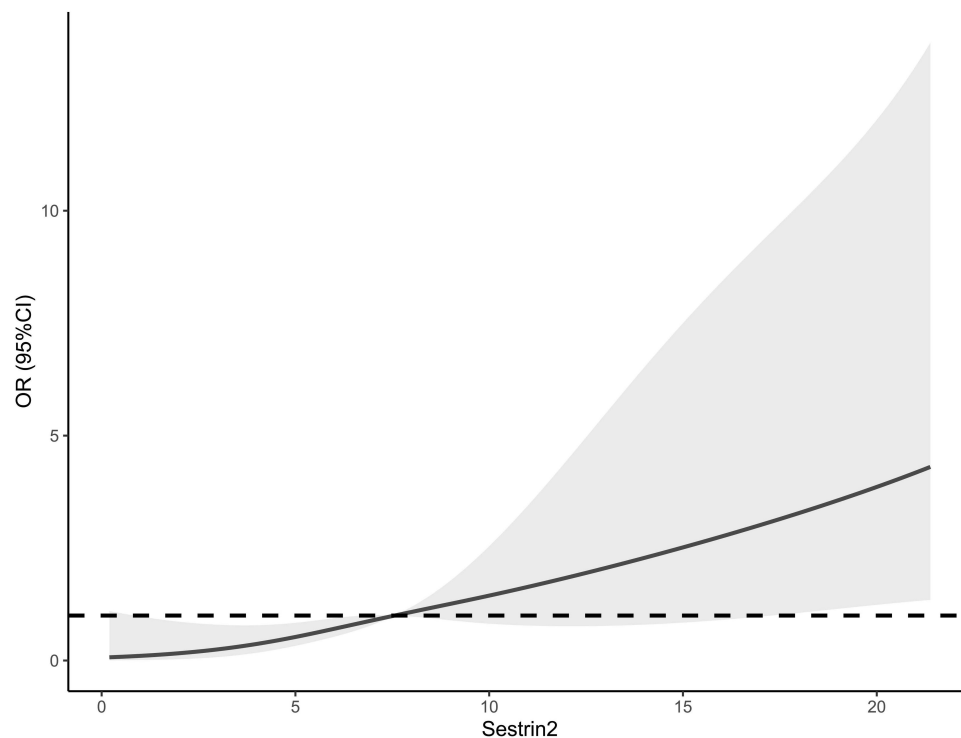


Figure 12 Restricted cubic spline describing a linear correlation between serum sestrin2 levels and risk of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. Serum sestrin2 levels were linearly related to risk of delayed cerebral ischemia.

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.

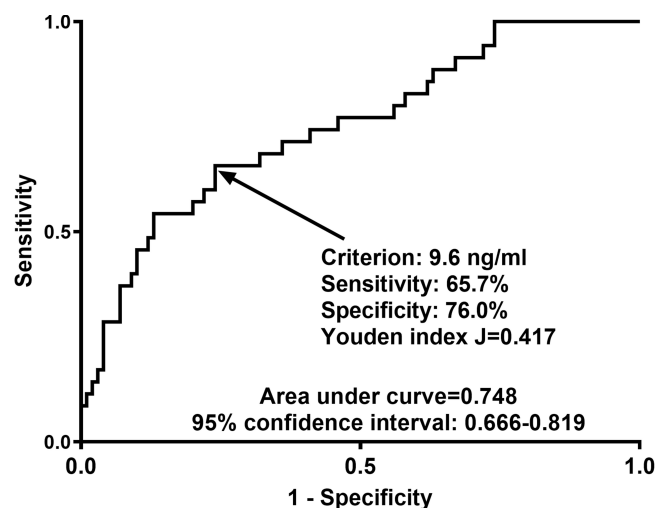


Figure 13 Receiver operating characteristic curve with respect to serum sestrin2 levels for predicting delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. Serum sestrin2 levels significantly discriminated risk of delayed cerebral ischemia and an optimal value was identified using the Youden method.

As displayed in Table 4, as opposed to patients without DCI development, those experiencing DCI exhibited dramatically increased Hunt-Hess scores ($P<0.001$), modified Fisher scores ($P<0.001$), serum sestrin2 levels ($P<0.001$) and blood glucose levels ($P<0.05$), as well as showed markedly raised percentages of intraventricular bleeding ($P<0.05$), acute hydrocephalus ($P<0.05$) and external ventricular drainage ($P<0.05$). The above-mentioned seven significant variables were entered into the binary logistic regression model, and subsequently it was found that Hunt-Hess scores (OR, 2.432; 95% CI, 1.225–4.856; $P=0.011$), modified Fisher scores (OR, 2.402; 95% CI, 1.130–5.230; $P=0.023$) and serum sestrin2 levels (OR, 1.079; 95% CI, 1.008–1.156; $P=0.029$) emerged as the three independent predictors of DCI following aSAH.

Table 4 Factors Associated with Delayed Cerebral Ischemia After Aneurysmal Subarachnoid Hemorrhage

	DCI	Non-DCI	P value
Demographic data			
Gender (male/female)	15/20	44/56	0.907
Age (years)	53.1±8.0	51.8±11.5	0.520
Body mass index (kg/m ²)	23.5±2.2	23.6±2.7	0.908
Adverse lifestyle habits			
Cigarette smoking	13 (37.1%)	28 (28.0%)	0.311
Alcohol consumption	9 (25.7%)	28 (28.0%)	0.794
Chronic diseases			
Hypertension	10 (28.6%)	15 (15.0%)	0.075
Diabetes mellitus	5 (14.3%)	9 (9.0%)	0.355
Dyslipidemia	9 (25.7%)	21 (21.0%)	0.564
Coronary heart disease	4 (11.4%)	3 (3.0%)	0.074
Medication history			
Statin use	6 (17.1%)	14 (14.0%)	0.652
Anticoagulant use	2 (5.7%)	5 (5.0%)	0.870
Antiplatelet use	5 (14.3%)	8 (8.0%)	0.321
Antihypertensive use	7 (20.0%)	14 (14.0%)	0.399
Hypoglycemic or insulin use	4 (11.4%)	7 (7.0%)	0.475
Vital signs			
Systolic arterial blood pressure (mmHg)	136.8±25.6	129.1±19.3	0.109
Diastolic arterial blood pressure (mmHg)	84.1±12.9	81.1±13.4	0.264
Mean arterial blood pressure (mmHg)	110.4±18.7	105.1±15.1	0.134
Severity scales			
Hunt-Hess scores	4 (3–4)	3 (2–3)	<0.001
Modified Fisher scores	3 (3–3)	2 (2–3)	<0.001
Aneurysm-related parameters			
Position (posterior/anterior circulation)	9/26	22/78	0.653
Shape (cystic/others)	27/8	86/14	0.222
Diameter (<10 mm/≥10 mm)	18/17	56/44	0.640
Treatments (clipping/endovascular)	17/18	36/64	0.190
External ventricular drain	10 (28.6%)	12 (12.0%)	0.022
Complications			
Acute hydrocephalus	11 (31.4%)	13 (13.0%)	0.014
Intraventricular bleeding	10 (28.6%)	12 (12.0%)	0.022
Pneumonia	10 (28.6%)	16 (16.0%)	0.105
Seizure	7 (20.0%)	10 (10.0%)	0.143
Biochemical indices			
Blood glucose levels (mmol/l)	14.2 (7.3–16.7)	9.0 (7.2–11.2)	0.029
Blood leukocyte count (×10 ⁹ /l)	9.5 (6.6–11.3)	7.5 (5.0–12.1)	0.216
Serum sestrin2 levels (ng/mL)	10.8 (7.3–12.9)	6.6 (3.5–9.6)	<0.001
Others			
Admission time (h)	11.9 (7.2–14.4)	8.4 (3.4–13.5)	0.185
Blood-collection time (h)	12.5 (7.5–15.4)	9.2 (4.3–14.4)	0.192

Notes: Variables were shown as number (percentage), mean + standard deviation or median (percentiles 25th–75th) as appropriate and statistical methods included the student t-test, Mann–Whitney test, Fisher's exact test and χ^2 test.

Abbreviation: DCI, delayed cerebral ischemia.

A model, in which the Hunt-Hess scores, modified Fisher scores and serum sestrin2 levels were combined using the combined logistic regression method, was built to predict DCI. A nomogram (Figure 14) was used to show the model visually. Using the Hosmer–Lemeshow goodness-of-fit test, the model was comparatively stable ($P=0.561$). The model was rather valid using the calibration curve analysis (Figure 15). Under the decision curve (Figure 16), the model was clinically beneficial. As

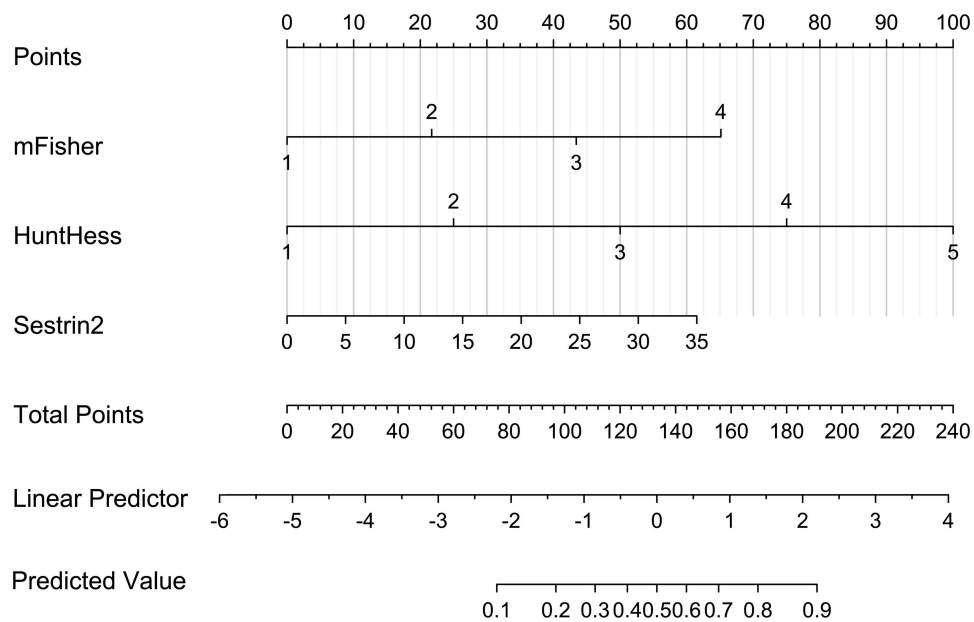


Figure 14 Nomogram displaying model for predicting delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. The model was composed of Hunt-Hess scores, modified Fisher scores and serum sestrin2 levels. Base on nomogram, risk of delayed cerebral ischemia could be displayed.

Abbreviation: mFisher, modified Fisher.

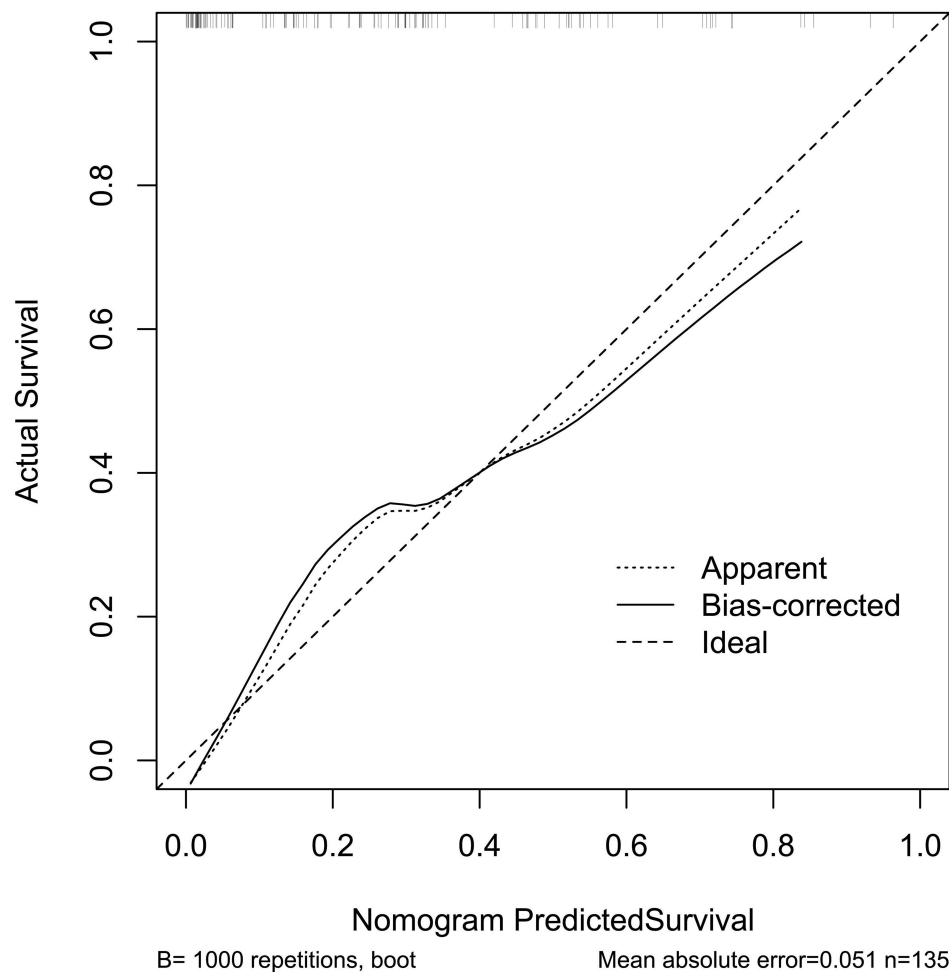


Figure 15 Calibration curve assessing prediction model for delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. The model, which consisted of Hunt-Hess scores, modified Fisher scores and serum sestrin2 levels, was relatively stable.

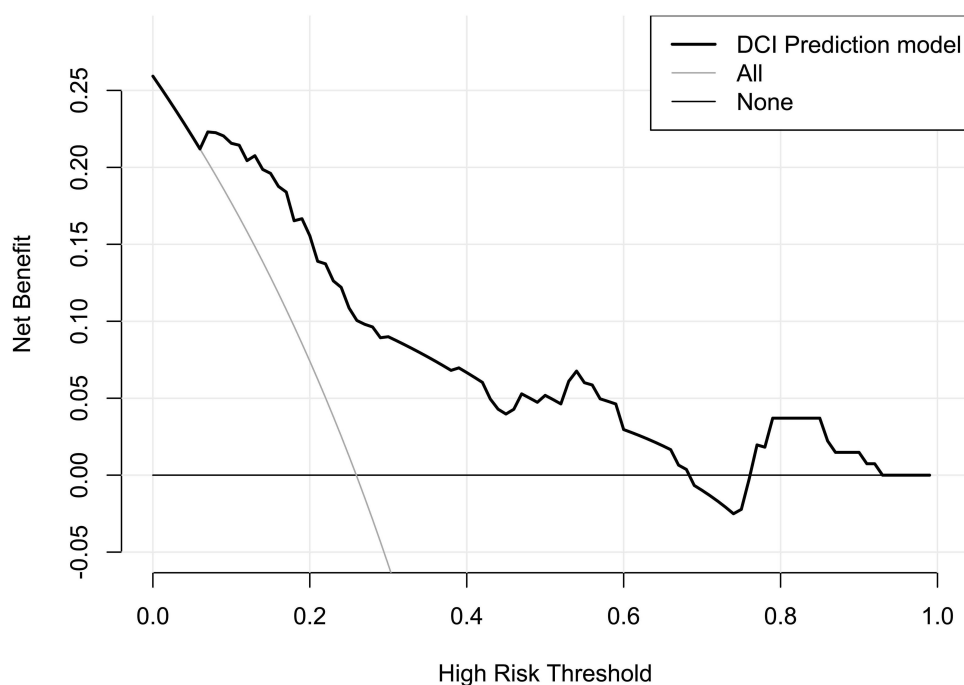


Figure 16 Decision curve evaluating prediction model for delayed cerebral ischemia following aneurysmal subarachnoid hemorrhage. The model, in which Hunt-Hess scores, modified Fisher scores and serum sestrin2 levels were entered, was clinically beneficial.

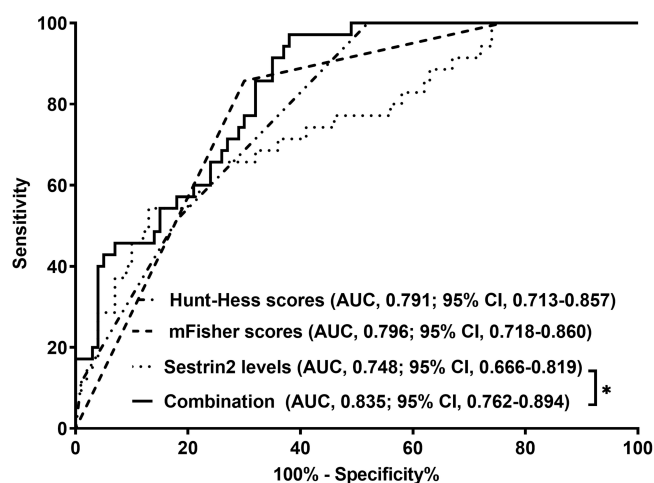


Figure 17 Predictive significance of prediction model for delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. The model, in which Hunt-Hess scores, modified Fisher scores and serum sestrin2 levels were forced, displayed markedly higher prognostic predictive performance than serum sestrin2 levels alone ($P < 0.05$). Asterisk indicates significant difference ($P < 0.05$).

Abbreviations: AUC, area under curve; 95% CI, 95% confidence interval.

regards predictive ability under the ROC curve (Figure 17), the model displayed significantly higher DCI predictive ability than serum sestrin2 levels alone ($P < 0.05$), but not than the Hunt-Hess scores and modified Fisher scores alone (both $P > 0.05$).

Discussion

To the best of our knowledge, this epidemiological study may be for the first time done to investigate serum sestrin2 levels after human aSAH and subsequently some interesting findings were discovered. There was a significant enhancement in serum sestrin2 levels after aSAH, which peaked at day 2 and thereafter gradually decreased. And serum sestrin2 levels of patients with aSAH at all time points were substantially higher than those of controls. Also, serum sestrin2 levels were independently

correlated with Hunt-Hess scores, modified Fisher scores and postinjury six-month GOSE scores, as well as were independently associated with DCI and poor prognosis at six months after aSAH. Noteworthy, serum sestrin2 levels significantly improved discriminatory ability of Hunt-Hess scores and modified Fisher scores alone, especially in prognostic prediction. The prediction model composed of serum sestrin2 levels, Hunt-Hess scores and modified Fisher scores showed high stability, accuracy, validity and clinical benefit. Presumably, serum sestrin2 may be a prognostic biomarker of clinical value.

Sestrin2 has been recognized as a stress response protein, which may be in possession of cytoprotective characteristics via anti-inflammation, anti-oxidation and anti-apoptosis.^{11,12} Therefore, its expressions may be easily elicited in acute or chronic neurologic disorders with inflammatory or oxidative feature.^{17–21} As hypothesized, in rats subjected to acute focal cerebral ischemia, sestrin2 expressions were obviously up-regulated in infarcted zone.²⁴ Similarly, sestrin2 expression was progressively enhanced in the hippocampal CA1 subfield within 24 hours in rats following transient global ischemia, reaching the maximal levels at 24 hours, and afterwards decreased till 48 hours.²⁵ Also, sestrin2 was markedly induced in mouse cerebral cortices exposed to traumatic brain injury.¹⁸ Consistently, sestrin2 was dramatically raised in injured brain tissues of mice after SAH and moreover, it was mainly located in neurons and microglia.¹⁹ Thus, acute brain injury could lead to production or release of sestrin2 from injured brain tissues. In some chronic neurologic diseases, such as Parkinson's disease, Alzheimer's disease and mild cognitive impairment, serum sestrin2 levels have been found to be significantly higher than those of healthy controls.^{20,21} Our study not only observed the change of serum sestrin2 levels in humans after aSAH, but also investigated its temporal change. Specifically, peripheral blood of aSAH patients was collected from 0.6 to 26.5 hours, with a median value of 9.4 hours. Among a small portion of patients, blood samples were obtained also in other time points, namely days 1, 2, 3, 5 and 7 after aSAH. Our data showed that serum sestrin2 levels were immediately increased during the early phase, peaked at day 2 and then gradually diminished. Its high levels still existed during 7 days after aSAH, when compared to healthy controls. In consideration of a significant elevation of its expressions in animal brain tissues after acute brain injury,^{18,19,24,25} it is theoretically possible that serum sestrin2 may at least partially originate from central nervous system.

A growing body of evidence suggests that sestrin2 may confer neuroprotective effects against various noxious insults. In experiments regarding ischemic brain injury, sestrin2 has been demonstrated to be brain-protective via promoting angiogenesis, lessening cerebral edema, diminishing cerebral infarct area and improving neurological function.²⁴ Also, using in vivo and in vitro models of traumatic brain injury, sestrin2 was confirmed to inhibit oxidative stress, reduce apoptosis, depress brain edema and diminish neurological deficit.¹⁸ Similarly, in an endovascular perforation SAH model of mice, sestrin2 obviously attenuated neuroinflammatory injury and oxidative stress, thereby ameliorating neurologic function.¹⁹ Contrarily, in another experiment, in which both oxygen-glucose deprivation/reoxygenation model of primary neurons and rat model of middle cerebral artery occlusion were built, sestrin2 significantly led to neuronal injury and enhanced the cerebral ischemia-reperfusion injury.²⁶ Although there is an inconsistency in experimental data with respect to protective or detrimental effects of sestrin2 in acute brain injury, the accumulating evidence has been strongly supportive of the notion that sestrin2 may be cytoprotective in acute brain injury diseases.^{17–19,24,25} In view of the present assumption of sestrin2's protective function, it is conceivably believed that increased sestrin2 levels should probably be a compensatory response to noxious stimuli.

In the current study, the registered prognostic parameters were DCI and poststroke six-month functional outcome; and the recorded severity indicators were Hunt-Hess scores and modified Fisher scores. In terms of statistical methods, univariate and multivariate analyses were sequentially employed to analyze all correlations and associations. Also, GOSE, Hunt-Hess scale and modified Fisher scale were not only regarded as the continuous variables, but also as the categorical variables. Statistical results pointed to the assumption that serum sestrin2 levels were independently correlated with illness severity indicated by Hunt-Hess scores and modified Fisher scores and were independently associated with poor prognosis reflected by DCI, 6-month GOSE scores after aSAH.

In our study, a potential prediction model of DCI or poor prognosis at six months after aSAH was established, which was visually displayed using a nomogram. The model was composed of serum sestrin levels, Hunt-Hess scores and modified Fisher scores, all of which have been demonstrated to be the independent predictors of DCI and poor prognosis in the current study. For the sake of assessment of model stability, efficiency and clinical benefit, a series of statistical methods were applied, including the Hosmer–Lemeshow goodness-of-fit test, calibration curve analysis, ROC curve analysis and decision curve analysis. Our data showed that the model was highly efficient, comparatively stable and clinically beneficial in prognostic discrimination. Thus, serum sestrin2 may aid in severity assessment and prognostic

prediction of aSAH, when combined with other severity scales, such as Hunt-Hess scale and modified Fisher scale, indicating that serum sestrin2 may be a prognostic biomarker of human aSAH.

Several strengths and weaknesses warrant to be mentioned in the current study. The strengths are that (1) Serum sestrin2, for the first time, was investigated with respect to its prognostic value in human aSAH and therefore was verified to be a potential prognostic biomarker of aSAH; (2) In view of statistical methods, all relations of serum sestrin2 levels to severity, DCI and prognosis of aSAH were demonstrated using multivariate analysis; and (3) Besides correlation analysis, two combination models were established to well predict DCI and long-term clinical outcomes of aSAH. The weaknesses are that (1) Although serum sestrin2 levels were determined at multiple time-points after aSAH, this study was devoid of enough patients to reveal optimal time-point, where serum sestrin2 levels would display highest prognostic predictive ability; (2) Although this study included more than one hundred patients for clinical investigation, this is still a single-center study. Thus, the final conclusions need to be validated via a larger cohort study in future.

Conclusions

To the best of our knowledge, this is a first series for determining relationship between serum sestrin2 levels and severity along with functional outcome of aSAH. Our study showed that (1) Serum sestrin2 levels are significantly elevated during the seven days after aSAH, reaching a highest level at day 2; (2) Admission serum sestrin2 levels are independently related to disease severity, DCI and functional outcome of aSAH; (3) The additive effects of serum sestrin2 levels on conventional severity scales (namely, Hunt-Hess scale and modified Fisher scale) are dominant in terms of DCI and outcome prediction. Hypothetically, serum sestrin2 may be considered as a prognostic determinant of clinical significance.

Abbreviations

aSAH, aneurysmal subarachnoid hemorrhage; AUC, area under the curve; CI, confidence interval; GOSE, Extended Glasgow Outcome Scale; OR, odds ratio; ROC, receiver operating characteristic; DCI, delayed cerebral ischemia.

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Disclosure

The authors report no conflicts of interest concerning the materials or methods used in this study, or the findings specified in this article.

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