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ORIGINAL RESEARCH

Clinical Value of Serum Secretoneurin Levels in Prediction of Delayed Cerebral Ischemia and Prognostic Analysis of Aneurysmal Subarachnoid Hemorrhage: A Prospective Cohort Study

Jiasen Ma[1], Xiuqin Huang[2], Yanping Hu[1], Bing Xu[1], Chunhua Jin[1]

[1]Emergency Department, Shaoxing People's Hospital, Shaoxing, Zhejiang Province, People's Republic of China; [2]Department of Plastic and Cosmetic Surgery, The 72nd Group Military Hospital of the People's Liberation Army of China, Huzhou, Zhejiang Province, People's Republic of China

Correspondence: Chunhua Jin, Emergency Department, Shaoxing People's Hospital, Shaoxing, Zhejiang Province, People's Republic of China, Email mjsjch@163.com

Background: Secretoneurin is a neuropeptide with several neuroprotective properties. Here, we discuss the importance of serum secretoneurin in assessing severity and predicting delayed cerebral ischemia (DCI) and functional outcomes following aneurysmal subarachnoid hemorrhage (aSAH).

Methods: A prospective cohort study of 167 patients with aSAH and 100 controls was performed to determine serum secretoneurin levels. Severity was reflected by the Hunt-Hess and modified Fisher scores. Prognostic parameters included DCI and poor 6-month prognosis (extended Glasgow outcome scale scores of 1–4). Univariate analysis followed by multivariate analysis was performed to determine the correlation between severity and prognosis.

Results: Compared to controls, patients exhibited a marked elevation in serum secretoneurin levels. Serum secretoneurin levels, which were independently correlated with Hunt-Hess scores and modified Fisher scores, independently predicted DCI and bad 6-month prognosis. Serum secretoneurin levels, which were linearly related to the risk of DCI and poor prognosis under a restricted cubic spline, effectively distinguished the risks under the receiver operating characteristic (ROC) curve. Subgroup analysis for prognosis or DCI prediction revealed no substantial interactions between serum secretoneurin levels and other variables, such as age, sex, hypertension, diabetes, alcohol consumption, and cigarette consumption. In addition, the prognosis model, in which serum secretoneurin, Hunt-Hess scale, and modified Fisher scale were merged, was graphically represented by a nomogram and performed well under the calibration, decision, and ROC curves.

Conclusion: Serum secretoneurin levels significantly increased after aSAH, which was intimately correlated with disease severity and independently associated with DCI and worse outcomes, indicating that serum secretoneurin may be a potential prognostic biomarker of aSAH.

Keywords: aneurysm, subarachnoid hemorrhage, secretoneurin, delayed cerebral ischemia, prognosis, outcome, severity

Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) is a severe health problem affecting people worldwide.[1] It can cause death and lead to neurological and psychiatric sequelae in survivors.[2] Although the mechanisms underlying acute brain injury after aSAH are rather complex, early brain injury and delayed cerebral ischemia (DCI) are two pivotal pathophysiological processes; and DCI can be clinically observed and its active treatment can markedly improve neurological outcomes of aSAH.[3] In the neurological field, Hunt-Hess scale is a clinical assessment system for reflecting consciousness status, and clinical symptoms and signs, as well as modified Fisher scale is a very common radiological scoring system; and both of them are frequently preferred for managing patients with aSAH and determining its

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severity.[4,5] Biochemical markers have recently attracted widespread attention with respect to their prognostic significance in aSAH.[6–8]

Secretoneurin, a member of the granin family, is a 31–42 amino acid neuropeptide derived from secretogranin II.[9] It has significantly elevated expression in response to hypoxia and subsequently harbors multiple functions, such as the induction of angiogenesis, regulation on neuroinflammation and neurotransmitter release, and stimulation on pituitary hormone release.[10–12] In experimental ischemic stroke, secretoneurin exerts neuroprotective effects by inhibiting microglial cell activation, enhancing neurogenesis and angiogenesis, and suppressing neuronal apoptosis.[13] It is broadly distributed in brain tissues and is present at high concentrations in cerebrospinal fluid.[14] Interestingly, increased levels of secretoneurin were observed both in the umbilical cord blood of neonates with hypoxic-ischemic encephalopathy[15] and in the peripheral blood of patients with acute ischemic stroke.[16] Univariate analysis showed that increased blood secretoneurin levels were closely associated with poor neurological outcomes in cardiac arrest patients after cardiopulmonary resuscitation[17] and were closely related to the severity and clinical outcome of patients with traumatic brain injury.[18] Previous evidence strongly indicates that secretoneurin may be a potential biomarker for acute brain injury. In this study, serum secretoneurin was quantified to assess its relationship with aSAH severity, DCI, and prognosis.

Methods

Study Design, Ethical Requirements and Participant Enrollments

This single-center prospective cohort study from February 2019 to March 2022, which was performed in accordance with the 2008 Revision of the Declaration of Helsinki and its later amends, was approved by the Institutional Review Committee at the Shaoxing People's Hospital (No. SH2019019). Written informed consent was obtained from patient representatives and controls. Patients were consecutively recruited according to the following criteria: (1) radiologically diagnosed aSAH; (2) pre-admission modified Rankin scale score of 2 or less; (3) age ≥ 18 years; (4) admission to the hospital within 24 h post-ictus; and (5) securing aneurysms within 48 h post-admission. Next, we excluded patients with other neurological diseases (eg, stroke, Moyamoya disease, arteriovenous malformation, and intracranial tumors), severe diseases in other organs (eg, cirrhosis, malignancies, and chronic obstructive pulmonary disease), or other specific conditions (eg, pregnancy, missed visits, unqualified samples, inadequate information, and unwillingness to participate). Simultaneously, a group of healthy controls was selected.

Data Obtainments and Outcome Assessment

The recorded data included demographic data, vascular risk factors, medications, vital signs, severity scales, aneurysm-related radiological parameters, complications, and surgical procedures. The demographic data included age and sex. Vascular risk factors included cigarette smoking, alcohol consumption, hypertension, and diabetes mellitus. The use of statins, anticoagulants, and antiplatelet agents was recorded. The arterial blood pressure was measured noninvasively. The clinical severity was appraised using the Hunt-Hess score. Radiological severity was evaluated using modified Fisher scores. Aneurysm-related parameters included location, shape, and size. Surgical procedures included clipping and endovascular intervention for treating ruptured aneurysms as well as external ventricular drainage for severe intraventricular bleeding and acute hydrocephalus. Seizures and pneumonia are the two common complications of aSAH. Head CT scans were conventionally performed on postoperative days 1, 7, 14, and 30, and the diagnosis of DCI complied with previous reports.[19] Extended Glasgow Outcome Scale (GOSE) scores six months after aSAH were recorded via clinic visits or telephone calls. A scores of 1–4 signified a bad prognosis.[20]

Secretoneurin Determination

Blood samples from patients and controls were drawn by venipuncture and placed into 5 mL tubes. Blood samples were centrifuged at 3000 × g for 15 min, and serum was aliquoted into Eppendorf tubes. The samples were preserved at −80 °C conditions until assayed. Serum secretoneurin levels were detected in batches using an enzyme immunoassay commercial kit (Peninsula Laboratories International, Inc., USA; Catalogue number: S-1387), following the manufacturer's instructions. The detection range was 0.05–50 ng/mL. The intra-assay coefficient of variation was < 10% and the inter-assay

coefficient of variation was < 15%. Determinations were performed in duplicate by the same technician, who was blinded to the clinical data, and the results are shown in ng/mL.

Statistical Analysis

The SPSS 28.0 (SPSS Corp., Armonk, NY, USA), R 3.5.1, (https://www.r-project.org) and MedCalc 20.1 (MedCalc Software, Mariakerke, Belgium) were used for statistical analyses. First, the normality of the measured data distribution was examined. Descriptive statistics are shown as means (standard deviations, SDs) for normally distributed numerical data, medians (lower-upper quartiles) for non-normally distributed numerical data, and numbers (percentages) for nominal data. Intergroup comparison methods included the Fisher's exact test, chi-square test, independent *t*-test, Mann–Whitney *U*-test, and Kruskal–Wallis test. Spearman's test was used for bivariate correlation analysis. Multivariate models, including logistic and linear regression models, were configured to independently determine the associative factors of serum secretoneurin levels, DCI, and poor prognosis. Linear correlations were explored using a restricted cubic spline. Interactive effects were determined using a subgroup analysis. Discriminative efficiencies were determined using a receiver operating characteristic (ROC) curve. Prediction models were established and graphically delineated using the nomograms. Its predictive ability was assessed under ROC receiver operating characteristic, calibration, and decision curves. By using the MedCalc 20.1 (MedCalc Software, Mariakerke, Belgium), 167 patients were sufficient for statistical analysis, whether for intergroup comparisons, ROC curve analysis or bivariate correlation analysis. Statistical significance was set at $P<0.05$.

Results

Participant Characteristics

A total of 212 patients with aSAH were screened, and 167 patients were finally retained for further analysis after 45 patients were excluded for the reasons outlined in Figure 1. A total of 100 healthy controls were recruited for the study. Some variables, namely age, sex, cigarette smoking, and alcohol consumption, were not significantly different between controls and patients (all $P<0.05$).

Among this cohort of patients, there were 62 males and 105 were female. They were aged from 27 to 72 years, with a mean value of 50.8 years (SD, 10.7 years). There were 49 cigarette smokers, 49 alcohol drinkers, 38 hypertensive patients, and 13 diabetic patients. A total of 24, 9, and 16 patients were orally administered statins, anticoagulants, and antiplatelet agents, respectively. Systolic arterial blood pressure varied from 73 to 188 mmHg, with a mean value of 129.3 mmHg (SD, 26.5 mmHg) and diastolic arterial blood pressure ranged from 42 to 105 mmHg, with a mean value of 75.9 mmHg (SD, 15.2 mmHg). Patients were admitted into hospital from 0.5 to 24.0 hours (median, 8.2 hours; 25th-75th percentiles, 3.7–12.2 hours) and blood was drawn from 1.0 to 25.0 hours (median, 9.1 hours; 25th-75th percentiles, 5.0–13.9 hours). The Hunt-Hess scores varied from 1 to 5 (median, 3; 25th-75th percentiles, 2–4); and scores of 1, 2, 3, 4, and 5 were found in 28, 36, 55, 35, and 13 patients, respectively. Modified Fisher scores varied from 1 to 4 (median, 2; 25th-75th percentiles, 2–3); and scores of 1, 2, 3, and 4 were obtained in 33, 55, 58, and 21 cases, respectively. Thirty-four aneurysms were located in the posterior circulation, and 133 in the anterior circulation. There were 140 cystic aneurysms, and the remainder were of other types. A total of 97 aneurysms had a diameter of <10 mm, and 70 aneurysms had a diameter of ≥10 mm. Sixty-three aneurysms were clipped via craniotomy and 104 were secured via endovascular intervention. Twenty-one patients had acute hydrocephalus, 25 had intraventricular hemorrhage, and 14 had intracerebral hemorrhage. External ventricular drainage was performed on 23 patients. Common complications were pneumonia (n = 26) and seizures (n = 15).

Serum Secretoneurin Levels and Its Correlation with aSAH Severity

As shown in Figure 2, serum secretoneurin levels were significantly higher in patients than in the controls ($P<0.001$). A strong positive correlation was observed between serum secretoneurin levels and Hunt-Hess scores ($P<0.001$; Figure 3A), and the levels were significantly elevated in the order of Hunt-Hess scores from 1 to 5 ($P<0.001$; Figure 3B). In addition, serum secretoneurin levels were strongly positively correlated with modified Fisher scores

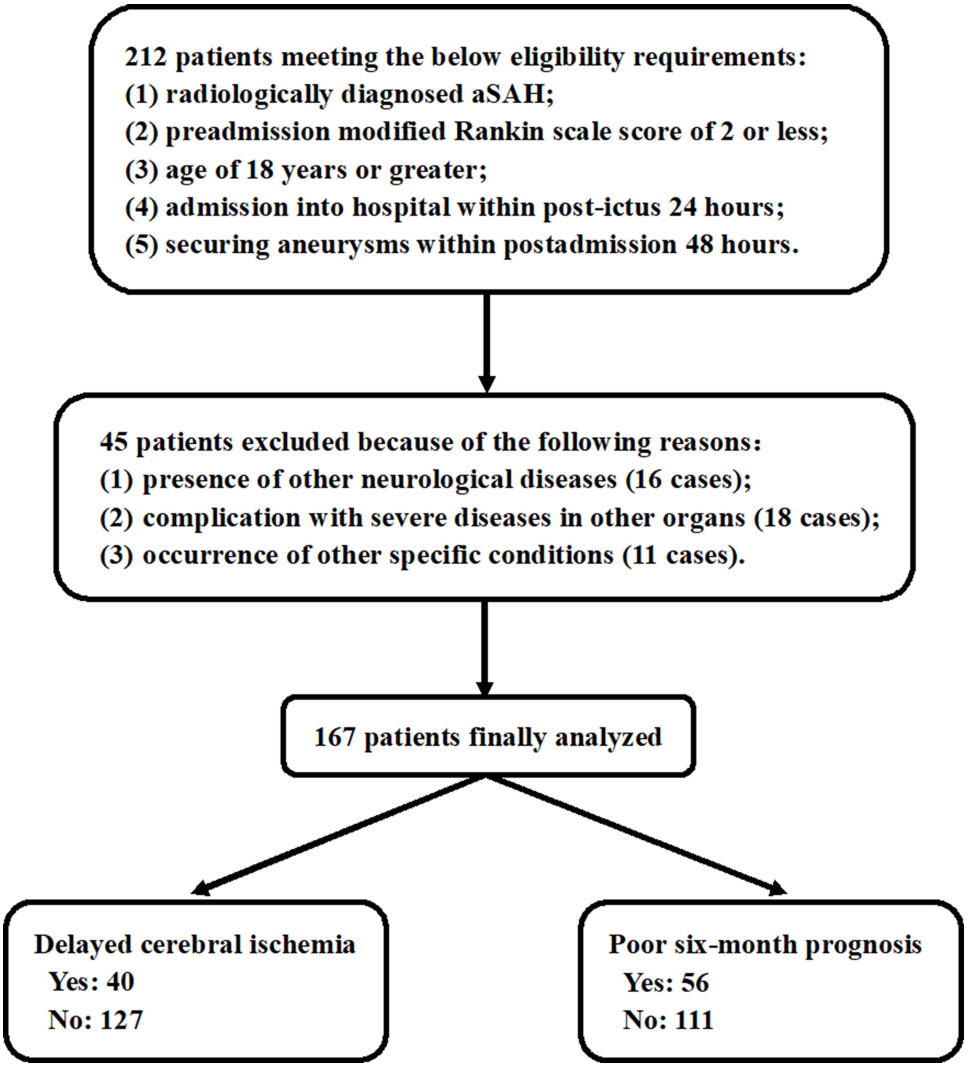

Figure 1 Flowing diagram for choosing eligible patients. A total of 212 patients with aneurysmal subarachnoid hemorrhage met the recruitment criteria, and 45 were excluded from this study according to the exclusion acquirements. Finally, 167 samples were analyzed. aSAH, indicates aneurysmal subarachnoid hemorrhage.

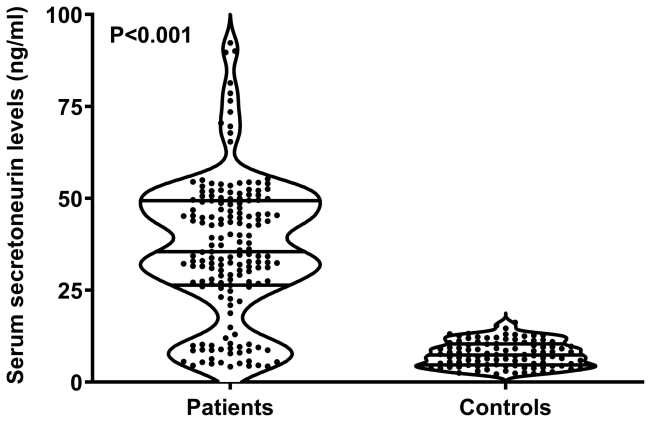

Figure 2 Serum secretoneurin levels between healthy controls and patients with aneurysmal subarachnoid hemorrhage. The levels were markedly higher in patients than in controls (P<0.001).

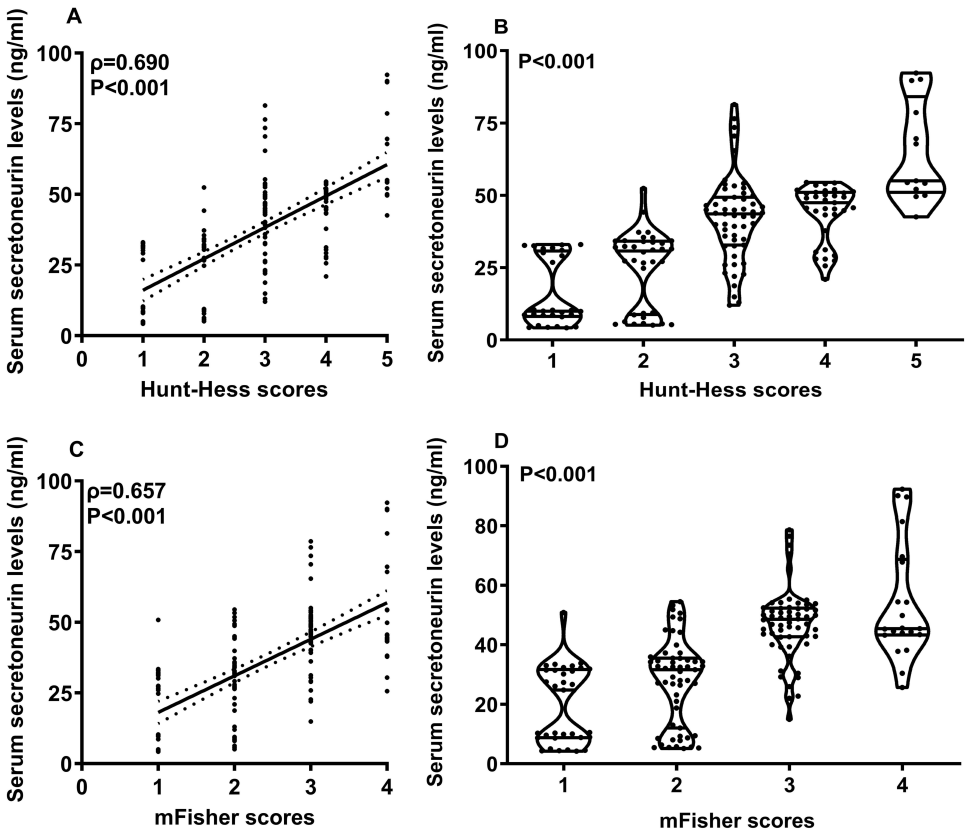

Figure 3 Serum secretoneurin levels and severity of aneurysmal subarachnoid hemorrhage. (**A**) Relationship between serum secretoneurin levels and Hunt-Hess scores after aneurysmal subarachnoid hemorrhage. Serum secretoneurin levels were significantly positively correlated with Hunt-Hess scores (P<0.001). (**B**)Serum secretoneurin levels in subgroups with different Hunt-Hess scores after aneurysmal subarachnoid hemorrhage. Serum secretoneurin levels were substantially increased in the order of Hunt-Hess scores from 1 to 5 (P<0.001). (**C**)Relationship between serum secretoneurin levels and modified Fisher scores following aneurysmal subarachnoid hemorrhage. (**D**)Serum secretoneurin levels were significantly positively correlated with modified Fisher scores (P<0.001).

(P<0.001; Figure 3C), and the levels were substantially lower in patients with a score of 1, followed by scores of 2 and 3, and were highest in those with a score of 4 (P<0.001; Figure 3D). Table 1 shows that serum secretoneurin levels were strongly correlated with intraventricular hemorrhage, acute hydrocephalus, and blood glucose levels (all P<0.05). The above-mentioned five significant variables in the univariate correlation analysis were incorporated into the multivariate linear regression model, and it was shown that the factors that were independently correlated with serum secretoneurin levels were Hunt-Hess scores (beta, 6.921; 95% CI, 4.404–9.438; VIF, 2.246; P=0.001) and modified Fisher scores (beta, 6.077; 95% CI, 2.984–9.169; VIF, 2.189; P=0.001).

Serum Secretoneurin Levels and DCI Risk After aSAH

A total of 40 patients developed DCI. In Figure 4A, DCI patients, as opposed to the other remainders, had significantly higher serum secretoneurin levels (P<0.001). Also, serum secretoneurin distinguished risk of DCI efficiently and using the Youden method, the levels above 45.2 ng/mL predicted DCI with medium-high sensitivity and specificity values (Figure 4B). Under a restricted cubic spline (Figure 5), the levels were linearly related to the DCI risk (P>0.05). Table 2 shows that patients with DCI had significantly higher Hunt-Hess scores and modified Fisher scores than those without (both P<0.001); patients with DCI, in contrast to those without, showed substantially elevated proportions of intraventricular hemorrhage and intracerebral bleeding (both P<0.01), as well as patients with suffering of DCI, as opposed to those not presenting with, exhibited pronouncedly higher serum secretoneurin levels and blood glucose levels (both P<0.05). Next, the six significant variables in the univariate analysis were forced into the binary logistic regression model, and it was verified that the modified Fisher scores (odds ratio, 2.060; 95% confidence interval, 1.132–3.748; P=0.010) and serum secretoneurin levels (odds ratio, 1.045; 95% confidence interval, 1.019–1.072; P=0.028) were

Table 1 Factors in Correlation with Serum Secretoneurin Levels After Aneurysmal Subarachnoid Hemorrhage

	ρ	*P* value
Gender (male/female)	0.080	0.306
Age (years)	−0.010	0.903
Cigarette smoking	−0.068	0.380
Alcohol drinking	−0.028	0.718
Hypertension	−0.024	0.763
Diabetes mellitus	0.047	0.550
Previous statins use	0.005	0.946
Previous anticoagulant use	−0.101	0.193
Previous antiplatelet use	−0.020	0.797
Systolic arterial blood pressure (mmHg)	−0.057	0.466
Diastolic arterial blood pressure (mmHg)	−0.097	0.214
Hunt-Hess scores	0.690	<0.001
Modified Fisher scores	0.657	<0.001
Aneurysmal position (posterior/anterior circulation)	0.025	0.749
Aneurysmal shape (cystic/others)	−0.081	0.298
Aneurysmal diameter (<10 mm/≥10 mm)	−0.076	0.328
Securing modality of aneurysms (clipping/endovascular intervention)	0.063	0.417
Acute hydrocephalus	0.167	0.031
Intraventricular bleeding	0.236	0.002
Intracerebral hemorrhage	0.144	0.064
External ventricular drain	0.067	0.389
Admission time after stroke (h)	0.125	0.106
Blood-sampling time after stroke (h)	0.104	0.180
Pneumonia	−0.005	0.947
Seizure	−0.050	0.520
Blood glucose levels (mmol/l)	0.229	0.003
Blood leukocyte count ($\times 10^9$/l)	0.071	0.364

Note: In use of Spearman test, bivariate correlations were reported.

independently associated with DCI. The Hosmer–Lemeshow test showed that the model was stable (P=0.429). In the subgroup analysis, no significant interactions were found between serum secretoneurin levels and other variables such as age, sex, and other vascular risk factors (all P>0.05; Figure 6). Next, serum secretoneurin and the modified Fisher scale were combined to form a model that was described by a nomogram (Figure 7). The model was assessed as having no obvious higher discriminatory efficiency under the calibration curve (Figure 8), decision curve (Figure 9), and ROC curve (Figure 10).

Serum Secretoneurin Levels and Poor Prognosis at Six Months After aSAH

Six months after aSAH, the GOSE scores ranged from 1 to 8 (median, 5; 25^{th} – 75^{th} percentiles, 4–7). GOSE scores from 1 to 8 were obtained for 11, 14, 14, 17, 31, 22, 21, and 37 patients, respectively. Serum secretoneurin levels were significantly positively correlated with GOSE scores (P<0.001; Figure 11A) and prominently increased from 1 to 8 (P<0.001; Figure 11B). A total of fifty-six patients had a poor prognosis six months after aSAH. Patients presenting with poor prognosis, in contrast to those with good prognosis, exhibited profoundly higher serum secretoneurin levels (P<0.001; Figure 12A). Additionally, serum secretoneurin levels substantially discriminated patients at risk of poor prognosis, and serum secretoneurin levels more than 37.2 ng/mL differentiated a poor prognosis with a maximum Youden index of 0.498 (Figure 12B). Using a restricted cubic spline, there was a clear linear relationship between serum secretoneurin levels and poor prognosis (P>0.05; Figure 13). In Table 3, as compared to patients with good prognosis, those with development of poor prognosis had significantly higher Hunt-Hess scores, modified Fisher scores, serum secretoneurin levels and blood glucose levels, as well as displayed markedly enhanced percentages of hypertension,

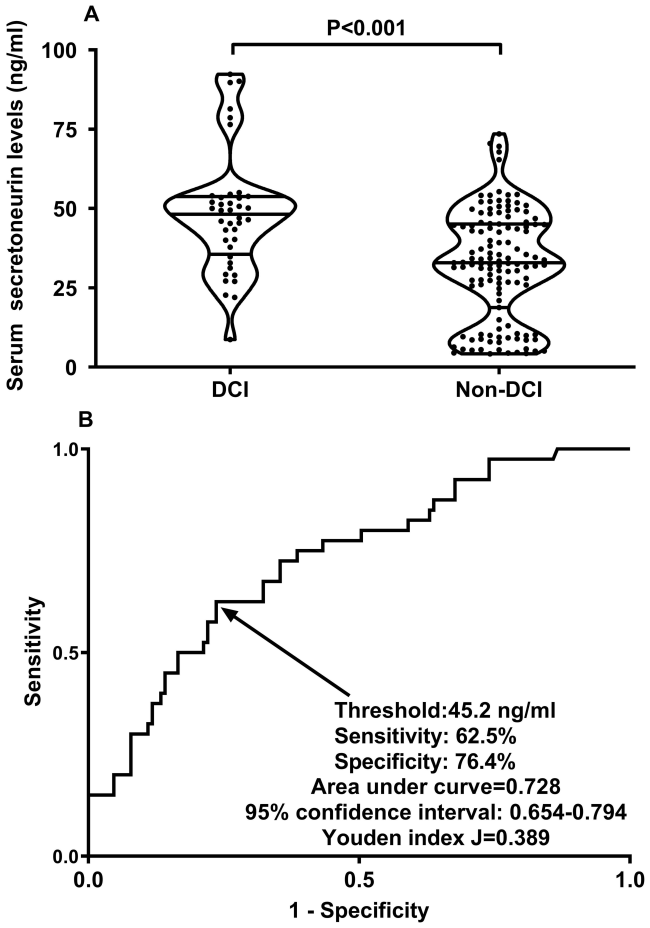

Figure 4 Serum secretoneurin levels predicting delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. (**A**) Difference in terms of serum secretoneurin levels across delayed cerebral ischemia following aneurysmal subarachnoid hemorrhage. Serum secretoneurin levels were significantly higher in patients with delayed cerebral ischemia than in those without (P<0.001). DCI means delayed cerebral ischemia. (**B**)Discrimination efficiency of serum secretoneurin levels for risk of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage under receiver operating characteristic curve. Delayed cerebral ischemia was efficiently predicted by serum secretoneurin levels and its optimal threshold value was chosen using the Youden method, which distinguished delayed cerebral ischemia with the medium-high sensitivity and specificity.

intraventricular hemorrhage and external ventricular drainage (all P<0.05). The above-mentioned seven significant variables in the univariate analysis were entered into the binary logistic regression model, and it was confirmed that Hunt-Hess scores (odds ratio, 2.241; 95% confidence interval, 1.363–3.684; P=0.004), modified Fisher scores (odds ratio, 2.372; 95% confidence interval, 1.326–4.240; P=0.010), and serum secretoneurin levels (odds ratio, 1.035; 95% confidence interval, 1.012–1.065; P=0.029) independently predicted a poor prognosis. The Hosmer–Lemeshow test showed that the model was stable (P=0.259). Subgroup analysis showed that serum secretoneurin levels were not significantly associated with age, sex, or other vascular risk factors (all P>0.05; Figure 14). The three independent predictors, namely, serum secretoneurin, Hunt-Hess scale, and modified Fisher scale, were merged into a prediction model, as shown (Figure 15). Using calibration (Figure 16), decision (Figure 17), and ROC curves (Figure 18), the model was comparatively stable, clinically beneficial, and efficient.

Discussion

To the best of our knowledge, this is the first study to confirm that serum secretoneurin levels are altered following aSAH. Moreover, serum secretoneurin levels were independently correlated with Hunt-Hess scores and modified Fisher scores, and were independently associated with DCI and poor six-month prognosis after aSAH. In addition, serum secretoneurin levels were linearly correlated with the risk of DCI and poor prognosis and had no significant interactions

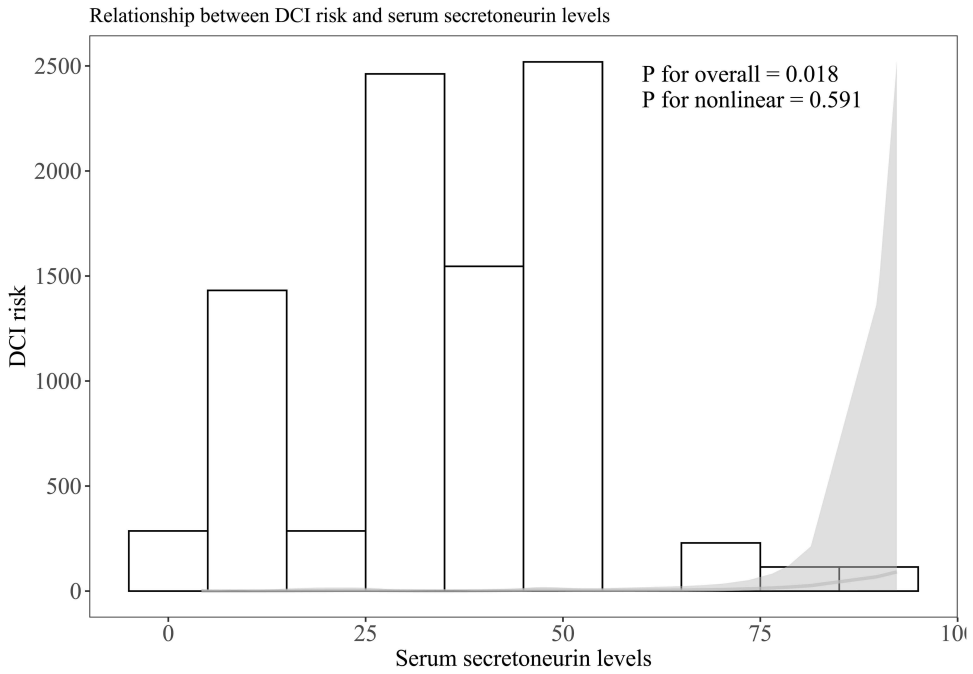

Figure 5 Restricted cubic spline delineating linear relationship between serum secretoneurin levels and risk of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. A linear relationship was observed between serum secretoneurin levels and the risk of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage (P>0.05). DCI means delayed cerebral ischemia.

with other variables, such as age, sex, hypertension, diabetes mellitus, alcohol drinking, and cigarette alcohol, for predicting DCI and six-month poor prognosis in aSAH. Interestingly, the prognosis prediction model, in which independent predictors were integrated, performed well using a series of statistical methods. These data strongly suggest that serum secretoneurin may be a potential biochemical marker for predicting the clinical outcomes of aSAH.

Table 2 Factors in Relation to Delayed Cerebral Ischemia After Aneurysmal Subarachnoid Hemorrhage

	Delayed Cerebral Ischemia		*P* value
	Presence	Absence	
Gender (male/female)	13/27	49/78	0.487
Age (years)	50.3±9.4	50.9±11.2	0.744
Cigarette smoking	11 (27.5%)	38 (29.9%)	0.769
Alcohol drinking	9 (22.5%)	40 (31.5%)	0.276
Hypertension	13 (32.5%)	25 (19.7%)	0.092
Diabetes mellitus	6 (15.0%)	7 (5.5%)	0.083
Previous statins use	7 (17.5%)	17 (13.4%)	0.518
Previous anticoagulant use	3 (7.5%)	6 (4.7%)	0.448
Previous antiplatelet use	7 (17.5%)	9 (7.1%)	0.065
Systolic arterial blood pressure (mmHg)	128.6±33.9	129.5±23.9	0.853
Diastolic arterial blood pressure (mmHg)	74.5±19.4	76.4±13.8	0.480
Hunt-Hess scores	4 (3–4)	3 (2–3)	<0.001
Modified Fisher scores	3 (3–3)	2 (1–3)	<0.001
Aneurysmal position (posterior/anterior circulation)	8/32	26/101	0.948
Aneurysmal shape (cystic/others)	32/8	108/19	0.450
Aneurysmal diameter (<10 mm/≥10 mm)	21/19	76/51	0.412
Securing modality of aneurysms (clipping/endovascular intervention)	18/22	45/82	0.276
Acute hydrocephalus	7 (17.5%)	14 (11.0%)	0.281
Intraventricular bleeding	12 (30.0%)	13 (10.2%)	0.002
Intracerebral hemorrhage	8 (20.0%)	6 (4.7%)	0.006

(*Continued*)

Table 2 (Continued).

	Delayed Cerebral Ischemia		*P* value
	Presence	Absence	
External ventricular drain	9 (22.5%)	14 (11.0%)	0.066
Admission time after stroke (h)	9.6 (4.9–12.5)	8.0 (3.3–12.0)	0.406
Blood-sampling time after (h)	10.2 (5.6–14.0)	9.0 (4.4–13.7)	0.477
Pneumonia	7 (17.5%)	19 (15.0%)	0.699
Seizure	4 (10.0%)	11 (8.7%)	0.758
Blood glucose levels (mmol/l)	14.3 (8.1–16.2)	9.1 (7.5–11.6)	0.006
Blood leukocyte count ($\times 10^9$/l)	9.2 (6.4–11.0)	7.2 (5.2–11.3)	0.318
Serum secretoneurin levels (ng/mL)	48.2 (36.3–53.7)	32.9 (19.9–45.1)	<0.001

Notes: Data were presented in form of count (proportion), mean + standard deviation or median (percentiles 25th-75th) as appropriate. Comparisons were fulfilled using the student *t* test, Mann–Whitney test, Fisher's exact test or $\chi 2$ test as appropriate.

Secretoneurin acts as a neuropeptide with extensive distribution in nervous tissues and functions as a neuroprotective factor by inducing angiogenesis, regulating neuroinflammation and neurotransmitter release, and stimulating pituitary hormone release under hypoxic conditions.[9–12] Specifically, its supplementation could dramatically rescue primary cortical cells against oxygen/glucose deprivation injury, its intraventricular injection greatly reduced cerebral infarction and improved motor function in rats with occluded right middle cerebral arteries, and intraperitoneal injection could

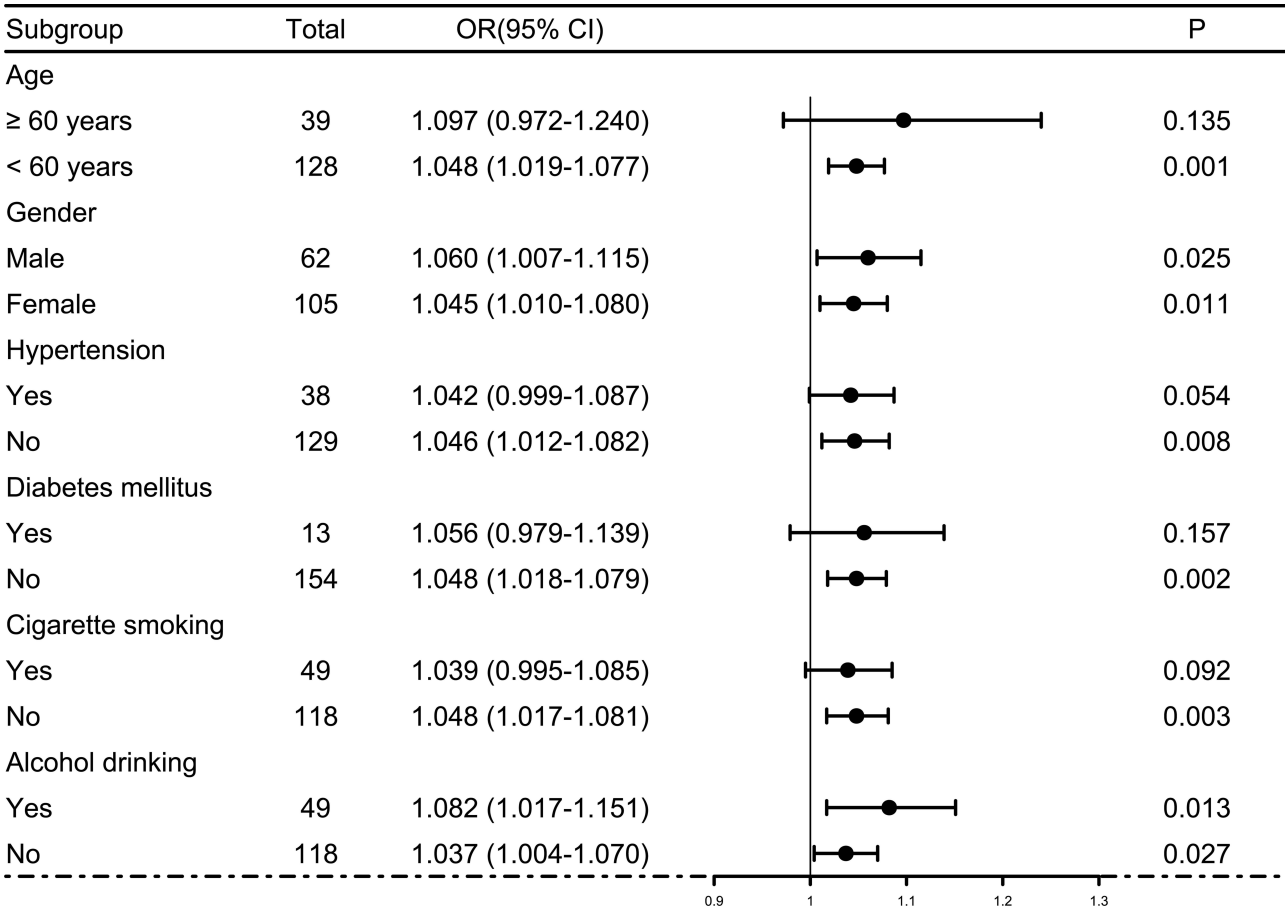

Figure 6 Subgroup analysis of delayed cerebral ischemia in aneurysmal subarachnoid hemorrhage. There were no significant interactions between serum secretoneurin levels and other variables, such as age, sex, hypertension, diabetes, smoking, and drinking (all P interaction > 0.05). OR indicates odds ratio; 95% CI, 95% confidence interval.

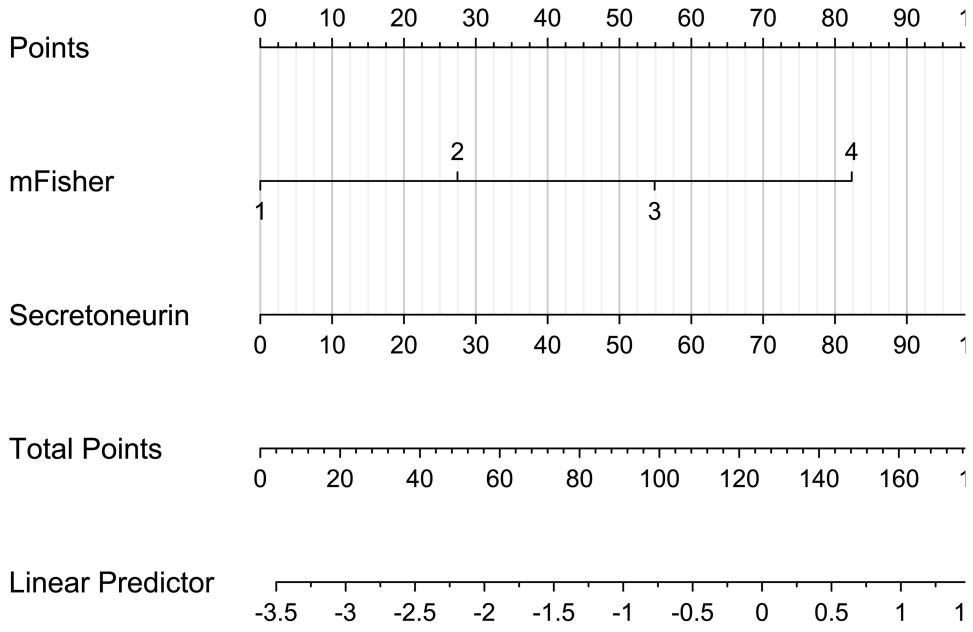

Figure 7 Nomogram describing prediction model of delayed cerebral ischemia following aneurysmal subarachnoid hemorrhage. Modified Fisher scores and serum secretoneurin levels were combined to determine the risk of delayed cerebral ischemia. mFisher indicates modified Fisher.

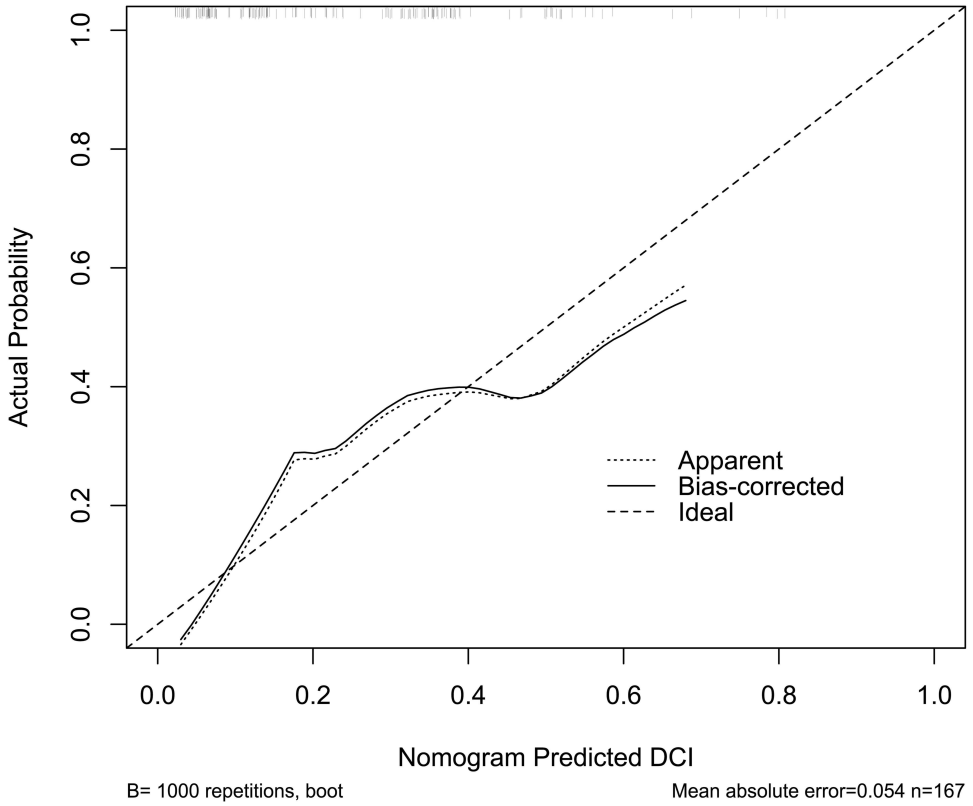

Figure 8 Calibration curve assessing stability of prediction model of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. The prediction model of delayed cerebral ischemia may take possession of strong stability. DCI indicates delayed cerebral infarction.

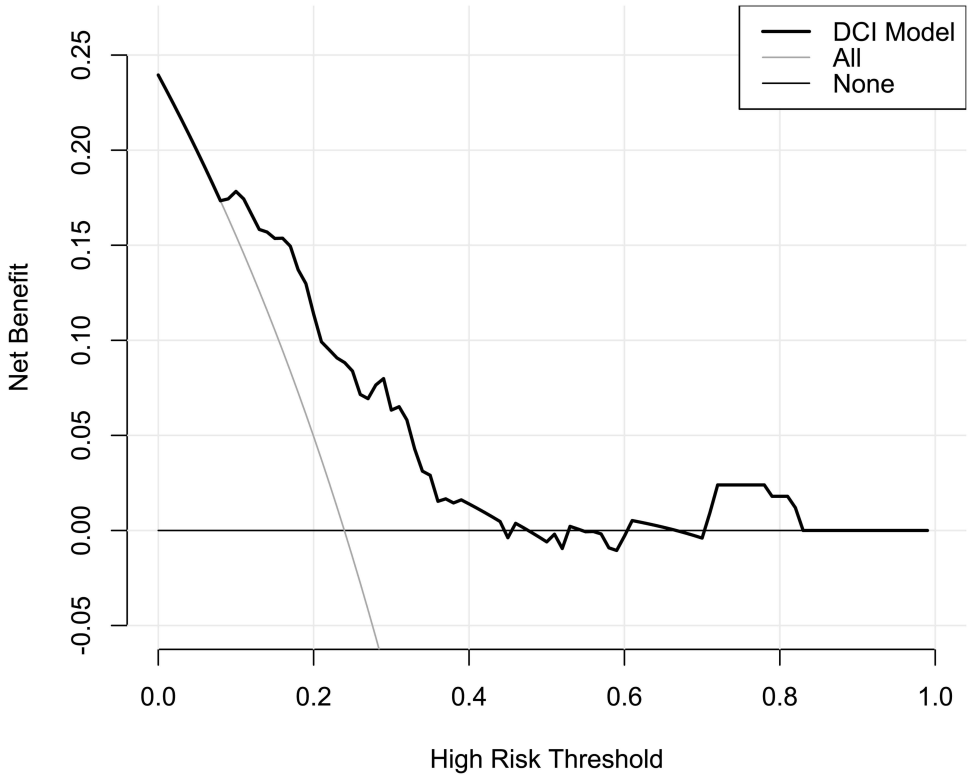

Figure 9 Decision curve evaluating clinical benefit of prediction model of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. The prediction model of delayed cerebral ischemia may be of clinical benefit. DCI indicates delayed cerebral infarction.

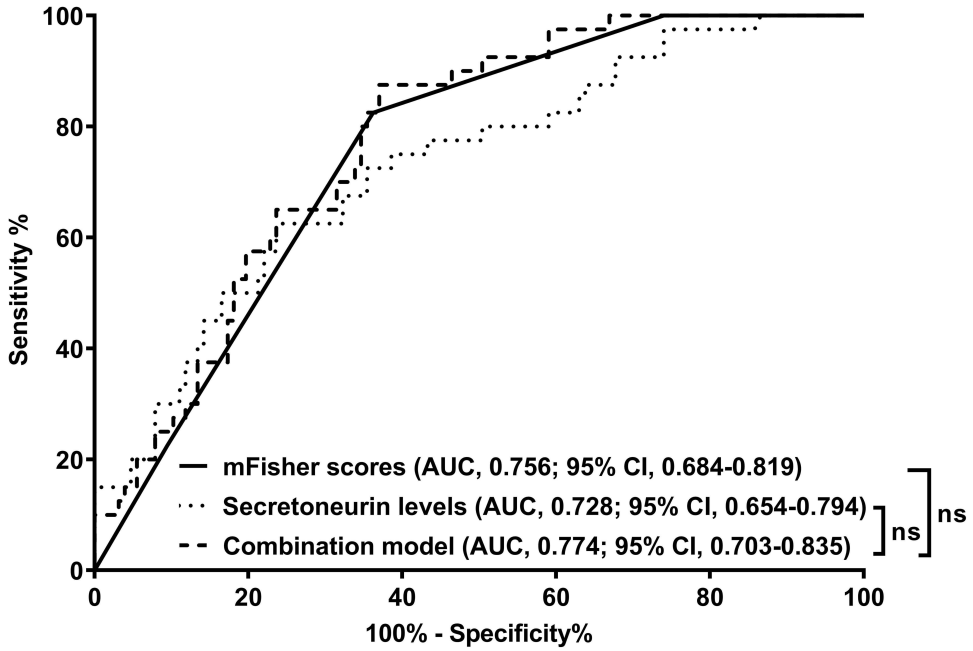

Figure 10 Receiver operating characteristic curves showing the predictive ability of prediction model of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. Compared to the modified Fisher scores and serum secretoneurin levels, the model did not display a significantly elevated predictive capability (both P>0.05). mFisher means modified Fisher; AUC, area under the curve; 95% CI, 95% confidence interval; ns, non-significant.

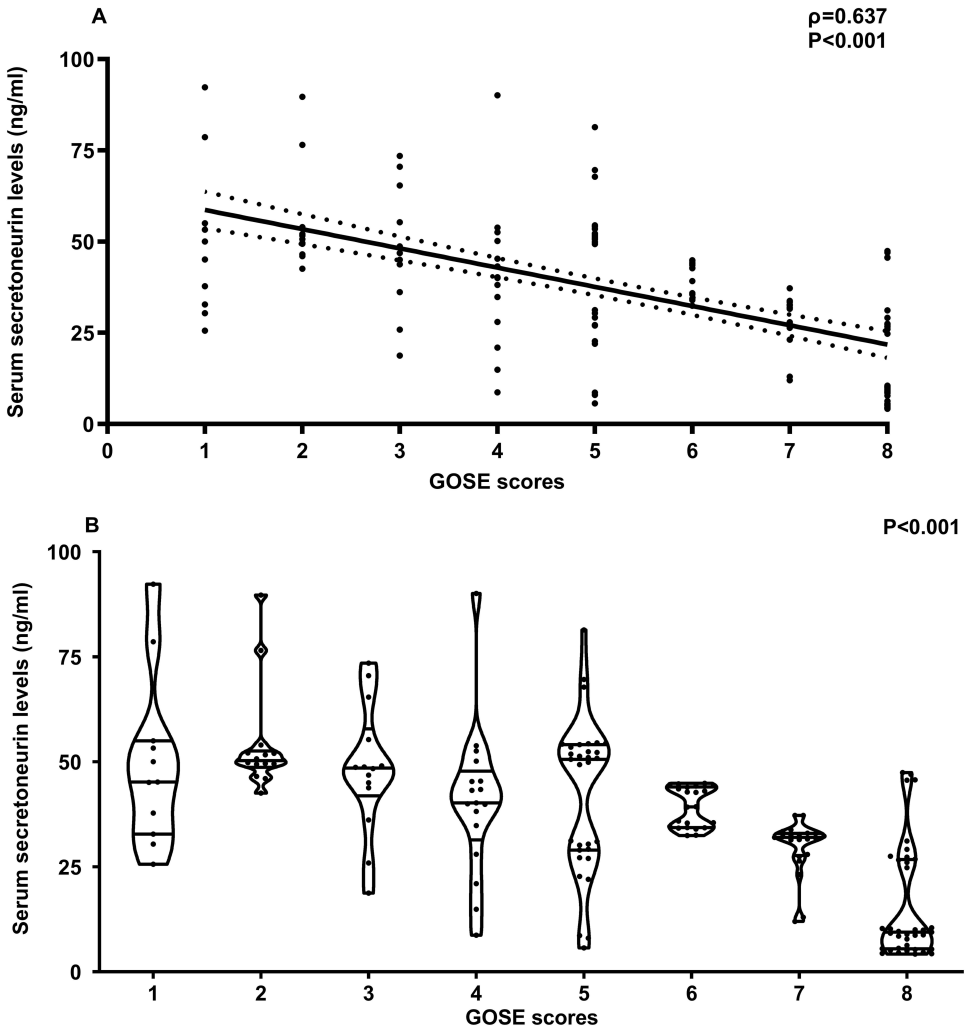

Figure 1 Serum secretoneurin levels and six-month extended Glasgow Outcome Scale scores after aneurysmal subarachnoid hemorrhage. (**A**)Relationship between serum secretoneurin levels and six-month extended Glasgow Outcome Scale scores after aneurysmal subarachnoid hemorrhage. Serum secretoneurin levels were markedly inversely correlated with six-month extended Glasgow Outcome Scale scores (P<0.001). GOSE indicates Extended Glasgow Outcome Scale.**(B)**Serum secretoneurin levels in subgroups with different six-month extended Glasgow Outcome Scale scores after aneurysmal subarachnoid hemorrhage. Serum secretoneurin levels were substantially decreased in the order of six-month extended Glasgow Outcome Scale scores from 1 to 8 (P<0.001). GOSE indicates Extended Glasgow Outcome Scale.

markedly enhance neurogenesis and angiogenesis in stroke mice.[13] Similarly, secretoneurin protects primary hippocampal neurons against oxygen/glucose deprivation damage, and in mice undergoing unilateral common carotid artery ligation or exposure to 8% oxygen/nitrogen, hemisphere administration of secretoneurin substantially represses neuronal apoptosis and inhibits microglial cell activation.[21] Thus, secretoneurin may protect neurons against hypoxia and ischemic insults.

Secretoneurin in neurons and endothelial cells is significantly upregulated in brain tissues of ischemic rats and humans.[13] High concentrations of secretoneurin are present in cerebrospinal fluid.[14] In addition, compared to healthy individuals, secretoneurin was found to be present at higher levels in the umbilical cord blood of neonates after hypoxic-ischemic encephalopathy[15] and in the peripheral blood of adult stroke patients.[16] Our data showed that the serum secretoneurin levels were substantially elevated after aSAH. Given that hypoxia and ischemia are two common pathophysiological mechanisms of aSAH,[22,23] secretoneurin is presumably elevated in these patients. In addition, considering the neuroprotective role of secretoneurin,[9–12] its elevation may be a compensatory response to hypoxic and ischemic injury following aSAH.

There was a substantial elevation in serum secretoneurin levels after cardiopulmonary resuscitation in cardiac arrest patients, which was significantly associated with poor neurological outcomes, as indicated by the Cerebral Performance

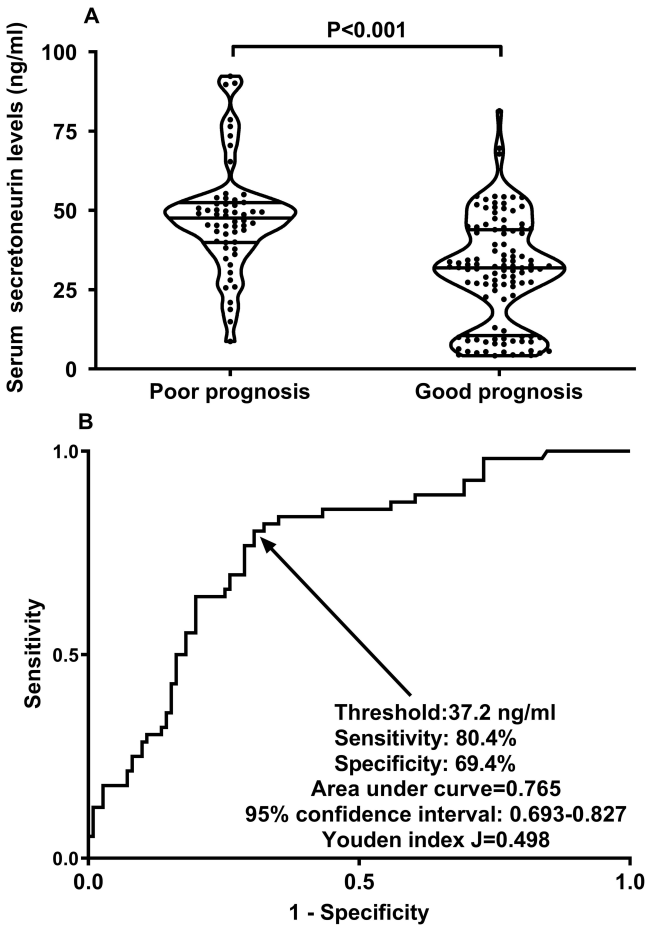

Figure 12 Serum secretoneurin levels for predicting six-month poor prognosis after aneurysmal subarachnoid hemorrhage. **(A)** Difference in terms of serum secretoneurin levels across six-month poor prognosis following aneurysmal subarachnoid hemorrhage. Serum secretoneurin levels were significantly higher in patients with six-month poor prognosis than in those without (P<0.001). **(B)**Discrimination efficiency of serum secretoneurin levels for risk of six-month poor prognosis after aneurysmal subarachnoid hemorrhage under receiver operating characteristic curve. Six-month poor prognosis was efficiently predicted by serum secretoneurin levels and its optimal threshold value was chosen using the Youden method, which distinguished six-month poor prognosis with the medium-high sensitivity and specificity.

Categories Scale at hospital discharge.[17] Consistently, serum secretoneurin levels were markedly higher in patients with moderate-to-severe traumatic brain injury than in healthy controls and were closely related to mortality at discharge in patients with traumatic brain injury.[18] Although the two preceding clinical studies offered results using only univariate analysis,[17,18] such data have suggested that serum secretoneurin may be a biomarker of acute brain injury.

In the current study, multivariate analysis was used to investigate the relationship between serum secretoneurin levels and stroke severity, as assessed using Hunt-Hess and modified Fisher scores. In addition, DCI and poor six-month prognosis were designated as the dependent variables. Multivariate analysis confirmed its independent correlation with disease severity and its association with DCI and poor prognosis. Furthermore, to be more scientific and reliable at the evidence level, linear correlations and interactions were assessed. Serum secretoneurin levels were significantly linearly related to DCI and poor prognosis, and there were no substantial interactions between serum secretoneurin levels and other variables such as age, sex, hypertension, diabetes, alcohol consumption, and cigarette smoking. Notably, the prognosis model, in which three independent predictors were incorporated, showed high clinical value via several statistical verifications, including ROC, decision, and calibration curve analyses. These results undoubtedly support the inference that secretoneurin may be a potential biomarker in relation to the severity, DCI, and prognosis of aSAH.

There are several advantages and weaknesses in this study. The advantages are that (1) to the best of our knowledge, serum secretoneurin is for the first time investigated in aSAH, and subsequently it was found that serum secretoneurin may be an excellent biomarker for assessing severity and forecasting DCI and poor prognosis following aSAH; and (2)

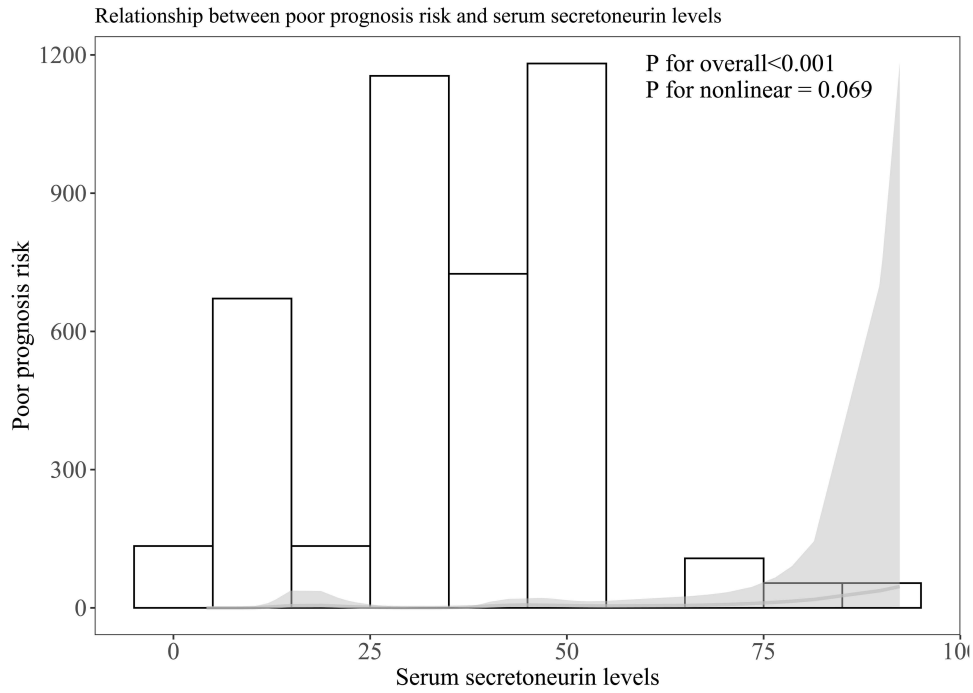

Figure 13 Restricted cubic spline delineating linear relationship between serum secretoneurin levels and risk of poor prognosis after aneurysmal subarachnoid hemorrhage. A linear relationship was observed between serum secretoneurin levels and the risk of poor prognosis after aneurysmal subarachnoid hemorrhage (P>0.05).

the potential of serum secretoneurin as a satisfactory biochemical marker was verified using numerous assessment methods, such as multivariate analysis, ROC curve analysis, calibration curve analysis, decision curve analysis, restricted cubic spline assessment and subgroup analysis approach. The weaknesses are that (1) serum secretoneurin levels were

Table 3 Factors in Relation to Neurological Functional Outcome at 6 Months After Aneurysmal Subarachnoid Hemorrhage

	Extended Glasgow Outcome Scale		*P* value
	Score 1–4	Score 5–8	
Gender (male/female)	17/39	45/66	0.198
Age (years)	51.5±11.6	50.4±10.3	0.559
Cigarette smoking	15 (26.8%)	34 (30.6%)	0.606
Alcohol drinking	18 (32.1%)	31 (27.9%)	0.572
Hypertension	18 (32.1%)	20 (18.0%)	0.040
Diabetes mellitus	8 (14.3%)	5 (4.5%)	0.034
Previous statin use	7 (12.5%)	17 (15.3%)	0.624
Previous anticoagulant use	3 (5.4%)	6 (5.4%)	1.000
Previous antiplatelet use	7 (12.5%)	9 (8.1%)	0.363
Systolic arterial blood pressure (mmHg)	128.2±27.0	129.8±26.4	0.724
Diastolic arterial blood pressure (mmHg)	75.9±16.1	76.0±14.9	0.977
Hunt-Hess scores	3 (3–4)	2 (1–3)	<0.001
Modified Fisher scores	3 (3–3)	2 (1–3)	<0.001
Aneurysmal position (posterior/anterior circulation)	12/44	22/89	0.807
Aneurysmal shape (cystic/others)	44/12	96/15	0.190
Aneurysmal diameter (<10 mm/≥10 mm)	31/25	66/45	0.612
Securing modality of aneurysms (clipping/endovascular intervention)	20/36	43/68	0.703
Acute hydrocephalus	11 (19.6%)	10 (9.0%)	0.050
Intraventricular bleeding	14 (25.0%)	11 (9.9%)	0.010
Intracerebral hemorrhage	8 (14.3%)	6 (5.4%)	0.074

(*Continued*)

Table 3 (Continued).

	Extended Glasgow Outcome Scale		*P* value
	Score 1–4	Score 5–8	
External ventricular drain	12 (21.4%)	11 (9.9%)	0.041
Admission time after stroke (h)	9.4 (5.3–12.3)	8.0 (3.2–12.0)	0.283
Blood-sampling time after stroke (h)	10.1 (5.9–14.2)	9.0 (3.7–13.5)	0.303
Pneumonia	12 (21.4%)	14 (12.6%)	0.138
Seizure	5 (8.9%)	10 (9.0%)	0.986
Blood glucose levels (mmol/l)	10.3 (7.8–15.6)	9.2 (7.4–11.7)	0.021
Blood leukocyte count ($\times 10^9$/l)	9.1 (6.7–11.0)	6.6 (5.0–11.5)	0.071
Serum secretoneurin levels (ng/mL)	47.6 (40.0–52.3)	31.9 (11.3–43.7)	<0.001

Notes: Data were presented in form of count (proportion), mean + standard deviation or median (percentiles 25th-75th) as appropriate. Comparisons were fulfilled using the student *t* test, Mann–Whitney test, Fisher's exact test or $\chi 2$ test as appropriate.

measured only at a time-point and it may better strengthen clinical significance to determine evolutionary trajectory of serum secretoneurin levels with time progression; and (2) although an enough sample size has been in application for statistical analysis, it is undoubtedly more valuable for the conclusions to be validated in a larger cohort study.

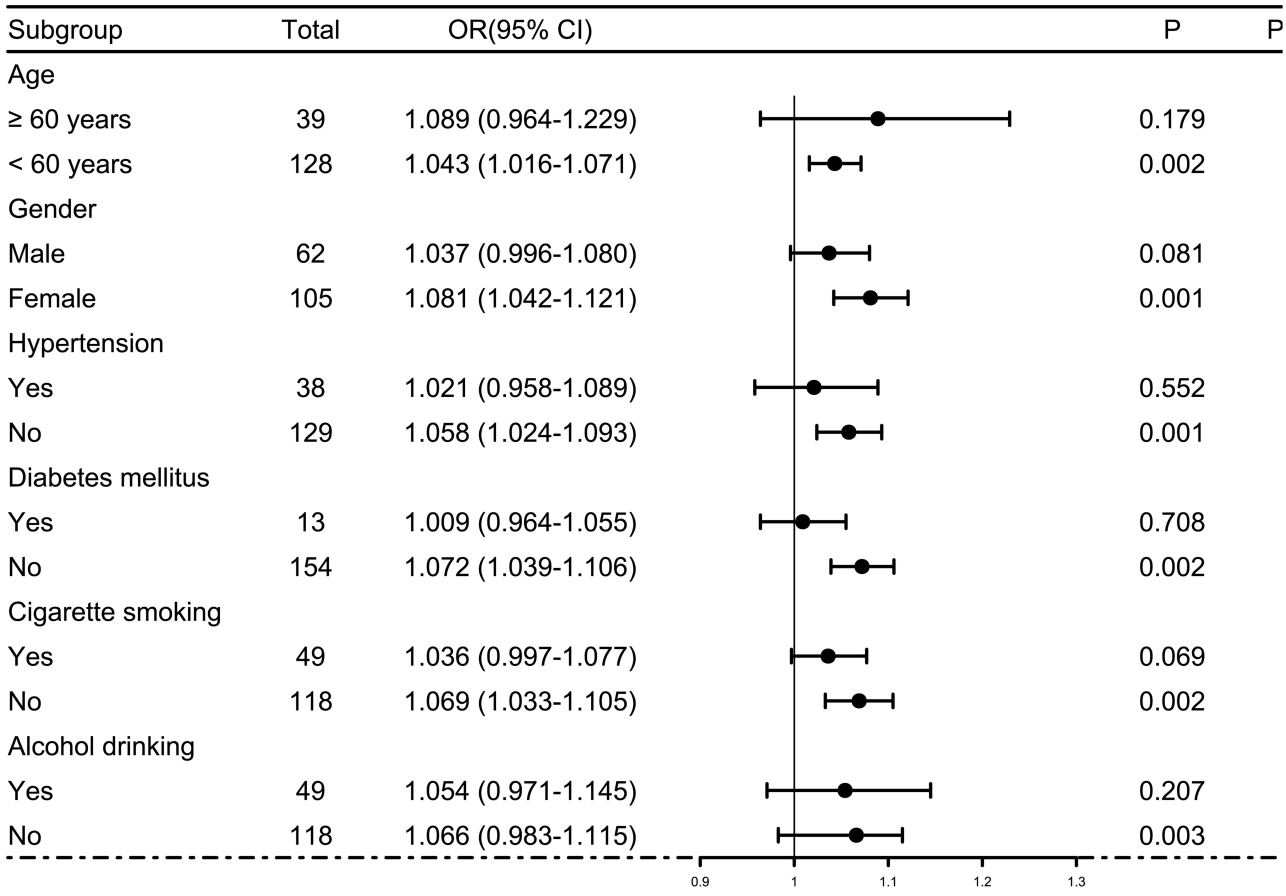

Figure 14 Subgroup analysis of poor prognosis after aneurysmal subarachnoid hemorrhage. No significant interactions were found between serum secretoneurin levels and other variables, such as age, sex, hypertension, diabetes, smoking, and drinking (all P interaction > 0.05). OR indicates odds ratio; 95% CI, 95% confidence interval.

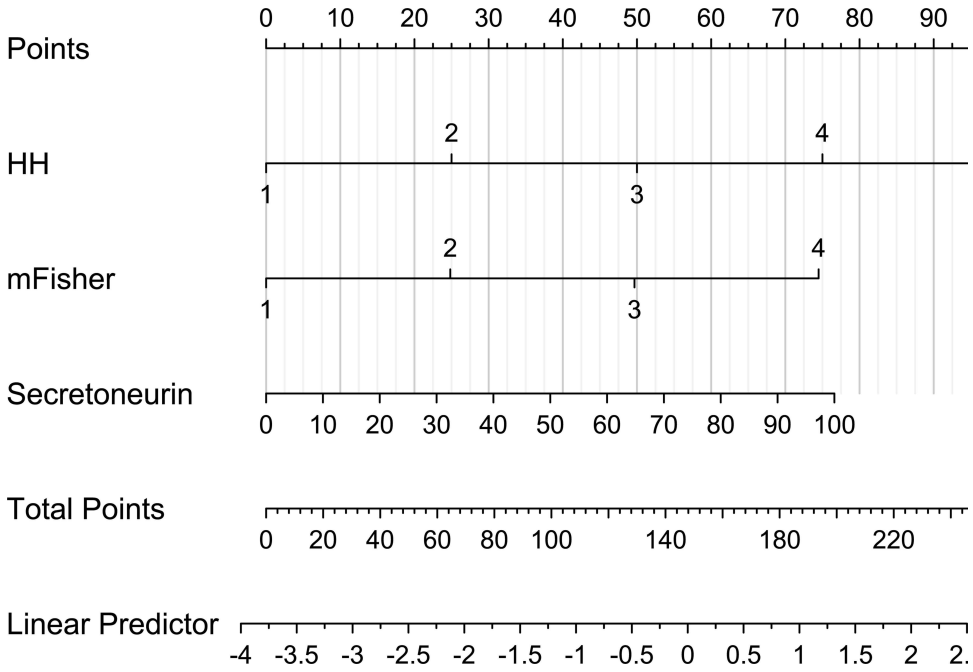

Figure 15 Nomogram describing prediction model of poor prognosis after aneurysmal subarachnoid hemorrhage. The Hunt-Hess scores, modified Fisher scores, and serum secretoneurin levels were integrated to assess the risk of poor prognosis. mFisher indicates modified Fisher; HH, Hunt-Hess.

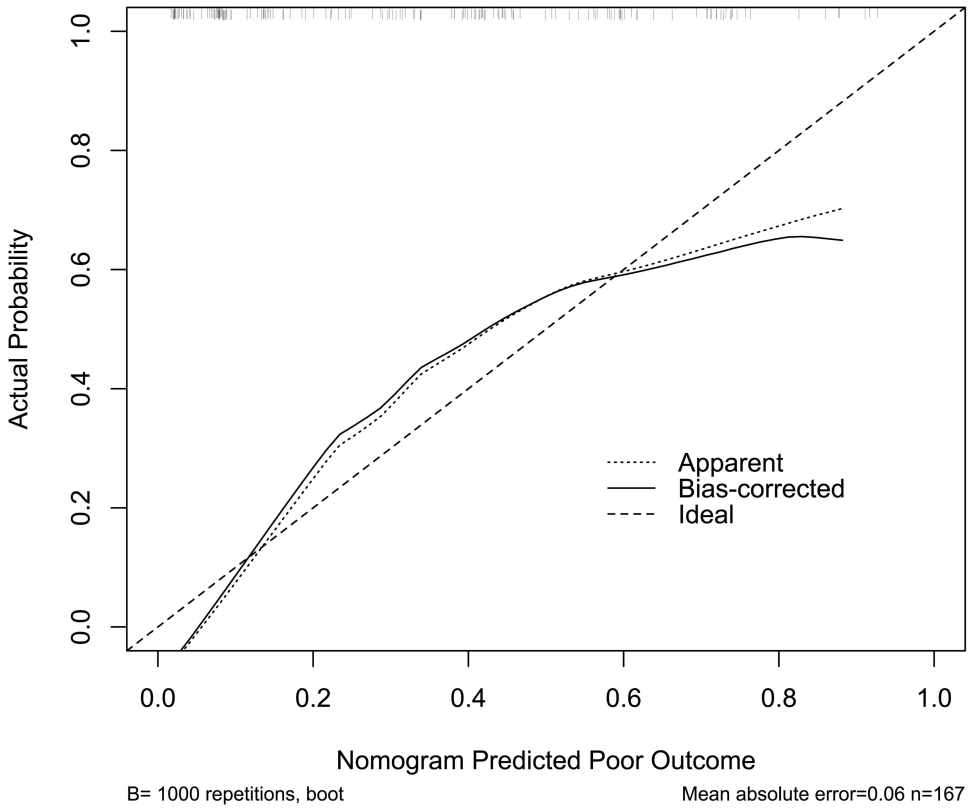

Figure 16 Calibration curve evaluating stability of prediction model of poor prognosis after aneurysmal subarachnoid hemorrhage. The prognosis prediction model may possess strong stability.

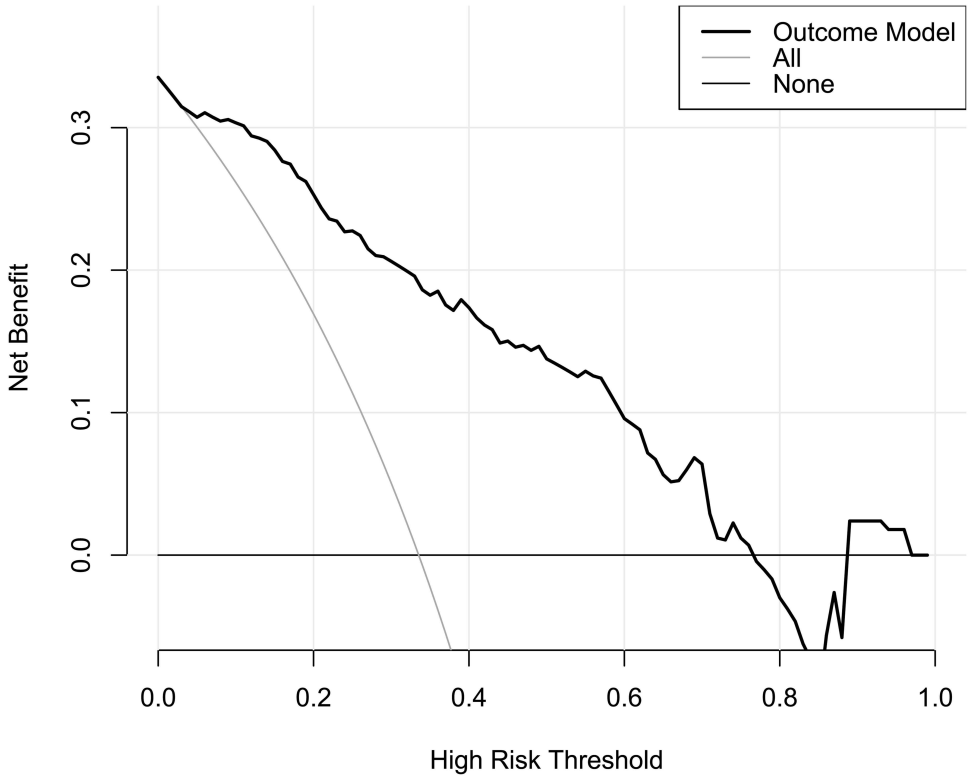

Figure 17 Decision curve evaluating clinical benefit of prognosis prediction model following aneurysmal subarachnoid hemorrhage. The prognosis prediction model may be clinically beneficial.

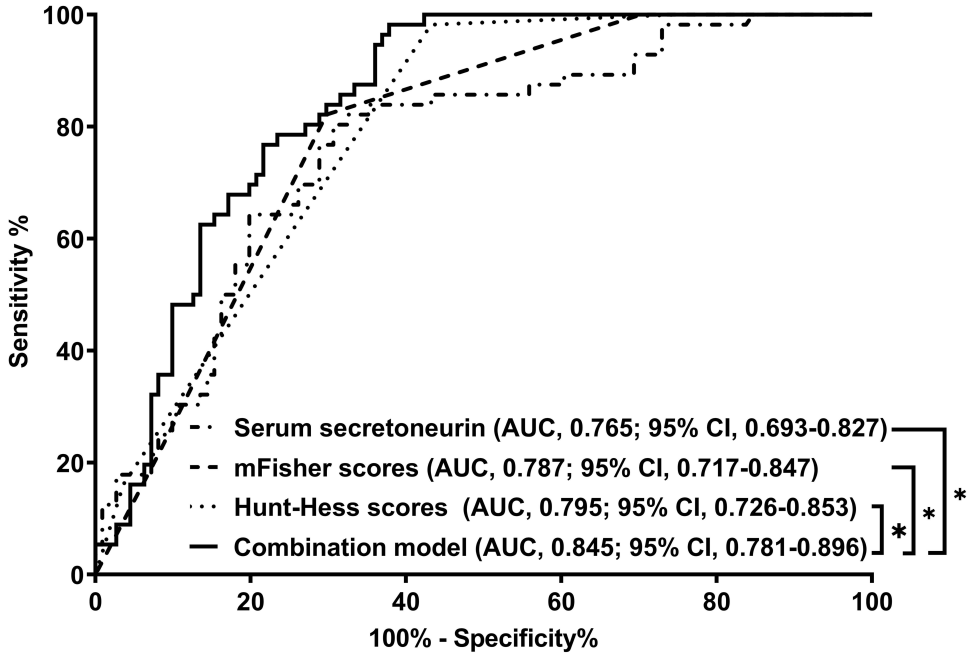

Figure 18 Receiver operating characteristic curves exhibiting the discriminatory ability of prognosis prediction model following aneurysmal subarachnoid hemorrhage. Compared to the modified Fisher scores, Hunt-Hess scores, and serum secretoneurin levels, the model displayed a significantly elevated predictive capability (all P<0.05). mFisher indicates modified Fisher; AUC, area under the curve; 95% CI, 95% confidence interval. *P <0.05.

Conclusions

In this study, we measured the serum secretoneurin levels in a group of patients with aSAH. While observing substantially increased serum secretoneurin levels after aSAH, we also observed an independent correlation between serum secretoneurin levels and the extent of brain injury. Additionally, serum secretoneurin has been demonstrated to be an independent predictor of DCI and poor prognosis six months after aSAH. Moreover, serum secretoneurin has efficient prognostic capability in aSAH. Taken together, serum secretoneurin may serve as a valuable biomarker for evaluating the severity and prognosis of neurological outcomes after aSAH.

Data Sharing Statement

The datasets generated and/or analyzed during the current study are not publicly available because they are personal data, but are available from the corresponding author upon reasonable request.

Consent for Publication

Not applicable.

Acknowledgments

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Disclosure

The authors declared no potential conflicts of interest in this work.

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