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Serum netrin-1 serves as a prognostic biomarker of aneurysmal subarachnoid hemorrhage

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ABSTRACT

Background: Netrin-1 exhibits anti-inflammatory properties. Netrin-1 could alleviate brain injury of subarachnoid hemorrhage (SAH) rat. This study was designed to discern the utility of serum netrin-1 as a biomarker for assessing the severity and prognosis of patients with aneurysmal SAH.

Methods: Netrin-1 concentrations were gauged in serum from 104 patients and 104 controls. Hemorrhagic clinical and radiological severity was assessed utilizing World Federation of Neurological Surgeons (WFNS) score, modified Fisher score, and Hunt Hess score. Glasgow Outcome Scale (GOS) score was recorded at 6 months after SAH. GOS score of 1–3 was considered as a poor outcome.

Results: Patients showed substantially lower serum netrin-1 concentrations than controls (median, 237.9 pg/ml; interquartile range, 189.6–271.2 pg/ml vs. median, 815.4 pg/ml; interquartile range, 581.8–990.4 pg/ml). Netrin-1 concentrations were independently correlated with WNFS score, modified Fisher score, Hunt Hess score and serum C-reactive protein concentrations ($t = -4.667$, -3.792, -4.304 and -3.549 respectively). Area under ROC curve was 0.837 (95% CI, 0.752–0.902) for predicting 6-month poor prognosis. Serum netrin-1 concentrations < 229.3 pg/ml emerged as an independent prognostic predictor (odds ratio, 14.316; 95% confidence interval, 5.032–40.726).

Conclusions: Serum netrin-1 might represent a potential biomarker for reflecting severity, inflammation and prognosis of human aneurysmal SAH.

1. Introduction

Netrin-1, a laminin-related protein, is implicated in the regulation of axon guidance and angiogenesis [1–5]. Accumulating evidence has shown that netrin-1 possesses anti-inflammatory properties [6–10]. Netrin-1 is an important regulator of blood-brain barrier maintenance that protects the central nervous system against inflammatory conditions such as multiple sclerosis and experimental autoimmune encephalomyelitis [11]. Also, netrin-1 could alleviate neuronal cellular apoptosis, inhibit brain inflammatory reaction, and maintain the integrity of blood-brain barrier in rats with ischemic stroke [12]. Aneurysmal subarachnoid hemorrhage (aSAH) is a common stroke type, where neuroinflammation plays an important role [13–17]. Recently, some experimental studies demonstrated that recombinant netrin-1 could attenuate neuroinflammation, ameliorate neuronal apoptosis, preserve blood-brain barrier integrity and subsequently, alleviate brain injury after animal SAH [18–21]. Therefore, netrin-1 is capable of exerting a neuroprotective effect on brain injury. Interestingly, decreased serum netrin-1 concentrations were revealed in humans with subclinical atherosclerosis [22], type 2 diabetes mellitus [23] and multiple sclerosis [24]. Moreover, elevated serum netrin-1 concentrations were associated with improved prognosis at 3 months after ischemic stroke [25], hinting that serum netrin-1 might be a potential prognostic biomarker for acute brain injury. To date, there is a paucity of data with respect to serum netrin-1 concentrations in aSAH patients. Therefore, we intended to prospectively investigate the relationship between serum netrin-1 concentrations and inflammation, severity in addition to prognosis at post-stroke 6 months in a group of humans with aSAH.

Abbreviations: aSAH, aneurysmal subarachnoid hemorrhage; WFNS, World Federation of Neurological Surgeons

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2. Methods

2.1. Study design, study population and ethics statement

We performed a prospective, observational study for 6-month prognostic assessment among first-ever aSAH patients at our hospital from May 2014 to May 2017. Exclusion criteria included admission time of > 24 h after the onset of stroke, time of > 48 h between admission and treatment of intracranial aneurysms, rebleeding following admission, multiple aneurysms, age of < 18 y, a history of neurological disease (e.g. ischemic or hemorrhagic stroke, severe head trauma and intracranial infection), use of antiplatelet or anticoagulant medication and steroid therapy, infection within recent 4 weeks and other systemic diseases (such as autoimmune diseases, hypertension, diabetes mellitus, uremia, liver cirrhosis, malignancy, chronic heart disease, and chronic lung disease). We chose a group of healthy individuals who constituted controls. The protocol of the current study was approved by the institutional ethics committees at our hospital prior to performance according to the Code of Ethics of the World Medical Association (Declaration of Helsinki). All controls and the relatives of patients gave their written informed consent.

2.2. Data collection

We collected demographic characteristics, clinical features, and medical history of all patients at enrollment. Stroke severity was assessed using World Federation of Neurological Surgeons (WFNS) score and Hunt-Hess score. Radiological severity of SAH was evaluated with utility of modified Fisher score. Aneurysms were confirmed using computerized tomography angiography or digital subtraction angiography. Information for aneurysm included its shape, size and position. Clipping or endovascular coiling was performed for aneurysms. Symptomatic cerebral vasospasm was defined according to several previous related studies [26–28]. Acute hydrocephalus and intraventricular hemorrhage might occur. If necessary, external ventricular drain was done. Glasgow outcome scale (GOS) score was recorded at 6 months after stroke. GOS score of 1–3 meant a poor outcome.

2.3. Serum netrin-1 determination

Blood samples were collected in Vacutainer® tubes and allowed to clot before centrifugation at 1000 × *g*, 20 min and 4 °C. The supernatants (serum) were transferred into a polypropylene tube and stored in aliquots at −80 °C. Serum netrin-1 concentrations were gauged with a quantitative sandwich enzyme linked immunosorbent assay detection kit purchased from Wuhan Huamei Biological Engineering Co., Ltd. Laboratory technicians who measured serum netrin-1 were blinded to baseline characteristics and clinical outcomes of the study participants. Every 3 months, quantifications were carried out in duplicate and the results were averaged.

2.4. Statistical analysis

The used statistical softwares included SPSS 19.0 for windows and MedCalc 9.6.4.0. Categorical variables were shown as frequency (percentage) and were compared using the x^2 test or Fisher's exact test as appropriate. Continuous variables (not normally distributed) were reported as median (interquartile range) and were compared with the Mann-Whitney *U* test or Kruskal-Wallis test as appropriate. Bivariate correlations were analyzed using Spearman's Coefficient test. Multiple linear regression analysis was used to examine associations between serum netrin-1 concentrations and other variables. Binary logistic regression analysis was utilized to investigate independent predictors of 6-month poor outcome, with reported odds ratio (OR) and 95% confidence interval (CI) values. Receiver operating characteristic (ROC) curve was conFig.d to determine some variables' discriminatory capability for poor outcome, with estimated area under curve (AUC) and 95% CI values. A 2 tailed *P* value < 0.05 was considered statistically significant.

Table 1
The clinical, laboratory and radiological characteristics of aneurysmal subarachnoid hemorrhage patients.

	Frequency (percentage) / median (interquartile range)
Gender (female)	61 (58.7%)
Age (y)	51.5 (36–61)
Body mass index (kg/m^2)	23.8 (22.6–25.4)
Smoking	43 (41.3%)
World Federation of Neurological Surgeons scores	3 (1–4)
Modified Fisher scores	2 (2–3)
Hunt Hess scores	2 (2–3)
Aneurysmal position (anterior circulation / posterior circulation)	86/18
Aneurysm shape (cystic)	89 (85.6%)
Aneurysm diameter (< 10 mm/≥10 mm)	45/59
Surgery (clipping/endovascular coiling)	57/47
Acute hydrocephalus	20 (19.2%)
Intraventricular hemorrhage	11 (10.6%)
External ventricular drain	22 (21.2%)
Symptomatic cerebral vasospasm	35 (33.7%)
Admission Time (h)	10.5 (8.4–14.7)
Blood- collecting Time (h)	15.0 (11.5–20.5)
Aneurysm-treating time (h)	25.5 (20.5–32.0)

Categorical variables were shown as frequency (percentage) and continuous variables were reported as median (interquartile range).

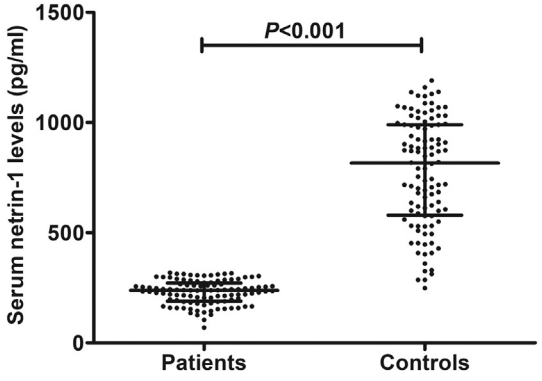

Fig. 1. Comparison of serum netrin-1 concentrations between healthy controls and patients with aneurysmal subarachnoid hemorrhage.

3. Results

3.1. Characteristics of study subjects

Initially, we enrolled a total of 141 aSAH patient. Afterwards, according to the exclusion criteria, thirty-seven patients were excluded. Among the excluded 37 patients, 3 patients had admission time of > 24 h after the onset of stroke, 4 patients had time of > 48 h between admission and treatment of intracranial aneurysms, 6 patients rebled following admission, 5 patients showed multiple aneurysms, 2 patient were aged at < 18 years, 5 patients had a history of neurological disease, 4 patients used antiplatelet or anticoagulant medication and steroid therapy, 2 patients were infected within recent four weeks and 6 patients suffered from other systemic diseases. Eventually, 104 (73.8%) patients were assessed in this study. The clinical, laboratory and radiological characteristics of the included patients were listed in Table 1. Simultaneously, we recruited a total of 104 healthy controls. They were aged at median value of 53 years (interquartile range, 34–62 years),

Table 2
The factors correlated with serum netrin-1 levels among aneurysmal subarachnoid hemorrhage patients using Spearman's Coefficient test.

	r value	*P* value
Gender (female/male)	0.183	NS
Age (y)	0.144	NS
Body mass index (kg/m^2)	0.140	NS
Smoking	0.026	NS
World Federation of Neurological Surgeons scores	−0.657	< 0.001
Modified Fisher scores	−0.575	< 0.001
Hunt Hess scores	−0.698	< 0.001
Aneurysmal position (anterior circulation / posterior circulation)	0.017	NS
Aneurysm shape (cystic)	0.190	NS
Aneurysm diameter (< 10 mm/≥10 mm)	0.041	NS
Surgery (clipping/endovascular coiling)	0.049	NS
Acute hydrocephalus	−0.278	0.004
Intraventricular hemorrhage	−0.228	0.020
External ventricular drain	−0.282	0.004
Symptomatic cerebral vasospasm	−0.301	0.002
Admission Time (h)	0.090	NS
Blood- collecting Time (h)	0.077	NS
Aneurysm-treating time (h)	0.032	NS
Systolic arterial pressure (mmHg)	−0.104	NS
Diastolic arterial pressure (mmHg)	−0.145	NS
Blood glucose (mmol/l)	−0.240	NS
Blood white blood cell count (×10^9/l)	−0.150	NS
Blood platelet count (×10^9/l)	0.169	NS
Serum C-reactive protein (mg/l)	−0.507	< 0.001

contained 63 females and 41 males, as well as had median body mass index of 23.6 kg/m^2 (interquartile range, 22.4–26.3 kg/m^2). There were not significant differences in age, gender percentage and body mass index between the controls and the aSAH patients. At 6 months after aSAH, GOS score ranged from 1 to 5 (median, 4; interquartile range, 2–5). There were 11 patients with GOS score 1, 15 patients with GOS score 2, 12 patients with GOS score 3, 38 patients with GOS score 4 and 28 patients with GOS score 5. By definition, 38 (36.5%) patients experienced a poor outcome (GOS score of 1–3).

3.2. Serum netrin-1 concentrations change after aSAH

Fig. 1 shows that serum netrin-1 concentrations were substantially lower in aSAH patients than in controls. Using bivariate correlation analysis, we found that serum netrin-1 concentrations were closely correlated with WFNS score, Hunt Hess scores, modified Fisher scores, aneurysmal shape, intraventricular hemorrhage, symptomatic cerebral vasospasm, acute hydrocephalus, external ventricular drain, blood glucose concentrations, blood platelet count and serum C-reactive protein concentrations (Table 2). Furthermore, we incorporated all preceding significant variables ($P < 0.1$) to multivariate linear regression model and thereby found that the variables in independent correlation with serum netrin-1 concentrations were WFNS score ($t = -4.667$, $P < 0.001$), Hunt Hess scores ($t = -4.304$, $P < 0.001$), modified Fisher scores ($t = -3.792$, $P < 0.001$) and serum C-reactive protein concentrations ($t = -3.549$, $P = 0.001$). Their correlations are depicted in Fig. 2. Also, WFNS score, Hunt Hess scores, modified Fisher

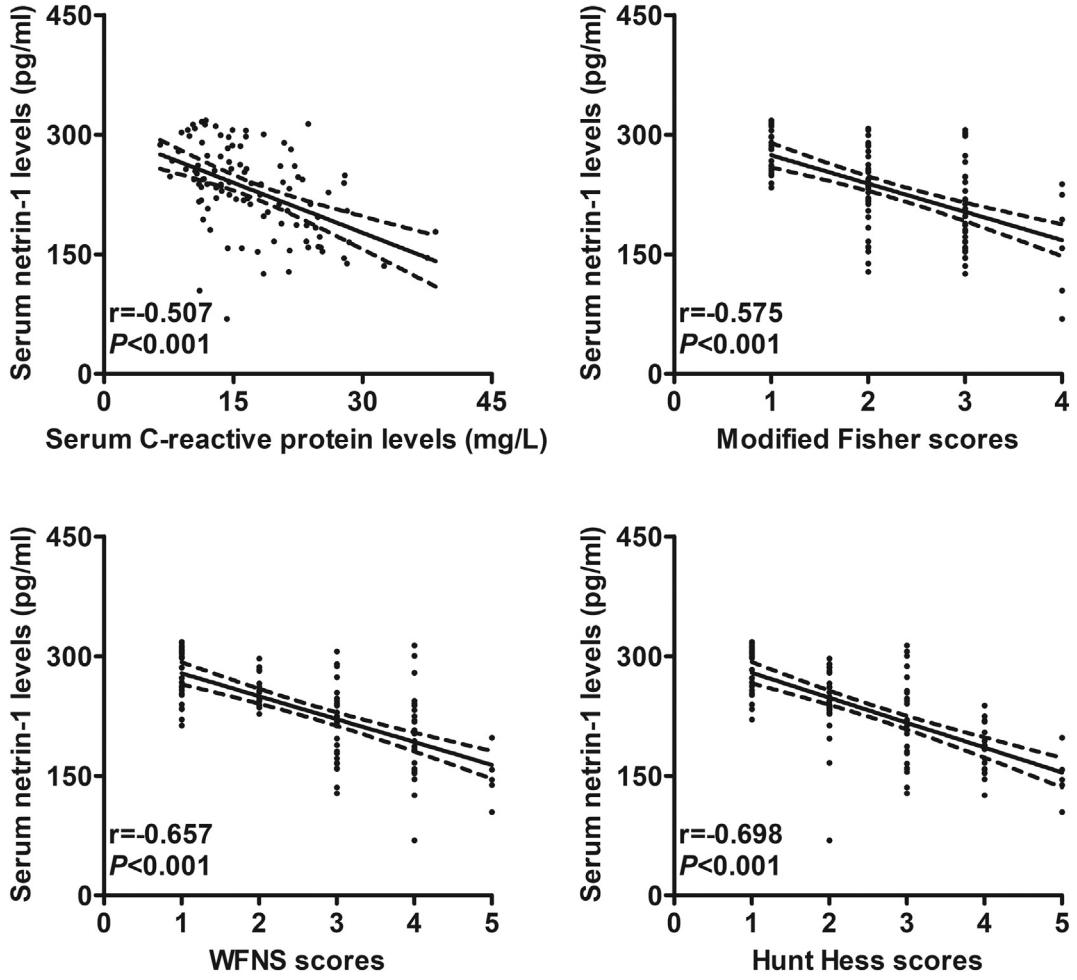

Fig. 2. Correlation of serum netrin-1 concentrations with World Federation of Neurological Surgeons (WFNS) scores, modified Fisher scores, Hunt Hess scores and serum C-reactive protein concentrations after aneurysmal subarachnoid hemorrhage.

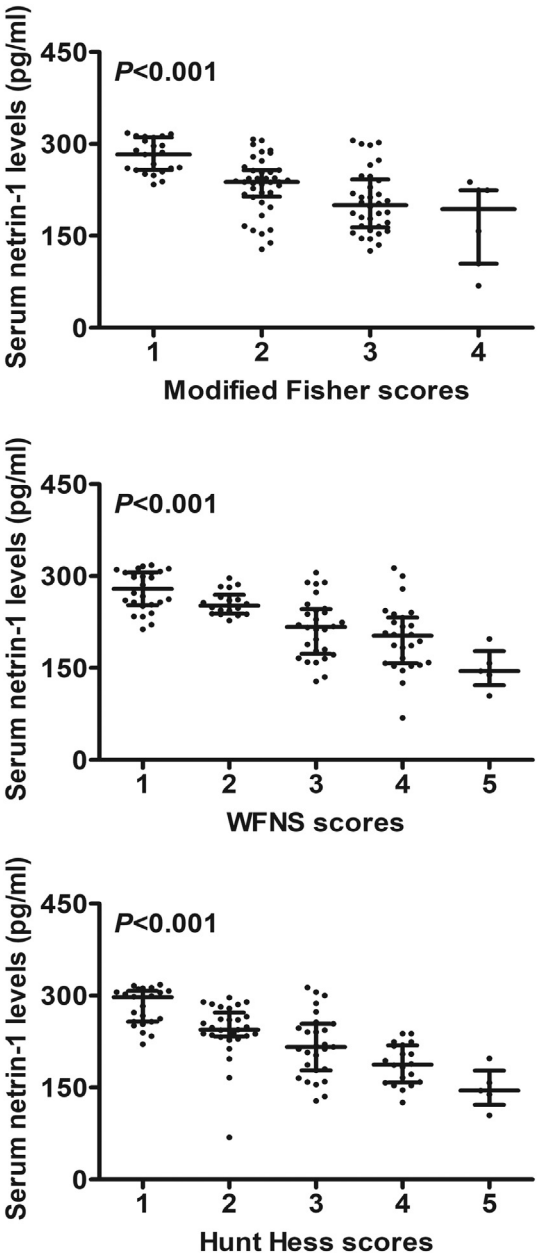

Fig. 3. Comparison of serum netrin-1 concentrations among different grades of severity indicated by World Federation of Neurological Surgeons (WFNS) scores, modified Fisher scores, and Hunt Hess scores following aneurysmal subarachnoid hemorrhage.

scores were considered as continuous variables and subsequently, Fig. 3 displays that serum netrin-1 concentrations were remarkably decreased with rising severity assessed by WFNS score, Hunt Hess scores, modified Fisher scores among this group of aSAH patients.

3.3. Outcome analysis of aSAH

In Fig. 4, serum netrin-1 concentration, whether as a categorical or continuous variable, was strongly and positively related to GOS scores at 6 months after stroke. Fig. 5 shows that serum netrin-1 concentration was profoundly declined in patients at risk of 6-month poor outcome, as compare with the remainders. Under ROC curve, serum netrin-1 concentration pronouncedly discriminated patients with the development of poor outcome at 6 months after SAH. In Fig. 5, an optimal cutoff value of serum netrin-1 concentration (229.3 pg/ml) was selected,

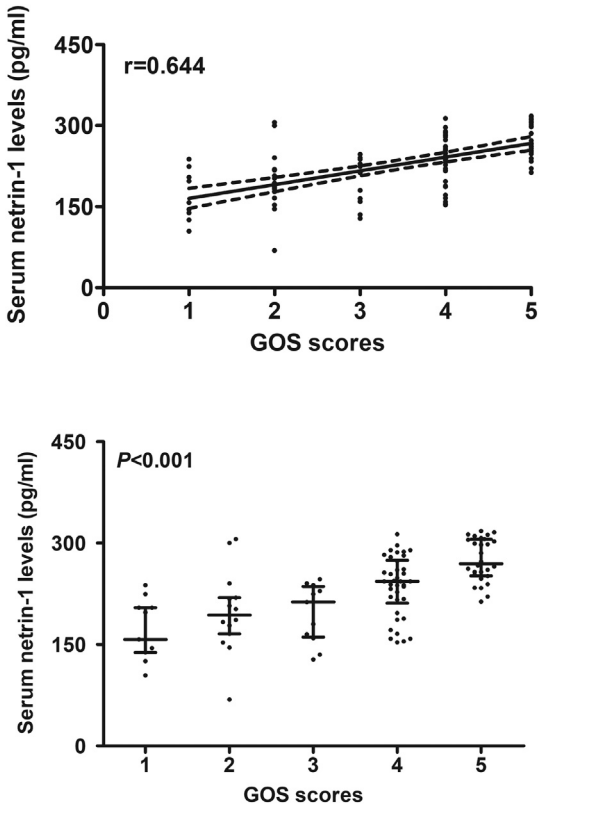

Fig. 4. Relationship between serum netrin-1 concentrations and Glasgow outcome scale (GOS) scores in patients with aneurysmal subarachnoid hemorrhage.

which yielded a sensitivity value of 81.6% and a specificity value of 77.3%. Its predictive ability was in the range of WFNS scores (AUC, 0.886; 95% CI, 0.809–0.940; $P = 0.235$), Hunt Hess scores (AUC, 0.883; 95% CI, 0.806–0.938; $P = 0.251$) and modified Fisher scores (AUC, 0.863; 95% CI, 0.782–0.923; $P = 0.565$). Moreover, in a combined logistic regression model, serum netrin-1 concentrations remarkably enhanced the AUCs of WFNS scores, Hunt Hess scores and modified Fisher scores to 0.917 (95% CI, 0.847–0.962; $P = 0.043$), 0.930 (95% CI, 0.863–0.971; $P = 0.023$) and 0.911 (95% CI, 0.839–0.958; $P = 0.029$) respectively.

We at first used univariate analysis to discern the parameters strongly associated with 6-month poor outcome, which were age, WFNS scores, modified Fisher scores, Hunt Hess scores, blood glucose concentrations, blood white blood cell count, blood platelet count, Serum netrin-1 concentrations < 229.3 pg/ml, serum C-reactive protein concentrations, existence of acute hydrocephalus, presence of intraventricular hemorrhage, occurrence of symptomatic cerebral vasospasm and application of external ventricular drain (Table 3 and Table 4). Next, the above-mentioned significant variables ($P < 0.1$) were included in the multivariate binary logistic regression model, showing that the independent variables were WFNS scores, modified Fisher scores, Hunt Hess scores and netrin-1 concentrations < 229.3 pg/ml, with OR values of 3.648 (95% CI, 1.256–10.597; $P = 0.007$), 7.223 (95% CI, 2.160–24.151; $P = 0.011$), 4.782 (95% CI, 2.239–10.213; $P = 0.019$) and 14.316 (95% CI, 5.032–40.726; $P = 0.010$) respectively.

4. Discussion

Netrin-1 was the first axon guidance molecule to be discovered in vertebrates and has a strong chemotropic function for axonal guidance, cell migration, morphogenesis and angiogenesis [1–5]. Expression of

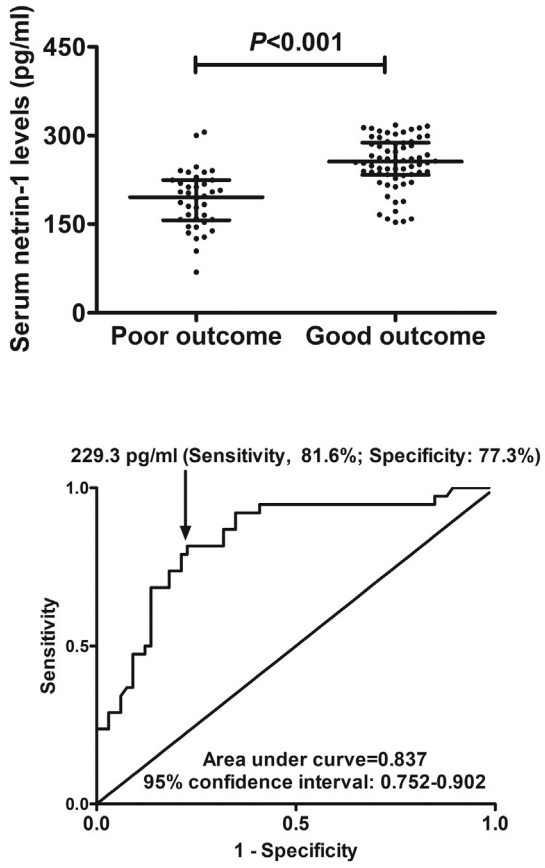

Fig. 5. Analysis of predictive value of serum netrin-1 concentrations for 6-month poor outcome (Glasgow outcome scale score of 1–3) after aneurysmal subarachnoid hemorrhage.

Table 4
The factors associated with poor outcome (Glasgow outcome scale score of 1-3) at 6 months following aneurysmal subarachnoid hemorrhage by univariate logistic regression analysis.

	Odds ratio	95% confidence interval	*P* value
Gender (female/male)	1.344	0.592–3.048	NS
Age (y)	1.033	1.002–1.064	0.034
Body mass index (kg/m^2)	1.133	0.937–1.369	NS
Smoking	1.476	0.658–3.311	NS
World Federation of Neurological Surgeons scores	6.764	3.229–14.169	< 0.001
Modified Fisher scores	11.346	4.573–28.150	< 0.001
Hunt Hess scores	6.180	3.119–12.246	< 0.001
Aneurysmal position (anterior circulation / posterior circulation)	1.129	0.397–3.210	NS
Aneurysm shape (cystic)	0.445	0.147–1.344	NS
Aneurysm diameter (< 10 mm/≥10 mm)	1.078	0.481–2.415	NS
Surgery (clipping/ endovascular coiling)	1.148	0.515–2.558	NS
Acute hydrocephalus	3.346	1.222–9.161	0.019
Intraventricular hemorrhage	3.500	0.952–12.867	NS
External ventricular drain	2.585	0.990–6.747	NS
Symptomatic cerebral vasospasm	3.778	1.601–8.914	0.002
Admission Time (h)	1.008	0.923–1.101	NS
Blood- collecting Time (h)	1.026	0.953–1.106	NS
Aneurysm-treating time (h)	1.012	0.963–1.064	NS
Systolic arterial pressure (mmHg)	1.000	0.986–1.015	NS
Diastolic arterial pressure (mmHg)	1.010	0.990–1.031	NS
Blood glucose (mmol/l)	1.107	1.029–1.190	0.006
Blood white blood cell count (×10^9/l)	1.088	0.976–1.213	NS
Blood platelet count (×10^9/l)	0.990	0.980–1.000	0.042
Serum C-reactive protein (mg/l)	1.097	1.026–1.173	0.006
Serum netrin-1 levels < 229.3 pg/ml	12.750	4.836–33.612	< 0.001

endogenous netrin-1 protein was significantly up-regulated in rat brain tissues after cerebral ischemia, which promoted neural stem cells migration from the subventricular zone [29]. Also, netrin-1 overexpression increased neurobehavioral test scores and reduced cerebral infarct volume following middle cerebral artery occlusion via inhibition of the Notch1 signaling pathway [30]. Similarly, netrin-1 improved functional recovery through autophagy stimulation by activating the AMP-activated protein kinase / mammalian target of rapamycin signaling pathway in rats with spinal cord injury [31]. Consistently,

Table 3
The factors related to poor outcome (Glasgow outcome scale score of 1-3) at 6 months after aneurysmal subarachnoid hemorrhage utilizing univariate analysis.

	Poor outcome	Good outcome	*P* value
Gender (female/male)	24/14	37/29	NS
Age (y)	54 (42–64)	50 (36–58)	0.048
Body mass index (kg/m^2)	24.1 (22.9–25.7)	23.7 (21.7–25.5)	NS
Smoking	18 (47.4%)	25 (37.9%)	NS
World Federation of Neurological Surgeons scores	4 (3–4)	2 (1–3)	< 0.001
Modified Fisher scores	3 (3–3)	2 (1–2)	< 0.001
Hunt Hess scores	4 (3–4)	2 (1–3)	< 0.001
Aneurysmal position (anterior circulation / posterior circulation)	31/7	55/11	NS
Aneurysm shape (cystic)	30 (79.0%)	59 (89.4%)	NS
Aneurysm diameter (< 10 mm/≥10 mm)	16/22	29/37	NS
Surgery (clipping/ endovascular coiling)	20/18	37/29	NS
Acute hydrocephalus	12 (31.6%)	8 (12.1%)	0.015
Intraventricular hemorrhage	7 (18.4%)	4 (6.1%)	0.048
External ventricular drain	12 (31.6%)	10 (15.2%)	0.048
Symptomatic cerebral vasospasm	20 (52.6%)	15 (22.7%)	0.002
Admission Time (h)	11.5 (9.0–13.2)	10.5 (8.5–14.7)	NS
Blood- collecting Time (h)	15.2 (12.9–20.6)	14.4 (11.1–19.1)	NS
Aneurysm-treating time (h)	26.5 (22.9–31.8)	25.4 (19.9–33.2)	NS
Systolic arterial pressure (mmHg)	131 (104–146)	128 (102–153)	NS
Diastolic arterial pressure (mmHg)	82 (66–100)	80 (64–97)	NS
Blood glucose (mmol/l)	14.9 (10.9–17.6)	10.9 (9.5–14.4)	0.040
Blood white blood cell count (×10^9/l)	12.2 (8.8–14.3)	8.7 (7.0–11.2)	NS
Blood platelet count (×10^9/l)	138 (98–183)	154 (134–186)	0.004
Serum C-reactive protein (mg/l)	18.5 (14.4–23.5)	14.5 (11.4–21.5)	0.008
Serum netrin-1 < 229.3 pg/ml	30 (79.0%)	15 (22.7%)	< 0.001

intranasal administration of exogenous recombinant netrin-1 attenuated neuronal apoptosis and improved neurological function in SAH rats [21]. In parallel, recombinant netrin-1 treatment attenuated neuroinflammation and neurological impairments through inhibiting microglia activation in rats after SAH [19]. Recently, it was verified that netrin-1 reduced lipopolysaccharide-induced interleukin-1βand interleukin-12βrelease in cultured astrocytes, knockdown of netrin-1 increased astrocyte activation in the mouse brain after middle cerebral artery occlusion, as well as injection of netrin-1 attenuated the release of interleukins and reduced infarct volume after brain ischemia [32]. Of note, experimental autoimmune encephalomyelitis mice showed significantly lower netrin-1 concentrations and higher tumor necrosis factor - alpha amounts in sera, spinal cord and cerebella than healthy control mice; multiple sclerosis patients showed significantly lower serum netrin-1 concentrations than controls; tumor necrosis factor -alpha serum concentrations were higher in multiple sclerosis patients compared with controls, and negatively correlated with netrin-1 concentrations [24]. The preceding data suggest that netrin-1 might play a neuroprotective role in SAH-related brain injury and anti-inflammatory property might at least in part contribute to its pathophysiological mechanisms. Our study revealed that declined serum netrin-1 concentrations were intimately and independently correlated with rising serum C-reactive protein concentrations using multivariate linear logistic regression analysis. Such results are prominently supportive of the notion that netrin-1 might be involved in brain inflammation following aSAH and it is also assumed that netrin-1 might exert an anti-inflammatory effect on brain injury after aSAH.

The above-mentioned data and analyses might have resulted in a hypothesis that circulating netrin-l concentrations could be used to assess severity and predict clinical outcome in some illnesses with acute brain injury. Intriguingly, China Antihypertensive Trial in Acute Ischemic Stroke has found that elevated serum netrin-1 was associated with a decreased risk of primary outcome (modified Rankin Scale score of ≥ 3) after adjustment for baseline National Institutes of Health Stroke Scale score and other potential confounders [25]. However, no controls have been set in that study. The current study added the controls and compared serum netrin-1 concentrations between aSAH patients and controls. Undoubtedly, serum netrin-1 concentrations were pronouncedly lower in aSAH patients than in healthy controls. We further evaluated its correlation with hemorrhagic severity reflected by clinical and radiological parameters, namely WFNS scores, Hunt-Hess scores and modified Fisher scores. A multivariate linear logistic regression model in our study confirmed the independent and inverse relation of serum netrin-1 concentrations to the preceding severity assessment systems, which is suggestive of the concept that determination of serum netrin-1 should enable clinicians to assess hemorrhagic severity early in aSAH patients.

In general, GOS score can be calculated to facilitate the assessment of neurological function in patients with aSAH [33–35]. Our study demonstrated that serum netrin-1 concentrations were positively correlated with GOS scores at 6 months after aSAH. Simultaneously, GOS scores were identified as a categorical variable. Multiple comparisons also revealed serum netrin-1 concentrations were substantially declined with rising worse prognosis, reflected by a low GOS score. According to the previous definition [33–35], in which GOS score of 1–3 was referred to as a poor prognosis, we did such a classification. In other words, GOS score was transformed into a binary variable. Thereby, we conFig.d a ROC curve to estimate predictive ability of serum netrin-1 concentrations for 6-month poor outcome after aSAH. Actually, serum netrin-1 concentrations discriminated patients at risk of 6-month poor outcome with a high area under curve (> 0.8). Moreover, an optimal cutoff value of serum netrin-1 concentration (229.3 pg/ml) was selected, generating a sensitivity value of 81.6% and a specificity value of 77.3%. More interestingly, its predictive performance was equivalent to those of WFNS score, Hunt Hess score and modified Fisher score, all of which are the three commonest determinants for poor prognosis of aSAH [33–36] and were found to be independently associated with 6-month poor prognosis following aSAH in the current study. In order to discern relationship between serum netrin-1 concentrations and post stroke 6-month poor prognosis, we chose serum netrin-1 concentration $<$ 229.3 pg/ml as a cutoff value and hereby all patients were dichotomized. Multivariable analysis showed that independent predictors associated with poor prognosis included WFNS score, Hunt Hess score and modified Fisher score, in addition to serum nerin-1 concentration $<$ 229.3 pg/ml. Collectively, this may be a novel finding that serum netrin-1 concentration should be strongly associated with clinical outcome and its measurement should be capable of serving as a promising prognostic biomarker for aSAH.

5. Conclusions

In this study, there are some novel findings that (1) serum netrin-1 concentrations are significantly lower in aSAH patients than in healthy controls, (2) serum netrin-1 concentrations were strongly and inversely correlated with WFNS scores, Hunt Hess scores and modified Fisher scores, (3) serum netrin-1 concentrations were closely and negatively related to serum C-reactive protein concentrations, (4) serum netrin-1 independently predicts poor prognosis at 6 months after aSAH, and (5) serum netrin-1 concentrations display a substantially high discriminatory capability for 6-month poor prognosis post stroke. Hence, it is presumed that serum netrin-1 might have the potential to be a useful prognostic biomarker for assessing hemorrhagic severity, inflammatory degree and clinical outcome of aSAH.

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