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Longitudinal Change of Plasma Retinol-Binding Protein 4 and its Relation to Neurological-Function Recovery, Relapse, and Death in Acute Ischemic Stroke Patients

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Retinol-binding protein 4 (RBP4) promotes dyslipidemia, insulin resistance, inflammation, and atherosclerosis, etc. which may participate in the progression of acute ischemia stroke (AIS). This study aimed to evaluate the longitudinal change of RBP4 after disease onset and its correlation with prognosis in AIS patients. Plasma RBP4 was measured by enzyme-linked immunosorbent assays in 402 AIS patients at admission, one day (D1), 3 days (D3), 7 days (D7), and 30 days (D30) after admission; and in 100 healthy controls after enrollment. The neurological-function recovery was evaluated by the modified Rankin Scale (mRS) at 3 months (M3); disease relapse and death were also recorded during a median 20-month follow-up in AIS patients. Our study revealed that RBP4 was elevated in AIS patients compared with healthy controls. RBP4 was related to a history of diabetes mellitus, a history of cardiovascular disease, and elevated National Institutes of Health Stroke Scale score in AIS patients. Longitudinally, RBP4 was increased from admission to D1/D3, then reduced gradually to D30 in AIS patients. Notably, RBP4 at admission and D1 was elevated in AIS patients with mRS > 2 compared to those with mRS ≤ 2. Meanwhile, RBP4 at admission, D1, D3, D7, and D30 were all higher in AIS patients occurred relapse than those without; RBP4 at D3, D7, and D30 were also higher in AIS patients who died later than those who survived. In conclusion, plasma RBP4 originally elevates and continuously decreases during disease, which forecasts neurological-function recovery status, relapse, and death risk of AIS.

Keywords: acute ischemia stroke; death; neurological-function recovery; relapse; retinol-binding protein 4
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Introduction

Acute ischemia stroke (AIS) accounts for nearly 90% of strokes, which is a serious brain dysfunction disease caused by focal cerebral ischemia (Barthels and Das 2020; Robba et al. 2022). It is reported that the risk factors of AIS contain hypertension, diabetes, hypertriglyceridemia, smoking, etc. (Shi et al. 2021). Although some progress has been made in these treatments in recent years, the disability and mortality rates of AIS are still rising, which causes heavy physical and financial burdens to those patients (GBD 2019 Stroke Collaborators 2021; Ma et al. 2021; Strilciuc et al. 2021; Wang et al. 2022). Therefore, it is important to explore a potential biomarker to reflect disease risk and prognosis for improving the management of AIS.

Retinol-binding protein 4 (RBP4) is a 21-kDa adipokine, which is mainly secreted by adipocytes (Ji et al. 2022). It is reported that RBP4 plays an important role in promoting dyslipidemia, insulin resistance, inflammatory reactions, and atherosclerosis, which is considered to potentially participate in the progression of cardio-cerebrovascular diseases (Nono Nankam and Bluher 2021; Ji et al. 2022). Additionally, even though some researches have explored the potential of RBP4 as a biomarker in AIS, the results of these studies exist some conflicts (Llombart et al. 2016; Rist et al. 2018; Zhu et al. 2018; Liu et al. 2021). For example, previous studies indicate that RBP4 can effectively distinguish patients with ischemic stroke from patients with hemorrhagic stroke and also can be used as a potential biomarker for predicting the early prognosis of

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AIS patients (Llombart et al. 2016; Zhu et al. 2018; Liu et al. 2021). However, another study discloses that there is no correlation between RBP4 and the risk of ischemic stroke (Rist et al. 2018). Therefore, a study with a larger sample size is required for further verification. Furthermore, the longitudinal change of RBP4 after disease onset, as well as its association with the risks of relapse and death in AIS patients during a long-term follow-up have not been reported.

Therefore, this study enrolled 402 AIS patients, aiming to evaluate the longitudinal change of RBP4 and its relationship with disease severity, as well as early and long-term prognosis in AIS patients.

Methods

Subjects

In this research, a total of 402 AIS patients who came to our hospital for treatment were enrolled from January 2019 to May 2022. The inclusion criteria were: 1) diagnosed as ischemia stroke via head computed tomography (CT) or magnetic resonance imaging (MRI); 2) over 18 years of age; 3) time since symptom to admission ≤ 24 h. The exclusion criteria were: 1) suffered from a transient ischemic attack; 2) had a history of traumatic brain injury, an intracranial mass, or brain surgery; 3) with tumors or severe functional impairment of other organs; 4) with a mental or serious psychological abnormality that could not cooperate in completing various examinations; 5) pregnant or lactating women. Besides, 100 healthy people who met the following criteria were recruited as healthy controls (HCs): 1) age and sex-matched with AIS patients; 2) without abnormality in recent physical examinations; 3) understood this study protocol and cooperated with blood samples collection. All subjects provided informed consent. The Ethics Committee of Cangzhou Central Hospital has permitted this study.

Collection and detection

Demographic data, histories of chronic disease, time since symptom to admission, the National Institutes of Health Stroke Scale (NIHSS) score (Deng et al. 2023), and treatment were collected from AIS patients. Additionally, for blood sample collection, those from AIS patients were obtained at admission, one day after admission (D1), 3 days after admission (D3), 7 days after admission (D7), and 30 days after admission (D30); while blood samples from HCs were gathered after enrollment.

For RBP4 detection, plasma was separated from the blood sample by using a plasma separator (BELLCO Inc., Mirandola, Emilia-Romagna, Italy). Then, the plasma RBP4 level was determined via the enzyme-linked immunosorbent assay (ELISA) method, and a commercial Human RBP4 Quantikine ELISA Kit was used (No. Cat. DRB400, Bio-Techne Inc., Minneapolis, MN, USA). The experimental procedure was conducted in strict accordance with the directions. Briefly, the experimental procedure was as follows: first, the standard or samples were added to the precoated microplate, which was incubated for 1 h at room temperature. Next, the secondary antibody conjugated was added for 1 h incubation at room temperature. After aspirating and washing, substrate solution was added to each well and further incubated for 30 min at room temperature. Finally, the stop solution was added to each well, and the intensity of the color was read at 450 nm within 30 min via Multiskan spectrum spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The samples were tested in triplicate.

Assessment and follow-up

The early prognosis of AIS patients was assessed by Modified Rankin Scale score at 3 months after the stroke (M3 mRS). The M3 mRS ≤ 2 was defined as a good prognosis, while the M3 mRS score > 2 was defined as a poor prognosis (Suzuki et al. 2021). Besides, AIS patients underwent routine follow-ups (median follow-up time, 20 months; range, 6-39 months). The last follow-up date was December 2022. Additionally, relapse and death were recorded. Accumulating relapse rate and accumulating death rate were also calculated. For analysis of accumulating relapse rate and accumulating death rate, a total of 41 patients lost to follow-up were censored at their last date of disease assessment.

Statistics

The Wilcoxon rank sum test was used for comparing RBP4 levels between groups. A receiver operating characteristic (ROC) curve was performed to show the ability in distinguishing AIS patients and HCs. The best cut-off point value was gained through finding the maximum of the sum of sensitivity and specificity. The spearman test was utilized for correlation analysis. The Friedman test was conducted for comparing RBP4 levels over time. Kaplan-Meier (K-M) curves were used to display the accumulating relapse rate and accumulating death rate in AIS patients. SPSS v.22.0 (IBM Inc., Amenk, NY, USA) and GraphPad Prism v.7.0 (GraphPad Software Inc., San Diego, CA, USA) were applied for data analysis and figure construction, respectively.

Results

Baseline features of AIS patients

Totally, 402 AIS patients with a mean age of 65.7 ± 9.5 years were enrolled, including 135 (33.6%) females and 267 (66.4%) males. The median [interquartile range (IQR)] time since symptom to admission was 5.0 (3.0-7.0) hours. Meanwhile, the mean value of the NIHSS score in AIS patients was 9.8 ± 5.4. There were 329 (81.8%) patients who were treated with thrombolysis and 73 (18.2%) patients who were treated with mechanical embolectomy. More specific characteristics of AIS patients were shown in Table 1.

Table 1. Clinical characteristics of acute ischemic stroke (AIS) patients.

Characteristics	AIS patients (n = 402)
Age (years), mean ± SD	65.7 ± 9.5
Sex, n (%)	
Female	135 (33.6)
Male	267 (66.4)
BMI (kg/m^2), mean ± SD	24.0 ± 2.7
History of smoke, n (%)	
No	223 (55.5)
Yes	179 (44.5)
History of hypertension, n (%)	
No	97 (24.1)
Yes	305 (75.9)
History of hyperlipidemia, n (%)	
No	237 (59.0)
Yes	165 (41.0)
History of diabetes mellitus, n (%)	
No	314 (78.1)
Yes	88 (21.9)
History of CKD, n (%)	
No	327 (81.3)
Yes	75 (18.7)
History of cardiovascular disease, n (%)	
No	278 (69.2)
Yes	124 (30.8)
Time since symptom to admission (h), median (IQR)	5.0 (3.0-7.0)
NIHSS score, mean ± SD	9.8 ± 5.4
Treatment, n (%)	
Thrombolysis	329 (81.8)
Mechanical embolectomy	73 (18.2)

SD, standard deviation; BMI, body mass index; CKD, chronic kidney disease; IQR, interquartile range; NIHSS, the national institutes of health stroke scale.

Comparison of RBP4 between AIS patients and HCs

RBP4 was increased in AIS patients compared with HCs [median (IQR): 34.4 (21.1-49.6) vs. 16.6 (11.4-23.1) μg/mL] ($P < 0.001$) (Fig. 1A). Additionally, RBP4 showed a good value to discriminate AIS patients from HCs with area under curve (AUC) of 0.803 [95% confidence interval (CI): 0.757-0.848]. Sensitivity and specificity were 0.624 and 0.870, respectively, at the best cut-off point (RBP4 value: 28.4 μg/mL) (Fig. 1B).

Relationship of RBP4 with histories of chronic disease and NIHSS score in AIS patients

Elevated RBP4 was associated with a history of diabetes mellitus ($P < 0.001$) and a history of cardiovascular disease ($P = 0.034$) in AIS patients. There was no relationship of RBP4 with a history of hypertension ($P = 0.084$), hyperlipidemia ($P = 0.190$), or chronic kidney disease (CKD) ($P = 0.613$) in AIS patients (Table 2). Furthermore, RBP4 was positively correlated with the NIHSS score in AIS patients ($r = 0.284$, $P < 0.001$) (Fig. 2).

RBP4 longitudinal change and its correlation with neurological-function recovery in AIS patients

RBP4 was elevated from admission to D1; subsequently, it was decreased from D1 to D30 continuously in AIS patients ($P < 0.001$) (Fig. 3). Moreover, the neurological-function recovery reflected by M3 mRS was evaluated, and the proportion of AIS patients with different mRS scores were shown in Supplementary Fig. S1. Briefly, there were 28.1% of AIS patients with M3 mRS > 2 and 71.9% of AIS patients with M3 mRS ≤ 2 (Fig. 4A). Interestingly, RBP4 at admission [median (IQR): 40.0 (26.8-53.8) vs. 32.3 (19.2-46.6) μg/mL] ($P = 0.001$) (Fig. 4B) and D1 [median (IQR): 47.3 (32.8-65.9) vs. 42.8 (24.7-63.4) μg/mL] ($P = 0.030$) (Fig. 4C) were elevated in patients with M3 mRS > 2 compared to those with M3 mRS ≤ 2. Furthermore, there was no difference in RBP4 at D3 [median (IQR): 44.5 (28.1-58.4) vs. 36.3 (23.3-61.2) μg/mL] ($P = 0.051$) (Fig. 4D), D7 [median (IQR): 30.0 (19.5-48.3) vs. 25.8 (16.0-44.4) μg/mL] ($P = 0.056$) (Fig. 4E), or D30 [median (IQR): 24.5 (19.0-34.9) vs. 22.7 (14.5-34.4)

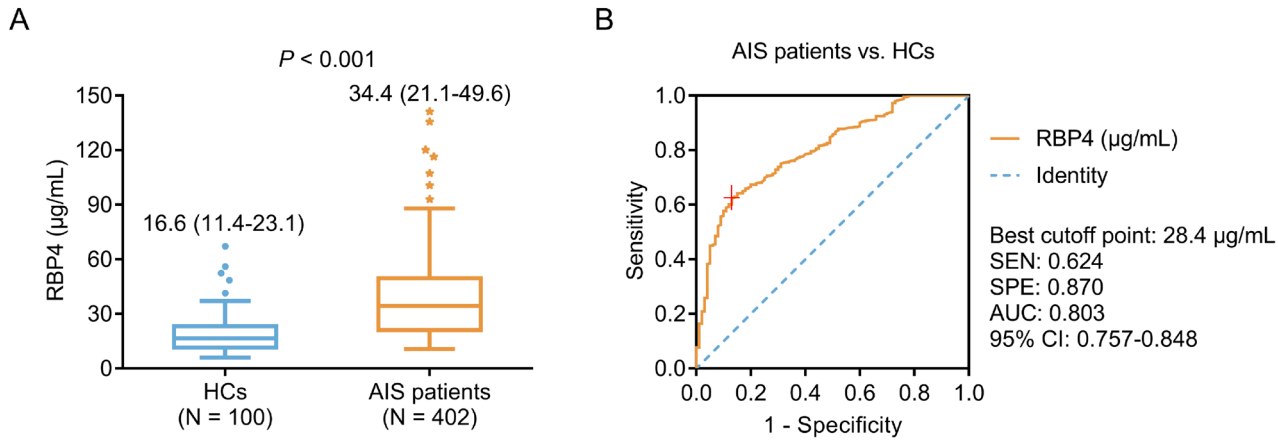

Fig. 1. Retinol-binding protein 4 (RBP4) in acute ischemic stroke (AIS) patients and healthy controls (HCs).
(A) Comparison of RBP4 between AIS patients and HCs. (B) ROC curve showing the value of RBP4 in differentiating AIS patients from HCs.

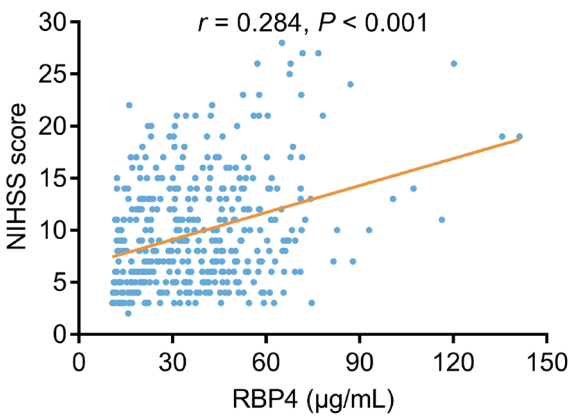

Fig. 2. Relationship of retinol-binding protein 4 (RBP4) with the National Institutes of Health Stroke Scale (NIHSS) score in acute ischemic stroke (AIS) patients.
The correlation of RBP4 with NIHSS score is shown in AIS patients.

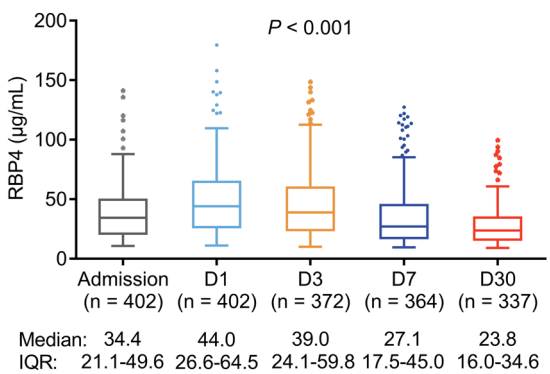

Fig. 3. Retinol-binding protein 4 (RBP4) change from admission to 30 days (D30) in acute ischemic stroke (AIS) patients.
The longitudinal change of RBP4 is shown from admission to one day (D1), 3 days (D3), 7 days (D7), and 30 days (D30) after admission in AIS patients.

Table 2. The relationship of retinol-binding protein 4 (RBP4) with histories of chronic disease in acute ischemic stroke (AIS) patients.

Items	RBP4 (μg/mL), median (IQR)	*P* value
History of hypertension		0.084
No	31.7 (18.6-45.2)	
Yes	34.6 (21.4-50.4)	
History of hyperlipidemia		0.190
No	33.2 (20.3-48.1)	
Yes	35.0 (21.8-50.5)	
History of diabetes mellitus		< 0.001
No	33.1 (19.5-46.1)	
Yes	44.1 (23.9-57.7)	
History of CKD		0.613
No	34.3 (21.1-48.4)	
Yes	34.5 (20.2-56.0)	
History of cardiovascular disease		0.034
No	33.9 (19.5-48.3)	
Yes	34.8 (23.1-51.0)	

IQR, interquartile range; CKD, chronic kidney disease.

μg/mL] (P = 0.050) (Fig. 4F) between patients with M3 mRS > 2 and patients with M3 mRS ≤ 2.

Association of RBP4 with relapse and death in AIS patients

There were 74 patients with relapse and 30 deaths during the entire study period. The 1-year, 2-year, and 3-year accumulating relapse rates were 6.6%, 20.7%, and 30.6% in AIS patients, respectively (Fig. 5A). In addition, the 1-year, 2-year, and 3-year accumulating death rates were 1.0%, 9.1%, and 19.0% in AIS patients, respectively (Fig. 5B).

RBP4 at admission [median (IQR): 41.6 (23.0-60.7)

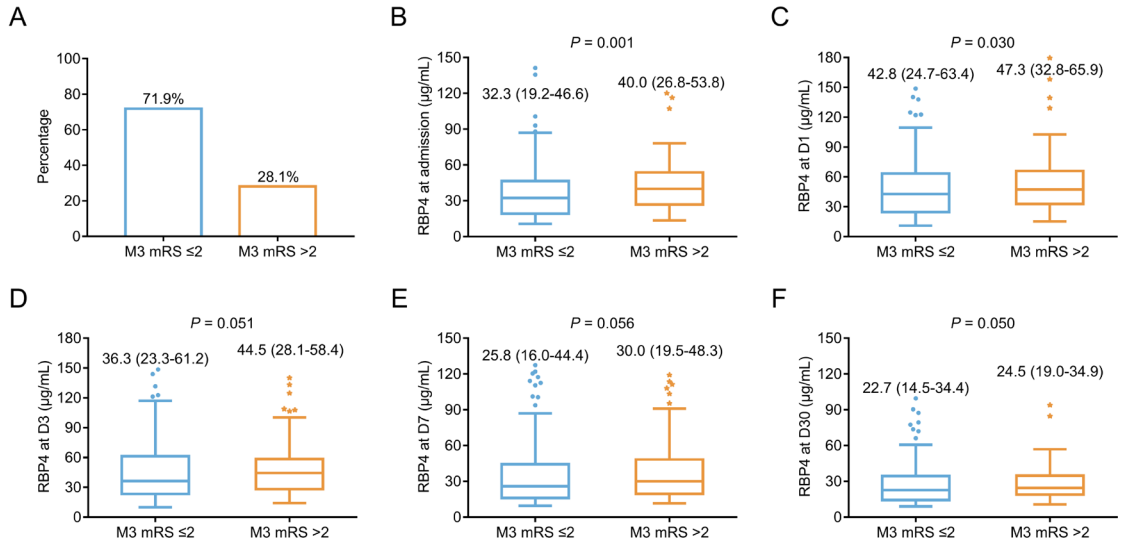

Fig. 4. Association of retinol-binding protein 4 (RBP4) with the Modified Rankin Scale score at 3 months after the stroke (M3 mRS) in acute ischemic stroke (AIS) patients.
(A) The percentage of AIS patients with M3 mRS ≤ 2 and M3 mRS > 2. (B-F) The association of RBP4 with M3 mRS at admission (B), one day (D1) (C), 3 days (D3) (D), 7 days (D7) (E), and 30 days (D30) (F) after admission in AIS patients.

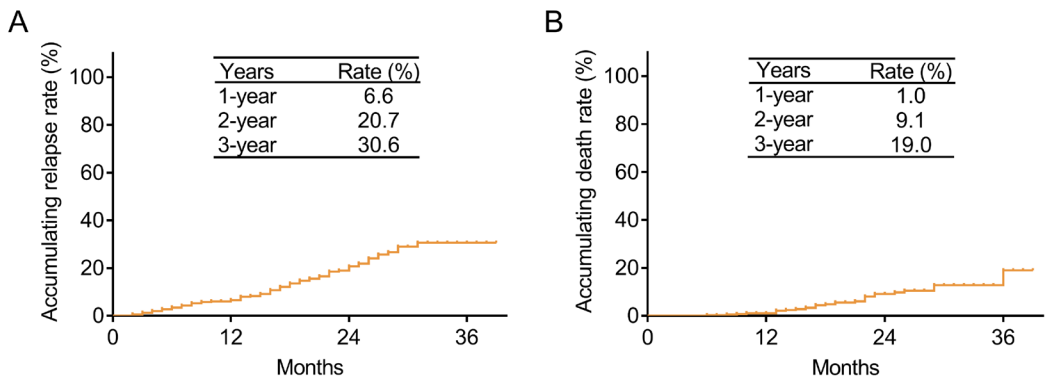

Fig. 5. Accumulating relapse rate and accumulating death rate in acute ischemic stroke (AIS) patients.
(A) The 1-year, 2-year, and 3-year accumulating relapse rates in AIS patients. (B) The 1-year, 2-year, and 3-year accumulating death rates in AIS patients.

vs. 33.5 (20.6-46.9) μg/mL] (P = 0.008), D1 [median (IQR): 50.9 (28.6-82.0) vs. 43.4 (25.9-62.5) μg/mL] (P = 0.039), D3 [median (IQR): 51.0 (25.5-82.9) vs. 37.6 (23.8-54.8) μg/mL] (P = 0.001), D7 [median (IQR): 42.1 (22.4-79.6) vs. 25.6 (16.4-40.1) μg/mL] (P < 0.001), and D30 [median (IQR): 37.7 (20.3-49.6) vs. 22.2 (14.6-31.6) μg/mL] (P < 0.001) were higher in patients experienced relapse than those did not (Table 3).

There was no difference in RBP4 at admission (P = 0.305) and D1 (P = 0.120) between patients who died and patients who survived. However, RBP4 at D3 [median (IQR): 49.9 (27.6-77.0) vs. 38.3 (23.7-57.5) μg/mL] (P = 0.018), D7 [median (IQR): 41.9 (24.3-7.7) vs. 26.4 (17.1-43.1) μg/mL] (P = 0.002), and D30 [median (IQR): 34.7 (22.8-47.1) vs. 22.7 (15.7-32.6) μg/mL] (P = 0.001) were higher in patients who died than patients who survived (Table 3).

Discussion

The results from this study were as follows: (1) RBP4 was increased in AIS patients compared to HCs, and it was related to a history of diabetes mellitus, a history of cardiovascular disease, and increased NIHSS score in AIS patients. (2) Longitudinally, RBP4 was elevated from admission to D1, and then reduced continuingly from D1 to D30 in AIS patients. (3) RBP4 at admission and D1 was linked with poorer early neurological-function recovery reflected by M3 mRS score in AIS patients. (4) Elevated RBP4 was associated with increased risks of relapse and death in AIS patients.

Table 3. The association of retinol-binding protein 4 (RBP4) with relapse and death in acute ischemic stroke (AIS) patients.

Items	Relapse		Death	
	RBP4 (μg/mL), median (IQR)	*P* value	RBP4 (μg/mL), median (IQR)	*P* value
Admission		0.008		0.305
No	33.5 (20.6-46.9)		34.5 (20.9-49.3)	
Yes	41.6 (23.0-60.7)		34.0 (22.3-66.9)	
D1		0.039		0.120
No	43.4 (25.9-62.5)		43.7 (26.0-63.4)	
Yes	50.9 (28.6-82.0)		48.5 (32.2-84.7)	
D3		0.001		0.018
No	37.6 (23.8-54.8)		38.3 (23.7-57.5)	
Yes	51.0 (25.5-82.9)		49.9 (27.6-77.0)	
D7		< 0.001		0.002
No	25.6 (16.4-40.1)		26.4 (17.1-43.1)	
Yes	42.1 (22.4-79.6)		41.9 (24.3-72.7)	
D30		< 0.001		0.001
No	22.2 (14.6-31.6)		22.7 (15.7-32.6)	
Yes	37.7 (20.3-49.6)		34.7 (22.8-47.1)	

IQR, interquartile range; D1, one day after admission; D3, 3 days after admission; D7, 7 days after admission; D30, 30 days after admission.

The dysregulation of RBP4 is considered to be associated with the occurrence and progression of some cardiocerebrovascular diseases, but it exists some controversy in stroke (Prentice et al. 2010; Rist et al. 2018; Liu and Che 2019). For example, one study suggests that RBP4 is not linked with ischemic stroke risk (Rist et al. 2018). Nevertheless, another study finds that RBP4 is elevated in stroke patients compared with non-stroke patients (Prentice et al. 2010). Moreover, one previous study observes that RBP4 is increased and related to high stroke severity in AIS patients (Liu and Che 2019). We hypothesized that the differences in sample sizes, populations, geographical locations, and assays for measuring RBP4 might lead to conflicting results in previous studies. Our study found that RBP4 was elevated in AIS patients compared to HCs, and was positively correlated with the NIHSS score in AIS patients. These findings indicated that RBP4 could be considered as a biomarker to predict the risk and severity of AIS. This might be because: (1) RBP4 promoted endothelial cell inflammation through the nuclear factor-kappaB (NF-κB)/nicotinamide adenine dinucleotide phosphate oxidase (NADPH) pathway (Flores-Cortez et al. 2022); meanwhile, inflammation played an important role in the pathogenesis of AIS (Jin et al. 2013). Thus, RBP4 might promote the occurrence and severity of AIS. (2) RBP4 induced oxidative stress by reducing endothelial mitochondrial function (Liu et al. 2014; Flores-Cortez et al. 2022); meanwhile, oxidative stress could induce brain injury, which was related to the pathogenesis of AIS (Hu et al. 2022; Pawluk et al. 2022), so RBP4 might accelerate the occurrence and severity of AIS. However, it is necessary to further clarify the specific mechanism of RBP4 to promote the development and progression of AIS. Additionally, in our study, the best cut-off point for RBP4 to discriminate AIS patients from HCs was 28.4 μg/mL (sensitivity and specificity were 0.624 and 0.870, respectively), which might be used clinically. However, due to the lack of validation cohort in our study, the cut-off we gave cannot be generalized and need to be further verified.

Although some studies have discussed the potential of RBP4 as a biomarker to detect the occurrence of AIS (Prentice et al. 2010; Liu and Che 2019), the longitudinal change of RBP4 after AIS onset has not been focused on so far. Therefore, our study further explored this issue and found that RBP4 was elevated from admission to D1, and then it was decreased continuingly from D1 to D30 in AIS patients. The possible explanation was as follows: RBP4 aggravated inflammation through inducing the production of pro-inflammatory cytokines, thus, it could reflect inflammation to a certain extent (Moraes-Vieira et al. 2014; Ren et al. 2022). Meanwhile, AIS patients might have increased inflammation at admission and at the initial stage of treatment, thus RBP4 was increased from admission to D1 (Jayaraj et al. 2019). With the benefits of treatment, inflammation in AIS patients might be relieved continually from D1 to D30, therefore, RBP4 gradually reduced from D1 to D30 in AIS patients (Yu et al. 2022).

RBP4 is considered to regulate oxidative stress and

induce inflammation, promoting neuronal loss, which may cause severe neurological dysfunction (Buxbaum et al. 2014; Wang et al. 2015; Flores-Cortez et al. 2022). Therefore, the correlation between RBP4 and neurological function outcomes in AIS patients should be emphasized. One study indicates that RBP4 is increased in AIS patients with poor functional outcomes compared to those with good functional outcomes (Zhu et al. 2018). Partly similar to the previous study, our study revealed that RBP4 at admission and D1 were associated with poorer early neurological-function recovery reflected by M3 mRS score in AIS patients. The possible reasons were as follows: (1) RBP4 destroyed neuron function, which might lead to poor neurological-function recovery of AIS (Steinhoff et al. 2021). (2) RBP4 promoted oxidative stress through reducing endothelial mitochondrial function (Flores-Cortez et al. 2022); meanwhile, oxidative stress caused neuroinflammatory reactions and glial immune responses, leading to permanent brain cell damage (Popa-Wagner et al. 2013). Therefore, RBP4 was associated with poor neurological-function outcomes in AIS patients. (3) RBP4 induced vascular inflammation (Farjo et al. 2012), which might cause imbalances in brain neuronal integrity and injury of brain function, leading to poor neurological-function recovery in AIS patients (Terrando and Pavlov 2018). Additionally, our study also found that elevated RBP4 was correlated with increased risks of relapse and death in AIS patients. This might be because: RBP4 might worsen the condition of AIS patients by promoting brain injury and inducing dyslipidemia, insulin resistance, inflammatory reaction, and cardiovascular diseases, causing relapse and death of AIS patients (ElHabr et al. 2021; Hopfinger et al. 2021; Steinhoff et al. 2021). Therefore, AIS patients with increased RBP4 might face poor short-term neurological-function recovery and high risks of relapse, and death.

There were some limitations in our study: (1) Our study was a single-center study, which led to the bias of selection. (2) The specific mechanism of RBP4 promoting AIS was unclear and should be investigated in further studies. (3) The mismatch between the number of AIS patients and the number of HCs might interfere with the statistical effect. (4) Our study did not include the disease controls. Future research should enroll disease controls to verify the abnormal expression of RBP4 in AIS patients.

In conclusion, plasma RBP4 initially increases then gradually decreases, which serves as a biomarker for monitoring disease severity and predicting neurological-function recovery condition, relapse, and death in AIS patients.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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Supplementary Files

Please find supplementary file(s);
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