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The value of serum ZAG levels in predicting the progression and prognosis of acute ischemic stroke

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Abstract

Objective: The aim of this study was to investigate the value of serum zinc-alpha2-glycoprotein (ZAG) levels in predicting the progression and prognosis of acute ischemic stroke (AIS). *Methods:* A total of 210 patients with AIS who were hospitalized for 72 hours were included in the case group, and 52 patients undergoing health check-ups at the hospital in the same period were included in the control group. Serum ZAG levels were measured early in the morning on the second day after admission via enzyme-linked immunosorbent assay (ELISA). For patients with AIS, those whose National Institutes of Health Stroke Scale (NIHSS) score progression was greater than 2 were regarded as the progression group, while those whose NIHSS score was less than 2 were regarded as the nonprogression group. Prognosis was assessed via the modified Rankin scale (mRS) score after 90 days: an mRS score > 2 was considered a poor prognosis. Logistic regression was used to analyze whether the serum ZAG level was an independent factor affecting the risk of disease progression and the long-term prognosis of AIS. Nomogram models were developed to predict the progression and prognosis of AIS. *Results:* The serum ZAG level was significantly lower in AIS patients than in controls. The binary logistic regression analysis revealed that the serum ZAG level [odds ratio (OR) 0.963, 95% confidence interval (CI): 0.948–0.979, $P < 0.01$] may be an independent factor for the risk of AIS onset. Subsequent single-factor analysis revealed that the serum ZAG level in the AIS progression group was lower than that in the nonprogression group. Binary logistic regression analysis also revealed that the serum ZAG level was an independent factor (OR 0.968, 95% CI: 0.947–0.991, P = 0.005) for the risk of AIS progression. Consistently, the serum ZAG level in the poor AIS prognosis group was lower than that in the good prognosis group, and binary logistic regression analysis revealed that the serum ZAG level was an independent risk factor for poor prognosis of acute cerebral infarction (OR 0.937, 95% CI: 0.905–0.969; $P < 0.01$). Nomogram models including the serum ZAG level to predict the progression and prognosis of AIS showed good prediction ability.
Conclusion: There is a close association between serum ZAG levels and the onset of AIS. A lower serum ZAG level may predict AIS progression and long-term poor prognosis.

Keywords: Zinc-alpha2-glycoprotein, acute ischemic stroke, adipokine, diagnosis, prognosis, nomogram

INTRODUCTION

Stroke is the second leading cause of mortality and the third leading cause of disability worldwide.[1] Acute ischemic stroke (AIS) is the most common type of stroke and leads to death and disability in patients. Therefore, finding effective methods to assess the progression and long-term prognosis of AIS, which can assist clinicians in making timely adjustments to patients' treatment methods, is particularly important.

Recent evidence has indicated that obesity is a highly modifiable risk factor for stroke. Adipokines, which are secreted by adipose tissue, have been implicated as potential mediators of the association between obesity and stroke.[2]

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However, although altered levels of adipokines are associated with stroke risk, their specific role in this relationship has not been established.[3]

Zinc-alpha2-glycoprotein (ZAG), encoded by the AZGP1 gene, is a novel adipokine synthesized by adipocytes and epithelial cells that has been demonstrated to play important roles in lipid metabolism, anti-inflammatory actions and the modulation of other adipokines.[4-10] ZAG can serve as a biomarker for various disorders, such as epilepsy, coronary atherosclerotic heart disease, diabetic nephropathy, several malignancies, and Cushing's syndrome.[11-14] A previous study revealed that ZAG may suppress epileptic seizures by reducing neuroinflammation through the inhibition of TGFβ-mediated ERK phosphorylation and by reducing the neuroinflammation mediated by TNFα and IL-6.[15] A separate investigation revealed a decrease in AZGP1 mRNA and ZAG protein levels in both epileptic patients and rat models[16], suggesting that the significance of ZAG in neurological disorders merits consideration.

On the basis of these findings, we hypothesized that the serum ZAG level might serve as a new biomarker for AIS onset. Therefore, in this study, we explored the relationship between serum ZAG levels and AIS.

METHODS

Participants

Patients with acute ischemic stroke diagnosed for the first time within 72 hours of the onset of stroke at the Stroke Centre of North Jiangsu People's Hospital from August 2022 to August 2023 and health check-ups were selected. Inclusion criteria: all study subjects were aged ≥18 years and <80 years, and all case group enrolments met the diagnostic criteria of the 2022 edition of the Chinese Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke. Exclusion criteria: 1) combined with heart failure, renal failure, liver function impairment, etc. 2) combined with infectious diseases and tumors. 3) history of major surgery or trauma in the past month. 4) history of cerebral infarction and transient ischemic attack. 5) combined with metabolic and immune diseases. 6) combined with a history of epilepsy. We included age- and sex-matched health check-ups of the hospital during the same period as the control group.

Ultimately, 210 patients with AIS were enrolled in the case group, as well as 52 cases were classified into the control group. All study subjects signed an informed consent form and passed the ethical review of North Jiangsu People's Hospital.

Data collection and outcome measures

Clinical data were collected in this study, including 1) baseline information: gender, age, body mass index (BMI), history of hypertension, history of diabetes, history of coronary heart disease (CHD), history of atrial fibrillation (AF), history of alcoholism, history of smoking, admission NIHSS score (National Institutes of Health Stroke Scale), TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification, and infarct volume; 2) laboratory indicators performed using fasting blood in the early morning of the next day:admission fasting blood glucose(FBG), glycosylated hemoglobin (HbA1c); total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), white blood cells (WBC), neutrophils (NEUT), lymphocytes (LYM), serum creatinine (SCR), blood urea nitrogen (BUN), etc.; and 3) imaging findings: all patients underwent head CT and MRI examination, and the cerebral infarction size was calculated according to the Pullicino formula.

The prognosis of patients after 90 days was assessed according to the modified Rankin Scale (mRS) through telephone follow-up after patients were admitted to the hospital, with 0≤Mrs≤2 being the good prognosis group, and mRS>2 being the poor prognosis group; patients with acute cerebral infarction who showed worsening symptoms of neurological deficits and an increase of NIHSS score by ≥2 within 7 days were considered as the progression group, and vice versa as the non-progression group. Eventually, a total of 210 patients were included in the acute cerebral infarction group, 52 patients in the control group, 38 patients in the progression group, 172 patients in the non-progression group, 40 patients in the poor prognosis group, and 170 patients in the good prognosis group.

For healthy controls, demographic information, epidemiological risk factors associated with vascular disease, and blood samples were collected.

Collection and processing of samples

The patients enrolled in the acute cerebral infarction group took 5ml blood samples from fasting elbow vein in the early morning of the second day, centrifuged at 3000r for 10 minutes to quickly and carefully separate the upper serum layer, placed in EP tubes, numbered and stored in -80° refrigerator to be measured, avoiding repeated freezing and thawing, and in the control group, the samples were taken in the early morning from fasting elbow vein and treated and stored in the same way. Serum ZAG concentrations were measured centrally at Jiangsu Province Su Bei People's Hospital by an enzyme-linked immunosorbent assay with a commercially available ELISA kit (Bosen, Jiangsu, China) according to the instructions, respectively.

Statistical method

SPSS27.0 statistical software was applied, and the measured data were tested for conformity with normal distribution by the Kolmogorov-Smirnov test. Data conforming to normal distribution were expressed as mean ± standard deviation, independent samples t-test was used for comparison between two groups, and one-way ANOVA was used for comparison of means between three groups and above; data not normally distributed were expressed as median M (Q25, Q75), Mann-Whitney U test was used for comparison between two groups, and Kruskal-Wallis H test was used for comparisons between three and more groups. Categorical information was expressed as frequency percentages, and the chi-square test was used for comparisons between groups. The data between groups were compared, and the influencing factors with $P<0.05$ were included in the binary logistic regression model to derive the independent factors for the progression and prognosis of cerebral infarction. Nomogram models were constructed by using Python software (version 3.10.6). Based on the results of the multivariate analysis, nomogram models were established to better predict the progression and prognosis of AIS. The research participants were randomly divided into a training set and an internal validation set at a ratio of 7:3.

RESULTS

Serum ZAG levels are lower in AIS patients

The clinical features of the enrolled patients are presented in Table 1. Serum ZAG levels were significantly lower in AIS patients [88.09 (79.17, 99.83) μg/mL, n=210] than in controls [101.57 (87.25, 131.24), n=52] ($P<0.01$) (Table 1). When demographic and clinical data were compared between the AIS and control groups, we observed significant between-group differences in the glycated hemoglobin (HbA1c) level, white blood cell (WBC), neutrophil (NEUT) count, lymphocyte (LYM) count and blood urea nitrogen (BUN) level ($P<0.05$).

Lower serum ZAG levels are independently associated with AIS

Factors with a P value <0.05 (serum ZAG level, HbA1c level, WBC count, NEUT count, LYM count and BUN level) were included in further binary logistic regression analysis. Multivariate analysis demonstrated that the serum ZAG level [odds ratio (OR) 0.963, 95% confidence interval (CI): 0.948–0.979, $P < 0.01$] was an independent risk factor for AIS (Table 1).

Serum ZAG levels decrease in parallel with AIS progression

Thirty-eight patients were included in the AIS progression group (with an increase in the National Institutes of Health Stroke Scale (NIHSS) score of more than 2 points within 7 days), and 172 patients were included in the nonprogression group (Table 2). The serum ZAG level of 86.53 (76.59, 92.27) μg/mL in the progression group was significantly lower than that of 88.92 (80.16, 101.05) μg/mL in the nonprogression group ($P < 0.05$). The admission NIHSS score, infarction size, high-density lipoprotein (HDL) level and NEUT count were significantly greater in the progression group ($P<0.05$). In addition, multivariate analysis demonstrated that the serum ZAG level (OR 0.968, 95% CI: 0.947–0.991, $P = 0.005$) was an independent factor for the risk of stroke progression (Table 2).

Lower serum ZAG levels predict poor long-term poor prognosis of AIS

Forty patients were included in the poor prognosis group [modified Rankin scale (mRS) score: >2 points], and 170 patients were included in the good prognosis group (mRS score: 0–2 points) on the basis of the 90-day mRS score (Table 3). The clinical data of the two groups are presented in Table 3. The serum ZAG level of 79.56 (70.28, 80.94) μg/mL in the poor prognosis group was significantly lower than that of 91.73 (81.72, 101.14) μg/mL in the good prognosis group ($P < 0.01$). Compared with the good prognosis group, the poor prognosis group had a higher admission NIHSS score, the different TOAST (Trial of ORG 10172 in Acute Stroke Treatment) classification, a larger infarction size and a higher NEUT count ($P < 0.05$), as shown in Table 3.

Table 1: Demographic and clinical characteristics, univariate and multivariate analysis of AIS group and healthy control group

Characteristics	AIS group n=210	Control group n=52	P value	Multivariate Univariate analysis analysis		
				OR (95%CI)	SE	P value
Male, n(%)	146(69.5%)	34(65.4%)	0.564	/	/	/
Age, y	65.60±11.35	65.96±9.79	0.816	/	/	/
BMI(kg/m2)	24.46(22.48, 26.79)	23.37(22.21,25.85)	0.217	/	/	/
Hypertension(%)	156(74.3%)	33(63.5%)	0.119	/	/	/
Diabetes mellitus(%)	76(36.2%)	16(30.8%)	0.463	/	/	/
AF(%)	17(8.1%)	2(3.8%)	0.290	/	/	/
CHD(%)	12(5.7%)	3(5.8%)	0.988	/	/	/
Smoking(%)	79(37.6%)	15(28.8%)	0.238	/	/	/
Drinking(%)	49(23.3%)	12(23.1%)	0.969	/	/	/
FBG, mmol/L	5.65(4.785,7.88)	5.335(4.650,5.884)	0.092	/	/	/
HbA1c	6.00(5.55,7.90)	5.70(5.30,6.975)	0.040	1.256(0.969-1.627)	0.132	0.084
WBC, 10^9/L	6.625(5.50,7.86)	6.12(4.77,7.15)	0.013	0.692(0.263-1.816)	0.493	0.454
NEUT, 10^9/L	4.55(3.46,5.81)	3.45(2.83,4.57)	<0.001	1.769(0.630-4.496)	0.527	0.279
LYM, 10^9/L	1.46(1.12,1.95)	1.68(1.32,1.95)	0.034	0.832(0.255-2.711)	0.603	0.760
BUN, mmol/L	5.75(4.86,7.21)	5.34(4.53,6.22)	0.015	1.131(0.917-1.396)	0.107	0.250
SCR, mmol/L	70.95(58.78,88.28)	73(57.03,85.45)	0.675	/	/	/
TC, mmol/L	4.16(3.58,4.92)	4.32(3.54,5.07)	0.917	/	/	/
TG, mmol/L	1.31(0.98,2.05)	1.24(0.99,1.94)	0.657	/	/	/
HDL, mmol/L	0.99(0.84,1.20)	1.03(0.93,1.24)	0.077	/	/	/
LDL, mmol/L	2.65(2.03,3.29)	2.74(1.96,3.43)	0.801	/	/	/
Serum ZAG, ug/mL	88.09(79.17,99.83)	101.57(87.25,131.24)	<0.001	0.963(0.948, 0.979)	0.008	<0.001

AIS, acute ischemic stroke; BMI, body mass index; AF, Atrial fibrillation; CHD, Coronary heart disease; FBG, fasting blood glucose; HbA1c, Glycosylated hemoglobin; WBC, white blood cells; NEUT, neutrophils; LYM,lymphocytes; BUN, blood urea nitrogen; Scre, serum creatinine; TC, Total cholesterol; TG, Triglyceride; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; ZAG,Zinc-Alpha2-Glycoprotein.

*Continuous variables are expressed as mean ± standard deviation or median (interquartile range). Categorical variables are expressed as frequency (%).

Table 2: Demographic and clinical characteristics, univariate and multivariate analysis of stroke progression group and nonprogression group

Characteristics	Non-progressive group n=172	Progress group n=38	P value	Multivariate Univariate analysis analysis		
				OR (95%CI)	SE	P value
Male, n(%)	123(71.5%)	23(60.5%)	0.183	/	/	/
Age, y	65.92±11.661	64.11±9.841	0.325	/	/	/
BMI(kg/m2)	24.48(22.49,26.73)	24.22(21.76,27.30)	0.729	/	/	/
Hypertension(%)	129(75%)	27(71.1%)	0.614	/	/	/
Diabetes mellitus(%)	64(37.2%)	12(31.6%)	0.513	/	/	/
AF(%)	14(8.1%)	3(7.9%)	0.960	/	/	/
CHD(%)	11(6.4%)	1(2.6%)	0.366	/	/	/
Smoking(%)	65(37.8%)	14(36.8%)	0.913	/	/	/
Drinking(%)	42(24.2%)	7(18.4%)	0.429	/	/	/
Admission NIHSS score	2(1,4)	3(2,7)	0.006	1.027(0.949-1.112)	0.041	0.508
TOAST classification(%)	/	/	0.448	/	/	/
LAA	75(43.6%)	20(52.6%)		/	/	/
SAA	43(25%)	7(18.4%)		/	/	/
CE	16(9.3%)	1(2.6%)		/	/	/
SOE	14(8.1%)	5(13.2%)		/	/	/
SUE	24(14%)	5(13.2%)		/	/	/
Infarction size, cm^3	0.65(0.23,4.00)	1.60(0.52, 7.50)	0.008	0.992(0.969-1.016)	0.012	0.502
FBG, mmol/L	5.65(4.73,7.96)	5.695(4.81,7.61)	0.721	/	/	/
HbA1c	6(5.5,7.98)	5.75(5.4,7.5)	0.204	/	/	/
WBC, 10^9/L	6.49(5.40,7.73)	7.20(6.04,8.61)	0.101	/	/	/
NEUT, 10^9/L	4.39(3.39,5.59)	5.39(3.59,6.85)	0.043	1.176(0.983-1.405)	0.091	0.076
LYM, 10^9/L	1.51(1.15,1.97)	1.34(1.00,1.58)	0.057	/	/	/
BUN, mmol/L	5.77(4.92,7.18)	5.53(4.55,8.21)	0.780	/	/	/
SCR, mmol/L	70.85(57.53,88)	73.05(62.48,90.63)	0.396	/	/	/
TC, mmol/L	4.11(3.57,4.78)	4.37(3.64,5.13)	0.087	/	/	/
TG, mmol/L	1.34(1,2.18)	1.13(0.85,1.62)	0.277	/	/	/
HDL, mmol/L	0.98(0.83,1.2)	1.09(0.92,1.43)	0.020	3.505(1.007-12.199)	0.636	0.049
LDL, mmol/L	2.61(1.98,3.27)	2.81(2.18,3.47)	0.373	/	/	/
Serum ZAG, ug/mL	88.92(80.16,101.05)	86.53(76.59,92.27)	0.011	0.968(0.947-0.991)	0.012	0.005

BMI, body mass index; AF, Atrial fibrillation; CHD, Coronary heart disease; NIHSS,National Institutes of Health Stroke Scale; TOAST, Trial of Org 10172 in Acute Stroke Treatment; LAA, large-artery atherosclerosis; SAA, small-arteryoclusion; CE, cardioembolism; SOE, stroke of other determined etiology; SUE, stroke of undetermined etiology; FBG, fasting blood glucose; HbA1c, Glycosylated hemoglobin; WBC, white blood cells; NEUT, neutrophils; LYM,lymphocytes; BUN, blood urea nitrogen; Scre, serum creatinine; TC, Total cholesterol; TG, Triglyceride; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; ZAG,Zinc-Alpha2-Glycoprotein.

*Continuous variables are expressed as mean ± standard deviation or median (interquartile range). Categorical variables are expressed as frequency (%).

Table 3: Demographic and clinical characteristics, Univariate and Multivariate analysis of good prognosis group and poor prognosis group

Characteristics	Good prognosis group n=170	Poor prognosis group n=40	P value	Multivariate Univariate analysis analysis		
				OR (95%CI)	SE	P value
Male, n(%)	121(71.2%)	25(62.5%)	0.283	/	/	/
Age, y	66(57,73)	69(60,75.25)	0.097	/	/	/
BMI(kg/m2)	24.49(22.04,26.73)	24.22(23.34,27.55)	0.558	/	/	/
Hypertension(%)	126(74.1%)	30(75%)	0.909	/	/	/
Diabetes mellitus(%)	64(37.6%)	12(30%)	0.365	/	/	/
AF(%)	13(7.6%)	4(10%)	0.624	/	/	/
CHD(%)	9(5.3%)	3(7.5%)	0.589	/	/	/
Smoking(%)	62(36.5%)	17(42.5%)	0.479	/	/	/
Drinking(%)	39(22.9%)	10(25.0%)	0.782	/	/	/
Admission NIHSS score	2(1,4)	6(3,12.5)	<0.01	1.331(1.159-1.528)	0.070	<0.01
TOAST classification(%)			0.024			
LAA	68(40%)	27(67.5%)		ref	ref	ref
SAA	16(9.4%)	1(2.5%)		3.689(0.687-19.805)	0.857	0.128
CE	42(24.7%)	8(20%)		0.948(0.053-16.912)	1.470	0.971
SOE	17(10%)	2(5%)		3.591(0.603-21.930)	0.910	0.160
SUE	27(15.9%)	2(5%)		0.823(0.080-8.465)	1.189	0.870
Infarction size, cm^3	0.61(0.24,2.56)	3.74(1.11, 24.84)	<0.01	1.041(1.005-1.079)	0.018	0.024
FBG, mmol/L	5.77(4.75,7.91)	5.49(4.81,7.69)	0.911	/	/	/
HbA1c	6.00(5.50,7.95)	5.49(4.81,7.69)	0.308	/	/	/
WBC, 10^9/L	6.49(5.44,7.52)	7.55(5.65,8.76)	0.053	/	/	/
NEUT, 10^9/L	4.39(3.40,5.59)	5.55(3.53,7.11)	0.028	0.944(0.741-1.203)	0.124	0.644
LYM, 10^9/L	1.50(1.17,2.00)	1.38(1.03,1.64)	0.078	/	/	/
BUN, mmol/L	5.71(4.855-7.138)	6.05(4.67,7.03)	0.883	/	/	/
Scre, mmol/L	70.85(59.08,88.58)	70.65(57.73,83.50)	0.901	/	/	/
TC, mmol/L	4.16(3.61,4.85)	4.13(3.32,5.18)	0.828	/	/	/
TG, mmol/L	1.30(1.00,2.18)	1.13(0.88,1.81)	0.088	/	/	/
HDL, mmol/L	0.98(0.830,2.10)	1.03(0.92,1.26)	0.144	/	/	/
LDL, mmol/L	2.65(2.010,3.27)	2.54(2.12,3.48)	0.605	/	/	/
Serum ZAG, ug/mL	91.73(81.72,101.14)	79.56(70.28,80.94)	<0.01	0.937(0.905-0.969)	0.018	<0.01

BMI, body mass index; AF, Atrial fibrillation; CHD, Coronary heart disease; NIHSS,National Institutes of Health Stroke Scale; TOAST, Trial of Org 10172 in Acute Stroke Treatment; LAA, large-artery atherosclerosis; SAA, small-arteryoclusion; CE, cardioembolism; SOE, stroke of other determined etiology; SUE, stroke of undetermined etiology; FBG, fasting blood glucose; HbA1c, Glycosylated hemoglobin; WBC, white blood cells; NEUT, neutrophils; LYM,lymphocytes; BUN, blood urea nitrogen; Scre, serum creatinine; TC, Total cholesterol; TG, Triglyceride; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; ZAG,Zinc-Alpha2-Glycoprotein; ref, reference.

*Continuous variables are expressed as mean ± standard deviation or median (interquartile range). Categorical variables are expressed as frequency (%)·

The results of multivariate analysis indicated that the independent risk factors related to functional prognosis at 90 days included the admission NIHSS score (OR 1.331, 95% CI: 1.159–1.528, $P < 0.01$), infarction size (OR 1.041, 95% CI: 1.005–1.079, $P = 0.024$) and serum ZAG level (OR 0.937, 95% CI: 0.905–0.969, $P < 0.01$).

The development of nomogram models for predicting the progression and prognosis of AIS

According to the above analysis, nomogram models were established to predict the progression and prognosis of AIS. The serum ZAG level (P <0.01) and HDL level ($P = 0.049$) were used to establish a nomogram model to predict the progression of AIS (Fig. 1a). The area under the curve (AUC) of the training set (n = 147) was 0.682, and that of the validation set (n = 63) was 0.721 (Figure 1b and c).

The admission NIHSS score ($P < 0.01$), infarction size ($P = 0.024$) and serum ZAG level ($P < 0.01$) were used to establish a nomogram model to predict the prognosis of AIS patients (Figure 2a). The AUC of the training set (n =147) was 0.834, and that of the validation set (n = 63) was 0.909 (Figure 2b and c). The AUC of the nomogram model established in this study was better than the AUC of any single risk factor.

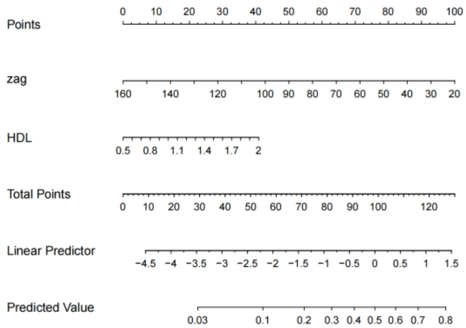
DISCUSSION

In this study, we show for the first time that the serum ZAG level is lower in AIS patients than healthy controls. The serum ZAG level on admission was an independent factor for the risk of AI occurence, progression and prognosis and had a certain clinical predictive value for clinical outcomes.

ZAG is a soluble protein encoded by the AZGP1 gene and has a molecular weight of around 40 kilodaltons (kDa). It is a glycoprotein produced by white adipose tissue, brown adipose tissue, and liver.[4] It is then released into bodily fluids, including serum, sweat, saliva, cerebrospinal fluid, and urine.[5] A previous study revealed the crystal structure of human ZAG, which has three introns and four exons, each encoding a signal peptide. The protein is composed of three distinct structural domains known as α1, α2, and α3. The enitre structure exhibits a significant resemblance to the heavy chain of class I MHC, which serves a valuable purpose in the immune system.

ZAG is involved in lipid metabolism, glucose metabolism, and the regulation of insulin sensitivity. Studies have confirmed that ZAG has anti-inflammatory effects.[7] The prevalence of large artery atherosclerosis (LAAS) among acute stroke treatment (TOAST) subtypes is relatively high in China.[17] The crucial role of inflammation in the formation and progression of atherosclerotic plaques is indisputable.[18] A study revealed that ZAG colocalizes with CD68-positive macrophage α3-AR in the core of atherosclerotic plaques in patients with coronary artery disease and is negatively correlated with anti-atherosclerotic factors, indicating that ZAG is linked to atherosclerosis and potentially to LAAS cerebral infarction.[7] ZAG was found to inhibit the lipopolysaccharide (LPS)-induced inflammatory response in macrophages via JNK/AP-1 signaling.[19] Leal *et al* reported that the serum ZAG level was inversely associated with markers of proatherogenic factors, such as TNF-α and vascular cell adhesion molecule 1 (VCAM-1).[20] Liu *et al*. reported that serum ZAG levels were decreased in Chinese patients with premature coronary artery disease.[21] Thus, it is reasonable to theorize that serum ZAG is not only a marker of stroke risk but also a risk factor for stroke disease progression and prognosis. In this study, the serum ZAG levels were lower in the AIS progression group and in the poor AIS prognosis group. Actually, the progression of disease may also be affected by the infarction site. In patients where the infarction foci were in the critical site, the disease may still progress despite low NIHSS scores, small infarct volume and high ZAG levels.

Previous studies have shown that neutrophils in peripheral blood can infiltrate the central nervous system (CNS) and produce proinflammatory factors such as IL-1β, reactive oxygen species (ROS), neutrophil elastase, and matrix metalloproteinase 9 (MMP-9).[22-23] ZAG overexpression has a significant inhibitory effect on MMP-2 and MMP-9.[24] It is theorized that serum ZAG decreases the permeability of the blood–brain barrier by inhibiting the levels of MMP-9. Another study showed a positive correlation between the severity of cerebral infarction and the number of pro-inflammatory microglia[25], and ZAG could facilitate the polarization of microglia from a pro-inflammatory (M1) state to an anti-inflammatory (M2) state and inhibit LPS-induced inflammatory response by suppressing JNK/AP-1 signaling.[7] On the basis of these findings, we theorized that the effect of serum ZAG levels on neurological deficits could be mediated by the following process: a decrease in serum ZAG levels leads to an increased rate of NEUT infiltration into the CNS. Moreover, a decrease in serum ZAG



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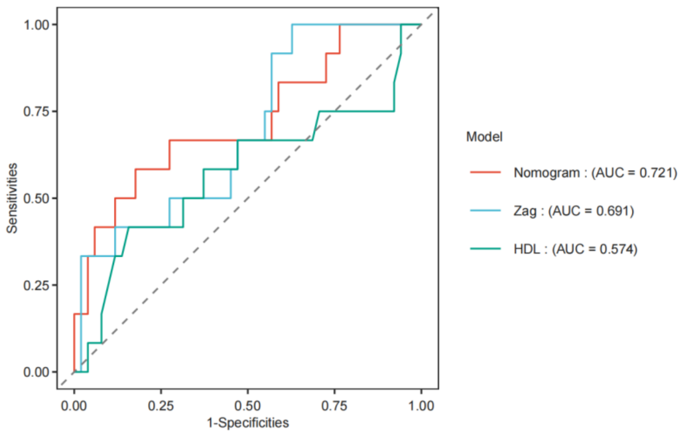

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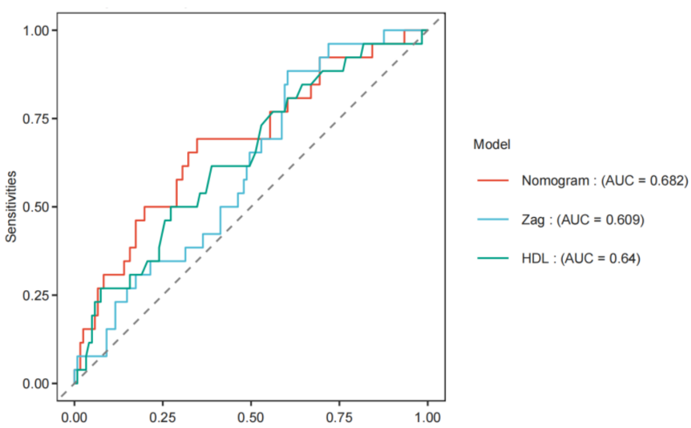

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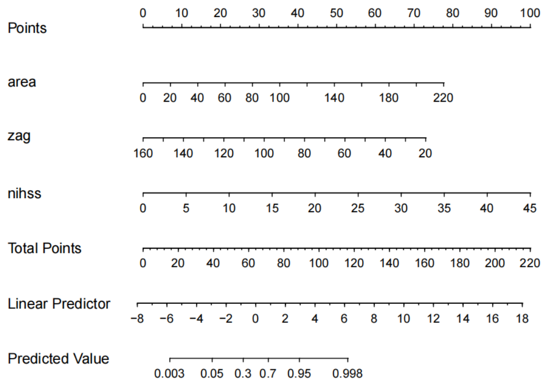
Figure 1. The nomogram for predicting progress of acute ischemic stroke

a, The nomogram for predicting the progress of AIS patients. Each of the independent predictors was projected upwards to the value of the "points" at the top level of the nomogram to obtain a score within the range of 0-100, and then the total score of these points was recorded to accurately predict the risk of progress in the AIS patients.
b, The receiver operating characteristic (ROC) curves of the nomogram, serum ZAG level ($P < 0.01$) and HDL level ($P = 0.049$) for progress prediction of AIS patients in the training set. The yaxis meant the truepositive rate of the risk prediction. The x-axis meant the false-positive rate of the risk prediction.
c, The ROC curves of the nomogram, serum ZAG level and HDL level for progress prediction of AIS patients in the testing set.
ZAG,Zinc-Alpha2-Glycoprotein; HDL, High-density lipoprotein; AUC, areas under the receiver operating characteristic curve.



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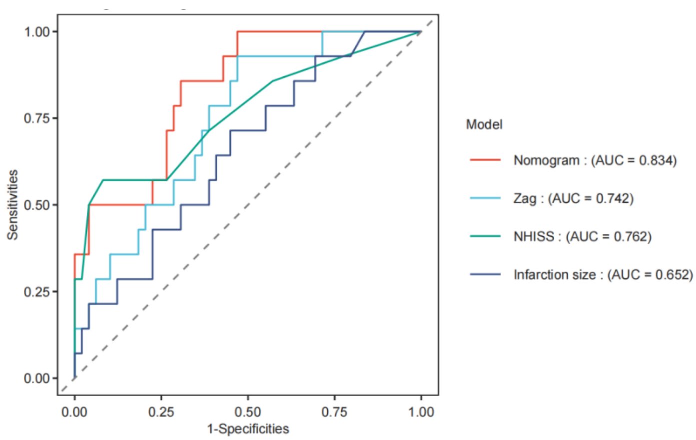

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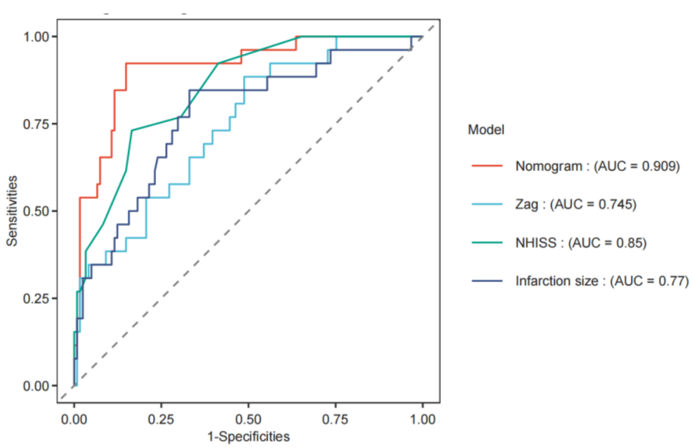

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Figure 2. The nomogram for predicting prognosis of acute ischemic stroke

a The nomogram for predicting the prognosis of AIS patients. Each of the independent predictors was projected upwards to the value of the “points” at the top level of the nomogram to obtain a score within the range of 0-100, and then the total score of these points was recorded to accurately predict the risk of prognosis in the AIS patients.
b, The receiver operating characteristic (ROC) curves of the nomogram, admission NIHSS score ($P < 0.01$), infarction size ($P = 0.017$) and serum ZAG level ($P < 0.01$) for prognosis prediction of AIS patients in the training set. The y-axis meant the truepositive rate of the risk prediction. The x-axis meant the false-positive rate of the risk prediction.
c, The ROC curves of the nomogram, admission NIHSS score ($P < 0.01$), infarction size ($P = 0.017$) and serum ZAG level ($P < 0.01$) for prognosis prediction of AIS patients in the testing set.
ZAG, Zinc-Alpha2-Glycoprotein; NIHSS, National Institutes of Health Stroke Scale scores; AUC, areas under the receiver operating characteristic curve.

levels promotes the conversion of microglia to a proinflammatory phenotype, resulting in increased release of proinflammatory factors such as MMP9, ROS, and IL-6. Consequently, this increase in proinflammatory factors leads to the disruption of the blood–brain barrier. The decrease in the serum ZAG level also leads to the weakening of ZAG binding to α3-AR in macrophages. This further weakens the anti-inflammatory effect exerted by JNK/AP-1 signaling, hence exacerbating postischemic inflammatory injury in AIS patients. The reduction in serum ZAG levels may also reduce its neuroprotective effects against oxidative stress and atherosclerosis, which in turn affects the pathophysiological process of acute cerebral infarction.

On the basis of the serum ZAG level and selected risk factors, we established nomogram models to predict the progression and prognosis of AIS. Especially, the prognosis prediction model showed great prediction ability for AIS, which is helpful for the early assessment of AIS patients and early-stage intervention.

This study had several limitations. First, the current study was a single-center study with a small sample size, which could result in selection bias. Second, serum ZAG levels were examined at only one time point, and the specific evolution of serum ZAG levels throughout different stages of acute cerebral infarction was not examined. Third, whether the protective effects of ZAG in cerebral infarction are simply due to its anti-inflammatory mechanism remains to be elucidated, and work is continuing in this field. Further experimentation on animals is needed to elucidate the molecular mechanisms of action of ZAG.

In conclusion, we show for the first time that serum ZAG levels are lower in AIS patients than in controls. We also found that lower serum ZAG levels may predict disease progression and the long-term poor prognosis of AIS. Thus, it is reasonable to theorize that the lower serum ZAG levels observed in our study may have been involved in the development and progression of AIS and that ZAG may be a novel marker of AIS onset.

DISCLOSURE

Ethics: This study was approved by the Ethics Committee of the Northern Jiangsu People's Hospital (Ethics No 2022ky299).

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Conflict of interest: None

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