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Plasma S100A1 protein: An effective prognostic biomarker for 3-month clinical outcome in acute ischemic stroke patients

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Abstract

Background: Plasma S100A1 protein is a novel inflammation biomarker associated with acute myocardial infarction and neurodegenerative diseases. This study aimed to investigate the predictive value of S100A1 protein for the three-month prognosis of acute ischemic stroke (AIS) patients and the potential pathophysiological mechanisms of AIS. *Methods:* A total of 206 people in a stroke center from April 2020 to February 2021 were studied. Clinically relevant data and blood indicators were recorded. The clinical outcome was disability or death at discharge or 90 days (defined as a modified Rankin Scale score of 3-6). The relationship between S100A1 protein, NF-κB p65, and IL-6 and functional outcomes was investigated by binary logistic regression analysis and further assessed by the receiver operating characteristic curve (ROC). The correlation between S100A1, NF-κB p65, and IL-6 was detected by Pearson or Spearman correlation analysis. *Results:* A total of 206 subjects were enrolled (Age, 67.17±10.74; 40.3% female). Patients with unfavorable outcome showed higher S100A1, NF-κB p65, and IL-6 than those with favorable outcome (S100A1, [252.72±25.15]vs[219.84±23.24], P<0.001; NF-κB p65, [4.45±0.71]vs[3.58±0.66], P<0.001; IL-6, [13.86±1.41]vs[12.18±1.73], P<0.001). In multivariate and ROC curve analysis, higher S100A1 (>227.155) (Area under the curve [AUC], 0.864; odds ratio [OR], 1.093; 95% confidence interval [CI], 1.052- 1.135; P <0.001) and higher NF-κB p65 (>3.685) (AUC, 0.807; OR, 6.416; 95% CI, 1.852- 22.229; P=0.003), higher IL-6 (>12.330) (AUC, 0.767; OR, 2.029; 95% CI, 1.136- 3.624; P=0.017) were independently associated with unfavorable outcome. The combined predictive value of the three indexes was higher than that of a single index. There was a significant statistical correlation between S100A1, NF-κB P65 and IL-6(P<0.001).
Conclusion: Higher S100A1 protein may be an independent risk factor for predicting the three-month poor prognosis in AIS patients. This protein may mediate inflammatory response through the NF-κB pathway during the pathogenesis of AIS.

Keywords: S100A1 calcium-binding protein, nuclear transcription factor κB phosphorylation 65, interleukin 6, acute ischemic stroke, Prognosis

INTRODUCTION

Acute ischemic stroke (AIS) is caused primarily by an interruption in cerebral blood flow, which induces severe neural injuries. It is one of the leading causes of death and disability worldwide.[1] According to the latest global burden of disease data, AIS was similarly one of the main reasons for the increase in the number of years of life lost and all age disability-adjusted life years in China.[2] The population incidence rate is 1.6% and the mortality rate is 1.1%.[3] In China's rural and remote locations, AIS was the leading cause of disability and death and accounted for 69.6% of all stroke types.[3-5] There were approximately two million new cases of brain infarction annually,

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with associated medical expenses totaling tens of billions of dollars.[6] The prevalence rate of stroke in the United States was also 2.5% to 3.7% of the population, and the cost of the disease was as high as US$28 billion.[7] Except for recombinant tissue plasminogen activator (rt-PA), a thrombolytic drug used to recanalize the occluded artery, most medical treatments have been demonstrated ineffective.[8] Therefore, reliable biological markers that can aid in determining the prognosis and outcome of stroke, particularly for hospitals located in remote locations with only the most basic equipment would be most needed.

The immune-inflammatory response is one of the pathophysiological foundations of AIS and plays a role in each stage of the AIS pathogenesis.[9] As a result, the immune-inflammatory response is also recognized as an important target for diagnosing and treating acute ischemic stroke. Firstly, stroke induces a localized neuroinflammatory response, where the inflammatory activation of glial, endothelial, and brain-invading cells contributes to the progression of the post-stroke lesion. Secondly, ischemic brain injury disrupts the homeostasis of the systemic immune system, resulting in enduring modifications.[9] When an ischemic stroke occurs, a massive variety of biochemical and inflammatory mediators, including cytokines, chemokines, and pro-inflammatory enzymes, are produced.[10,11] S100A1 protein is a recently identified novel inflammatory marker and a member of the S100 protein family. It is expressed in endothelial cells and can bind to specific proteins both intracellularly and extracellularly, participating in signaling, neurotransmitter transfer, and the formation of specific enzymes.[12,13] According to previous studies, the S100A1RyR complex was formed when the S100A1 protein bound to RyR receptors, controlling intracellular Ca2+ homeostasis and contributing to the pathophysiological processes of Alzheimer's disease.[14] S100B protein, another member of the S100 protein family, and S100A1 protein had similar biological behavior.[15] According to research by Ercole *et al*., S100B protein could accurately predict the severity of acute cerebral infarction and its prognosis.[16] Considering the potential involvement of S100A1 and S100B proteins in a shared signaling pathway in vivo[16-18], it was reasonable to speculate whether S100A1 protein could be used to prognosticate the outcome of AIS. Previous studies have shown that S100A1 might regulate the inflammatory response and oxidative stress response of H9C2 cells through TLR4 /NF-κB pathway.[19] Hypoxic cardiomyocytes released endogenous S100A1, which activated the NF-κB p65 protein through binding to TLR4 receptors, leading to an upregulation of pro-inflammatory cytokines, including IL-6. It was rational to hypothesize that the involvement of S100A1 protein in a similar pathophysiological mechanism occurs in AIS. As far as we are aware, no research has been done on the significance of the S100A1 protein for AIS.

In this study, our objective was to retrospectively analyze S100A1, NF-κB p65, and IL-6 values in AIS patients with different prognostic outcomes and to determine the diagnostic efficacy of these three values for the 3-month prognosis of AIS patients. Simultaneously, the mechanism of plasma S100A1 protein in the pathogenesis of AIS was discussed.

METHODS

Study population

This retrospective study was conducted from April 2020 to February 2021 at the Brain Center, Department of Neurology, Subei People's Hospital affiliated to Yangzhou University, China. A total of 206 patients were enrolled (Age, mean ± standard deviation, 67.17±10.74; 40.3% female, 59.7%male). Inclusion criteria were as follows: 1) Patients with acute cerebral infarction of the first onset confirmed by cranial CT/MRI who met the diagnostic criteria of the Chinese Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke 2018[20,21]; 2) Age greater than 18 years and less than 90 years; 3) Signed informed consent from the patient or family. Exclusion criteria were: 1) Patients with encephalomalacia of the brain, as indicated by head CT or MRI, unrelated to AIS; 2) Recurrent stroke or patients with severe infection, severe liver, kidney, heart, and respiratory system diseases, pregnancy, and known malignancies; 3) Patients with Alzheimer's disease or epileptic seizures; 4) Patients with rheumatoid arthritis and other autoimmune diseases; 5). Patients with various secondary cerebral hemorrhages, intracranial and extracranial injuries, or brain tumor hemorrhages; 6) Patients with acute myocardial infarction or previous serious cardiovascular and cerebrovascular adverse events. 7). Patients receiving intravenous thrombolysis and mechanical thrombolysis in other hospitals or our hospital. Two well-trained senior neurologists completed all the inclusion and exclusion criteria. This study was approved by

the Ethics Committee of Subei People's Hospital affiliated to Yangzhou University in China, and all the selected patients were signed with informed consent by their legal representatives or close relatives.

Baseline assessments and laboratory measurements

We gathered information about the research participants, such as their demographic characteristics, risk factors, clinical data, blood routine indexes, biochemical indicators, and laboratory parameters. Demographic characteristics included age and sex. Risk factors comprised body mass index (BMI), smoking, alcohol drinking, hypertension, diabetes, hyperlipidemia, atrial fibrillation (AF), and coronary heart disease (CHD). Clinical data contained admission diastolic blood pressure (BP), admission systolic BP, admission NIHSS (range, 0-42, with a higher score suggesting a more severe neurologic impairment), and TOAST type (including Large-artery atherosclerosis [LAA], cardioembolism [CE], small-artery occlusion [SAO], and others). Blood routine indexes contained blood glucose (BG), red blood cell (RBC) count, hematocrit count, leukocyte count, neutrophil count, lymphocyte count, monocyte count, red blood cell distribution width (RDW), Platelet distribution width (PDW), and platelets (PLT) count. Biochemical indicators included total cholesterol (TC), triglycerides (TG), low-density lipoproteins (LDL), high-density lipoproteins (HDL), uric acid (UA), creatinine, alanine transaminase (ALT), aspartate transaminase (AST), and homocysteine. Routine blood indicators were detected and analyzed by Sysmex XE-2100 automatic blood cell analyzer from Japan. Biochemical indexes were detected and analyzed by the Modular PP automatic biochemical analyzer manufactured by Roche, Germany. Plasma levels of S100A1 protein, NF-κB p65, and IL-6 were detected using ELISA kits from Beijing Green Source Boulder Biotechnology Co. The measurements were performed by two senior examiners strictly following the instructions of the ELISA double antibody sandwich assay. The final value was calculated as the average of the two measurements. Fasting venous blood samples were collected from AIS patients in the early morning of the second day of admission. All blood samples were tested by our senior examiners in a sterile, sealed clinical laboratory.

Clinical outcome

The clinical outcomes of all patients were assessed by two experienced neurologists. All patients underwent a clinical evaluation of the modified Rankin Scale (mRS) at three months. A good clinical outcome was defined as an mRS score of 0-2. Adverse clinical outcomes were defined as an mRS score of 3-6.

Statistical analysis

Statistical analysis was performed using SPSS.26 software. The Kolmogorov-Smirnov test was used to assess the normal distribution. The mean and standard deviation ($x \pm s$) of continuous variables that fit the normal distribution was used as the unit of expression, and a t-test was used to compare the two groups. Continuous variables not subject to normal distribution were expressed as the median and interquartile range (25th to 75th percentile), and the Mann-Whitney U test was used for comparison between the two groups. Enumeration data were expressed in percentage (%), and a comparison between the two groups was made by the Chi-square test or Fisher exact test. Univariate and multivariate Logistic regression analysis was used to select the independent influencing factors related to the prognosis of AIS patients. The odds ratio (OR) and 95% confidence interval (CI) were calculated as the estimated values of each endpoint. The markers (S100A1, NF-κB p65, IL-6, and their composite) were assessed using the Receiver Operating Characteristic curve (ROC). Maximum Youden Index, the optimal cutoff, sensitivity, and specificity were calculated. Pearson or Spearman correlation analysis was used to investigate the correlation between plasma levels of S100A1, NF-κB p65, and IL-6. A significance level of $P < 0.05$ (two-tailed) was applied.

RESULTS

Comparison of baseline characteristics of AIS patients with different prognosis.

The data on the baseline characteristics of patients with AIS of various prognosis were summarized in Table 1. A total of 206 subjects were enrolled (Age, mean ± standard deviation, 67.17±10.74; 40.3% female, 59.7%male). 130 patients showed a favorable prognosis at three months (mRS score 0-2; age, 65.53±10.03; 42.3% female; 57.7% male). Unfavorable 3-month outcome was found for the other 76 patients (mRS score

Table 1: Comparison of baseline characteristics of acute ischaemic stroke patients with different prognosis

Characteristics	Patients (N=206)	Favorable (N=130)	Unfavorable (N=76)	*P*-Value
Demographics				
Age (years)	67.17±10.74	65.53±10.03	69.96±11.40	0.004
Female (n) (%)	83(40.3)	55(42.3)	28(36.8)	0.440
Risk factors				
BMI(kg/m^2)	24.27±3.10	24.26±3.15	24.30±3.04	0.932
Smoking(n) (%)	77(37.4)	50(38.5)	27(35.5)	0.674
Alcohol drinking(n) (%)	51(24.8)	32(24.6)	19(25.0)	0.951
Hypertension(n) (%)	136(66.0)	80(61.5)	56(73.7)	0.076
Diabetes(n) (%)	37(26.2)	5(25)	8(25.8)	0.992
Hyperlipidemia (n) (%)	60(29.1)	39(30.0)	21(27.6)	0.718
AF(n) (%)	33(16.0)	21(16.2)	12(15.8)	0.945
CHD (n) (%)	29(14.1)	21(16.2)	8(10.5)	0.262
Clinical data				
Systolic BP(mmHg)	149.26±17.46	148.45±17.87	150.64±16.76	0.386
Diastolic BP(mmHg)	83.62±11.41	83.98±11.21	83.00±11.78	0.554
Admission NIHSS	3.00(2.00,5.00)	2.00(1.00,3.25)	4.00(2.25,11.00)	<0.001
TOAST type				
LAA(n) (%)	91(44.2)	56(43.1)	35(46.1)	0.678
CE(n) (%)	24(11.7)	15(11.5)	9(11.8)	0.948
SAO(n) (%)	76(36.9)	52(40.0)	24(31.6)	0.227
Others(n) (%)	15(7.3)	7(5.4)	8(10.5)	0.171
Blood routine indexes				
BG(mmol/L)	6.40±2.30	6.58±2.51	6.09±1.86	0.137
RBC(10^{12}/L)	4.64±0.83	4.59±0.79	4.73±0.90	0.258
Hematocrit(%)	40.14±5.68	40.05±6.34	40.29±4.36	0.745
Leukocytes(10^9/L)	7.58±4.92	7.80±5.95	7.22±2.23	0.421
Neutrophils(10^9/L)	5.48±2.27	4.78±2.20	6.67±1.88	<0.001
Lymphocytes (10^9/L)	1.55±0.65	1.61±0.71	1.43±0.51	0.050
Monocytes(10^9/L)	0.48±0.22	0.48±0.26	0.47±0.16	0.846
RDW (%)	12.71±1.26	12.72±1.30	12.71±1.19	0.985
PDW (%)	14.83±4.96	15.06±4.90	14.44±5.08	0.389
PLT(10^9/L)	186.61±55.97	184.52±56.10	190.17±55.96	0.486
Biochemical indicators				
TC(mmol/L)	4.08±0.83	3.99±0.67	4.24±1.04	0.073
TG(mmol/L)	1.55±0.79	1.33±0.72	1.92±0.77	<0.001
LDL(mmol/L)	2.44±0.69	2.39±0.58	2.53±0.86	0.194
HDL(mmol/L)	1.07±0.31	1.18±0.23	0.87±0.32	<0.001
UA(μmol/L)	329.35±84.22	317.41±77.97	349.77±90.90	0.007
Creatinine (μmol/L)	63.50(52.90, 76.33)	61.35(52.78,67.43)	71.90 (54.75, 82.98)	0.007
ALT(u/L)	25.00(20.00,35.00)	24.00(18.00,33.00)	27.00(21.25,38.00)	0.014
AST(u/l)	25.00(20.75,30.00)	23.00(19.00,27.25)	27.00(24.00,34.00)	<0.001
Homocysteine(μmol/L)	10.00(6.00,13.00)	9.00(5.00, 12.25)	11.00(8.00,14.00)	0.001
Laboratory parameters				
S100A1(pg/ml)	231.97±28.71	219.84±23.24	252.72±25.15	<0.001
NF-κB p65(ng/ml)	3.90±0.79	3.58±0.66	4.45±0.71	<0.001
IL-6(pg/ml)	12.80±1.81	12.18±1.73	13.86±1.41	<0.001

Abbreviations: BMI, Body mass index; AF, Atrial fibrillation; CHD, Coronary heart disease; TOAST, Trial of Org 10172 in acute stroke treatment; LAA, Large-artery atherosclerosis; CE, Cardioembolism; SAO, Small-artery occlusion lacunar; BP, Blood pressure; NIHSS, National Institutes of Health Stroke Scale; BG, Blood glucose; RBC, Red blood cell; RDW, Red blood cell distribution width; PDW, Platelet distribution width; PLT, Platelet; TC, Total cholesterol; TG, Triglycerides; LDL, Low-density lipoproteins; HDL, High-density lipoproteins; UA, Uric Acid; ALT, Alanine transaminase; AST, Aspartate transaminase; NF-κB p65, Nuclear factor kappa-B p65; IL-6, Interleukin-6.

3-6; age, 69.96±11.40; 36.8% female; 63.2% male). There were no significant differences in risk factors between the two groups. The age and admission NIHSS scores of patients with favorable prognosis were significantly lower than that of those with unfavorable prognosis (age, [65.53±10.03] vs[69.96±11.40], P=0.004; admission NIHSS, [median, 2.00; Interquartile range, IQR, 1.00-3.25]vs[4.00; IQR, 2.25-11.00], P<0.001). There were no significant differences in female, admission systolic BP, admission diastolic BP, and TOAST type between the two groups(P>0.05). HDL counts were significantly increased in the favorable group([1.18±0.23] vs[0.87±0.32], P<0.001). At the same time, the peripheral blood neutrophil counts, TG, UA, creatinine, ALT, AST, and homocysteine were significantly increased in the unfavorable group (neutrophils, [6.67±1.88]vs[4.78±2.20], P<0.001; TG, [1.92±0.77]vs[1.33±0.72], P<0.001; UA, [349.77±90.90]vs[317.41±77.97], P=0.007; creatinine, [71.90; IQR, 54.75-82.98] vs[61.35; IQR, 52.78-67.43], P=0.007; ALT, [27.00; IQR, 21.25-38.00]vs[24.00; IQR, 18.00-33.00], P=0.014; AST, [27.00; IQR, 24.00-34.00]vs[23.00; IQR, 19.00-27.25], P<0.001; homocysteine, [11.00; IQR, 8.00-14.00]vs[9.00; IQR, 5.00-12.25], P=0.001). The overall S100A1, NF-κB p65, and IL-6 values of the unfavorable group were higher than those of the favorable group(S100A1, [252.72±25.15]vs[219.84±23.24], P<0.001; NF-κB p65, [4.45±0.71]vs[3.58±0.66], P<0.001; IL-6, [13.86±1.41]vs[12.18±1.73], P<0.001) (Figure 1).

Univariate and multivariate logistic regression analysis for the outcome

The prognosis of patients with AIS at three months was taken as the dependent variable. Age, Admission NIHSS, Neutrophils, TG, HDL, UA, Creatinine, ALT, AST, Homocysteine, S100A1, NF-κB p65, and IL-6 were taken as independent variables. Univariate Logistic regression analysis showed that the values of S100A1, NF-κB p65, and IL-6 were correlated with the prognosis of 90d, and their unadjusted OR values were 1.071 (95%CI, 1.050-1.093; P<0.001), 5.884 (95%CI, 3.464- 9.995; P < 0.001), 1.863 (95%CI, 1.515- 2.291; P < 0.001). In multivariate logistic regression analysis adjusted for other indicators, S100A1, NF-κB p65, and IL-6 were still associated with adverse outcomes (S100A1: OR, 1.093; 95%CI, 1.052- 1.135; P < 0.001; NF-κB p65: OR, 6.416; 95%CI, 1.852- 22.229; P= 0.003; IL-6: OR, 2.029; 95%CI, 1.136- 3.624; P=0.017). Levels of S100A1, NF-κB p65, and IL-6 may be independent risk factors for predicting prognosis. Besides, Neutrophils, HDL, Creatinine, and Homocysteine may also be independent predictors of the prognosis of AIS patients (Table 2).

Prognostic analysis of S100A1, NF-κB p65, and IL-6 level on patients with AIS

According to the ROC curve, the optimal cutoff value of the S100A1 level predicted 90-Days prognosis of patients with AIS was 227.15, the sensitivity (*Se*) was 89.5%, the specificity (*Sp*) was 67.7%, Youden Index was 0.572, and the area under the curve (AUC) was 0.864 (95%CI, 0.810- 0.918). Besides, the optimal cutoff value of NF-κB p65 level was 3.68, *Se* was 86.8%, *Sp* was 61.5%, Youden Index was 0.484, and AUC was 0.807 (95%CI, 0.748- 0.865). The optimal cutoff value of the IL-6 level was 12.330, *Se* was 86.8%, *Sp* was 58.5%, Youden Index was 0.453, and AUC was 0.767 (95%CI, 0.704- 0.831). The optimal cutoff value of the three combined indexes was 0.274, *Se* was 92.1%, *Sp* was 80.8%, Youden

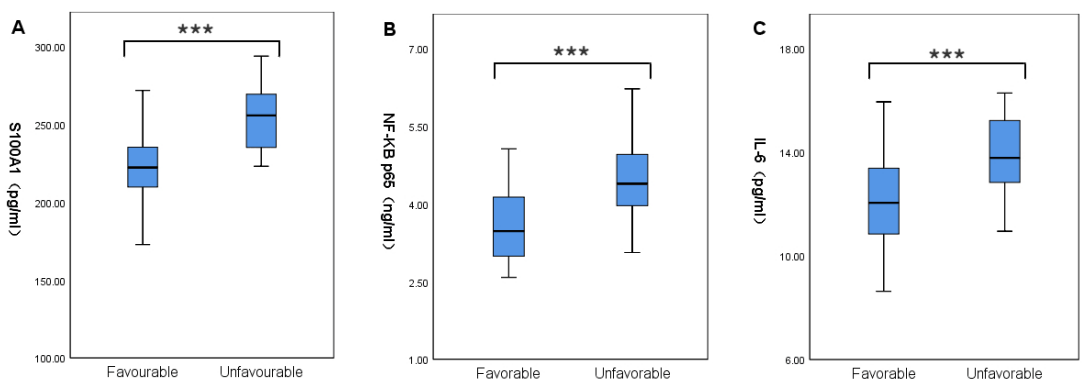

Figure 1. Comparison of S100A1(A), NF-κB p65(B), and IL-6 (C) among patients with different prognosis.

Table 2: Univariate and multivariate logistic regression analysis for the outcome

	Univariate Analysis			Multivariate Analysis		
	OR	**95%CI**	***P***	**OR**	**95%CI**	***P***
Age	1.043	1.013-1.074	0.005	1.057	0.985- 1.135	0.124
Admission NIHSS	1.109	1.050-1.172	<0.001	0.992	0.904- 1.089	0.872
Neutrophils	1.643	1.351-1.998	<0.001	1.800	1.243- 2.606	0.002
TG	2.832	1.843-4.351	<0.001	1.935	0.771- 4.860	0.160
HDL	0.010	0.002- 0.041	<0.001	0.001	0.000- 0.016	<0.001
UA	1.005	1.001-1.008	0.009	1.007	0.998- 1.015	0.121
Creatinine	1.017	1.002-1.031	0.024	0.930	0.890- 0.971	0.001
ALT	1.022	1.004-1.042	0.019	0.953	0.880- 1.031	0.227
AST	1.052	1.021-1.084	0.001	1.059	0.958- 1.171	0.263
Homocysteine	1.084	1.031- 1.140	0.002	1.118	1.002- 1.248	0.046
S100A1	1.071	1.050- 1.093	<0.001	1.093	1.052- 1.135	<0.001
NF-κB p65	5.884	3.464- 9.995	<0.001	6.416	1.852- 22.229	0.003
IL-6	1.863	1.515- 2.291	<0.001	2.029	1.136- 3.624	0.017

Abbreviations: NIHSS, National Institutes of Health Stroke Scale; TG, Triglycerides; HDL, High-density lipoproteins; UA, Uric Acid; ALT, Alanine transaminase; AST, Aspartate transaminase; NF-κB p65, Nuclear factor kappa-B p65; IL-6, Interleukin-6.

Index was 0.729, and AUC was 0.909 (95%CI, 0.865- 0.952). Therefore, the prognostic accuracy of the S100A1 level in AIS patients at three months was higher than that of NF-κB p65 and IL-6. At the same time, the combined predictive value of the three indexes was higher than that of a single index (Table 3, Figure 2).

Correlation analysis among the levels of plasma S100A1 protein, NF-κB p65, and IL-6

There was a significant statistical correlation between S100A1, NF-κB P65 and IL-6. There was a positive correlation between plasma S100A1 protein and NF-κB p65 with statistical significance (R =0.461, *P*<0.001) (Figure 3.A). In addition, there was also a positive correlation between NF-κB p65 and IL-6 with statistical significance (R =0.523, *P*<0.001) (Figure 3.B). Lastly, there was similarly a positive and statistically significant correlation between plasma S100A1 protein and IL-6 (R = 0.501, *P*<0.001) (Figure 3.C).

DISCUSSION

In this study, we first assessed the predictive value of plasma S100A1 protein, NF-κB p65, and IL-6 levels at admission for the prognosis of patients with AIS. The results revealed a positive association between higher levels of S100A1, NF-κB p65, and IL-6 and adverse outcomes at three months, suggesting their potential as independent

Table 3: The diagnostic efficacy of related indicators and their combinations for poor prognosis in patients with acute ischemic stroke

Prediction	**AUC**	***Jmax***	**Cut off**	***SE* (%)**	***Sp* (%)**	**95%CI**
S100A1	0.864	0.572	227.155	89.5	67.7	0.810- 0.918
NF-κB p65	0.807	0.484	3.685	86.8	61.5	0.748- 0.865
IL-6	0.767	0.453	12.330	86.8	58.5	0.704- 0.831
S100A1+ IL-6+ NF-κB p65	0.909	0.729	0.274	92.1	80.8	0.865- 0.952

Abbreviations: AUC, Area under the curve; *Jmax*, Maximum Youden Index; *Se*, Sensitivity; *Sp*, Specificity; CI, Confidence interval; NF-κB p65, Nuclear factor kappa-B p65; IL-6, Interleukin-6.

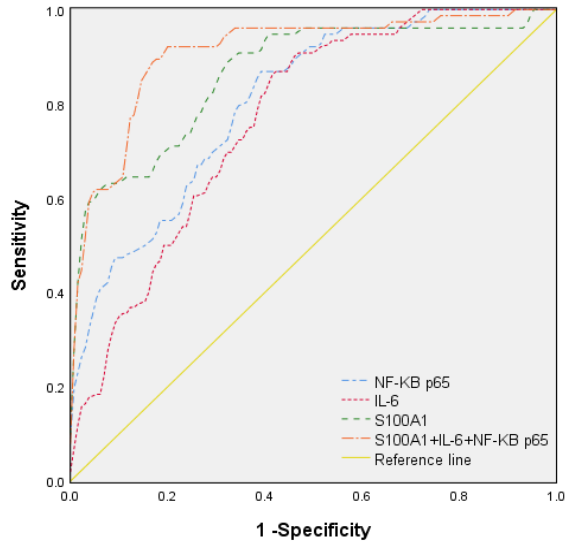

Figure 2. ROC curve analysis of the predictive value of S100A1, NF-κB p65, and IL-6 and their comprehensive indicators on the 3-month unfavorable prognosis of patients with acute ischemic stroke.

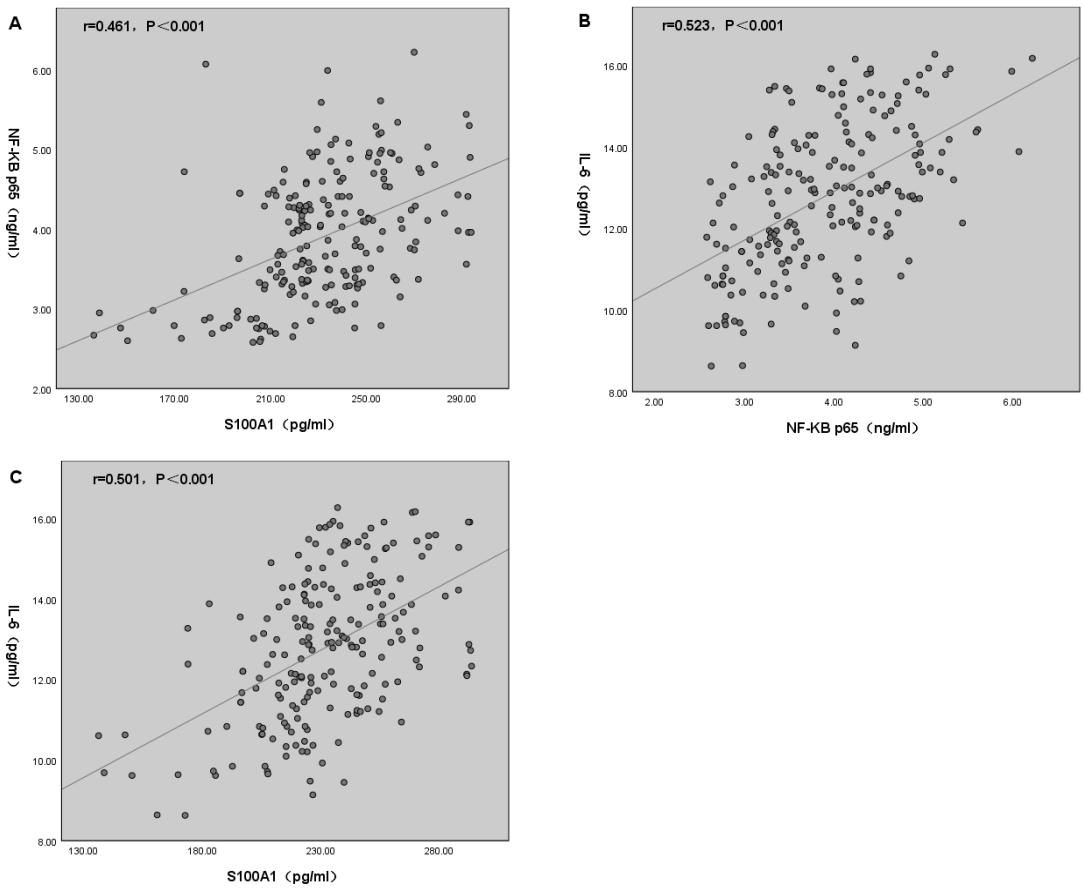

Figure 3. Correlation analysis among the levels of plasma S100A1 protein, NF-κB p65, and IL-6.

Abbreviations: NF-κB p65, Nuclear factor kappa-B p65; IL-6, Interleukin-6; r, Linear correlation coefficient. There was a statistically significant correlation between the two-factor levels(P<0.05).

risk factors for the 90-day prognosis of AIS patients. Additionally, the combined prediction value of the three indicators was likewise found to be stronger than that of the individual index in this study. Moreover, this study also discovered a correlation between plasma S100A1 protein levels, IL-6, and NF-B p65. It is reasonably speculated that the S100A1 protein may mediate inflammatory responses through the NF-κB pathway in the pathophysiological process of AIS.

In various pathophysiological stages of AIS, inflammation, atherosclerosis, and abnormal coagulation all play significant roles.[22-24] S100A1 protein is a new inflammatory marker and a subtype of protein member of the S100 protein family. [13]. This protein is a calcium-binding protein primarily found in vertebrates due to its ability to bind calcium ions.[25,26] The S100 protein family currently consists of 25 members with a high degree of sequence and structural similarity. In diverse tissues, they participate in intracellular and extracellular regulatory processes.[27] S100 proteins can induce neuronal death through apoptotic programs and activate or inhibit cellular inflammation in the nervous system by acting both intracellularly and extracellularly.[28] S100 proteins have emerged as promising diagnostic markers for the identification and monitoring of diverse diseases. In recent years, S100A1 protein has received much research attention for several systemic disorders, including acute myocardial infarction[29], heart failure[30,31], and malignancy.[32-34] Therefore, we ventured to speculate whether S100A1 protein also had an analogous monitoring role in AIS patients.

In this study, clinical data were first compared between patients with AIS with different prognosis, and statistically significant differences were found in plasma S100A1, NF-κB p65, and IL-6 ($P<0.001$). S100A1 is a calcium-binding protein that belongs to the S100 family and was extensively expressed in cardiac tissues. It exhibits superior diagnostic sensitivity compared to conventional cardiac biomarkers such as cardiac troponin T and creatine kinase-MB. According to a research by Li *et al*., higher plasma S100A1 concentrations were significantly associated with a worse outcome in patients with acute myocardial infarction (AMI) following the initial PCI.[35] This was also mainly consistent with the conclusion in this study that S100A1 levels were significantly elevated in the AIS patients with an unfavorable prognosis compared to those with a favorable prognosis. We analyzed the plasma levels of S100A1, NF-B p65, and IL-6 in patients with different AIS prognosis in Figure 1. We discovered that the overall levels of all three were higher in the unfavorable prognosis group than in the favorable prognosis group. The three levels represented, to some extent, the inflammatory level of the organism. In other words, higher values represented a more pronounced inflammatory reaction in the patient's body. The findings of this research unequivocally demonstrated that the values in the group with a poor prognosis were significantly greater than those in the group with a good prognosis. This suggests that the subset of AIS patients with a worse prognosis may experience a more intense inflammatory response.

After multifactorial logistic regression analysis, it was shown that S100A1 was an independent predictor of poor prognosis in AIS patients and that patients who possessed higher levels of S100A1 protein had a significantly increased risk of poor prognosis. According to Li *et al*., individuals with acute non-ST-segment acute coronary syndrome(NSTE-ACS) might have higher S100A1 levels, which might indicate a worse prognosis and a greater likelihood of 30-day adverse cardiac events.[36] This is consistent with the conclusions reached in this article. Similarly, the S100A1 protein also demonstrates precise prognostic capabilities for malignant tumors. It is abundantly expressed in many cancers, including papillary thyroid, breast, cervical, and hepatocellular carcinoma.[34,37-39] There are currently 25 known members of the S100 protein family, 19 of which are found in group A on chromosome 1q21.[40] These family members share striking structural and biochemical similarities.[28] S100A8 / A9 protein, also known as calmodulin, is expressed mainly in activated neutrophils and monocytes/macrophages and is a non-specific activating inflammatory marker of phagocytosis.[41] Previous studies have shown that higher calprotectin levels were associated with 3-month mortality, hemorrhagic transformation, and lower 3-month functional independence. These findings indicate that elevated calprotectin levels are indicative of a worse prognostic outcome.[42] In a large multicenter cohort study, high plasma S100A8/A9 concentrations at baseline were found to be independently associated with increased risks of adverse clinical outcomes three months after acute ischemic stroke.[43] This is the same conclusion as in this paper that S100A1 protein may be an independent risk factor for short-term adverse prognosis. Consistent with previous research, a recent study demonstrated

significantly elevated plasma calprotectin levels in patients with a poor prognosis of AIS compared to those with a favorable prognosis. Moreover, higher protein levels proved to be an effective predictor of unfavorable short-term prognosis.[44] The biological behavior of plasma S100B protein and S100A1 protein seems to be quite similar, and there is substantial structural and sequence commonality.[15] Evidence from previous studies on S100B protein also showed analogous findings to ours. Moreover, the S100B protein is progressively gaining recognition as a potential therapeutic target for both neurological and non-neurological disorders.[45] Previous studies have shown that measurement of serum S100B concentrations after acute stroke could effectively predict short-term functional neurological prognosis.[46,47] At the genetic level, the Chinese population's polymorphisms in the S100B gene may contribute to susceptibility to AIS by promoting serum S100B expression.[48] However, another study discovered that the neurological prognosis of AIS patients was not independently predicted by baseline serum S100B protein concentrations. This can be due to the study's tiny experimental sample size. This gap is filled by research that comprised ten case-control studies examining the relationship between AIS and S100B blood levels.[49] Although this study demonstrated that the higher the serum S100B level, the more serious the condition and the worse the prognosis of AIS patients. Nevertheless, subgroup analysis based on ethnicity demonstrated statistically insignificant serum S100B levels among Caucasians and Asians with AIS, indicating that racial and geographic variables might also potentially influence the prognosis of this protein. But despite this, this study still demonstrates that plasma S100A1 protein may be a valid independent, influential factor in predicting adverse prognosis in AIS patients. Furthermore, we have derived some interesting conclusions in Table 2 that neutrophils, HDL, creatinine, and homocysteine may also be independent predictors of prognosis in AIS patients. Neutrophils are the most abundant granulocyte type, accounting for more than half of all human leukocytes, and are by far the most recognized inflammatory factor; increased levels of this factor represent a more intense inflammatory response of the body. HDL, a well-accepted anti-atherogenic plasma lipoprotein, is a favorable biomarker. Our study found that elevated HDL levels might indicate a more favorable prognosis. Consistent with the findings of this study, previous prognostic studies on creatinine, homocysteine, and AIS also showed that the increase in their levels could effectively predict the poor prognosis of patients.[50-52]

Additionally, the diagnostic efficacy of plasma S100A1, NF-κB p65, and IL-6 for poor prognosis in patients with AIS was also analyzed in this study. The analysis in our study based on these three factors tended to be similar to previous studies but often showed differences in each value. These discrepancies may be derived from differences in patient populations, regions, and the disease itself. Importantly, all three inflammation-based biomarkers can predict poor prognosis in AIS patients. From the results in Table 3 and Figure 2, the area under the curve of plasma S100A1 protein (AUC=0.864) was much larger than that of NF-κB p65 and IL-6(NF-κB p65, AUC=0.807; IL-6, AUC=0.767). These results indicated that plasma S100A1 protein exhibited significantly superior diagnostic efficacy compared to the other two parameters. As anticipated, the aggregate predictive prognostic value of all three indicators (AUC=0.909) was higher than that of a single indicator. Therefore, it suggested that the combined predictors had a higher degree of accuracy when predicting the prognosis of AIS patients. In terms of the degree of correlation between the three, they did not present a high degree of correlation with each other. This also means that each biomarker contains independent information, which is why this information leads to higher predictive accuracy when combined.

Last but not least, the association between plasma S100A1, NF-κB p65, and IL-6 was also examined in this study. It turned out that there was some correlation between these three. All three were statistically significantly different from one another, with moderate correlations maintained at around 0.5 for the correlation coefficients. This may be due to the complex pathophysiological mechanisms of cerebral infarction and the small number of studied samples. As previously demonstrated, S100A1 may control the TLR4/NF-B pathway to regulate the inflammatory response of H9C2 cells. Necrosis of hypoxic cardiac tissue released endogenous S100A1 to activate TLR4, which in turn induced IL-6 production by binding to TLR4 and thus stimulating NF-κB p65.[19] This same perspective was shown in the research by Rohde[53]. Through a negative feedback mechanism in monocytes from patients with primary thrombocytopenia (PT), IL6 can reduce NF-κB -mediated production of S100A protein levels.[54] In other words, S100A can increase IL-6 expression via activating the NF-κB pathway. Along the same lines, the NF-κB

pathway is activated by the S100A protein in studies of multiple sclerosis (MS), which can increase the release of inflammatory cytokines from the microglia[55]. Recent research has also demonstrated that the S100A protein, a modulator of signaling pathways connected to inflammation-induced proliferation, can efficiently create inflammatory factors by activating NF-κB.[56] In conclusion, it is reasonable to speculate that plasma S100A1 protein may mediate the synthesis of the inflammatory cytokine IL-6 through the NF-κB pathway during the pathophysiology of AIS, which also explains well the correlation between these three values.

There were several limitations to this report. First, the sample size of our study was limited; it was a single-center study, with retrospective analysis, which requires expanded sample size and multi-center prospective study in the future. Second, due to the measurement of plasma S100A1 protein levels at a single time point, the detection of dynamic changes in these levels was precluded. Consequently, future work on this protein's dynamic monitoring and long-term follow-up should be enhanced. Third, the possible influence of non-specific inflammatory biomarkers such as C-reactive protein and interleukin-1 had not been excluded. Further controlled studies are required. Fourth, animal studies are still needed to confirm whether plasma S100A1 protein actually increases the production of the inflammatory cytokine IL-6 through the NF-B pathway in AIS patients. Finally, it was impossible to totally rule out the potential that further unidentified confounders could affect the outcomes.

In conclusion, our study indicated that plasma S100A1 protein, NF-κB p65, and IL-6 were all substantially related to a poor prognosis in AIS patients. Patients with unfavorable outcomes showed higher S100A1, NF-κB p65, and IL-6 than those with favorable outcomes. Furthermore, higher S100A1, NF-κB p65 and IL-6 can be independent predictive parameters for the poor prognosis of patients. Concurrently, the comprehensive prediction efficiency of these three biomarkers was more substantial. Increases in these three values may mean that AIS patients benefited less after three months, whereas those with lower levels benefited more. It is plausible to assume that this protein may mediate the inflammatory response through the NF-κB pathway during the pathophysiology of AIS, given the association between plasma S100A1 protein levels and IL-6 and NF-κB p65. Considering our findings and previous studies, plasma S100A1 protein shows promise as an inclusion criterion for future clinical diagnostic trials in AIS. Moreover, targeting a reduction in this protein level may offer a unique therapeutic approach to enhance prognosis.

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DISCLOSURE

Data availability: The original contributions presented in the study are included in the article/ Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics: The studies involving human participants were reviewed and approved by Jiangsu Subei People's Hospital affiliated to Yangzhou University. The patients/participants provided their written informed consent to participate in this study.

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