

and release proteolytic enzymes, which damage the blood-brain barrier and promote the accumulation of inflammatory factors in the ischemic area, aggravating brain tissue damage [5,6]. Albumin plays important role in maintaining colloid osmotic pressure balance and influencing microvascular integrity and inflammatory pathways, including neutrophil adhesion [7]. Some studies have shown that the neurological impairment in IS patients may be related to the decreased protection of albumin on ischemic brain tissue [8], and the serum albumin level has a predictive role in the prognosis of acute IS [9]. The neutrophil/albumin ratio (NAR) is a comprehensive inflammation biomarker, which has been used in many clinical studies. For example, in the association study between NAR and delayed cerebral ischemia (DCI) in the early stage of aneurysmal subarachnoid hemorrhage (aSAH), NAR was found to be positively correlated with the severity of subarachnoid hemorrhage, which can be used as a new predictive biomarker for DCI after aSAH [10]. NAR is a potential prognostic biomarker for mortality in patients with cardiogenic shock (CS), and its predictive value is more sensitive than neutrophil percentage or serum albumin level alone [11]. At present, there is no study on the correlation between NAR and the severity of acute IS and early neurological deterioration (END) occurrence after acute IS. Therefore, we performed a retrospective cohort study to analyze the expressive changes of five accessible and inexpensive indexes NAR, leukocyte count/albumin ratio (LAR), albumin, neutrophil count and leukocyte count in patients with acute IS. To determine the relationship between the five indexes and the severity of neurological function injury and END occurrence within acute IS, to better timely treatment of IS and reduce mortality and improve the prognosis.

Materials and Methods

Study Population

This was a retrospective, observational study. 299 consecutive patients with acute IS were enrolled from November 2016 to October 2021 at the Affiliated Hospital of Guilin Medical College. 56 age- and sex-matched individuals with no neurological or psychiatric diseases found in medical examinations were included as normal controls. This study was approved by the Ethics Review Committee of Affiliated Hospital of Guilin Medical College and consent was obtained from all participants prior to enrollment. The inclusion criteria were as follows: (1) hospital admission within 48 hours of first stroke onset; (2) symptoms consistent with the 2014 Chinese Acute Ischemic Stroke Diagnostic Criteria with the responsible lesions identified via DWI [12]; (3) the age of onset is more than 18 years old; (4) National Institutes of Health Stroke Scale (NIHSS) score [12] measured within 24 hours after admission; (5) detection of blood biochemical indicators within 24 hours of admission; (6) the score of

WORSEN was calculated according to Miyamoto et al [13], and (7) early neurological deterioration (END) was defined as an increase in two or more NIHSS points, an increment of at least one point in motor power, or description of fluctuating of clinical symptoms in medical reports during the first 7 days after admission [14]. The exclusion criteria were as follows: (1) hemorrhagic stroke, transient ischemic attack (TIA), multiple sclerosis (MS), intracranial infection or other diseases; (2) patients who accepted intravenous thrombolysis and/or mechanical thrombectomy; (3) previous history of IS, TIA, cerebral hemorrhage, serious infection, major surgery, or more severe trauma; (4) severe heart, liver, kidney, lung, digestive tract and other important organ damage, blood, immune diseases and tumors. These patients take many types of medications, which may have adverse factors affecting the inflammatory response; (5) history of major trauma, surgery, blood transfusion, blood donation or immunization in the past six months; (6) pregnant women, dementia, persons with neurological disabilities, and persons with severe psychological disorders; (7) Treatment with hormone drugs, immunosuppressive drugs, or nonsteroidal anti-inflammatory drugs before or after the onset. The drugs have an impact on the immune response and inflammatory response in the body; (8) pneumonia, bloodstream infection, urinary infection, infectious diarrhea, catheter-related infection, and/or sinusitis; (9) early discharge and/or incomplete clinical data.

Risk Factor Assessments

The related risk factors considered in the present investigation were summarized in Table 1, hypertension: an arterial systolic blood pressure ≥ 140 mmHg and/or arterial diastolic blood pressure ≥ 90 mmHg without the use of blood pressure-lowering drugs measured twice by continuous monitoring of arterial blood pressure [15]. A previous diagnosis of hypertension and continued use of the relevant drugs to control blood pressure were also used to define hypertension; diabetes: random venous blood glucose > 11.1 mmol/L, fasting blood glucose > 7.0 mmol/L, or OGTT 2-h blood glucose > 11.1 mmol/L and accompanying symptoms of diabetes [16]. A previous diagnosis of diabetes and the current use of blood glucose control drugs were also used to define diabetes; dyslipidemia: (1) total cholesterol (TC) ≥ 6.22 mmol/L, (2) triglyceride (TG) ≥ 2.26 mmol/L, or (3) low-density lipoprotein (LDL-C) ≥ 4.14 mmol/L [17]; smoking: (1) previous or current regular smoking habit and smoking > 10 cigarettes/day and (2) smoking duration > 1 year or smoking cessation < 10 years [18], and drinking: daily consumption of alcohol with an average alcohol intake > 50 g/day [19].

Laboratory Measurements

The clinical information on the normal controls and the patients is summarized in Table 2. According to the NIHSS score, the observation group was divided into a mild IS

group ($1 \leq \text{NIHSS score} \leq 4$), moderate IS group ($5 \leq \text{NIHSS score} \leq 15$), severe IS group ($16 \leq \text{NIHSS score}$). Fasting blood samples were collected within 24 hours after admission and were measured at the Affiliated Hospital of Guilin Medical College by using an automated analytical platform (Beckman Coulter AU5800: Beckman Coulter Inc. Brea, CA, USA). HDL-C, LDL-C, TC, TG and albumin were measured using blood samples drawn at approximately 7 a.m. after an overnight fast. White blood cell count, neutrophil count and lymphocyte count in EDTA-anticoagulated whole-blood samples from venipuncture were determined with automated particle counters within the first 24h after admission. NAR and LAR were calculated as the ratio of neutrophil count to albumin and leukocyte count to albumin.

Statistical Analysis

Statistical analyses were performed using standard statistical software (SPSS 22.0, IBM Corp., Armonk, NY, USA). The measurement data were represented by ($x \pm s$). Logistic regression analysis was performed to evaluate the related risk factors on IS, adjusting for baseline variables when a $p < 0.1$ was found in the univariate analysis. One-way ANOVA and Kruskal-Wallis tests were performed to analyze the differences among four groups followed by the Kolmogorov-Smirnov test. Pearson's correlation analysis was performed to analyze the Correlation analysis between the levels of the neutrophil count, albumin, NAR, leukocyte count and LAR and the NIHSS score. We calculated the sensitivity and specificity of different levels of NIHSS

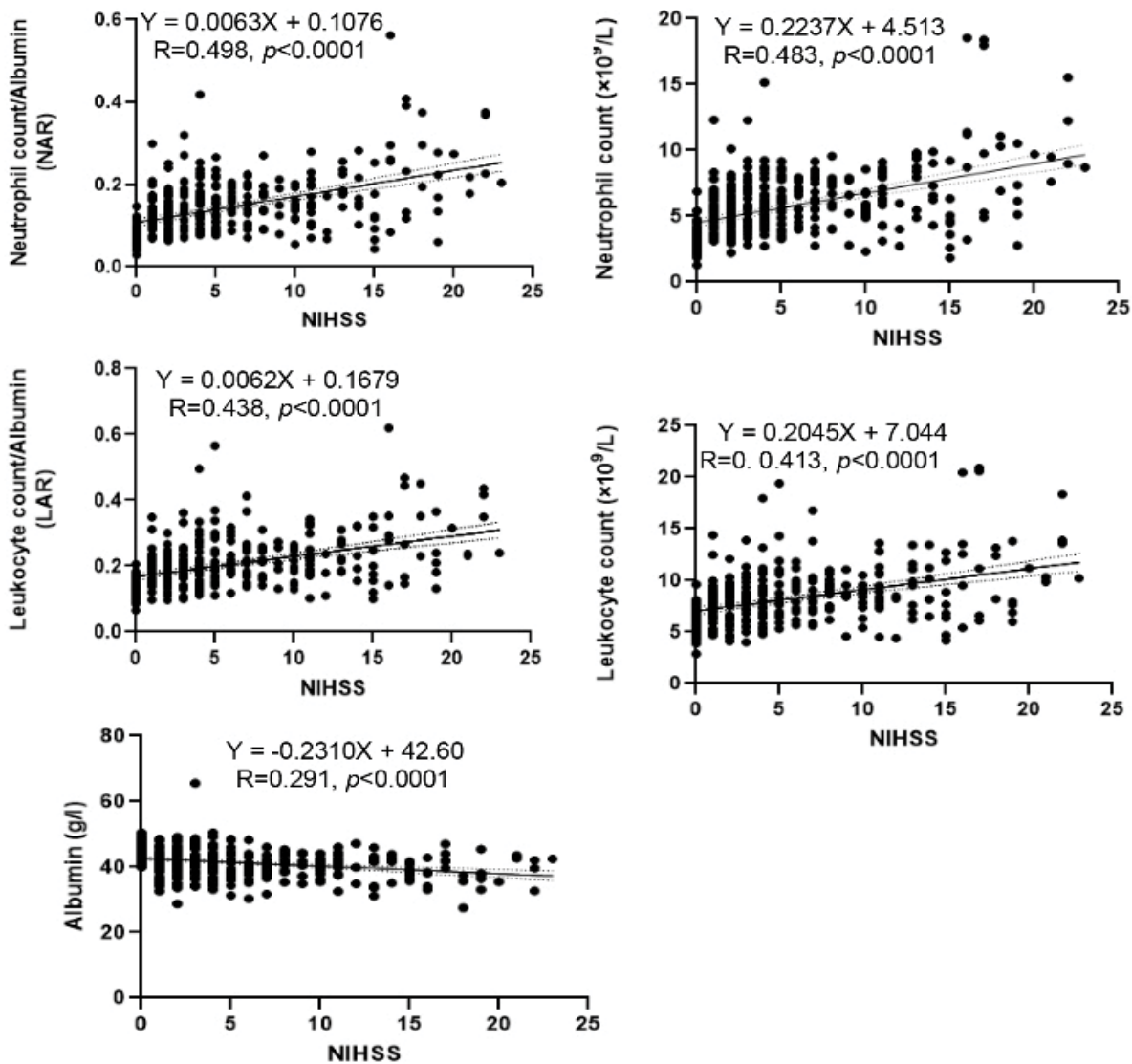


Figure 1: Correlation analysis between the levels of neutrophil count, albumin, NAR, leukocyte count and LAR and the NIHSS score.

and WORSEN scores, NAR and LAR for the prediction of END by using receiver operating characteristic (ROC) curves. Differences with a probability value of $p < 0.05$ were considered statistically significant.

Results

Logistic Analysis of the related Risk Factors of Acute IS

299 acute IS patients and 56 normal controls were included in this study. The results of the logistic analysis showed that gender ($p < 0.05$, OR=0.334), smoking ($p < 0.01$, OR=0.161), drinking ($p < 0.01$, OR=11.653), diabetes mellitus (DM, $p < 0.01$, OR=0.099), hypertension ($p < 0.01$, OR=0.051), and coronary heart disease (CHD, $p < 0.05$, OR=10.360) were all related to the onset of IS (Table 1).

Laboratory Indicators Analysis

IS patients were evaluated according to NIHSS score, there

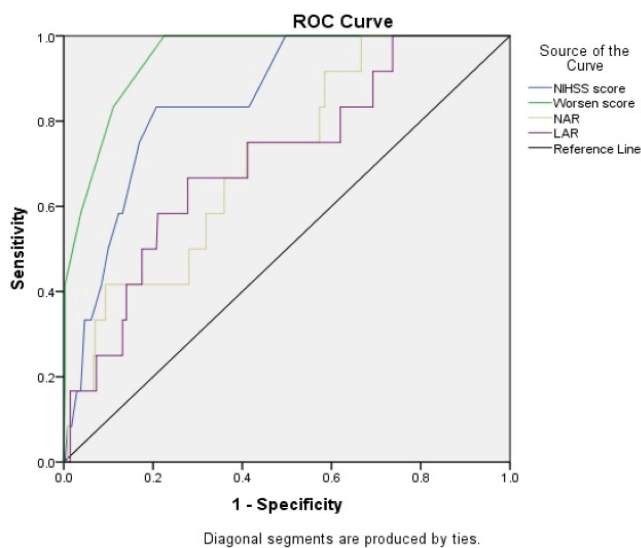


Figure 2: The predictive value of WORSEN score, NIHSS score, NAR and LAR for END using ROC.

were 100 cases in the mild IS group (1 point \leq NIHSS score \leq 4 points), 81 cases in the moderate IS group (5 points \leq NIHSS score \leq 15 points), and 23 cases in the severe IS group (16 points \leq NIHSS score). The results of laboratory indicators were presented in Table 2. Compared with the normal controls, the levels of the neutrophil count, neutrophil ratio, leukocyte count and lymphocyte count were significantly higher, meanwhile, albumin levels showed significantly lower in the three IS groups, but no significant differences were found among them. The levels of NAR, LAR, NIHSS score and WORSEN score were remarkably higher than in the normal controls, meanwhile, with a significant difference among the three IS groups. About the results of serum lipid examination, only the level of HDL-C was remarkably lower in the three IS groups than in the normal controls.

Correlation Analysis between the Levels of the NAR, LAR, Albumin, Neutrophil Count and Leukocyte Count and the NIHSS Score

The correlation analysis showed that the five indexes were significantly correlated with the NIHSS score (Figure 1), the levels of neutrophil count ($R=0.483$, $p < 0.0001$), NAR ($R=0.498$, $p < 0.0001$), leukocyte count ($R=0.413$, $p < 0.0001$) and LAR ($R=0.438$, $p < 0.0001$) were positively correlated with the NIHSS score. On the other hand, the level of albumin was negatively correlated with the NIHSS score ($R=0.291$, $p < 0.0001$).

Predictive Value of WORSEN score, NIHSS score, NAR and LAR for Early Neurological Deterioration (END) using ROC

In Figure 2, the area under the ROC curve (AUC) of the WORSEN score for the prediction of END was 0.95 (95%CI 0.91-0.98). The AUC of the NIHSS score for the prediction of END was 0.85 (95%CI 0.76-0.94). Meanwhile, The AUC of the NAR and LAR for the prediction of END was 0.71 (95%CI 0.58-0.85) and 0.71 (95%CI 0.56-0.85), respectively.

Table 1: Logistic analysis of the related risk factors of acute IS. * $p < 0.05$, ** $p < 0.01$. AF: Atrial fibrillation, CHD: Coronary heart disease, DM: diabetes mellitus, IS: ischemic stroke.

Patient characteristics	Normal	IS	OR	95% C.I.		p
	(n=56)	(n=299)		Lower	Upper	
Males [n(%)]	29 (51.78)	224 (74.92)	0.334	0.123	0.909	0.032*
Smoking [n(%)]	19 (33.93)	138 (46.15)	0.161	0.045	0.576	0.005**
Drinking [n(%)]	22 (39.29)	74 (24.75)	11.653	3.273	41.482	0.000**
DM [n(%)]	2 (3.57)	68 (22.74)	0.099	0.02	0.479	0.004**
Hypertension [n(%)]	9 (16.07)	227 (75.92)	0.051	0.021	0.125	0.000**
AF [n(%)]	0	31 (10.37)	0	0	0	0.997
CHD [n(%)]	3 (5.35)	7 (2.34)	10.36	1.668	64.334	0.012*

AF- Atrial fibrillation; CHD- Coronary heart disease; DM- diabetes mellitus; IS- ischemic stroke, * $p < 0.05$, ** $p < 0.01$.

Table 2: Comparison of laboratory indicators among each group. ^a*p*<0.05 vs. normal, ^b*p*<0.05 vs. mild IS, ^c*p*<0.05 vs. moderate IS. HDL-C: High-density lipoprotein cholesterol, IS: ischemic stroke, LDL-C: low-density lipid-cholesterol.

Clinical data	Normal (n=56)	Mild IS (n=147)	Moderate IS (n=127)	Severe IS (n=25)	P
Age at examination (years)	66.09±7.95	65.35±11.27	67.65±11.22	73.56±8.74 ^{ab}	0.007
Neutrophil count (×10 ⁹ /L)	3.34±1.02	5.59±1.71 ^a	6.18±1.92 ^a	9.67±4.37 ^{abc}	0
Albumin (g/l)	45.11±2.46	41.38±4.88 ^a	40.15±3.68 ^a	38.81±4.67 ^{ab}	0
Neutrophil count/Albumin (NAR)	0.074±0.022	0.138±0.047 ^a	0.156±0.052 ^{ab}	0.253±0.116 ^{abc}	0
Neutrophil ratio	0.55±0.08	0.72±0.09 ^a	0.73±0.11 ^a	0.82±0.11 ^{abc}	0
Leukocyte count (×10 ⁹ /L)	6.07±1.29	7.93±2.01 ^a	8.67±2.49 ^a	11.61±4.62 ^{abc}	0
Leukocyte count/Albumin (LAR)	0.135±0.029	0.195±0.060 ^a	0.219±0.071 ^{ab}	0.303±0.122 ^{abc}	0
Lymphocyte count (×10 ⁹ /L)	2.10±0.62	1.45±0.56 ^a	1.60±0.75 ^a	1.44±1.19 ^a	0
Triglycerides (mmol/L)	1.49±0.97	1.60±1.02	1.45±0.97	1.30±0.60	0.419
Cholesterol (mmol/L)	4.73±0.83	4.43±1.08	4.56±1.00	4.10±0.93	0.04
HDL-C (mmol/L)	1.40±0.38	1.19±0.33 ^a	1.18±0.31 ^a	1.15±0.32 ^a	0
LDL-C (mmol/L)	2.91±0.72	2.99±0.98	3.13±0.94	2.75±0.89	0.188
NIHSS score	0	2.40±1.09 ^a	8.97±3.21 ^{ab}	18.60±2.20 ^{abc}	0
WORSEN score	0	1.11±0.87 ^a	2.31±1.54 ^{ab}	4.24±1.36 ^{abc}	0

HDL-C- High-Density Lipoprotein Cholesterol; IS- Ischemic Stroke; LDL-C- Low-Density Lipoprotein Cholesterol; ^a*p*<0.05 vs. normal, ^b*p*<0.05 vs. mild IS, ^c*p*<0.05 vs. moderate IS.

Discussion

Many clinical studies have focused on how to find effective, easily available and inexpensive blood-based biomarkers for the early prediction of IS severity. In this study, we examined differences in the expression levels of several plasma biomarkers in the mild, moderate and severe IS groups, and found the expressive differences are indicative of the diverse pathological mechanisms underlying IS. Our results showed that gender, smoking, drinking, DM, hypertension, and CHD were related to the onset of IS (Table 1). The results of laboratory indicators showed that the levels of the NAR, LAR, neutrophil count, leukocyte count, NIHSS score and WORSEN score were remarkably higher, and albumin remarkably lower in the three IS groups. Meanwhile, NAR, LAR, NIHSS score and WORSEN score showed significant differences among the three IS groups (Table 2). Moreover, the correlation analysis showed that the five indexes (NAR, LAR, albumin, neutrophil count and leukocyte count) were significantly correlated with the NIHSS score (Figure 1). WORSEN score, NIHSS score, NAR and LAR showed a good predictive value in identifying patients with END after acute IS (Figure 2). After IS, the levels of serum leukocytes and neutrophils increased (Table 2), because within 1-6 hours of the onset of acute IS, a large number of leukocytes, main neutrophils, adhere to the post-capillary venules and capillary walls of ischemic tissue. At the same time, oxidative damage and proteolysis of vascular endothelial cells promote the aggregation of leukocytes and red blood cells around ischemic foci, and aggravate the microcirculation disorder and blood hypercoagulability, and further reduce cerebral blood flow [20,21]. Then, over

the next 6 to 24 hours, these neutrophils migrate from the damaged vessel wall to the ischemic cortical area. Infiltrating leukocytes and resident brain cells, including neurons and glial cells, release proinflammatory mediators such as cytokines, chemokines, and oxygen-nitrogen free radicals, exacerbating the evolution of brain tissue damage and leading to increased mortality [22-24]. Some studies have found that leukocyte count can be used as independent predictors of IS [25]. Our results further suggested that the level increases of NAR and LAR were associated with different severity of IS (Table 2, Figure 1). Serum albumin offers neuroprotective effects through antagonizing thrombosis, stagnation and leukocyte adhesion within the postcapillary microcirculation in the early reperfusion phase of stroke [26]. Some studies reported that the level of serum albumin is closely related to the occurrence and development of IS [27, 28]. Our result also showed that the albumin levels were significantly lower in the three IS groups, especially in the severe IS group (Table 2, Figure 1). Which suggested that the serum albumin level changes may be used to assess the severity of acute IS in elderly patients. Moreover, albumin treatment has been found to improve neurological function in rats with focal IS and clinical IS patients [29,30]. In acute IS patients, END not only has a high incidence of 5-40% [31], but is also associated with poor prognosis [32]. Our results showed that the initial NIHSS score and WORSEN score had good predictive values for END (Figure 2), which were consistent with previous studies [14]. We also found that LAR and NAR had good predictive values for END (Figure 2). Thus, inflammation may be one of the risks of END in patients with acute IS.

Limitations

This study was subject to several limitations. First, this study had a retrospective case-control design and was performed within a small area of China. Second, relatively few patients and controls were included, especially in the severe IS groups, so the validity of our results remains to be tested. Finally, no equal numbers of males and females were achieved in the study.

Conclusions

In summary, the present study is the first to analyze the changes of NAR, LAR, albumin, neutrophil count and leukocyte count levels in patients with acute IS, and found that the NAR and LAR levels were correlated with the severity of IS. Attention should be paid to the condition changes of IS hospitalized patients with high NAR, LAR, neutrophil count and leukocyte count levels but low albumin level, hoping that the prognosis can be improved by early active and effective treatment. Effective blood markers and NIHSS score and WORSEN score for IS may occur during the END provides the clinical basis, which helps to improve the treatment of acute IS clinical decision. This study further explored the early assessment of the severity of IS and END occurrence, and provided a clinical reference for large-scale marker screening and combination.

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Conflicts of Interest

The authors have no financial conflicts of interest.

Availability of Data and Material

Researchers can request for access to anonymized data from the present study for well-defined research questions that are consistent with the overall research agenda for the cohort. Please contact the corresponding author.

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