Interleukin-6 and Neutrophil-to-Lymphocyte Ratio indicated the Severity and 3-Month Functional Outcome in Patients with First-ever Anterior Circulation Acute Ischemic Stroke

Running head: IL-6 and NLR correlate with 3-month outcome of AIS

Yayin Luo, MD, [a] Guangxiang Yu, MD, [a] Qiuyi Zhang, MM, [a] Geng Chang, MD, [a]

Zhe Wang, MD, [a,#] Jie Han, MD, [a,#]

[a] Department of Neurology, The First Affiliated Hospital of Dalian Medical University, Dalian, China;

Corresponding Author's information:

Jie Han, MD, Department of Neurology, The First Affiliated Hospital of Dalian Medical University;

Address: No.222, Zhongshan Road, Xigang District, Dalian 116011, China.

Tel: +8618098876278; E-mail: hjj.427@163.com

[#] Zhe Wang and Jie Han are co-corresponding authors.

Received：December25，2023 ；Accepted：December31,2023

Abstract

Objectives

To investigate the correlation between interleukin-6 (IL-6) level in serum and the neutrophil-to-lymphocyte ratio (NLR) with the severity and 3-month functional outcome of patients after first-ever anterior circulation acute ischemic stroke (AIS).

Methods

Serum IL-6 and NLR were measured on admission from patients who suffered from the first-ever anterior circulation AIS. We classified our patients into minor stroke (NIHSS ≤ 5) and non-minor stroke groups (NIHSS > 5). The baseline clinical characteristics and laboratory data of each group were compared. The 3-month functional outcome after stroke was defined as either good outcome with modified Rankin Scale (mRS) value 0-2 or poor outcome with mRS value 3-6. To further unravel the

risk factors, logistic regression analysis was applied to our laboratory data from the patients with poor outcome.

Results

Data from 152 patients indicated that IL-6 and NLR in non-minor stroke patients were largely increased than those in minor stroke ones ($p<0.001$). Moreover, compared to those with 3-month good outcome, patients with poor outcome had higher serum IL-6 levels and NLR at admission ($p<0.001$). Univariate logistic regression analysis defined both elevated IL-6 and NLR as risk factors for AIS patients with 3-month poor outcome. Multivariate logistic regression correlated a 1 pg/mL increase in IL-6 level with a 62% odds ration increase of a 3-month poor outcome (OR 1.616, 95%CI 1.306-1.999, $p<0.01$), suggesting elevated IL-6 level as an independent parameter to predict poor outcome after adjusting for confounding factors. The best predictive IL-6 cut-off value was 6.345pg/mL (sensitivity: 96.9%, specificity: 95.4%, AUC: 0.973). In spearman correlation analysis, IL-6 was positively correlated with NLR ($r=0.585$, $p<0.001$). Furthermore, IL-6 and NLR showed a positive correlation with infarct volume ($r=0.962$, $p<0.001$ and $r=0.596$, $p<0.001$, respectively).

Conclusion

IL-6 and NLR levels were associated with the severity and infarct volume of stroke, and high IL-6 level is an independent indicator to predict 3-month poor functional outcome among patients suffering from first-ever anterior circulation AIS.

Keywords

Anterior circulation acute ischemic stroke; Interleukin-6; Neutrophil-to-lymphocyte ratio; Short-term outcome

Introduction

Ischemic stroke is a major global health burden with significant morbidity and mortality [1]. In accordance with the Global Burden of Diseases in 2017, it also becomes the leading factor in China which causes death and disability-adjusted life years[2]. More suddenly and rapidly, acute ischemic stroke (AIS) leads to brain injury, followed by inflammatory response. Inflammation is an important factor affecting the progression and prognosis of AIS, leading to abnormal secretion of a large number of inflammatory mediators and promote the progress of neurological deficit in the early stage of stroke. Biomarkers are essential for identifying and reducing the incidence of poor outcome in high-risk populations. However, simple biomarkers for rapid identification of high-risk populations with poor outcome are still rare. The activated peripheral

immune cells, such as neutrophils and lymphocytes, can pass the damaged blood-brain barrier and accumulate specifically where the brain injury is [3]. In addition, it is confirmed that interleukins are important factors involved in the inflammatory response to ischemic stroke [4]. So, peripheral immune cells and interleukins may become potential biomarkers.

Interleukin-6 (IL-6), a proinflammatory cytokine, is widely known as a biomarker of systemic inflammation. The occurrence and prognosis of many diseases, such as coronary heart disease, hypertension, and brain injury, is closely related to high levels of IL-6 [5-7]. Previous evidence suggests the association of an increased serum IL-6 levels with a higher risk of stroke [8]. Furthermore, the neuroinflammation, affecting damage during and recovery process after ischemic stroke, is also related to IL-6 level [9]. Some studies have investigated whether IL-6 level is associated with outcome of ischemic stroke [10-13]. Compared to the healthy controls, data from 11 AIS patients showed indeed an increase level of IL-6 at the acute phase of stroke, which was further linked to the low efficiency of prognosis after 1 year [11]. On the contrary, another study found that IL-6 levels showed a significant negative correlation with both final neurological impairment and infarct size[12]. Thus, the previous results were controversial and the sample size was small.

Neutrophil-to-lymphocyte ratio (NLR) is another marker which is easily-measurable to determine systemic inflammation. Compared to the neutrophil or lymphocyte counts, NLR may reflect the immune and inflammatory state of the body accurately. It has been shown in many studies to be associated with cardiovascular diseases [14], autoimmune disease activity [15], and survival of cancer patients [16]. Meanwhile, some studies have begun to focus on the correlation between NLR and ischemic stroke [17,18]. NLR was associated with intracranial artery stenosis and was a risk factor to predict ischemic stroke[17]. Luo *et al*. demonstrated that early NLR played an important role in distinguishing mild ischemic stroke with possible disability at the early-stage of stroke [18].

Up to date, the clinical studies on the association of IL-6 and NLR with ischemic stroke are few and not systematic. Especially the combination of IL-6 and NLR to predict the short-term outcome of anterior circulation AIS is not well explored. Therefore, by analyzing the data from patients with first-ever anterior circulation AIS, we studied the influence of serum IL-6 levels and NLR on their severity and short-term functional outcome to provide evidence for guiding and optimizing clinical practice in the future.

Materials and Methods

Enrolling Patients

Consecutive patients were enrolled with first-ever anterior circulation AIS within 48 hours of symptoms onset in our hospital from June 2021 to July 2022. The following inclusion were applied to select the patients: (1) ≥18 years old; (2) within 48 hours of onset; (3) anterior circulation AIS confirmed by Magnetic Resonance Imaging (MRI). We excluded some patients based on the following criteria: (1) any disease history in central nervous system; (2) complicated with hematologic disease, malignant tumors, or autoimmune disease; (3) important organ failure, like heart failure, severe liver insufficiency, and renal insufficiency; (4) serious infection history, trauma or surgery time ≤ 4 weeks; (5) incomplete clinical data; (6) loss to follow-up due to diverse reasons.

All study here and data collection was approved by the Ethics Committee in our hospital with written informed consent from patients in this study.

Collecting baseline data

Demographic information and medical history of participants within 24 hours of admission were documented through face-to-face interviews. Within 24 hours of admission, different biochemical parameters in serum were quantified after overnight fasting, including total cholesterol (TC), triglyceride (TG), fasting glucose, blood urea nitrogen (BUN), serum creatinine (Scr), uric acid (UA), homocysteine (HCY), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). On admission, our experienced neurologists assessed the stroke severity in order to group the patients using the National Institute of Health Stroke Scale (NIHSS) score. Consequently, participants were categorized into minor (NIHSS≤5) and non-minor (NIHSS>5) subgroups [19].

Serum IL-6 and NLR collection

Fasting peripheral venous blood for IL-6 and NLR analysis was collected in the morning of the next day after admission, and immediately sent to the hospital clinical laboratory for testing. The serum IL-6 levels were measured using IMMULITE IL-6 kit (SIEMENS Healthineers, Germany). Blood cell counts were tested by flow cytometry. NLR was calculated as neutrophils / lymphocytes. The laboratory measurements were performed blinded to clinical findings.

Neuroimaging

All participants completed cranial MRI scans within 72 hours of onset. The cerebral infarct volumes were manually measured on diffusion-weighted MR imaging (DWI), which was further analyzed using

PACS software according to the ABC/2 formula (ellipsoid) [20]by two radiologists who were blinded to the clinical outcomes. The slice was selected with the largest lesion. Using a ruler tool on this slice, we firstly measured the longest lesion axis (A). Then we drew a second line (B) perpendicular to (A) at the widest dimension. Last, we computed a third axis (C) by multiplying slice thickness (6mm) by the number of analyzed slices.

Functional outcomes

Two neurologists blinded to baseline clinical characteristics assessed the 3-month functional outcomes through structured telephone interviews or outpatient service according to the modified Rankin Scale (mRS) score, which was further classified into good outcome group with mRS value 0-2 or poor outcome group with mRS value 3-6 [21].

Statistical analysis

All statistical analysis was conducted by SPSS version 26.0. We considered the *p* value less than 0.05 as statistically significant. Continuous variables with a normal distribution tested by Kolmogorov-Smirnov tests were represented as mean value with standard deviation (SD), while non-normal distribution was represented as median value with interquartile range [IQR] indicated. Two-group difference was tested by the Mann-Whitney *U*-test or the independent-samples *t*-test. Categorical variables were represented as frequency and percentage, which were analyzed using Fisher's exact test or chi-square test. Spearman correlation analysis was used to test the bivariate correlation. The association between IL-6, NLR, clinical parameters and poor functional outcome was analyzed by univariate and multivariate logistic regression analysis. Multivariable models were built using forward selection of covariates significant to a p-value <0.2 in univariable testing. The area under the receiver-operating characteristic (ROC) curve (AUC) was used to determine the best cut-off value.

Results

Characterizing the baseline of participants

First of all, we recorded and analyzed the baseline demographic and clinical characteristics (Table 1). The mean age of the total 152 enrolled patients was 68.27 ± 9.16 years. Among the 152 patients, 96 (63.2%) were males. 72 patients were classified into the minor stroke group. The non-minor stroke group included the other 80 patients who were significantly older and had larger infarct volumes compared to the 72 patients with minor stroke. No statistical difference in gender was identified. In addition, no significant difference of the previous medical history between the two groups was observed, including coronary heart disease, hypertension, diabetes, atrial fibrillation, and smoking and drinking.

3-month functional outcome

Two groups were formed from the participants in accordance with the mRS at 3 months. 65/152 (42.8%) patients got poor functional outcome. The group with poor outcome was characterized with higher age (72.83 vs. 64.86, p<0.001), leukocytes (9.79 vs. 6.86, p<0.001), neutrophils (7.60 vs. 4.41, p<0.001), HCY levels (13.20 vs. 11.67, p=0.024), infarct volume (198.90 vs. 10.20, p<0.001), but lower lymphocytes levels (1.38 vs. 1.65, p=0.002) compared to the group with good outcome (Table 2).

IL-6 and NLR levels in different groups

Among the total study population, the median admission serum IL-6 level was 5.44 pg/mL (IQR 2.88–16.85) and NLR was 3.43 (IQR 2.37–5.70). The results revealed that the patients with non-minor stroke had significantly higher IL-6 level and NLR at admission than those with minor stroke (Table 1 and Figure 1). Meanwhile, IL-6 and NLR of patients were significantly higher when comparing the poor outcome group to the good outcome group (Table 2 and Figure 2).

Logistic regression analysis in the poor outcome group

The association between the IL-6 level, NLR and the 3-month poor outcome in anterior circulation AIS patients was tested using univariate and multivariate logistic regression analysis. Both elevated IL-6 and NLR were risk factors for 3-month poor outcome in univariate logistic regression analysis. In multivariate logistic regression, high IL-6 remained to be an independent indicator to predict the poor outcome after adjusting for age, leukocytes, neutrophils, lymphocytes, NLR and HCY. The result of multivariate logistic regression suggested a correlation between a 1 pg/mL increase in IL-6 level and a 62% odds ration increase of a 3-month poor outcome (OR 1.616, 95%CI 1.306-1.999, p<0.001) (Table 3).

Predictive value of IL-6 for 3-month poor outcome

Next, we assessed the viability of IL-6 levels in predicting poor outcome using ROC curve analysis. To determine the possible predictive value, we measured the area under the ROC curve (AUC). The AUC value (0.973) indicated that IL-6 had good predictive value. The cut-off value of IL-6 was 6.345 pg/mL with a 96.9% sensitivity and a 95.4% specificity (Figure 3).

Correlation between IL-6 and NLR and infarct volume

In spearman correlation analysis, IL-6 was positively and significantly correlated with NLR (r=0.585, p<0.001). More importantly, IL-6 and NLR showed a positive correlation with infarct volume (r=0.962, p<0.001 and r=0.596, p<0.001, respectively).

Discussion

In this study, we revealed that the serum IL-6 levels and NLR at admission were associated with severity and 3-month functional outcome in patients with first-ever anterior circulation AIS. Our findings indicated that IL-6 levels and NLR were increased in accord with stroke severity, poor outcome after 3 months, and cerebral infarct volume.

It is well established that ischemic stroke is a multifactorial and dynamic process, and the pathophysiological mechanism after cerebral infarction is complicated. Immune inflammation has been recognized as an important contributor to the pathophysiological mechanism of stroke etiology and progression [22]. Neutrophils are a key member of the innate immune system, which can promote inflammatory response after AIS and aggravate brain injury, while lymphocytes are considered as the immunomodulator of brain protection and contribute to a great extent to the recovery of inflammation. We found a significant difference of NLR by comparing the minor stroke group to the non-minor stroke group. Moreover, high NLR was detected in the poor outcome group. It is consistent with previous studies [18,23].

Neutrophils and monocytes were activated and recruited by IL-6, which plays an important role in the secretion of adhesion molecules and other inflammatory transmitters by vascular endothelial cells [4]. Jeon *et al.* used dogs as experimental models and found that the expression of IL-6 in ischemic lesions was increased [24]. Clinical studies demonstrated that IL-6 level in the AIS patients was increased in the peripheral blood at the acute phase [25,26]. Further studies positively correlated the serum IL-6 level with the severity of ischemic stroke and cerebral infarct volume [10,27]. Smith *et al*. found that the poor prognosis of the stroke patients was likely due to the peak plasma IL-6 level [10]. Shenhar-Tsarfaty *et al.* reported that a potential use of high-sensitivity IL-6 to predict the one-year survival of AIS patients at the early stage and defined 6.47pg/ml as high risk for patients [28]. Another study indicated that serum IL-6 levels on admission was a potential marker of early neurological deterioration of endovascular therapy [29]. Thus, IL-6 both in central nervous system and systemic circulation increase in AIS, and it may be related to the development and prognosis of stroke. Our results suggested a positive correlation between serum IL-6 levels and several parameters, i.e. stroke severity, infarct volume and 3-month poor outcome.

From the results above, we hypothesized that IL-6 and NLR may predict the short-term (e.g. 3-month) outcome among AIS patients. Univariate and multivariate logistic regression analysis indicated that IL-6 level could be used to predict the 3-month

poor outcome, in agreement with the study of Li *et al*. [30]. However, previous studies reported that NLR was a prognostic marker in AIS[18,23,31], this may be due to different confounding factors included. Large and multi-center studies are needed for further research.

In our study, a positive correlation also appeared between IL-6, NLR and cerebral infarct volume. The size of the irreversibly damaged region is positively associated with the severity of the clinical deficits. A recent study revealed that, when patients had moderate-to-large infarcts, a linear association could be observed between the infarct volume and the poor outcome, which was not applied to patients with small infarcts [32]. Larger infarct volume may cause a stronger systemic inflammatory response in both the central nervous system and the periphery, which possibly caused elevated IL-6 levels and NLR shortly after stroke. Current results suggest that elevated IL-6 and NLR levels, reflecting a larger infarct volume, may serve as helpful serum markers to evaluate the severity of stroke. It requires further research to understand whether the relationship between infarct volume and IL-6 levels is causal.

Taken together, inflammation should be a potential therapeutic target in the whole process management of AIS. The results may provide research basis for the intervention treatment of IL-6 from the perspective of inhibiting inflammatory response. Our work was conducted in a single-center. Furthermore, the sample size was relatively limited. Especially considering that the serum IL-6 levels and NLR were only tested once on admission, the effects of dynamic changes of IL-6 and NLR on outcome remain unclear. Therefore, to further study the long-term effect of IL-6 level and NLR on the prognosis of AIS and even understand the underlying mechanism, it requires more and better-designed longitudinal studies in the future.

Conclusion

In conclusion, IL-6 levels and NLR were related to the severity and infarct volume of the anterior circulation AIS, and high IL-6 level can be used to independently predict the 3-month poor functional outcome in patients with the first-ever anterior circulation AIS.

Ethical approval and consent

The study was approved by the Ethics Committee in the First Affiliated Hospital of Dalian Medical University with written informed consent from patients in this study.

Acknowledgments

We thank ikann-editorial team for their excellent assistance with writing and all the participants in the

study.

References

1. Disease, G.*, et al.* Global, regional, and national incidence, prevalence, andyears lived with disability for 354 diseases and injuries for195 countries and territories, 1990–2017: a systematicanalysis for the Global Burden of Disease Study 2017. *Lancet Publishing Group* (2018).
2. Zhou, M.*, et al.* Mortality, morbidity, and risk factors in China and its provinces, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 394, 1145-1158 (2019).
3. Shi, K.*, et al.* Global brain inflammation in stroke. *The Lancet Neurology* 18, 1058-1066 (2019).
4. Zhu, H.*, et al*. Interleukins and Ischemic Stroke. *Front Immunol* 13, 828447 (2022).
5. Erta, M., Quintana, A. & Hidalgo, J. Interleukin-6, a major cytokine in the central nervous system. *Int J Biol Sci* 8, 1254-1266 (2012).
6. Su, J.H.*, et al*. Interleukin-6: A Novel Target for Cardio-Cerebrovascular Diseases. *Front Pharmacol* 12, 745061 (2021).
7. Yuan, S., Lin, A., He, Q.Q., Burgess, S. & Larsson, S.C. Circulating interleukins in relation to coronary artery disease, atrial fibrillation and ischemic stroke and its subtypes: A two-sample Mendelian randomization study. *Int J Cardiol* 313, 99-104 (2020).
8. Jenny, N.S.*, et al.* Inflammatory cytokines and ischemic stroke risk: The REGARDS cohort. *Neurology* 92, e2375-e2384 (2019).
9. Armstead, W.M.*, et al.* Release of IL-6 After Stroke Contributes to Impaired Cerebral Autoregulation and Hippocampal Neuronal Necrosis Through NMDA Receptor Activation and Upregulation of ET-1 and JNK. *Transl Stroke Res* 10, 104-111 (2019).
10. Craig J Smith, H.C.E., Carole M Gavin,, Rachel F Georgiou, A.V., Elisa M Barberan, Gregory J del Zoppo, John M Hallenbeck, Nancy J Rothwell, Stephen J Hopkins; & Tyrrell, P.J. Peak plasma interleukin-6 and other peripheral markers of inflammation in the first week of ischaemic stroke correlate with brain infarct volume, stroke severity and long-term outcome. *BMC Neurology* 4(2004).
11. Waje-Andreassen, U.*, et al.* IL-6: an early marker for outcome in acute ischemic stroke. *Acta Neurol Scand* 111, 360-365 (2005).
12. Sotgiu, S.*, et al.* Inflammatory biomarkers in blood of patients with acute brain ischemia. *European journal of neurology: the official journal of the European Federation of Neurological Societies*, 13 (2006).
13. Wang, Y.*, et al.* Interleukin-6 as Predictor of One-Year Cognitive Function After Ischemic Stroke or TIA. *Neuropsychiatr Dis Treat* 18, 391-399 (2022).
14. Angkananard, T., Anothaisintawee, T., McEvoy, M., Attia, J. & Thakkinstian, A. Neutrophil Lymphocyte Ratio and Cardiovascular Disease Risk: A Systematic Review and Meta-Analysis. *Biomed Res Int* 2018, 2703518 (2018).
15. Gao, M.Z.*, et al.* Red blood cell distribution width and neutrophil to lymphocyte ratio are correlated with disease activity of dermatomyositis and polymyositis. *J Clin Lab Anal* 32(2018).
16. Guo, J., Chen, S., Chen, Y., Li, S. & Xu, D. Combination of CRP and NLR: a better predictor of postoperative survival in patients with gastric cancer. *Cancer Manag Res* 10, 315-321 (2018).

17. Huang, L.Y., *et al.* Associations of the neutrophil to lymphocyte ratio with intracranial artery stenosis and ischemic stroke. *BMC Neurol* 21, 56 (2021).
18. Luo,Y.,*etal.* Early neutrophil-to-lymphocyte ratio is a prognostic marker in acute minor stroke or transient ischemic attack. *Acta Neurol Belg* 121, 1415-1421 (2021).
19. Asdaghi, N., Yavagal, D.R., Wang, K., Mueller-Kronast, N. & Romano, J.G. Patterns and Outcomes of Endovascular Therapy in Mild Stroke: A Florida-Puerto Rico Collaboration. *Stroke* 50(2019).
20. Sims, J.R., *et al.* ABC/2 for rapid clinical estimate of infarct, perfusion, and mismatch volumes. *Neurology* 72, 2104-2110 (2009).
21. Gong, Y., et al. Association between serum uric acid to serum creatinine ratio and poor functional outcomes in patients with acute ischemic stroke. Eur J Neurol 29, 3307-3316 (2022).
22. Hallenbeck, A.n.C.J. The Harms and Benefits of Inflammatory and Immune Responses in Vascular Disease. *Stroke* 37(2)**:** 291-293(2006).
23. Xue, W*, et al* Influence of neutrophil-to-lymphocyte ratio and mean platelet volume on severity and short-term prognosis of acute ischemic stroke. *Am J Transl Res* 14, 4066-4073 (2022).
24. Jeon, J.H., *et al.* Canine model of ischemic stroke with permanent middle cerebral artery occlusion: clinical features, magnetic resonance imaging, histopathology, and immunohistochemistry. *J Vet Sci* 16, 75-85 (2015).
25. An, S.A., *et al.* Limited clinical value of multiple blood markers in the diagnosis of ischemic stroke. *Clin Biochem* 46, 710-715 (2013).
26. Mechtouff, L., et al. Association of Interleukin-6 Levels and Futile Reperfusion After Mechanical Thrombectomy. *Neurology 96, e752-e757* (2021)
27. Shaafi, S., *et al.* Interleukin-6, a reliable prognostic factor for ischemic stroke. *Iran J Neurol* 13, 70-76 (2014).
28. Shenhar-Tsarfaty, S., *et al.* Interleukin-6 as an early predictor for one-year survival following an ischaemic stroke/transient ischaemic attack. *Int J Stroke* 5, 16-20 (2010).
29. Deng, Q.W., *et al.* Inflammatory Factors as Potential Markers of Early Neurological Deterioration in Acute Ischemic Stroke Patients Receiving Endovascular Therapy - The AISRNA Study. *J Inflamm Res* 14, 4399-4407 (2021).
30. Li, X., *et al.* The Prognostic Value of Serum Cytokines in Patients with Acute Ischemic Stroke. *Aging Dis* 10, 544-556 (2019).
31. Xue, J., *et al.* Neutrophil-to-Lymphocyte Ratio Is a Prognostic Marker in Acute Ischemic Stroke. *J Stroke Cerebrovasc Dis* 26, 650-657 (2017).
32. Ospel, J.M., *et al.* Strength of Association between Infarct Volume and Clinical Outcome Depends on the Magnitude of Infarct Size: Results from the ESCAPE-NA1 Trial. *AJNR Am J Neuroradiol* 42, 1375-1379 (2021).

Table 1 Comparison of characteristics between two groups based on NIHSS score

Characteristics	Total (*n*=152)	Minor stroke (*n*=72)	Non-minor stroke (*n*=80)	*p*-value
Demographic characteristics				
Age, y, mean ± SD	68.27±9.16	65.10±6.34	71.13±10.33	<0.001*
Sex, male, n (%)	96 (63.2)	50 (69.4)	46 (57.5)	0.228
Past medical history				
Hypertension, n (%)	112 (73.7)	57 (79.2)	55 (68.8)	0.145
Diabetes mellitus, n (%)	66 (43.4)	34 (47.2)	32 (40.0)	0.370
Coronary heart disease, n (%)	27 (17.8)	12 (16.7)	15 (18.8)	0.737
Atrial fibrillation, n (%)	57 (37.5)	23 (31.9)	34 (42.5)	0.180
Smoking, n (%)	59 (38.8)	28 (38.9)	31 (38.8)	0.986
Alcohol intake, n (%)	35 (23.0)	16 (22.2)	19 (23.8)	0.563
Clinical and laboratory parameters				
SBP, mean ± SD, mmHg	153.69±20.01	151.96±18.44	155.25±21.32	0.313
DBP, mean ± SD, mmHg	86.40±13.24	86.32±12.14	86.48±14.23	0.943
Leukocytes, median (IQR), 10^9/L	7.76 (6.33-9.98)	6.64 (5.41-7.80)	8.94 (7.33-11.77)	<0.001*
Neutrophils, median (IQR), 10^9/L	5.47 (4.02-7.59)	4.31 (3.53-5.83)	6.64 (5.04-9.18)	<0.001*
Lymphocytes, median(IQR), 10^9/L	1.51 (1.18-2.07)	1.75 (1.26-2.26)	1.43 (1.15-1.85)	0.013*
NLR	3.43 (2.37-5.70)	2.53 (1.64-3.53)	4.64 (3.16-7.45)	<0.001*
IL-6, median (IQR), pg/mL	5.44 (2.88-16.85)	2.86 (2.27-3.78)	15.85 (7.41-28.98)	<0.001*
FBG, median (IQR), mmol/L	6.15 (5.31-8.20)	6.11 (5.11-8.93)	6.15 (5.46-7.58)	0.709
TC, median (IQR), mmol/L	5.01 (4.21-5.64)	5.02 (4.20-5.63)	5.01 (4.21-5.68)	0.987
TG, median (IQR), mmol/L	1.30 (1.01-1.73)	1.40 (1.08-1.78)	1.22 (0.85-1.70)	0.062
HDL, median (IQR), mmol/L	1.12 (0.97-1.33)	1.15 (0.96-1.31)	1.09 (0.97-1.42)	0.839
LDL, median (IQR), mmol/L	2.79 (2.28-3.43)	2.93 (2.40-3.44)	2.68 (2.24-3.35)	0.406
HCY, median (IQR), umol/L	12.19 (9.79-16.15)	11.84 (9.15-15.85)	12.49 (10.56-16.55)	0.085
Infarct volume, median (IQR), cm^3	21.85 (5.98-179.95)	2.48 (1.21-4.28)	176.60(60.63-273.55)	<0.001*
TOAST classification, n (%)				0.121
Large artery atherosclerosis	90 (59.2)	48 (66.7)	42 (52.5)	
Cardioembolic	55 (36.2)	20 (27.8)	35 (43.8)	
small artery occlusion	7 (4.6)	4 (5.6)	3 (3.8)	
Intravenous thrombolysis, n (%)	33 (21.7)	14 (19.4)	19 (23.8)	0.478

* *p*-value<0.05; SD, standard deviation; IQR, interquartile range; SBP, systolic blood pressure; DBP, diastolic blood pressure; NLR, neutrophil-to-lymphocyte ratio; FBG, fasting blood glucose; TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; HCY, homocysteine; IL-6, interleukin-6; NIHSS, National Institutes of Health Stroke Scale; TOAST, the Trial of ORG 10172 in Acute Stroke Treatment; Minor stroke, NIHSS≤5; Non-minor stroke, NIHSS>5.

Table 2 Characteristics of the Patients According to 3-month Functional Outcome

Characteristics	Good outcome（*n*=87）	Poor outcome（*n*=65）	*p*-value
Demographic characteristics			
Age, y, mean ± SD	64.86±6.59	72.83±10.13	<0.001*
Sex, male, n (%)	61 (70.1)	35 (53.8)	0.077
Past medical history			
Hypertension, n (%)	66 (75.9)	46 (70.8)	0.481
Diabetes mellitus, n (%)	40 (46.0)	26 (40.0)	0.462
Coronary heart disease, n (%)	15 (17.2)	12 (18.5)	0.846
Atrial fibrillation, n (%)	28 (32.2)	29 (44.6)	0.117
Smoking, n (%)	35 (40.2)	24 (36.9)	0.679
Alcohol intake, n (%)	20 (23.0)	15 (23.1)	0.686
Clinical and laboratory parameters			
SBP, mean ± SD, mmHg	152.14±18.89	155.77±21.39	0.270
DBP, mean ± SD, mmHg	86.61±12.17	86.12±14.64	0.824
Leukocytes, median (IQR), 10^9/L	6.86 (5.47-8.36)	9.79 (7.73-12.25)	<0.001*
Neutrophils, median (IQR), 10^9/L	4.41 (3.56-5.78)	7.60 (5.56-9.85)	<0.001*
Lymphocytes, median (IQR), 10^9/L	1.65 (1.27-2.25)	1.38 (1.07-1.75)	0.002*
NLR	2.67 (1.69-3.56)	5.56 (3.89-8.18)	<0.001*
IL-6, median (IQR), pg/mL	3.12 (2.48-4.36)	20.30 (10.39-33.50)	<0.001*
FBG, median (IQR), mmol/L	5.95 (5.08-8.48)	6.33 (5.49-7.71)	0.328
TC, median (IQR), mmol/L	5.02 (4.24-5.60)	4.93 (4.13-5.71)	0.960
TG, median (IQR), mmol/L	1.40 (1.07-1.73)	1.22 (0.85-1.72)	0.137
HDL, median (IQR), mmol/L	1.14 (0.97-1.31)	1.07 (0.97-1.39)	0.880
LDL, median (IQR), mmol/L	2.88 (2.36-3.42)	2.65 (2.24-3.45)	0.689
HCY, median (IQR), umol/L	11.67 (9.35-15.81)	13.20 (10.72-17.26)	0.024*
Infarct volume, median (IQR), cm^3	10.20 (4.10-13.30)	198.90 (114.55-321.40)	<0.001*
TOAST classification, n (%)			0.080
Large artery atherosclerosis	57 (65.5)	33 (50.8)	
Cardioembolic	25 (28.7)	30 (46.2)	
small artery occlusion	5 (5.8)	2 (3.2)	
Intravenous thrombolysis, n (%)	19 (21.8)	14 (21.5)	0.684

* *p*-value<0.05; SD, standard deviation; IQR, interquartile range; SBP, systolic blood pressure; DBP, diastolic blood pressure; NLR, neutrophil-to-lymphocyte ratio; FBG, fasting blood glucose; TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; HCY, homocysteine; IL-6, interleukin-6; NIHSS, National Institutes of Health Stroke Scale; TOAST, the Trial of ORG 10172 in Acute Stroke Treatment.

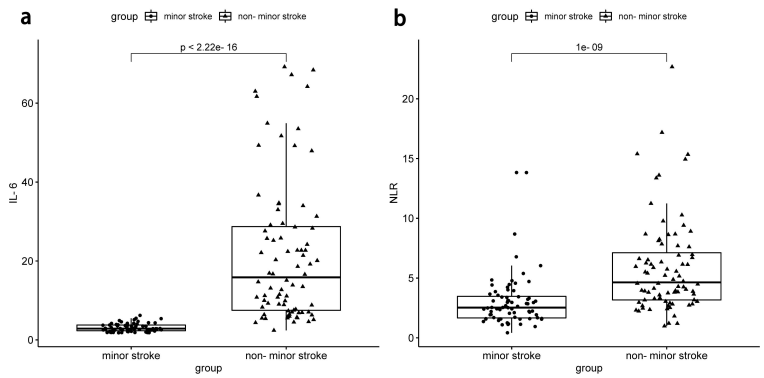

Fig. 1 IL-6 and NLR levels in different severities. IL-6 (a) and NLR (b) in patients with non-minor stroke were significantly higher than those with minor stroke

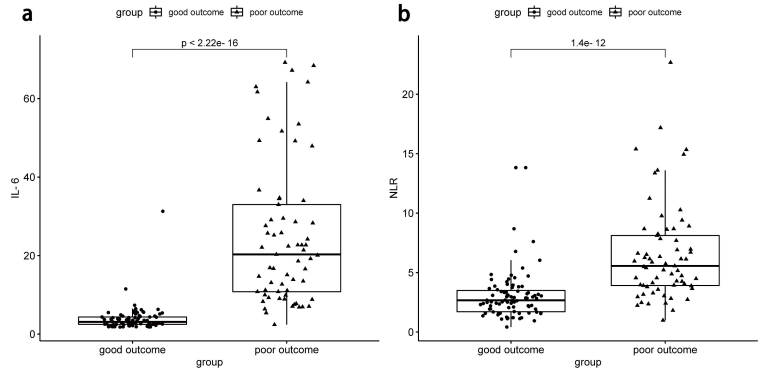

Fig. 2 IL-6 and NLR levels in different outcome. IL-6 (a) and NLR (b) in patients with 3-month poor outcome were significantly higher than those with good outcome

Table 3 The Univariate and Multivariate Logistic Regression Analysis of Risk factors for 3-month poor outcome

Risk factors	Unadjusted OR (95% CI)	*p*-value	Adjusted OR (95% CI)	*p*-value
Age	1.12 (1.070-1.172)	<0.001		
Leukocytes	1.648 (1.378-1.970)	<0.001		
Neutrophils	1.982 (1.576-2.494)	<0.001		
Lymphocytes	0.449 (0.260-0.775)	0.004		
NLR	1.613 (1.334-1.950)	<0.001		
IL-6	1.660(1.370-2.012)	<0.001	1.616 (1.306-1.999)	<0.01
HCY	1.045(0.988-1.105)	0.125		

OR, odds ratio; CI, confidence interval; NLR, neutrophil-to-lymphocyte ratio; IL-6, interleukin-6; HCY, homocysteine.

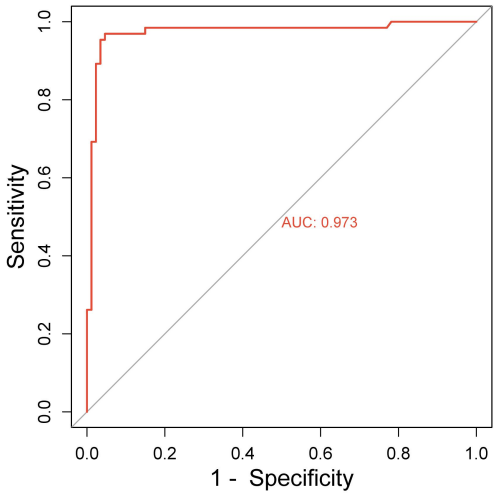

Fig. 3 ROC curve of IL-6 for predicting 3-month poor outcome in AIS patients. The optimal cut-off value was 6.345pg/mL (sensitivity: 96.9%, specificity: 95.4%, AUC: 0.973, p<0.001)