Neutrophil-to-lymphocyte Ratio as a Risk Determinant of Hemorrhage Transformation in Acute Ischemic Stroke

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Abstract

Objective: The purpose of this study was to investigate the clinical significance between neutrophil-to-lymphocyte ratio (NLR) and classification of non-thrombolytic hemorrhagic transformation (HT) in acute ischemic stroke (AIS), to unravel new diagnostic approach.

Methods: We recruited and selected 636 patients who did not undergo thrombolytic therapy between May 2018 and April 2019 at the Affiliated Hospital of Xuzhou Medical University. The laboratory and clinical data were collected within 24 h after the onset of AIS. Based on the status of HT development during hospitalization, all participants were divided into three groups, namely, the non-HT (NHT) group, hemorrhagic infarction (HI) group, and parenchymal hematoma (PH) group.

Results: Multivariate logistic regression analysis showed that NLR and the ischemic lesion diameter are independent risk factors of HI and PH, while the score of National Institutes of Health Stroke Scale (NIHSS) and cardioembolism are considered to be independent risk factors for PH only. Receiver operating characteristic (ROC) analysis determined that the optimal cutoff values of NLR in HI group and PH group were 3.75 and 3.97, respectively. The optimal cutoff value can be used as the critical value for the unfavorable outcome.

Conclusion: NLR values were significantly increased and correlated with both HI and PH groups and NLR could be used as a predictor of both HI and PH.

Keywords: Acute ischemic stroke, Hemorrhage transformation, Neutrophil-to-lymphocyte ratios

1 Introduction

Hemorrhagic transformation (HT) occurs predominantly in acute ischemic stroke (AIS) and is a common complication of antiplatelet therapy (thrombolyis, anticoagulation, antiplatelet aggregation, etc.) [1]. HT can be classified as hemorrhagic infarction (HI) and parenchymal hematoma (PH). HI is a heterogeneous hyperdensity inhabiting a section of an ischemic infarct zone on the computed tomography (CT) images, whereas PH is a further homogeneous, dense hematoma with significant effect. Each of them has two subtypes: HI type 1 (HI1) and HI type 2 (HI2) for HI and PH type 1 (PH1) and PH type 2 (PH2) for PH [2].

The initial CT/magnetic resonance imaging (CT/magnetic resonance imaging [MRI]) exhibits no hemorrhage despite the fact that hemorrhage
transformation is detected during follow-up CT/MRI [3], which probably occurs in 10 – 40% of AIS cases [4]. Given the incidence of HT, the treatment of AIS could be challenging. Therefore, early prediction of HT would be beneficial for designing a proper treatment plan for patients with AIS, consequently avoiding adverse outcomes and poor prognosis [5].

Recent studies suggested that inflammatory mediators have significant clinical significance in AIS and HT [6,7]. Neutrophil-to-lymphocyte ratio (NLR) is a rather new risk determinant factor of the inflammatory response that has been shown to be associated with the severity and prognosis of cardiovascular events, ischemic stroke, cancer, and bacterial infectious disease [8-11]. In addition, differential white blood cell counts and specific cell types such as neutrophils, lymphocytes, and monocytes could be predictors of cardiovascular disease [12]. There is substantial evidence that neutrophil counts may be an independent prognostic factor for both acute and chronic cardiovascular diseases, and this is supported by several laboratory studies that explain the different mechanisms of such association [13]. The rise in monocyte count was also found to carry an increased risk for cardiovascular diseases. A recent and intriguing study has found that NLR is more predictive than total white blood cell count or neutrophil count as a marker of cardiovascular disease and is emerging as an independent and useful prognostic parameter for cardiovascular disease [14].

NLR may have a predictive advantage for several reasons. Particularly, NLR is unlikely to be affected by various physiological conditions such as dehydration and exercise, despite these conditions may affect the absolute number of the individual cell type [15]. Second, and most prominently, NLR is the ratio of two different types of immune cells which serve in complementary immune pathways [16]. The neutrophil count reflects ongoing inflammation, whereas the lymphocyte count reflects the regulatory immune pathway [17]. In light of this line of evidence, NLR is a predictive marker of cardiovascular disease [12]. On the other hand, whether NLR is a predictive factor for different types of HT is still uncertain.

Besides, these studies, as mentioned earlier, mainly investigated the relationship between NLR and the occurrence of HT in patients receiving thrombolytic therapy [16]. Although thrombolytic therapy such as recombinant tissue plasminogen activator rtPA is considered the first-line approach for the management of AIS, it is undoubtedly a risk factor for HT. The patients who arrived at the emergency department with 4.5 h after onset of the symptoms are eligible for the rtPA, while those who come later than 4.5 h will not receive the rtPA, which considered to be the patients without thrombolytic therapy [18]. Nevertheless, it is still indefinite whether NLR association with HT could also be predicted in patients who did not receive thrombolytic treatment. Therefore, we aimed to explore the association of NLR with different types of HT in Chinese patients with AIS who did not receive thrombolytic therapy.

2 Methods

2.1 Study participants

The data in this study were obtained from the Neurology Department of the Affiliated Hospital of Xuzhou Medical University, Jiangsu. The study protocol was designed following local and international ethics criteria for human research. The ethics committee of the hospital approved the study (XYFY2018-KL038-01), and the written informed consent was obtained from the patients or their family members.

In the present study, 1342 patients were recruited and screened in the beginning. A total of 636 patients with AIS were selected and enrolled according to inclusion criteria. According to the indications of hemorrhage, the selected patients were divided into three groups – non-HT (NHT), HI, and PH groups. Of the 78 patients with HT, 45 had HI while the remaining 33 had PH, and 558 patients were identified as NHT. The study participants arrived at the hospital within 24 h after symptom onset from May 2018 to April 2019. All of these patients underwent brain CT/MRI scan within 24 h after the onset of AIS followed by regular MRI after admission or immediate brain CT scan in case of abrupt neurological deterioration. In general, the patients who did not arrive at the emergency department with 4.5 h
after the onset of the symptoms are not eligible to receive rtPA treatment, and thus, they were considered to be the patients without thrombolytic therapy [18] who were the subjects of interest in the present study.

The patient selection was conducted in accordance with the inclusion criteria. The patients who meet the following requirements were included: (1) The patients who meet the diagnostic criteria for AIS of the 2018 Guidelines for the Early Management of Patients With AIS [19], (2) the patients with complete blood tests and brain CT/MRI examination within 24 h after the onset of AIS, and (3) the patients with no HT found in the first brain CT/MRI. In the present study, the European Cooperative Acute Stroke Study classification method, a widely used AIS classification of HT, which divides HT into HI type and PH type, was used [2].

A total of 706 patients were excluded from this study, in accordance with the following exclusion criteria: (1) The patients with HT on baseline CT scan, (2) the patients without follow-up imaging, (3) the patients with blood cell count beyond 24 h after the onset of AIS, (4) the patients with a history of previous stroke or coronary heart disease, (5) the patients with a history of immune system disease or cancer, (6) the patients with infectious disease within 1 week before admission, and (7) the patients with intracranial hemorrhage, hematological diseases, intracranial mass or severe heart, liver, kidney, and lung or other diseases (Figure 1).

2.2 Baseline data collection

General clinical data of the included patients, including demographic data (age, gender), vascular risk factors (smoking, alcohol, hypertension, and diabetes), systolic blood pressure (SBP), diastolic blood pressure (DBP), National Institutes of Health Stroke Scale (NIHSS) score, and stroke subtypes which were classified according to the criteria of the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) were collected retrospectively. Aside from being user friendly, TOAST classification results are consistent to the observers, and allow researchers to report treatment responses in relevant subtypes of ischemic stroke patients [20]. In regards to the NIHSS, individuals with ≤ 8 points were categorized as having a mild stroke, 9 – 15 points as moderate, and ≥ 16 points as severe [21]. Data regarding the use of medications such as antiplatelet drugs, anticoagulants, lipid-lowering agents, and laboratory results such as white blood cells (WBC), neutrophils, and lymphocytes were collected. A fully automatic hemocytometer was used for counting the blood cells, whereas the activated partial thromboplastin time, international normalized ratio (INR), and fibrinogen were analyzed using the Sysmex CS-5100 coagulation analyzer (Siemens Healthcare Diagnostics, Erlangen, Germany).

Figure 1. Patient selection process.
2.3 Statistical analysis

Statistical processing of data was performed using Statistical Package for the Social Sciences (SPSS) version 23. Kolmogorov–Smirnov was used to assess the normal distribution of the quantitative variables, and the relevant data were not of normal distribution.

Kruskal–Wallis test was used to compare the data of quantitative variables between groups. The data are expressed as median (interquartile range). The differences of the categorical data between groups were analyzed using Pearson’s Chi-squared test, Chi-squared test with Yates correction for continuity, or Fisher’s exact test. The categorical data are expressed as count (percentage).

The variables that were found to have \( P < 0.05 \) in both HI and PH groups, as shown in the preliminary univariate regression analysis, were included in the multivariate logistic regression analysis to identify the independent predictors of different types of HT. Receiver operating characteristic (ROC) analysis was used to evaluate whether NLR can predict HI and PH, and to determine the optimal cutoff values. In the ROC curves, the area under the curve (AUC) represents the prediction accuracy of NLR. The AUC value is between 0 and 1. A higher AUC value indicates a higher level of prediction accuracy of NLR. The Youden Index, which is a measure of diagnostic accuracy, calculated using the formula of sensitivity + specificity −1, is commonly used to determine the optimal cutoff values. Optimal cutoff values were determined for diagnosis purpose. A variable is considered having better diagnostic value if its numerical value is greater than the optimal cutoff value.

In this study, \( P < 0.05 \) was considered statistically significant.

3 Results

3.1 Comparison of baseline data

A total of 1342 patients were admitted to the Neurology Department of the Affiliated Hospital of Xuzhou Medical University between May 2018 and April 2019. In this study, a total of 706 patients were excluded from the study, and 636 patients were eventually included in the study.

The demographic, clinical, and laboratory data of the patients are shown in Table 1. The AIS patients who were diagnosed with HT accounted for 12.26% of the included patients, of which, patients who were classified in HI group and PH group accounted for 7.07% and 5.19% of the included patients, respectively. HT group contained a smaller proportion of males, had a higher initial NIHSS score, higher systolic blood pressure on admission, larger ischemic lesion diameter, and a higher rate of cardioembolic stroke compared to the NHT group.

3.2 Multivariate logistic regression analysis

The variables that are statistically significant \( (P<0.05) \) in the univariate logistic regression analysis such as male, SBP, NIHSS score, ischemic lesion diameter, WBC, NLR, SVO, and cardioembolism were analyzed in multivariate logistic regression analysis. Since NLR was obtained by determining the ratio of neutrophils to lymphocytes, there is a covariate relationship between both variables. Therefore, only NLR was selected for the regression analysis. The results showed that the NLR was the independent predictor of HI and PH \( (P < 0.001) \), while NIHSS score \( (P < 0.001) \) was also the independent predictor of PH. On the other hand, ischemic lesion diameter and SVO were statistically significant in the HI group \( (P < 0.05) \) (Table 2).

3.3 Predictive values of NLR for HI and PH

The ROC curves of the predictive values of NLR for HI and PH are presented in Figure 2. The optimal cutoff value of NLR in the HI group was 3.75, indicating that the patient with a NLR value exceeding 3.75 stands a higher chance of having HI. The Youden Index, which is a measure of diagnostic accuracy, as determined from the ROC analysis of NLR in HI group was 0.47. Moreover, the sensitivity and specificity in the prediction of HI-type HT were 73.3% and 73.8%, respectively. With relatively large AUC value at 0.773, NLR is able to accurately predict the development of HI in the patients with AIS (Figure 2A).

On the other hand, the optimal cutoff value of NLR in the PH group was 3.97, while the Youden Index was 0.61, which is larger than that of HI group. The sensitivity and specificity in the prediction of PH-type HT were 84.8% and 76.5%, respectively, and the AUC value was 0.864 (Figure 2B).

In view of these findings, NLR can be regarded as a risk determinant for both HI and PH. Nevertheless, in a comparative sense, NLR appears
Table 1. Baseline characteristics of patients

| Characteristics                  | NHT group (n=558) | HI group (n=45) | PH group (n=33) | $\chi^2/Z$ | P       |
|----------------------------------|-------------------|----------------|----------------|-----------|---------|
| Demographics                     |                   |                |                |           |         |
| Age (years)                      | 66 (56 – 74)      | 66 (54 – 77)   | 65 (63 – 75)   | 2.035     | 0.362   |
| Male                             | 369 (66.13)       | 23 (51.11)     | 15 (45.45)     | 9.268     | <0.05   |
| History                          |                   |                |                |           |         |
| Hypertension                     | 355 (63.62)       | 24 (53.33)     | 20 (60.61)     | 1.952     | 0.377   |
| Diabetes                         | 164 (29.39)       | 13 (28.89)     | 10 (30.30)     | 0.019     | 0.991   |
| Smoking                          | 116 (26.24)       | 7 (15.56)      | 3 (9.10)       | 3.236     | 0.198   |
| Alcohol consumption              | 100 (17.92)       | 4 (8.89)       | 9 (28.57)      | 3.915     | 0.141   |
| Age (years)                      | 66 (56 – 74)      | 66 (54 – 77)   | 65 (63 – 75)   | 2.035     | 0.362   |
| Male                             | 369 (66.13)       | 23 (51.11)     | 15 (45.45)     | 9.268     | <0.05   |
| Hypertension                     | 355 (63.62)       | 24 (53.33)     | 20 (60.61)     | 1.952     | 0.377   |
| Diabetes                         | 164 (29.39)       | 13 (28.89)     | 10 (30.30)     | 0.019     | 0.991   |
| Smoking                          | 116 (26.24)       | 7 (15.56)      | 3 (9.10)       | 3.236     | 0.198   |
| Alcohol consumption              | 100 (17.92)       | 4 (8.89)       | 9 (28.57)      | 3.915     | 0.141   |
| Admission                        |                   |                |                |           |         |
| NIHSS score (points)             | 3 (1 – 7)         | 8 (5 – 19)     | 21 (17 – 28)   | 99.989    | <0.001  |
| Mild stroke                      | 454 (81.36)       | 23 (51.11)     | 2 (6.06)       | 9.268     | <0.05   |
| Moderate stroke                  | 66 (11.83)        | 9 (20.00)      | 5 (15.15)      | 1.952     | 0.377   |
| Severe stroke                    | 38 (6.81)         | 13 (28.89)     | 26 (78.79)     | 9.268     | <0.05   |
| Ischemic lesion diameter (mm)    | 13.66 (9.15 – 23.51) | 43.82 (25.23 – 72.48) | 67.16 (35.64 – 92.54) | 126.551 | <0.001  |
| SBP (mmHg)                       | 144 (130 – 160)   | 153 (137 – 170)| 159 (140 – 171)| 13.214   | <0.001  |
| DBP (mmHg)                       | 85 (80 – 95)      | 87 (80 – 95)   | 90 (80 – 96)   | 0.491     | 0.782   |
| Laboratory tests                 |                   |                |                |           |         |
| WBC ($\times 10^9$/L)            | 6.70 (5.50 – 8.13) | 7.70 (6.65 – 8.70) | 8.30 (7.20 – 9.75) | 28.841   | <0.001  |
| Neutrophil ($\times 10^9$/L)     | 4.42 (3.45 – 5.72) | 6.05 (4.55 – 6.78) | 6.72 (5.35 – 8.30) | 50.171   | <0.001  |
| Lymphocyte ($\times 10^9$/L)     | 1.60 (1.30 – 2.00) | 1.10 (0.85 – 1.45) | 1.20 (0.90 – 1.40) | 60.360   | <0.001  |
| NLR                              | 2.75 (1.93 – 3.85) | 4.91 (3.23 – 8.22) | 6.21 (4.30 – 8.51) | 81.620   | <0.001  |
| APTT (g/L)                       | 27.3 (25.40 – 30.30) | 26.00 (23.70 – 28.15) | 26.20 (25.20 – 29.90) | 8.593    | <0.05   |
| INR                              | 0.96 (0.90 – 1.04) | 0.97 (0.89 – 1.01) | 0.98 (0.91 – 1.05) | 0.488    | 0.783   |
| Fibrinogen (g/L)                 | 2.84 (2.41 – 3.48) | 3.02 (2.46 – 4.04) | 2.90 (2.69 – 3.45) | 3.402    | 0.183   |
| TOAST classification             | 103.309           | 93.10          | 87.20          | 103.309   | <0.001  |
| LAA                              | 154 (27.60)       | 22 (48.90)     | 11 (33.30)     | −9.14     | <0.05   |
| SVO                              | 286 (51.30)       | 1 (2.20)       | 1 (3.00)       | 65.48     | <0.001  |
| CE                               | 15 (2.70)         | 8 (17.80)      | 12 (26.40)     | 91.75     | <0.001  |
| SUE                              | 93 (16.70)        | 10 (22.20)     | 8 (17.80)      | 2.01      | 0.37    |
| SOE                              | 5 (0.90)          | 4 (8.90)       | 1 (3.00)       | 17.67     | <0.001  |
| Use of medications               |                   |                |                |           |         |
| Antiplatelet drugs               | 496 (88.89)       | 43 (95.56)     | 31 (93.94)     | 2.687     | 0.261   |
| Anticoagulants                   | 314 (53.40)       | 27 (60.00)     | 18 (54.55)     | 0.287     | 0.867   |
| Lipid-lowering agents            | 550 (98.56)       | 44 (97.78)     | 32 (96.97)     | 0.645     | 0.724   |

The quantitative data are expressed as median (interquartile range) and the categorical data are expressed as count (percentage). $\chi^2$: Chi-squared value; Z, Z-score; P, P-value; NHT, non-hemorrhagic transformation; HI, hemorrhagic infarction; PH, parenchymal hematoma; NIHSS, National Institutes of Health Stroke Scale; SBP, systolic blood pressure; DBP, diastolic blood pressure; WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; TOAST, Trial of ORG 10172 in Acute Stroke Treatment; APTT, activated partial thromboplastin time; INR, international normalized ratio; LAA, large artery atherosclerosis; SVO, small-vessel occlusion, CE, cardioembolism; SUE, stroke of undetermined etiology; SOE, stroke of other determined etiology.

to be more reliable in favor of the prediction of PH over HI in patients with AIS.

4 Discussion

HT is a common complication of AIS. Previous studies reported that the incidence of HT of AIS ranges from 10% to 40% [4]. This transformation is known to be an inflammatory reaction accompanied by increased permeability of the blood-brain barrier, ischemia-reperfusion injury, and the collateral circulation development in the AIS [3]. Inflammatory response plays a significant role in
Table 2. Identification of independent predictors of hemorrhagic infarction and parenchymal hematoma using multivariate logistic regression analysis

| Variables                  | HI group |          |          |          | PH group |          |          |          |
|----------------------------|----------|----------|----------|----------|----------|----------|----------|----------|
|                            | OR       | 95% CI   | P        | OR       | 95% CI   | P        | OR       | 95% CI   | P        |
| Male                       | 0.983    | 0.954 – 1.012 | 0.254 | 1.456    | 0.496 – 4.277 | 0.495 |          |          |          |
| SBP                        | 1.009    | 0.992 – 1.025 | 0.308 | 1.007    | 0.983 – 1.031 | 0.575 |          |          |          |
| NIHSS score                | 1.010    | 0.963 – 1.059 | 0.695 | 1.117    | 1.054 – 1.184 | <0.001 |          |          |          |
| Ischemic lesion diameter   | 1.022    | 1.007 – 1.037 | <0.05 | 1.019    | 1.000 – 1.038 | 0.052 |          |          |          |
| WBC                        | 0.818    | 0.660 – 1.014 | 0.066 | 0.916    | 0.733 – 1.145 | 0.440 |          |          |          |
| NLR                        | 1.493    | 1.260 – 1.770 | <0.001 | 1.475    | 1.161 – 1.873 | <0.001 |          |          |          |
| SVO                        | 0.046    | 0.005 – 0.420 | <0.05 | 0.248    | 0.026 – 2.326 | 0.222 |          |          |          |
| CE                         | 2.970    | 0.928 – 9.504 | 0.067 | 3.447    | 0.909 – 13.076 | 0.069 |          |          |          |

OR, odds ratio; CI, confidence interval; P, P-value; HI, hemorrhagic infarction; PH, parenchymal hematoma; NIHSS, National Institutes of Health Stroke Scale; WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; SVO, small-vessel occlusion; CE, cardioembolism.

Figure 2. Predictive values of neutrophil-to-lymphocyte ratio (NLR) for hemorrhagic infarction (HI) and parenchymal hematoma (PH). (A) The optimal cutoff value of NLR in the HI group which was associated with high Youden Index was 3.75. In regard to predicting HI-type HT, the sensitivity and specificity were 73.3% and 73.8%, respectively, and the area under the ROC curve (AUC) was 0.773 (95% CI: 0.700 – 0.847, P < 0.001). (B) The optimal cutoff value of NLR in the PH group which had a Youden Index comparatively larger than the HI group was 3.97. In regard to predicting PH-type HT, the sensitivity and specificity were 84.8% and 76.5%, respectively, and the AUC was 0.864 (95% CI: 0.800 – 0.928, P < 0.001). CI, confidence interval.

the HT of AIS [22]. In line with these observations, we noted a 12.26% incidence of HT in the current study.

Previous studies elucidated the mechanism involving neutrophils leading to HT of AIS in which various Chemokines, including C-X-C motif chemokine ligand 1 (CXCL1) released by ischemic tissues act on neutrophils in the bone marrow, further initiating their recruitment and activation [23,24]. Furthermore, these activated neutrophils quickly enter the site of infarcted tissues and thereby release a variety of chemical factors, including cytokines, reactive oxygen species, and elastase. In addition, these chemical factors promote the decomposition of extracellular matrix by destroying the integrity of neurovascular units that result in vascular damage [25]. Moreover, these chemical factors also participate in the destruction of the blood-brain barrier and the occurrence of HT [23,26]. Neutrophils are also an essential source of matrix metallopeptidase 9 (MMP-9) within 24 h after AIS [27]. MMP-9 can act directly on tight junction proteins to increase endothelial cell permeability and the enzyme may also be absorbed by endothelial
cells in the basement membrane [4,28] which further leads to the decomposition and destruction of the blood-brain barrier, which associates closely with the occurrence of HT [28,29]. In addition to the pro-inflammatory mechanisms, neutrophil-derived neutrophil extracellular traps (NETs) have also been portrayed as another potential mechanism [26]. Studies of ischemic mouse models suggested that individual components of NETs can aggravate cerebral ischemia-reperfusion injury [30], and in contrast, the mice lacking NETs were protected from myocardial and hepatic ischemia-reperfusion injury [31,32]. These results suggested that NETs may be involved in a mechanism that causes damage to the blood-brain barrier.

The current study found that peripheral blood lymphocyte counts in patients with hemorrhagic transformation were significantly reduced, which was due to the stress state of the body after the onset of AIS. During the stress state, activation of the hypothalamic-pituitary-adrenal axis and sympathetic nervous system leads to increased release of glucocorticoids and catecholamines [33]. Furthermore, the immunoinhibitory cytokine interleukin-10 can be stimulated by glucocorticoids and catecholamines, prompting the immune system to produce inhibitory responses. These mechanisms lead to a reduction of peripheral blood lymphocyte which can increase the production of pro-inflammatory cytokines that aggravate ischemic injury [33]. In addition, some experimental evidence suggests that some subtypes of lymphocytes, mainly the regulatory T cells and B cells, have regulatory functions of inducing neuroprotection during inflammation [34,35], while other subtypes such as cytotoxic T lymphocytes are significant sources of cytotoxic substances [36,37]. Thus, it is not clear which subtypes of lymphocytes play a dominant role in the pathophysiology of HT. Nonetheless, we believe that the reduction of regulatory B cells and regulatory T cells is responsible for the pathophysiology of HT, which is consistent with previous studies [38,39].

Our study showed that NLR is an independent risk determinant for HT of AIS. The NLR takes into consideration the specific and nonspecific immune responses. It is a convenient, practical, and stable indicator of a complex inflammatory state. Furthermore, the advantage of using NLR is that it is less likely to be affected by various physiological conditions as compared with other inflammatory markers [40]. The previous study has shown that elevated value of NLR is independently associated with HT, and when NLR exceeds 4.5, the risk of HT increases by 1.97 times after covariate adjustment [38]. Another study demonstrated that an increase in post-stroke NLR in patients with PH or symptomatic intracranial hemorrhage (sICH) after intravenous thrombolysis indicated that PH or sICH patients had significantly higher NLR than non-PH or non-sICH patients [39]. However, there are no relevant studies on whether the NLR values of patients with different types of HT are higher than those without HT. The data in our study showed that NLR was significantly increased in both HI and PH types of HT. In addition, NLR can be used as an independent risk determinant for HI and PH types of HT in patients without receiving thrombolytic therapy.

Furthermore, the data of our study showed that NLR could independently predict the occurrence of HI and PH. This implies that the inflammatory pathway may be a potential therapeutic target. Besides, assessing the changes in the levels of inflammation-related biomarkers can provide us an understanding of the disease progression. NLR reflects the balance of neutrophils and lymphocytes, which fully reflects the immune status of the body. In this sense, NLR is superior to independent neutrophil count or lymphocyte count in predicting the incidence of HT [38]. On the other hand, NLR helps discriminate the HT from non-HT cases on the basis of a larger quotient value between the two leukocyte types in the inflammatory event such as hemorrhage transformation in which neutrophils become excessively activated, and the inflammatory cytokines released by neutrophils trigger lymphocyte apoptosis [39]. Furthermore, consistent with previous studies [39], we observed higher neutrophil counts and lower lymphocyte counts in the HI and PH groups as compared to NHT group in the present study. Therefore, the prediction of different types of HT using NLR can improve the clinical recognition of HT and assist the treatment planning.

There are a few limitations to this study. First, the sample size of the HT group was relatively small which weakened the power of the present study. Therefore, further studies with larger sample
size are needed. Second, patients in the present study came from a single center and therefore, the results cannot be generalized to the entire Chinese population. Furthermore, some patients, especially those with severe AIS who died before being admitted to the hospital, were not included in the study. Thus, more prospective and multicenter studies are required to confirm our research results.

Third, the patient’s previous medical history before admission and the history of prior medications, especially antiplatelet drugs, anticoagulants, and lipid-lowering drugs, were not taken into account and analyzed. In the future, a prospective study involving multiple centers should be conducted to evaluate the predictive value of NLR at different time points in the different types of HT.

5 Conclusion

Higher NLR is associated with a greater risk for both HI and PH in Chinese patients. The patients who have high NLR values should be treated carefully to prevent the development of hemorrhage transformation.

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Conflicts of interest

Shuang Ma has received grant support from the National Institutes of Health China (GN-2018R009) for research unrelated to this manuscript. We do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Author contributions

SM and DG conceived and designed experiments. SM collected data. SM, SK, BM, JL, YM, ZS, and XC carried out the experiments. LW, ZW, and WW analyzed the data. SM and BM drafted the manuscript. All the authors read and approved the publication of the final manuscript.

References

[1] Yaghi, S.; Boehme, A.K.; Dibu, J.; Guerrero, C.R.L.; Ali, S.; Martin-Schild, S.; Sands, K.A.; Noorian, A.R.; Blum, C.A.; Chaudhary, S.; Schwamm, L.H.; Liebeskind, D.S.; Marshall, R.S.; Willey, J.Z. Treatment and Outcome of Thrombolysis-Related Hemorrhage: A Multicenter Retrospective Study. JAMA Neurol., 2015, 72(12), 1451-7.

[2] Hacke, W.; Kaste, M.; Fieschi, C.; Toni, D.; Lesaffre, E.; von Kummer, R.; Boyesen, G.; Bluhmki, E.; Höxter, G.; Mahagne, M.H. Intravenous Thrombolysis with Recombinant Tissue Plasminogen Activator for Acute Hemispheric Stroke. Eur. Coop. Acute Stroke Study JAMA, 1995, 274(13), 1017-25.

[3] Alvarez-Sabin, J.; Maistera, O.; Santamarina, E.; Kase, C.S. Factors Influencing Haemorrhagic Transformation in Ischaemic Stroke. Lancet Neurol., 2013, 12(7), 689-705.

[4] Jackling, G.C.; Liu, D.; Stavoma, B.; Ander, B.P.; Zhan, X.; Lu, A.; Sharp, F.R. Hemorrhagic Transformation after Ischemic Stroke in Animals and Humans. J. Cereb. Blood Flow Metab., 2014, 34(2), 185-99.

[5] Lu, G.; He, Q.; Shen, Y.; Cao, F. Potential biomarkers for predicting hemorrhagic transformation of ischemic stroke. Int. J. Neurosci., 2018, 128(1), 79-89.

[6] Tokgoz, S.; Keskin, S.; Kayrak, M.; Seyithanoglu, A.; Ogmenul, A. Is neutrophil/lymphocyte ratio predict to short-term mortality in acute cerebral infarct independently from infarct volume? J. Stroke Cerebrovasc. Dis., 2014, 23, 2163-8.

[7] Duan, Z.; Wang, H.; Wang, Z.; Hao, Y.; Zhi, W.; Yang, D.; Zhou, Z.; Liu, W.; Lin, M.; Shi, Z.; Lv, P.; Wan, Y.; Xu, G.; Xiong, Y.; Zhu, W.; Liu, X. Neutrophil-Lympocyte Ratio Predicts Functional and Safety Outcomes after Endovascular Treatment for Acute Ischemic Stroke. Cerebrovasc. Dis. 2018, 45(5-6), 221-7.

[8] Afari, M.E.; Bhat, T. Neutrophil to Lymphocyte Ratio (NLR) and Cardiovascular Diseases: An Update. Expert. Rev. Cardiovasc. Ther., 2016, 14(5), 573-7.

[9] Celikbilek, A.; Ismailogullari, S.; Zararziz, G. Neutrophil to Lymphocyte Ratio Predicts Poor Prognosis in Ischemic Cerebrovascular Disease. J. Clin. Lab. Anal., 2014, 28(1), 27-31.

[10] Li, M.X.; Liu, X.M.; Zhang, X.F.; Wang, W.L.; Zhu, Y.; Dong, J.; Cheng, J.W.; Liu, Z.W.; Ma, L.; Lv, Y. Prognostic Role of Neutrophil-to-lymphocyte Ratio in Colorectal Cancer: A Systematic Review and Meta-analysis. Int. J. Cancer, 2014, 134(10), 2403-13.

[11] de Jager, C.P.; van Wijk, P.T.; Mathoera, R.B.; de Jongh-Leuveninck, J.; van der Poll, T.; Wever, P.C. Lymphocytopenia and Neutrophil-lymphocyte Count Ratio Predict Bacteremia better than Conventional Infection Markers in an Emergency Care Unit. Crit Care, 2010, 14(S), R192.

[12] Kim, J.H.; Lim, S.; Park, K.S.; Jang, H.C.; Choi, S.H. Total and Differential WBC Counts are Related with Coronary Artery Atherosclerosis and Increase the Risk for Cardiovascular Disease in Koreans. PLoS One. 2017, 12(7), e0180332.

[13] Shah, A.D.; Denaxas, S.; Nicholas, O.; Hingorani, A.D.; Hemingway, H. Neutrophil Counts and Initial Presentation of 12 Cardiovascular Diseases: A CALIBER Cohort Study. J. Am. Coll. Cardiol., 2017, 169(9), 1160-9.

[14] Mazza, M.G.; Lucchi, S.; Rossetti, A.; Clerici. M. Neutrophil-lymphocyte Ratio, Monocyte-lymphocyte Ratio and Platelet-lymphocyte Ratio in Non-affective Psychosis: A Meta-analysis and Systematic Review. World J. Biol. Psychiatry, 2019, 1, 1-13.

[15] Park, C.S. Inflammation in Cardiovascular Disease. Korean Circ. J., 2017, 47(5), 314-5.

[16] Aurelian, S.V.; Adrian, M.; Andercou, O.; Bruno, S.; Alexandru, O.; Catalin, T.; Dan, B. Neutrophil-to-Lymphocyte Ratio: A Comparative Study of Rupture to Nonruptured Intracranial Abdominal Aortic Aneurysms. Ann. Vasc. Surg., 2019, 58, 270-5.

[17] Lin, L.; Piao, M.; Jiang, X.; Houning, L.V.; Zhao, N.; Yang, F.; Sun, C. Does Neutrophil-to-lymphocyte Ratio Predict 1-year Mortality in Patients with Primary Biliary Cholangitis? Results from a Retrospective Study with Validation Cohort. BMJ Open, 2017, 7(7), e015304.

[18] Kikuchi, K.; Tanaka, E.; Murai, Y.; Tanchaoren, S. Clinical Trials in Acute Ischemic Stroke. CNS Drugs, 2014, 28, 929-38.
Rosell, A.; Cuadrado, E.; Ortega-Aznar, A.; Hernandez-Guillamon, M.; Rosenberg, G.A.; Yang, Y. Vasogenic Edema Due to tight Junction Ho, W.M.; Reis, C.; Akyol, O.; Applegate, R.; Stier, G.; Martin, R.; Garcia-Culebras, A.; Duran-Laforet, V.; Pena-Martinez, C.; Cai, Z., Zhao, B., Deng, Y., Shangguan S1, Zhou F1, Zhou W1, Li Benakis, C., Garcia-Bonilla, L., Iadecola, C., Anrather, J. The Jickling, G.C.; Liu, D.; Ander, B.P.; Stamova, B.; Zhan, X.; Sharp, F.R. Targeting Neutrophils in Ischemic Stroke: Translational Insights from Experimental Studies. J. Cereb. Blood Flow Metab., 2015, 35(6), 888-901. Benakis, C., García-Bonilla, L., Iadecola, C., Anrather, J. The Role of Microglia and Myeloid Immune Cells in Acute Cerebral Ischemia. Front Cell. Neurosci., 2014, 8, 461. 2013, 33(44), 17350-62. Ren, X.; Akiyoshi, K.; Dziennis, S.; Vandenbark, A.A.; Herson, P.S.; Hern, P.D.; Offner, H. Regulatory B Cells Limit CNS Inflammation and Neurologic Deficits in Murine Experimental Stroke. J. Neurosci., 2011, 31(23), 8556-63. Liesz, A.; Hu, X.; Kleinshnitz, C.; Offner, H. Functional Role of Regulatory Lymphocytes in Stroke: Facts and Controversies. Stroke, 2015, 46(5), 1422-30. Kim, J.Y.; Kawabori, M.; Tenari, M.A. Innate Inflammatory Responses in Stroke: Mechanisms and Potential Therapeutic Targets. Curr. Med. Chem., 2014, 21(18), 2076-97. Song, Q.; Li, Y.; Wang, Y.; Weim, C.; Liu, J.; Liu, M. Increased Neutrophil-to-lymphocyte Ratios are Associated with Greater Risk of Hemorrhagic Transformation in Patients with Acute Ischemic Stroke. Curr. Neurovasc. Res., 2018, 15(4), 326-35. Guo, Z.; Yu, S.; Xiao, L.; Dynamic Change of Neutrophil to Lymphocyte Ratio and Hemorrhagic Transformation after Thrombolysis in Stroke. J. Neuroinflammation, 2016, 13(1), 199. Wu, Y.; Chen, Y.; Yang, X.; Chen, L.; Yang, Y. Neutrophil-to-Lymphocyte Ratio (NLR) and Platelet-to-Lymphocyte Ratio (PLR) were Associated with Disease Activity in Patients with Systemic Lupus Erythematosus. Int. Immunopharmacol., 2016, 36, 94-9. [20] Adams, H.P. Jr.; Biller, J. Classification of Subtypes of Ischemic Stroke: History of the Trial of org 10172 in Acute Stroke Treatment Classification. Stroke, 2015, 46(5), e114-7. [21] Muchada, M.; Rubiera, M.; Rodriguez-Luna, D.; Pagola, J.; Flores, A.; Kallasm, J.; Sanjuan, E.; Meler, P.; Alvarez-Sabin, J.; Ribo, M.; Molina, C.A. Baseline National Institutes of Health Stroke Scale-adjusted Time Window for Intravenous Tissue-type Plasminogen Activator in Acute Ischemic Stroke. Stroke, 2014, 45(4), 1059-63. [22] Nemeth, T.; Mocsai, A.; Lowell, C.A. Neutrophils in Animal Models of Autoimmune Disease. Semin. Immunol., 2016, 28(2), 174-86. [23] Jickling, G.C.; Liu, D.; Ander, B.P.; Stamova, B.; Zhan, X.; Sharp, F.R. Targeting Neutrophils in Ischemic Stroke: Translational Insights from Experimental Studies. J. Cereb. Blood Flow Metab., 2015, 35(6), 888-901. [24] Benakis, C., Garcia-Bonilla, L., Iadecola, C., Anrather, J. The Role of Microglia and Myeloid Immune Cells in Acute Cerebral Ischemia. Front Cell. Neurosci., 2014, 8, 461. [25] Cai, Z., Zhao, B., Deng, Y., Shangguan S1, Zhou F1, Zhou W1, Li X1, Li Y2, Chen G1. Notch Signaling in Cerebrovascular Diseases (Review). Mol Med Rep., 2016, 14(4), 2883-98. [26] Garcia-Culebras, A.; Duran-Laforet, V.; Pena-Martinez, C.; Ballesteros, I.; Pradillo, J.M.; Diaz-Guzmán, J.; Lizasoain, I.; Moro, M.A. Myeloid Cells as Therapeutic Targets in Neuroinflammation after Stroke: Specific Roles of Neutrophils and Neutrophil-platelet Interactions. J. Cereb. Blood Flow Metab., 2018, 38(12), 2150-64. [27] Ho, W.M.; Reis, C.; Akyol, O.; Applegate, R.; Stier, G.; Martin, R.; Zhang, J.H. Pharmacological Management Options to Prevent and Reduce Ischemic Hemorrhagic Transformation. Curr. Drug Targets, 2017, 18(12), 1441-59. [28] Rosenberg, G.A.; Yang, Y. Vasogenic Edema Due to tight Junction Disruption by Matrix Metalloproteinases in Cerebral Ischemia. Neurosurg. Focus, 2007, 22(5), E4. [29] Rosell,A.;Cuadrado,E.;Ortega-Aznar,A.;Hernandez-Guillamon,M.; Lo, E.H.; Montaner, J. MMP-9-Positive Neutrophil Infiltration is Associated to Blood-brain Barrier Breakdown and Basal Lamina Type IV Collagen Degradation During Hemorrhagic Transformation after Human Ischemic Stroke. Stroke, 2008, 39(4), 1121-6. [30] De Meyer, S.F.; Suidan, G.L.; Fuchs, T.A.; Monestier, M.; Wagner, D.D. Extracellular Chormatin is an Important Mediator of Ischemic Stroke in Mice. Arterioscler. Thromb. Vasc. Biol., 2012, 32(8), 1884-91. [31] Savchenko, A.S.; Borisoff, J.I.; Martinod, K.; De Meyer, S.F.; Gallant, M.; Erpenbeck, L.; Brill, A.; Wang, Y.; Wagner, D.D. VWF-mediated Leukocyte Recruitment with Chormatin Decondensation by PAD4 Increases Myocardial Ischemia/Reperfusion Injury in mice. Blood, 2014, 123(1), 141-8. [32] Huang,H.;Tohme,S.;Al-Khafaji,A.B.;Damage-associatedMolecular Pattern-activatedNeutrophil Extracellular Trap Excacerates Sterile Inflammatory Liver Injury. Hepatology, 2015, 62(2), 600-14. [33] Woiciechowsky, C.; Asadullah, K.; Nestler, D.; Eberhardt, B.; Platzner, C.; Schöning, B.; Glöckner, F.; Lanksch, W.R.; Volk, H.D.; Döcke, W.D. Sympathetic Activation Triggers Systemic Interleukin-10 Release in Immunodepression Induced by Brain Injury. Nat. Med., 1998, 4(7), 808-13. [34] Liesz, A.; Zhou, W.; Na, S.Y.; Boosting Regulatory T Cells Limits Neuroinflammation in Permanent Cortical Stroke. J. Neurosci., 2013, 33(44), 17350-62. [35] Ren, X.; Akiyoshi, K.; Dziennis, S.; Vandenbark, A.A.; Herson, P.S.; Hern, P.D.; Offner, H. Regulatory B Cells Limit CNS Inflammation and Neurologic Deficits in Murine Experimental Stroke. J. Neurosci., 2011, 31(23), 8556-63. [36] Liesz, A.; Hu, X.; Kleinshnitz, C.; Offner, H. Functional Role of Regulatory Lymphocytes in Stroke: Facts and Controversies. Stroke, 2015, 46(5), 1422-30. [37] Kim, J.Y.; Kawabori, M.; Tenari, M.A. Innate Inflammatory Responses in Stroke: Mechanisms and Potential Therapeutic Targets. Curr. Med. Chem., 2014, 21(18), 2076-97. [38] Song, Q.; Li, Y.; Wang, Y.; Weim, C.; Liu, J.; Liu, M. Increased Neutrophil-to-lymphocyte Ratios are Associated with Greater Risk of Hemorrhagic Transformation in Patients with Acute Ischemic Stroke. Curr. Neurovasc. Res., 2018, 15(4), 326-35. [39] Guo, Z.; Yu, S.; Xiao, L.; Dynamic Change of Neutrophil to Lymphocyte Ratio and Hemorrhagic Transformation after Thrombolysis in Stroke. J. Neuroinflammation, 2016, 13(1), 199. [40] Wu, Y.; Chen, Y.; Yang, X.; Chen, L.; Yang, Y. Neutrophil-to-Lymphocyte Ratio (NLR) and Platelet-to-Lymphocyte Ratio (PLR) were Associated with Disease Activity in Patients with Systemic Lupus Erythematosus. Int. Immunopharmacol., 2016, 36, 94-9. ©2020 Inno Science Press