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ORIGINAL ARTICLE

Temporal dynamics of peripheral neutrophil and lymphocytes following acute ischemic stroke

Ashley B. Petrone[1,2,3,4] · Rhae D. Eisenman[4] · Kelsey N. Steele[4] · Lindsey T. Mosmiller[4] · Ogaga Urhie[4] · Matthew J. Zdilla[1,5,6]

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Abstract

Background The immune response to acute ischemic stroke (AIS) is implicated in diagnosis, prognosis, and intervention; however, the temporal dynamics of leukocytes following AIS are poorly understood. The purpose of this study was to characterize peripheral leukocyte dynamics following AIS among individuals with poor and favorable outcomes.

Methods A retrospective chart review was conducted among patients with a diagnosis of AIS who were treated at a comprehensive stroke center across a 3-year timeframe. Groups were defined according to stroke outcomes. Patients with poor outcomes were distinguished from those with favorable outcomes by discharge National Institute of Health Stroke Score, infarct size, and Modified Rankin Scale. Leukocyte counts were compared among controls and AIS outcome groups.

Results The neutrophil-lymphocyte ratio (NLR) calculated at 48–72 h post-AIS was identified as the strongest predictor of outcome. NLR was significantly higher in the poor outcome group (8.68 ± 0.93) compared with both the favorable outcome (4.5 ± 0.51, $p = 0.009$) and control group (4.33 ± 0.66, $p < 0.001$). Patients with a 48–72 h NLR ≥ 4.58 were 5.58 times more likely to have a poor outcome than AIS patients with an NLR < 4.58.

Conclusions The results of this study improve the understanding of the immune response in AIS. Low neutrophil count relative to high lymphocyte count at 48–72 h post-AIS should be considered a predictor of a favorable stroke outcome. Conversely, high neutrophil count relative to low lymphocyte count at 48–72 h post-AIS should be considered a predictor of a poor stroke outcome.

Keywords Stroke · Immune · Leukocyte · Neutrophil · Neutrophil-lymphocyte ratio

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✉ Ashley B. Petrone
abpetrone@hsc.wvu.edu

Rhae D. Eisenman
rdeisenman@mix.wvu.edu

Kelsey N. Steele
knsteele@mix.wvu.edu

Lindsey T. Mosmiller
ltmosmiller@mix.wvu.edu

Ogaga Urhie
ourhie@mix.wvu.edu

Matthew J. Zdilla
mzdilla@westliberty.edu

[1] Department of Pathology, Anatomy, and Laboratory Medicine, West Virginia University, One Medical Center Drive, Morgantown, WV 26506, USA

[2] Department of Neurology, West Virginia University, One Medical Center Drive, Morgantown, WV 26506, USA

[3] Department of Anesthesia, West Virginia University, One Medical Center Drive, Morgantown, WV 26506, USA

[4] West Virginia University School of Medicine, One Medical Center Drive, Morgantown, WV 26506, USA

[5] Department of Natural Sciences & Mathematics, West Liberty University, 208 University Drive, West Liberty, WV 26074, USA

[6] Department of Graduate Health Sciences, West Liberty University, 208 University Drive, West Liberty, WV 26074, USA

Introduction

Acute ischemic stroke (AIS) results in local brain damage, which activates immune cells within the brain and the periphery in an attempt to limit the extent of damage [1]. Neutrophils have been traditionally viewed as harmful following stroke, in both preclinical and clinical models. In preclinical models, inhibition or depletion of neutrophils prior to induced-middle cerebral artery occlusion results in reduced infarct volume and improved functional outcome [2–9]. In clinical models, increased neutrophil counts within 24 h of AIS are associated with larger infarct volumes and worse functional outcomes [10–16].

Based upon the data from clinical and preclinical models, several clinical trials have evaluated the use of compounds that inhibit neutrophil migration into the brain following AIS, but all of these trials have shown no improvement in AIS outcome. Further, several have shown an increase in adverse events, including increased rate of infection and hemorrhagic transformation [17]. A potential pitfall among the clinical trials was a disregard for the timing of dosing relative to the onset of the stroke.

Few studies have evaluated the temporal change of leukocyte numbers over the acute phase of AIS [18–21]. To date, there have been no studies that characterized peripheral leukocyte counts, specifically neutrophils and lymphocytes, in the acute phase following AIS and compared the patterns in AIS patients stratified by clinical outcomes.

The neutrophil-lymphocyte ratio (NLR) has been widely reported as a prognostic biomarker for AIS, as an increased NLR has been associated with increased infarct volume and worse functional outcome following AIS [15, 22–29]. However, there have been no studies evaluating the prognostic value of the NLR obtained beyond 24 h of AIS.

The purpose of this study is to identify the temporal change in peripheral leukocyte counts—neutrophil, lymphocyte, and NLR—over the course of 72 h post-AIS. We hypothesize that AIS patients with a poor outcome will have a distinct peripheral leukocyte pattern in the acute phase following AIS compared with AIS patients with a favorable outcome. Second, we hypothesize that AIS patients with poor outcome will have an excessive and/or sustained increase in neutrophil count, as well as a sustained decrease in lymphocyte count, leading to an elevated NLR, compared with AIS patients with a favorable outcome.

Materials and methods

Study design

This study received institutional review board approval at West Virginia University. A retrospective chart review of a random sample of patients treated at a local comprehensive stroke center (Morgantown, WV) from 2012 to 2015 with a diagnosis of AIS (ICD9:434.91, ICD10:I63) was performed. The exclusion criteria for selection were summarized in Table 1. Signs of infection were defined as any known infection within 14 days of admission, antibiotic use on admission, fever upon admission, suspected/diagnosed infection at any point in hospitalization, and antibiotic prescription during hospitalization. Immunosuppressant use was screened for common corticosteroids or biologic immunosuppressants. In addition, patients must have had a white blood cell differential performed at three-time points following their last known normal, 0-24 h, 24-48 h, and 48-72 h, and a Modified Rankin Scale score recorded 60-90 days post-AIS.

The following data were collected from patient medical records: past medical history, National Institute of Health Stroke Scale (NIHSS) upon presentation to the emergency department and at discharge, white blood cell differentials at 0-24 h, 24–48 h, and 48–72 h from last seen normal, neuroimaging (CT or MRI), and Modified Rankin Scale score recorded 60–90 days post-AIS. Neutrophil and lymphocyte counts were recorded from the white blood cell differential in addition to neutrophil-lymphocyte ratio.

The control population ($n = 24$) consisted of patients with peripheral venous disease who were seen in clinic for a non-emergent appointment with white blood cell differential performed. This control group was matched to the AIS subject cohort for past medical history.

Infarct volume calculation

Infarct volume was calculated from CT or MRI images using the iPlan Cranial 3.0 software (BRAINLAB ©). Infarct volumes were calculated by two blinded investigators, under the supervision of a neuroradiologist.

AIS outcome grouping

Patients who experienced AIS were sorted into a poor outcome group and a favorable outcome group, respectively. Poor outcome was defined by having at least one of the following criteria: (1) Discharge NIHSS > 14 (severe), (2) large

Table 1 Study exclusion criteria

- Age < 18 years or > 89 years
- Unknown last known normal
- Symptom onset > 24 h prior to admission
- Pregnant women
- History of cancer or autoimmune disease
- Sign of infection at admission or during hospitalization
- Immunosuppressant use
- History of substance abuse
- Hospitalization or surgery within 30 days of admission

infarct = volume greater than sample median (~ 40 cm^3) or (3) a Modified Rankin Scale score > 2 at 60–90 days post-AIS. For the study population, $n = 33$ AIS patients were classified as poor outcome and 39 as favorable outcome.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics ® (Version 24) software, and p values < 0.05 were considered statistically significant. Grubb's outlier testing was used to detect any significant outliers in all study variables and was removed prior to analysis. For each continuous variable, a Shapiro-Wilk test was used to test for normality. An independent samples T test or one-way ANOVA was used to detect mean differences for normally distributed variables; otherwise, a Mann-Whitney U test or Kruskal-Wallis one-way ANOVA was performed. A repeated measures ANOVA was used to detect an effect of time on neutrophil count, lymphocyte count, and NLR. For all comparisons, linear regression analysis was used to assess for the effect of confounding variables, such as age, gender, medical history for each study variable. Receiver operating characteristic (ROC) curve analysis, with binary logistic regression, was used to determine the strength of the NLR as a predictor of outcome.

Results

Clinical characteristics

The total study population consisted of 96 participants: $n = 24$ control subjects, $n = 39$ AIS favorable outcome and $n = 33$ AIS poor outcome. The demographic information for the study population is shown in Table 2. There were no significant differences in demographics between the control group and the total AIS group; however, the favorable and poor outcome AIS groups were significantly different for two variables—hypertension and infarct volume. The proportion of patients with hypertension in the poor outcome AIS group was significantly higher than the favorable outcome group ($x^2 = 8.7$, $p = 0.014$), and expectedly, the mean infarct volume in the poor outcome AIS group was significantly higher than the favorable outcome group ($U = 6.5$, $p = 0.022$).

Neutrophil count

There was a statistically significant interaction between outcome group and time with regard to neutrophil count, (F (1.787, 119.738) = 9.716, $p < 0.0005$, partial $\eta^2 = 0.127$, $\varepsilon = 0.894$).

At the initial baseline evaluation post-stroke, neutrophil count was significantly higher in both the favorable outcome ($6.9 \pm 0.44 \times 10^3$ cells/uL, $p = 0.014$) and the poor outcome group ($7.5 \pm 0.48 \times 10^3$ cells/uL, $p < 0.001$) compared with control ($4.9 \pm 0.42 \times 10^3$ cells/uL). There was no difference between neutrophil count between the favorable and poor outcome AIS groups at baseline ($p = 0.949$). At 24–48 h, neutrophil count was significantly higher in both the favorable outcome ($6.6 \pm 0.40 \times 10^3$ cells/uL, $p = 0.002$) and the poor outcome group (8.7 ± 0.49 cells/uL, $p < 0.001$) compared with control ($4.9 \pm 0.42 \times 10^3$ cells/uL). Further, neutrophil count was significantly higher in the poor outcome group compared to favorable outcome ($p = 0.004$). At 48–72 h, neutrophil count in the poor outcome group ($9.01 \pm 0.42 \times 10^3$ cells/uL) was significantly higher than both the favorable outcome ($6.5 \pm 0.40 \times 10^3$ cells/uL, $p < 0.001$) and the control group ($4.9 \pm 0.42 \times 10^3$ cells/uL, $p < 0.001$); however, there was no significant difference between the favorable outcome group and control ($p = 0.144$) (Fig. 1).

Lymphocyte count

There was a moderate interaction between outcome group and time on lymphocyte count (F (2,132) = 2.453, $p = 0.09$, partial $\eta^2 = 0.036$).

At baseline, lymphocyte counts did not differ between any of the groups ($p = 0.164$). At 24–48 h, lymphocyte count was

Table 2 Study population characteristics

	Control ($n = 24$)	Favorable outcome AIS ($n = 39$)	Poor outcome AIS ($n = 33$)	P value
Age (median ± SD years)	72 ± 6	65 ± 14	73 ± 12	0.149
Sex (% female)	54	54	52	0.975
Hypertension (%)	83	77	100	*0.014*
Heart disease (%)	63	54	64	0.665
Diabetes (%)	38	31	49	0.311
Atrial fibrillation (%)	33	28	55	0.061
Dyslipidemia (%)	54	77	73	0.149
Prior stroke (%)	29	26	27	0.955
Infarct volume (median ± SD cm^3)	–	22 ± 14	109 ± 19	*0.022*

Italicized values are p-values < 0.05

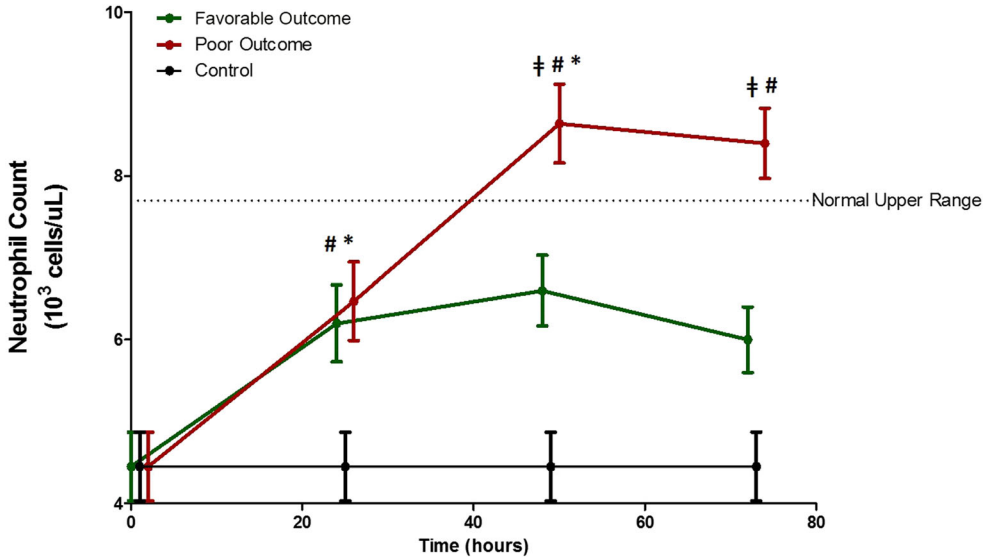

Fig. 1 Temporal neutrophil dynamics post-AIS by outcome group. Neutrophil count (mean ± SEM) is plotted over time at three intervals. Control is displayed in black, favorable outcome acute ischemic stroke (AIS) in green, and poor outcome AIS in red. The gray dashed line represents the upper limit of normal neutrophil count in a healthy individual. Statistical significance is set at $p < 0.05$, and *represents a significant different between control and favorable outcome AIS, #represents a significant different between control and poor outcome AIS, and ‡represents a significant different between poor and favorable outcome AIS groups

significantly higher in the favorable outcome ($1.74 \pm 0.12 \times 10^3$ cells/uL, $p = 0.003$) compared with the poor outcome group ($1.24 \pm 0.08 \times 10^3$ cells/uL); however, neither the favorable outcome ($p = 0.083$) nor the poor outcome group ($p = 0.840$) differed from control. At 48–72 h, lymphocyte count was significantly higher in the favorable outcome ($1.77 \pm 0.11 \times 10^3$ cells/uL, $p = 0.001$) compared with both the poor outcome group ($1.23 \pm 0.08 \times 10^3$ cells/uL, $p = 0.001$) and control ($1.39 \pm 0.08 \times 10^3$ cells/uL, $p = 0.034$). There was no significant difference between the poor outcome group and control ($p = 0.998$) (Fig. 2).

Neutrophil-lymphocyte ratio

There was a statistically significant interaction between outcome group and time on NLR, (F (4,174) = 3.123, $p = 0.016$, partial $\eta^2 = 0.067$).

At baseline, NLR was significantly higher in poor outcome group (6.77 ± 0.85, $p < 0.001$) compared with control (4.33 ± 0.66); however, there was no significant difference between control and favorable outcome (4.93 ± 0.47, $p < 0.001$). At 24–48 h, NLR was significantly higher in the poor outcome group (8.41 ± 0.91) compared with both the favorable outcome (4.93 ± 0.47, $p = 0.001$) and control group (4.33 ± 0.66, $p < 0.001$). There was no significant difference between the favorable outcome group and control at 24–48 h ($p = 0.918$). Similarly, at 48–72 h, NLR was significantly higher in the poor outcome group (8.68 ± 0.93) compared to both the favorable outcome (4.5 ± 0.51, $p = 0.009$) and control group (4.33 ± 0.66, $p < 0.001$). There was no significant difference between the favorable outcome group and control at 48–72 h ($p = 0.537$). (Fig. 3).

NLR as a predictor of poor outcome

A ROC curve was generated to assess the ability of the NLR, at baseline and 48–72 h, to accurately predict poor outcome in AIS (Supplemental Fig. 1. The 48–72 h NLR was a strong predictor of poor outcome (AUC = 0.804), whereas the baseline NLR was an overall weak predictor of poor outcome (AUC = 0.583). The study used the ROC curve to identify the optimal cut point value for prediction, maximizing both sensitivity and specificity. At 48–72 h, an NLR ≥ 4.58 conferred 76% sensitivity and 66% specificity, and at baseline, an NLR of ≥ 4.29 conferred 55% sensitivity and 56% specificity. Using these optimal values, we used binomial logistic regression to determine the ability of the NLR to predict poor outcome, when controlling for age, gender, cardiovascular risk factors, and infarct volume.

A binomial logistic regression was performed to determine the effects of age, gender, cardiovascular risk factors, infarct volume, baseline NLR, and 48–72 h NLR on the likelihood that AIS patients will have a poor outcome. Of the eleven predictor variables, after forward variable selection, only infarct volume and 48–72 NLR remained in the model as statistically significant predictor variables (Table 3). Further, AIS patients with a 48–72 h NLR ≥ 4.58 were 5.58 times more likely to have a poor outcome than AIS patients with an NLR < 4.58 (Table 3).

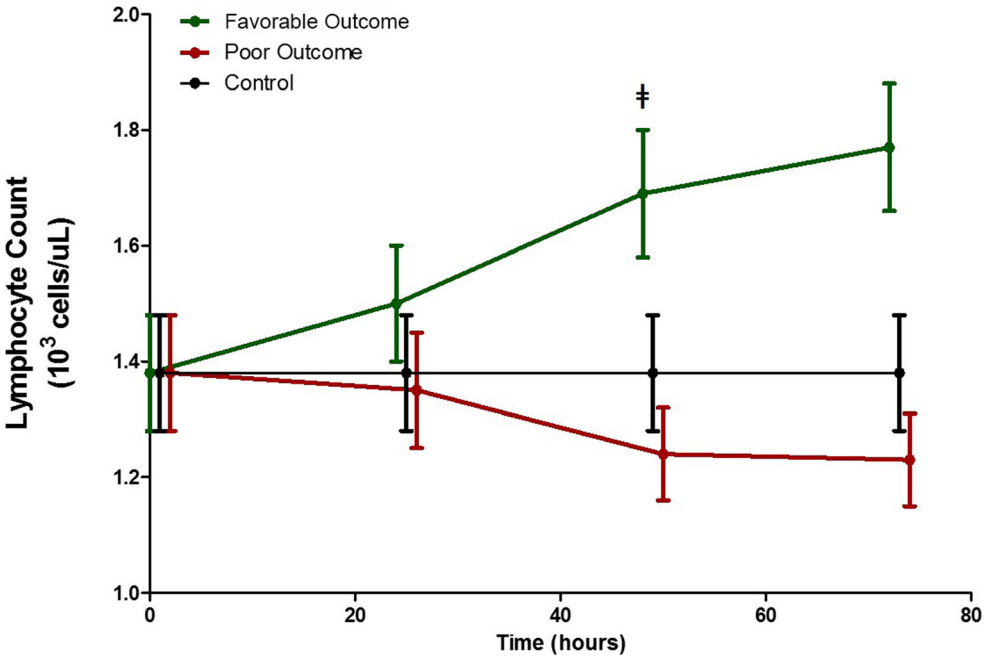

Fig. 2 Temporal lymphocyte dynamics post-AIS by outcome group. Lymphocyte count (mean ± SEM) is plotted over time at three intervals. Control is displayed in black, favorable outcome acute ischemic stroke (AIS) in green, and poor outcome AIS in red. Statistical significance is set at $p < 0.05$, and ‡represents a significant different between poor and favorable outcome AIS groups

Infarct volume—neutrophil and correlations

There was a significant positive correlation between infarct volume and neutrophil count ($r = 0.317$, $p = 0.012$). Further, when AIS were grouped into small (< 40 cm^3) and large (≥ 40 cm^3) infarct volume groups, neutrophil count was significantly higher in the large infarct group ($8.3 \pm 0.59 \times 10^3$ cells/uL) compared with the small infarct group ($6.5 \pm 0.49 \times 10^3$ cells/uL), $p = 0.024$).

Next, patients were grouped into four groups, based on both infarct volume and outcome (favorable outcome, small infarct ($n = 21$) vs. favorable outcome, large infarct ($n = 11$) vs. poor outcome, small infarct ($n = 11$) vs. poor outcome, large infarct ($n = 21$)). At baseline, there were no significant differences in neutrophil count between any of the groups ($p = 0.301$). At 24–48 h, neutrophil count was highest in the large infarct, poor outcome group compared with the other groups ($9.0 \pm 0.61 \times 10^3$ cells/uL). Further, while neutrophil

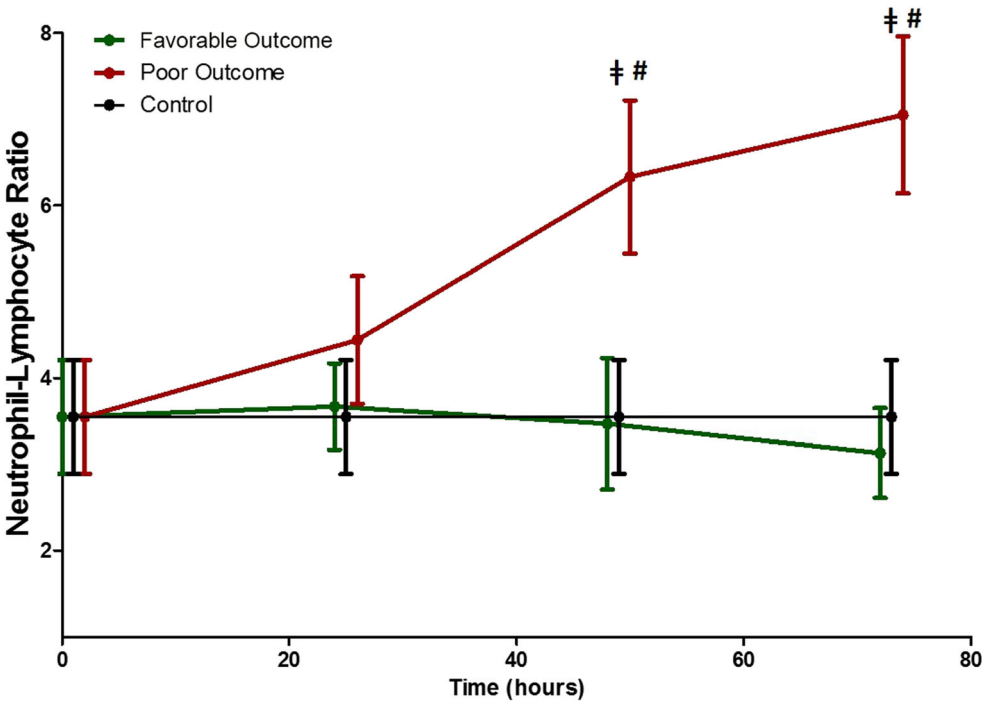

Fig. 3 Change in neutrophil-lymphocyte ratio post-AIS by outcome group. Neutrophil-lymphocyte ratio (NLR) is plotted over time at three intervals. Control is displayed in black, favorable outcome acute ischemic stroke (AIS) in green, and poor outcome AIS in red. The dashed line represents the upper limit of normal neutrophil count in a healthy individual. Statistical significance is set at $p < 0.05$, and #represents a significant different between control and poor outcome AIS, and ‡represents a significant different between poor and favorable outcome AIS groups

Table 3 Logistic regression

	B	S.E	Wald	Df	Sig	Exp (B)	95% CI for Exp (B) Lower. Upper	
48–72 h. NLR	0.184	0.097	3.59	1	0.048	1.2	1.19	1.45
Infarct volume	0.020	0.006	10.139	1	0.001	1.02	1.01	1.03
48-72 h. NLR ≥ 4.58	1.72	0.569	10.693	1	0.001	5.58	1.99	15.64

count in large infarct, poor outcome group was not significantly higher compared with the large infarct, favorable outcome group ($7.8 \pm 0.79 \times 10^3$ cells/uL, $p = 1.000$) nor the small infarct, poor outcome group ($7.5 \pm 0.73 \times 10^3$ cells/uL, $p = 0.757$), neutrophil count was significantly higher in the large infarct, poor outcome group compared with small infarct, favorable outcome group ($6.1 \pm 0.54 \times 10^3$ cells/uL, $p = 0.004$). Similarly, at 48–72 h, neutrophil count was highest in the large infarct, poor outcome group compared with the other groups ($8.9 \pm 0.57 \times 10^3$ cells/uL). Further, while neutrophil count in large infarct, poor outcome group was not significantly higher compared with the large infarct, favorable outcome group ($6.9 \pm 0.42 \times 10^3$ cells/uL, $p = 0.232$) nor the small infarct, poor outcome group ($8.2 \pm 0.44 \times 10^3$ cells/uL, $p = 1.000$), neutrophil count was significantly higher in the large infarct, poor outcome group compared to small infarct, favorable outcome group ($6.5 \pm 0.64 \times 10^3$ cells/uL, $p = 0.014$) (Fig. 4).

The neutrophil-infarct ratio (NIR) was calculated as a means to quantify the neutrophil counts as a product of infarct volume. Because of the aforementioned differences in neutrophil count at 48–72 h, the study calculated the NIR using the neutrophil counts at 48–72 h. The median NIR in the large infarct volume group ($16.5 \pm 8.9 \times 10^3$ cells/uL per cm^3) was higher than the small infarct volume group ($7.8 \pm 5.7 \times 10^3$ cells/uL per cm^3). Further, median NIR in the large infarct, poor outcome group ($17.2 \pm 10.9 \times 10^3$ cells/uL per cm^3) was higher than the large infarct, favorable outcome group ($10.5 \pm 18.6 \times 10^3$ cells/uL per cm^3). Similarly, median NIR in the small infarct, poor outcome group ($8.8 \pm 18.3 \times 10^3$ cells/uL per cm^3) was higher than the small infarct, favorable outcome group ($6.9 \pm 4.0 \times 10^3$ cells/uL per cm^3) (not shown).

Discussion

This study is the first to identify a temporal leukocyte profile associated with a favorable outcome versus a poor outcome following AIS.

Similar to previous studies, this study has confirmed that peripheral neutrophil count is elevated in stroke patients, regardless of outcome, compared with control subjects. This study has elaborated upon previous studies by comparing peripheral leukocyte counts between control, favorable outcome AIS, and poor outcome AIS groups, rather than comparing control with total stroke, as has been done traditionally. At baseline, peripheral neutrophil count is significantly higher in both AIS groups compared with controls; there was no significant difference in neutrophil count between favorable and poor outcome AIS groups. This is an important result that suggests that an initial rise in neutrophil count is seen in the favorable outcome group, thus may represent a critical, physiological response to injury rather than a pathological one.

We hypothesize that there is a certain peripheral neutrophil response that is proportional the amount of brain damage, or infarct volume, and both a failure to respond at correct magnitude or an excessive neutrophil response may be detrimental. This concept was strengthened by the use of the neutrophil-infarct ratio (NIR) in this study. To our knowledge, no other published study has attempted to utilize this ratio as a biomarker in AIS, nor any other disease state, but we believe the NIR is a measure that captures the proportionality of the peripheral neutrophil response with respect to infarct volume. While this was a secondary objective in this manuscript, and thus should viewed as preliminary evidence, we showed that the median NIR in the poor outcome group was higher than the favorable outcome group. The study did not establish an "optimal" NIR associated with favorable outcome, suggesting that AIS patients with a poor outcome likely have a peripheral neutrophil response excessive relative to infarct volume. Future studies are warranted to scrutinize this result.

We also found that at 48–72 h post-AIS, neutrophil count in the poor outcome group was significantly higher than both the favorable outcome and the control group; however, there was no significant difference between the favorable outcome group and control. This indicates that while both the favorable and poor outcome AIS groups have an initial increase in neutrophil count, the response decreases to the level of control in the favorable outcome group by 48–72 h post-AIS, but remains significantly, likely excessively, elevated in the poor outcome group. Overall, these results are in line with our initial hypothesis that increases in neutrophil count would be excessive and/or sustained for a longer time period than in the favorable outcome group.

Similarly, this study has yielded a temporal pattern in neutrophil-lymphocyte ratio (NLR) that distinguishes favorable versus poor outcome AIS patients. In agreement with previous literature, an elevated NLR is associated with poor outcome; however, this study suggests that, similar to

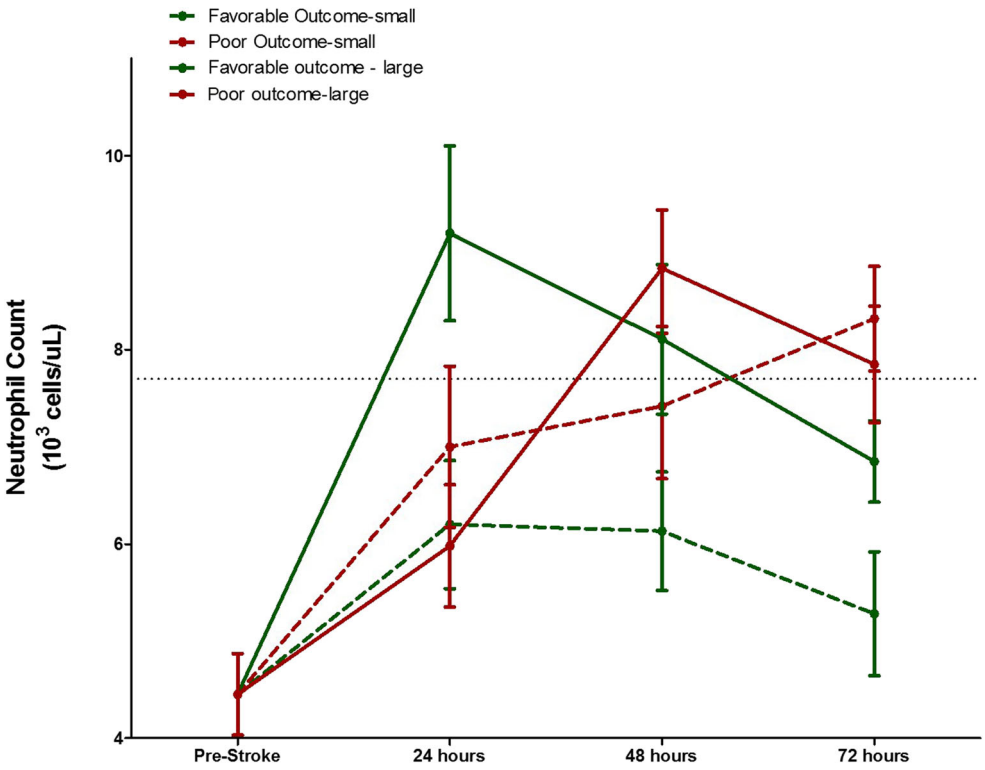

Fig. 4 Neutrophil dynamics by outcome group and infarct volume. Neutrophil count (mean ± SEM) is plotted at three intervals. Favorable outcome acute ischemic stroke (AIS) with small infarct is represented as an open circle, favorable outcome acute AIS with large infarct as a filled circle, poor outcome acute AIS with small infarct as an open triangle, and poor outcome acute AIS with large infarct as a filled triangle

neutrophil count, the prognostic value of the NLR is altered at different time points' post-AIS. In contrast to previous studies, the NLR at baseline was not a significant predictor of outcome. The difference can likely be attributed to the definition of poor outcome used in this study versus those in the past. Previous studies evaluating the use of the baseline NLR as a prognostic biomarker for outcome have defined outcome using either NIHSS (stroke severity), MRS (functional outcome), or infarct volume. Because this study combined all three criteria to determine outcome status in this study, it is likely that the study populations would not be identical in outcome grouping. The novel outcome classification utilized in this study encompasses multiple factors that have been traditionally used to categorize outcome, rather than a single factor, and allows for more robust outcome stratification. To our knowledge, this is the first study that has supported the use of the NLR at 48–72 h rather than baseline. We posit that the NLR at time of discharge would have similar a prognostic value to the NLR at 48–72 h, and recommend that this notion be tested in future studies.

While the purpose of this study was to characterize the temporal dynamics of leukocytes following AIS, not to investigate the molecular mechanisms regulating this response, we hypothesize the following pathophysiologic mechanism may underlie our findings. Following AIS, dying brain cells in the infarct and penumbra release danger associated molecular patterns and other signals that stimulate neutrophil migration and infiltration into the brain [1, 30–32]. Neutrophil infiltration occurs within minutes to hours of AIS onset, but typically resolves within 24 h [32–35]. While neutrophils are the most abundant immune cell to migrate into the brain in the acute phase of AIS, adaptive immune cell activation and subsequent infiltration into the brain is delayed, and several studies have reported peak lymphocyte infiltration within 24–72 h of AIS [33, 36–39].

Neutrophils have been shown release several enzymes or other inflammatory mediators that suppress lymphocyte activation. For example, neutrophils can release serine proteases that cleave interleukin-2 and interleukin-6 receptors from the surface of T lymphocytes, which are required for activation [40]. Further, neutrophils have also been shown to release the enzyme arginase 1, which can downregulate expression of the T cell receptor, through L-arginine depletion [41]. The role of lymphocytes in AIS is quite complex, given the number of distinct T lymphocyte populations, including CD4+ helper T cells, which can be further divided into TH_1, TH_2, and regulatory T cells, and CD8+ cytotoxic T cells. Depending on the subtype, T lymphocytes have been shown to play either a beneficial or detrimental role in AIS, or most commonly CD8+ T cells and TH_1 cells are considered detrimental, whereas TH_2 and regulatory T cells may be beneficial [42]. Similarly, several phenotypes of neutrophils have been isolated from the blood, and different populations of suppressive versus non-suppressive neutrophils are likely present in the absolute neutrophil count [35, 43, 44] . Taken altogether, our hypothesis is that sustained neutrophil expression beyond 24 h post-AIS suppresses the beneficial adaptive immunity

lymphocyte response that should peak from 24 to 72 h post-AIS, leading to excessive inflammation and poor outcome. Future studies are required to validate this hypothesis, through isolation and temporal quantification of specific neutrophil and lymphocyte populations' post-AIS.

Summary

This study has characterized the peripheral leukocyte dynamics following AIS and identified a leukocyte pattern associated with favorable versus poor outcome following AIS. Poor outcome AIS patients have an excessive and/or extended peripheral neutrophil count for 72 h post-AIS, compared with favorable outcome AIS patients. Also, poor outcome AIS patients have a decreased lymphocyte count, leading to an increased NLR. Though an elevated baseline NLR at baseline has been traditionally used as a predictor of poor outcome, our findings suggest that the NLR calculated at 48–72 h post-AIS was the strongest predictor of outcome in this study. These results may shed important light on the failure of the previous clinical trials that targeted neutrophils in AIS and strengthen the theory that these failures may have been at least partially attributed to an improper timing of administration. Furthermore, this study contributes to a more thorough understanding of the temporal immune response to AIS, promoting more successful development of immunomodulatory compounds in the future. Lastly, the NIR may be more informative with regard to intention to treat, rather than neutrophil count alone; however, this requires validation in future studies.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics statement Research Involving Human Participants: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee, and this study received approval from the WVU Institutional Review Board.

Informed consent This study was a retrospective chart review, thus did not require informed consent.

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