Early Neutrophilia Is Associated With Volume of Ischemic Tissue in Acute Stroke

Brian H. Buck, MD; David S. Liebeskind, MD; Jeffrey L. Saver, MD; Oh Young Bang, MD, PhD; Susan W. Yun, BSc; Sidney Starkman, MD; Latisha K. Ali, MD; Doojin Kim, MD; J. Pablo Villablanca, MD; Noriko Salamon, MD; Tannaz Razinia, BSc; Bruce Ovbiagele, MD

Background and Purpose—Few data exist on the relationship between differential subpopulations of peripheral leukocytes and early cerebral infarct size in ischemic stroke. Using diffusion-weighted MR imaging (DWI), we assessed the relationship of early total and differential peripheral leukocyte counts and volume of ischemic tissue in acute stroke.

Methods—All included patients had laboratory investigations and neuroimaging collected within 24 hours of stroke onset. Total peripheral leukocyte counts and differential counts were analyzed individually and by quartiles. DWI lesions were outlined using a semiautomated threshold technique. The relationship between leukocyte quartiles and DWI infarct volumes was examined using multivariate quartile regression.

Results—173 patients met study inclusion criteria. Median age was 73 years. Total leukocyte counts and DWI volumes showed a strong correlation (Spearman rho = 0.371, P < 0.001). Median DWI volumes (mL) for successive neutrophil quartiles were: 1.3, 1.3, 3.2, and 20.4 (P for trend < 0.001). Median DWI volumes (mL) for successive lymphocyte quartiles were: 3.2, 8.1, 1.3, and 1.5 (P = 0.004). After multivariate analysis, larger DWI volume remained strongly associated with higher total leukocyte and neutrophil counts (both probability values < 0.001), but not with lymphocyte count (P = 0.4971). Compared with the lowest quartiles, DWI volumes were 8.7 mL and 12.9 mL larger in the highest quartiles of leukocyte and neutrophil counts, respectively.

Conclusions—Higher peripheral leukocyte and neutrophil counts, but not lymphocyte counts, are associated with larger infarct volumes in acute ischemic stroke. Attenuating neutrophilic response early after ischemic stroke may be a viable therapeutic strategy and warrants further study. (Stroke. 2008;39:355-360.)

Key Words: diffusion-weighted imaging ■ inflammation ■ ischemic ■ leukocytosis ■ magnetic resonance imaging ■ stroke

In stroke patients, little information is available regarding the relationship of circulating leukocyte subtypes and the extent of ischemic brain injury, before the presence of substantial established infarction. The objective of this study was to examine total and differential leukocyte counts in the first 24 hours after stroke symptom onset. We hypothesized that a rise in leukocyte counts and specifically the neutrophil fraction would be associated with the volume of bioenergetically compromised brain tissue as measured with diffusion-weighted (DWI) MRI.

Methods

Study Population
This is a secondary analysis of a prospectively maintained database of consecutive ischemic stroke patients admitted to a university stroke program. Criteria for inclusion in this study were: age ≥18

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From the Division of Neurology (B.H.B.), Sunnybrook Health Sciences Centre, University of Toronto, Canada; the Stroke Center and Department of Neurology (B.H.B., D.S.L., J.L.S., O.Y.B., S.W.Y., S.S., L.K.A., D.K., T.R., B.O.), University of California, Los Angeles; the Department of Neurology (O.Y.B.), Samsung Medical Center, Sungkyunkwan University, South Korea; the Stroke Center and Department of Radiology (J.P.V., N.S.), University of California, Los Angeles; and the Department of Emergency Medicine (S.S.), University of California, Los Angeles.
Correspondence to Brian H. Buck MD, FRCP(C), Division of Neurology, Sunnybrook Health Sciences Centre, 2075 Bayview Avenue, room A421, Toronto, Ontario, Canada M4N3M5. E-mail brianhbuck@gmail.com
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years, diagnosis of acute ischemic stroke, multimodal MRI performed within 24 hours of last known well time, peripheral leukocyte count and differential performed on admission blood work, and admission during an 18-month period beginning January 1, 2004. The study was approved by the hospital Institutional Review Board.

MRI Methods and Image Analysis
Patients were imaged before receiving any reperfusion therapy with a 1.5-T Siemens Vision scanner (Siemens Medical System) using a protocol detailed previously,10 which included DWI, perfusion weighted imaging, gradient-recalled echo examination, and fluid attenuated inversion recovery imaging (FLAIR). DWI was performed with 2 levels of diffusion sensitization (b=0 and 1000 s/mm²) with the following parameters: 5 to 7 mm slice thickness, no gap, and 17 to 20 slices. DWI lesion volumes were measured with MIPAV software (Medical Image Processing, Analysis and Visualization, version 3.0, National Institutes of Health, Bethesda, Md). Raters outlined regions of acute diffusion abnormality on the b=1000 image, consulting apparent diffusion coefficient and FLAIR sequences to distinguish acute from nonacute diffusion change. Acute diffusion lesions were defined on a slice-by-slice basis using a semiautomatic threshold approach by a rater blinded (BHB) to all clinical information. Lesion volumes were calculated by multiplying slice thickness by the total lesion area. To assess interrater reliability, lesion volumes were measured by a second rater (OYB) on a randomly selected subset of 19 patients.

Statistical Methods
Admission total leukocyte, lymphocyte, and neutrophil counts were collapsed into quartiles. Patient characteristics and other relevant admission variables were compared across quartiles to look for any baseline differences. Nonnormally distributed continuous variables were compared across quartiles according to median values and tested for statistical significance using the Kruskal–Wallis rank sum test. Differences in categorically variables were compared using a χ² test. Where multiple post-hoc tests were performed as with the comparisons of stroke subtypes between leukocyte, lymphocyte, and neutrophil quartiles a Bonferroni method was used to maintain an α of 0.05. Interrater reliability for DWI measurements was assessed by calculation of the intraclass correlation coefficient with greater than 0.80 set as the threshold for good agreement.

The distribution of DWI volumes was assessed for deviation from normality and was skewed to the left, with more small than large lesion volume patients in the study population. Because of the non-Gaussian distribution for DWI lesion volumes, median (or quartile) regression was selected to examine the relationship between leukocyte counts, DWI volumes, and clinical outcomes, because it is less sensitive than parametric regression methods to extreme outliers.11

Median regression was used to examine the association of admission leukocyte, lymphocyte, and neutrophil counts with DWI volume. The regression analysis was performed in 2 stages. First the bivariate relationship between WBC quartiles and imaging was examined. Next, multivariate median was used to correct for potential confounding variables. Covariates were selected based on prior literature,12,13 and included the following categorical variables: history of atrial fibrillation, previous stroke, history of statin use, diabetes, and the stroke subtype; and continuous variables: age, blood glucose, temperature, time to MRI scan, and systolic blood pressure. Statistical analysis was performed using STATA version 9.1 software (Stata Corporation).

Results
One hundred seventy-three of 322 patients met the study criteria. Reasons for exclusion were: presented outside of 24 hours, 85; no leukocyte count or MRI was available, 64. Of the 173 patients, 163 patients had a differential performed on the total leukocyte count. Among the 173 patients, median age was 73 (range 24 to 100) and 101 (50.5%) were women. Racial distribution was 66% white, 12% black, 13% Asian, and 0.6% other. Ethnic distribution was 9% Hispanic and 91% not Hispanic. The median NIHSS on presentation was 4 (interquartile [IQR] range 2 to 12; full range 0 to 38) and the mean±SD time between last known well time and the MRI scan was 10.7±7.9 hours (range 0.7 to 24). Stroke subtypes classified using the modified TOAST criteria were: 41% cardioembolic, 17.4% large vessel atherothromboembolic, 25.4% small vessel occlusion, 13.3% stroke of undefined etiology, and 5.8% other etiology. On discharge, 94 (54.3%) had poor outcome defined as discharge Rankin ≥2.

Leukocyte count was measured at a mean±SD of 9.6±7.3 hours after stroke onset. The mean±SD total peripheral leukocyte, neutrophil, and lymphocyte counts were: 8.73±3.36, 6.17±3.15, and 1.83±0.83×10⁶ cells/L, respectively. The mean±SD time between the MRI and leukocyte collection was 1.2±5.3 hours.

Table 1 shows population demographic information, risk factors, and admission clinical characteristics by quartile of leukocyte count. Patients with high leukocyte counts were more often Hispanic and tended to have higher baseline stroke severity NIH Stroke Scale scores. There were no other differences in the premorbid risk factor profile, use of antithrombotic and statin medication, admission blood pressure, and temperature across leukocyte quartiles. Additionally, the time between stroke onset and collection of MRI and leukocyte bloodwork did not differ between quartiles.

Across all patients, the median DWI lesion volume was 2.9 mL (IQR 0.52 to 19.75 mL, full range 0 to 279.0 mL), and there was good interrater reliability in the measurement of DWI lesion volumes (intraclass correlation coefficient=0.985). The distribution of DWI volumes was positively skewed reflecting a larger proportion of patients with smaller lesion volumes (skewness=3.4, kurtosis=13.2). Median DWI volumes (IQR) for small vessel, cardioembolic, large vessel, other, and unknown TOAST subgroups were, respectively: 0.38 mL (0.26 to 0.97), 7.24 mL (1.89 to 35.17), 5.12 mL (1.19 to 12.51), 15.86 mL (1.22 to 63.24), and 10.84 mL (0.62 to 51.37).

Association Between Total Leukocyte Quartiles and Stroke Subtype
The distribution of TOAST stroke subtypes differed across leukocyte quartiles and neutrophil quartiles (P<0.001) but not lymphocyte quartiles. The percentage of patients with small vessel, cardioembolic, large vessel, other, and unknown classifications within each leukocyte and neutrophil quartile are shown in Figure 1. Pairwise comparisons of the proportions of patients with a given stroke subtype were performed across leukocyte and neutrophil quartiles. In the lowest leukocyte quartile, 50% of strokes were classified as small vessel occlusion, which was a greater proportion than in the 3 higher leukocyte quartiles (19%, 23% and 9%, respectively; P<0.05). The proportions of cardioembolic and large vessel strokes mechanisms did not differ between leukocyte quartiles. Across neutrophil quartiles, the proportion of small vessel occlusion was larger in the lowest compared with the highest quartile (43% versus 8%, P<0.05). None of the other differences were significant.
Association of Total Leukocyte, Lymphocyte, and Neutrophil Quartiles With DWI Lesion Volume

The median (IQR) DWI lesion volumes by quartile of total leukocyte count, neutrophil count, and lymphocyte count are shown in Table 2, including both univariate and adjusted multivariate analyses. DWI volume was positively correlated with total leukocytes (Spearman rho = 0.371, \(P = 0.001\)) and neutrophils (Spearman rho = 0.415, \(P = 0.001\)) but not lymphocytes. On bivariate median regression analysis, higher leukocyte quartiles were significantly associated with larger DWI lesion volumes (Table 2). Patients in the highest leukocyte quartile had significantly larger median lesion volumes than the lower 3 quartiles. None of the 3 lower quartiles differed significantly from each other.

Both neutrophil and lymphocyte counts were significantly associated with DWI lesion volume. The results for the neutrophil count were similar to that of the total leukocyte count. The volume in the highest quartile was significantly larger than the lower 3 quartiles. DWI volumes in the lower 3 neutrophil quartiles did not differ significantly from each other. The pattern for the lymphocyte count was different and showed the DWI lesion volume in the second quartile to be significantly larger than the other 3 quartiles.

The multivariate estimated effect sizes of total leukocyte and neutrophil quartiles on DWI lesion volumes showed a similar pattern to the bivariate analysis. Elevated leukocyte and neutrophil count remained positively associated with DWI lesion volumes. Figure 2 shows the multivariate adjusted median DWI volumes, IQR, and 95% CI for each of the neutrophil quartiles. Pairwise comparison showed that the difference in DWI volumes in the highest neutrophil quartile was significantly larger than the lower 3 quartiles (\(P = 0.001\)).
The difference in DWI volumes across the other 3 neutrophil quartiles was not significant. After adjusting for covariates, the association between lymphocyte counts and DWI volume was no longer significant.

**Discussion**

We found that in ischemic stroke patients evaluated within 24 hours of symptom onset, elevated peripheral total leukocyte and neutrophil counts were associated with larger volumes of ischemic tissue as measured by DWI MRI. The volumes of DWI abnormality in patients in the highest total leukocyte and neutrophil quartiles were substantially larger than in the lowest quartile. This effect partially reflected the larger proportion of patients with small vessel stroke noted in the lowest total leukocyte and neutrophil quartiles; however, even after adjusting for stroke mechanism and other potentially confounding variables there remained a robust finding.

**Table 2. Infarct Volumes According to Quartiles of Total Leukocyte, Lymphocyte, and Neutrophile Count (×10⁹ cells/L) for Univariate and Multivariate Models**

<table>
<thead>
<tr>
<th></th>
<th>Univariate DWI Volume (mL) Median (IQR)</th>
<th>Multivariate* Δ DWI Volume (mL) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total leukocyte</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1</td>
<td>&lt;6.5</td>
<td>0.9 (0.3,2.0)</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>6.5–7.9</td>
<td>2.9 (0.5,10.4)</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>7.91–10.2</td>
<td>3.7 (0.7,21.1)</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>&gt;10.2</td>
<td>15.1 (1.4,53.6)</td>
</tr>
<tr>
<td>†P value for trend</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1</td>
<td>&lt;4.1</td>
<td>3.2 (0.8,25.4)</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>4.1–5.4</td>
<td>8.1 (2.2,21.3)</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>5.41–7.5</td>
<td>1.3 (0.4,6.0)</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>&gt;7.5</td>
<td>1.5 (0.5,14.0)</td>
</tr>
<tr>
<td>†P value for trend</td>
<td></td>
<td>0.004</td>
</tr>
<tr>
<td>Neutrophil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1</td>
<td>≤1.2</td>
<td>1.3 (0.4,4.9)</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>1.21–1.7</td>
<td>1.3 (0.4,5.0)</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>1.71–2.3</td>
<td>3.2 (0.5,20.6)</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>&gt;2.3</td>
<td>20.4 (8.0,53.6)</td>
</tr>
<tr>
<td>†P value for trend</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

†Two-sided P value based on univariate and multivariate quartile regression.

*Effect estimates adjusted for diabetes, atrial fibrillation, previous stroke, statin pretreatment, antithrombotic pretreatment, stroke mechanism, age, blood glucose, temperature, systolic blood pressure, and time to MRI.

IQR indicates interquartile range; DWI, diffusion-weighted imaging; ref, referent quartile.
of increased DWI volume in the highest total leukocyte and neutrophil quartiles. Analysis of differential subpopulations of peripheral leukocytes indicated that the total leukocyte effect was largely attributable to the positive association between the neutrophil fraction and DWI volume because we did not find any independent relationship between DWI volumes and lymphocyte counts. This effect did not appear to be present across the full range of neutrophil counts, but rather there was a threshold between the third and fourth quartiles above which elevation in neutrophil counts was associated with significantly larger infarct volumes.

These results are consistent with previous studies that have reported a relationship between lesion size and elevated markers of systemic inflammatory response including WBC, body temperature, C-reactive protein, and additional inflammatory biomarkers. Studies that have correlated the inflammatory cell response with infarct volumes have almost exclusively used CT or T2-weighted MRI. There have been studies that have looked at inflammatory markers in relation to DWI lesion volumes in small numbers of patients. This study is distinct in that DWI MRI was used to determine volumes of ischemic tissue early after onset. DWI MRI demarcates brain tissue in which there has been a failure of cellular energy-dependent processes attributable to cerebral hypoperfusion and predicts final infarct volume well, albeit imperfectly. Our finding that total leukocyte and neutrophil counts are associated with DWI lesion volumes suggests this inflammatory response occurs early, before the development of large volumes of infarcted or necrotic tissue.

Several mechanisms may contribute to the association between leukocyte count and DWI lesion volume. Atherosclerosis is increasingly being viewed as a chronic inflammatory disease, and one possibility is that an elevation in leukocyte count predates the stroke onset and reflects the burden of atherosclerotic disease. Leukocytosis has been associated with degree of atherosclerosis, and is a risk factor for cardiovascular events and stroke. It is related to stroke subtype, and consequently may affect DWI lesion volumes. Furthermore, there is evidence leukocytosis may be associated with plaque destabilization and the induction of acute thrombotic events. This study provides some support for an interplay of these mechanisms. We found a greater proportion of patients with small vessel occlusion in the lowest (versus highest) total leukocyte quartiles, whereas the upper 3 quartiles trended toward larger proportions of patients with cardioembolic and large vessel atherosclerotic mechanisms. This finding accords with a population-based study that found that stroke-free individuals with the highest quartile of leukocyte count have an overall increased risk of stroke compared with the lowest quartile, and the risk is more pronounced for atherosclerotic and cardioembolic subtypes. However, because monocyte-macrophages and lymphocytes are the major immune cells implicated in atherosclerotic lesions, our finding that elevated neutrophils were correlated with larger DWI volumes cannot fully be explained by an association with premorbid atherosclerotic burden.

Another possibility is that increased admission leukocyte counts reflect the presence of premorbid infection. After infection the incidence of stroke exceeds the general age-matched stroke incidence by 2-to 3-fold, possibly as a result of increased platelet leukocyte aggregation and platelet activation. We attempted to control for clinically-manifest concurrent infection by adjusting for body temperature in the multivariate analyses, and this did not affect the relationship noted between neutrophil counts and DWI lesion volume.

Elevation in leukocyte counts and specifically the neutrophil population may have occurred after the onset of stroke, with greater inflammatory response contributing to greater ischemic brain injury. The present finding that only the neutrophil quartile and not lymphocytes predicted DWI lesion volume is in keeping with the known temporal profile of inflammatory cell involvement in the hyperacute phases of stroke. Prior studies have shown that neutrophils are among the earliest inflammatory cell type to show substantial up-regulation in gene expression and to infiltrate the ischemic brain. Recruitment of neutrophils can be detected as early as 5 hours after stroke onset and peaks at 24 hours.

It must also be acknowledged that the association of neutrophilia and lesion volume we detected may be an epiphenomenon. Large infarcts may provoke a greater stress and inflammatory response as a secondary reaction to the initial brain injury.

This study has some limitations. This is a cross-sectional study, and the cause and effect relationship between total leukocyte and neutrophil quartiles and DWI lesion volume cannot be determined. Serial measures of leukocytes and lesion volume during the first hours after stroke onset could potentially better illuminate the potential role of inflammatory cells in the propagation of ischemic injury, but are logistically challenging to accomplish. To control for premorbid infection we used body temperature. Systemic infection may occur without elevations in body temperature, and future studies should include additional biomarkers of inflammation such as high sensitivity CRP. Initial DWI lesion volume was analyzed, not final T2-weighted lesion volume, as late T2 studies were not obtained routinely in these patients. However, multiple studies have demonstrated that early DWI lesion volumes correlate well with final T2 lesion volumes.

In conclusion, in acute ischemic stroke early elevation of total leukocyte count and neutrophil count is associated with larger volume of early ischemic tissue. Part of this effect is attributable to an association of small vessel stroke mechanism with low leukocyte count, but even after adjustment for this and other factors, the association of inflammatory cells and early lesion volume remains robust. To date, clinical trials of agents that modulate inflammation in acute stroke have been disappointing. Determining whether suppressing the response of neutrophils can help reduce the propagation of ischemic damage should continue to be an important goal of future investigations.

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Disclosures
None.
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