Association of Leukocyte Count With Progression of Aortic Atheroma in Stroke/Transient Ischemic Attack Patients

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Background and Purpose—Leukocyte count is an independent predictor of stroke. We investigated the association between leukocyte count and progression of aortic atheroma over 12 months in stroke/transient ischemic attack (TIA) patients.

Methods—Consecutive ischemic stroke and transient ischemic attack patients underwent 12-month sequential transesophageal echocardiography and were assessed for total and differential leukocyte counts on admission. Paired aortic plaque images were assessed for several parameters, including changes in grade, intimal-medial thickness (IMT), and cross-sectional area. Multivariate linear and logistic regressions were used to calculate the effect of leukocyte count on the change in aortic atheromas over 12 months.

Results—Of the 115 participants (mean±SD age, 64.6±11.9 years; 53.1% men; 73.4% white, 24.2% black, and 2.3% Asian), 45 (35%) showed clinically significant progression of aortic atheromas (maximal change in IMT >0.70 mm over 12 months). The mean admission leukocyte count was higher in the progression group compared with the no-progression group (8.6±2.2 vs 7.3±2.2×10⁹/L respectively, *P*=0.002). Each unit increase in leukocyte count was associated with a 0.26-mm increase in aortic arch IMT over 12 months (*P*=0.006). After adjustment for other atherosclerosis risk factors, the relation persisted (mean increase in aortic arch IMT per unit increase in leukocyte count=0.27 mm, *P*=0.007). Each unit increase in leukocyte count was associated with an increased risk of significant progression of aortic atheromas (adjusted odds ratio=1.33; 95% CI, 1.09 to 1.61).

Conclusions—In stroke/transient ischemic attack patients, leukocyte count is independently associated with the progression of aortic atheroma over 12 months (>0.70 mm), which is associated with cardiovascular risk. (Stroke. 2007;38:000-000.)

Key Words: aorta • atherosclerosis • inflammation • leukocytes • transesophageal echocardiography

Atherosclerosis is one of the major mechanisms of coronary heart disease and stroke, a leading cause of morbidity and mortality worldwide.¹ Recent evidence suggests that atherosclerosis is an inflammatory disease.² Leukocytes (WBCs), including macrophages and lymphocytes, play an important role in the initiation and propagation of the atherosclerotic process. WBC count has been shown to be associated with carotid atherosclerosis³ and its progression,⁴ as well as with the risk of coronary heart diseases⁵,⁶ and stroke.⁷,⁸ In the Northern Manhattan Stroke Study, WBC count was predictive of first ischemic stroke, after adjustment for vascular risk factors.⁹

Thoracic aortic atheroma (TAA) is an increasingly recognized stroke risk factor.¹⁰⁻¹² A recent transesophageal echocardiography (TEE) study demonstrated an association between WBC count and TAA.¹³ The study was limited by a single measurement of TAA in a stroke-free population. Information that we gained from a previous study demonstrated that aortic atheroma has a high rate of progression.¹⁴ In our prior study, we did not measure the effect of WBC count on aortic atheroma progression, and there were 2 major limitations with respect to TEE measurement of aortic atheromas. First, plaque progression assessment based on categorical severity grades has a “ceiling effect” in patients with severe-grade aortic plaque (intimal-medial thickness [IMT] ≥4 mm). Second, a reliable method had not been used to ensure the measurement of plaque thickness at the same location at baseline and follow-up. To counter these limitations, we used the methodological details discussed herein to assess TAA and to test the association between WBC count and aortic atheroma progression.

Subjects and Methods

Consecutive stroke/transient ischemic attack (TIA) patients underwent TEE within 1 month of symptom onset as part of their stroke evaluation.
work-up. All eligible patients had measurable plaque \( \geq 1 \text{ mm} \) in the ascending aorta, aortic arch, or descending thoracic aorta on index TEE. Exclusion criteria were age <18 years, intracerebral hemorrhage, subarachnoid hemorrhage, coma, conditions limiting life expectancy to <12 months (eg, end-stage cancer), clinical signs of obvious infection, concomitant immunosuppressive agent use (eg, prednisone), and no aortic atheroma on baseline TEE. Of the stroke/TIA patients (N=307), 167 had evidence of measurable aortic atheroma, and 125 of 167 eligible patients consented to a follow-up TEE at 12 months, based on a protocol approved by the institutional review board. Of the remaining 42 of 167 who did not consent, 35 refused and 7 were deceased before their follow-up 12-month TEE. Adequate paired aortic images were obtained in 117 of 125 patients (78 strokes, 39 TIA), allowing detailed plaque measurements. Of these patients, 115 of 117 without any obvious signs of infection had complete blood counts as part of their stroke risk assessment at the time of their initial presentation to the hospital/clinic for the index stroke/TIA. All patients had a fasting lipid profile and C-reactive protein measured as part of their initial stroke risk assessment. Stroke risk factors, stroke, and TIA were defined on the basis of previously described criteria. The medication history, including daily statin therapy, was collected at the time of the protocol-mandated 12-month TEE.

TEE Assessment of Aortic Atheroma

A comprehensive TEE with detailed imaging of the thoracic aorta was performed with a Hewlett-Packard 21364A omniplane probe. Imaging and quantification of aortic atheromas were conducted according to previously described methods. In brief, the proximal and midascending aorta were imaged at a probe depth of \( \approx 30 \text{ cm} \) with a multiplane angle of 100° to 150° to view the vessel in the long axis. The descending thoracic aorta was examined by advancing the probe to the distal esophagus, imaging the aorta in cross section (at 0°), and then slowly withdrawing the probe to image the proximal segments. As the transducer reached the aortic arch plaque, the multiplane angle was rotated to between 0° and 90° to acquire sequential short-axis views. Digital images were acquired of the diseased areas in each segment of the aorta with annotation of the distance of the transducer from the incisors. Identical locations in the aorta were evaluated on the 1-year examination by using the depth of the transducer, plaque morphology, and surrounding anatomic landmarks for guidance. Some of these patients had fever (mean \( \pm 3 \text{D temperature}, 36.4 \pm 0.4 \text{C} \)) or TEE evidence of valvular vegetation suggestive of infective endocarditis.

Two observers, masked to the clinical data and order of TEE, independently quantified atheromas on the index and 12-month follow-up TEE examinations. At both time points, IMT was measured as the maximal thickness of the intimal and medial layers as a continuous variable. It was also graded as mild (<1 mm), moderate (1 to 3.9 mm), or severe ( \( \geq 4 \text{ mm} \) ) according to the clinical criteria of Amarenco et al. Finally, cross-sectional plaque area was measured by tracing the outline of the atheroma as described by Khoury et al and Ti et al. Paired aortic plaque images were assessed for several parameters, including changes in grade, IMT, and cross-sectional area. These changes were assessed in the ascending, arch, and descending segments as well as the maximal change among these segments. There was good interobserver reliability between the 2 observers in the assessment of aortic plaque progression in the ascending aorta ( \( \kappa = 0.77 \) ), aortic arch ( \( \kappa = 0.85 \) ), and descending thoracic aorta ( \( \kappa = 0.86 \) ). Excellent intraobserver reliability was noted for the first ( \( \kappa = 0.93 \) to 1.00) and second ( \( \kappa = 0.91 \) to 0.94) observers. Receiver operator characteristics curve analysis was used to determine the parameter that was best correlated with cardiovascular events and to determine a clinically significant “cutoff” for that parameter. Plaques noted on the index TEE were also assessed for morphological features including heterogeneous-in-
Risk Factors | Progression (n=45) | No Progression (n=70) | P Value
--- | --- | --- | ---
Age, y (mean±SD) | 68±11 | 62±12 | 0.007
Body mass index | 27.9±5.3 | 29.8±5.9 | 0.12
Black race | 9 (20%) | 19 (27%) | 0.52
Men | 27 (60%) | 35 (50%) | 0.39
Hypertension | 39 (87%) | 52 (74%) | 0.17
Diabetes | 11 (24%) | 19 (27%) | 0.92
Current smoker | 13 (29%) | 13 (19%) | 0.29
Ever smoker | 25 (56%) | 35 (50%) | 0.70
Alcohol use | 8 (18%) | 13 (19%) | 0.91
Coronary artery disease | 16 (36%) | 20 (29%) | 0.56
Atrial fibrillation | 7 (15%) | 4 (6%) | 0.15
Peripheral vascular disease | 7 (15%) | 13 (19%) | 0.87
TIA | 15 (33%) | 24 (34%) | 0.92
Statin use | 23 (51%) | 43 (61%) | 0.37
Total cholesterol, mg/dL | 185.6±46.2 | 186.3±36.4 | 0.93
LDL, mg/dL | 105.7±43.0 | 107.3±34.7 | 0.83
HDL, mg/dL | 52.7±17.5 | 52.7±16.6 | 0.99
C-reactive protein, mg/dL* | 1.23±0.43 | 0.70±0.07 | 0.37
WBCs, ×10^9/L | 8.6±2.2 | 7.3±2.2 | 0.002
Days between index stroke/ TIA and WBC count | 2.8±7.3 | 3.0±7.2 | 0.91

* Logarithmically transformed C-reactive protein values compared the progression (n=35) vs the no-progression group (n=50) by t test.

Table 1. Baseline Clinical Characteristics of Stroke/TIA Patients With and Without Significant Progression of Aortic Arch Atheroma (>0.70 mm over 12 Months)

with current smoking but not with age, sex, hypertension, diabetes mellitus, hypercholesterolemia, or rate of statin use. The mean WBC count among current smokers was 8.92±2.19×10^9/L compared with a mean WBC of 7.54±2.36×10^9/L among current nonsmokers (P=0.008). There was no significant difference (P=0.14) in the mean WBC count among patients with stroke (8.18±2.50×10^9/L) versus those with TIA (7.52±2.09×10^9/L).

A significant correlation (R=0.40, P<0.0001; R^2=0.16, P<0.0001) was noted between the admission WBC count and change in aortic arch plaque IMT over 12 months (the Figure). In a univariate linear-regression model, WBC count was the most strongly associated with IMT (0.262-mm increase in IMT per unit increase in WBC, P=0.006). After adjustment for age and sex, the association was stronger (0.296-mm increase in IMT per unit increase in WBC, P=0.002). After further adjustment for the other conventional atherosclerotic risk factors of hypertension, diabetes mellitus, current cigarette smoking, and hypercholesterolemia, the association was only slightly attenuated (0.274-mm increase in IMT per unit increase in WBC, P=0.007).

After adjustment for age, sex, hypertension, diabetes mellitus, hypercholesterolemia, and current smoking, WBC count remained associated with significant aortic plaque progression as defined earlier (adjusted odds ratio [OR] per unit increase in WBC=1.33; 95% CI, 1.09 to 1.61; Table 2). The association did not change after including stroke severity (National Institutes of Health Stroke Scale score on admission) in the model (adjusted OR per unit increase in WBC=1.32; 95% CI, 1.09 to 1.61). In analyses stratified by sex, the magnitude of the association was greater in men (adjusted OR per unit increase in WBC count=1.70; 95% CI, 1.19 to 2.42; in women, adjusted OR=1.18; 95% CI, 0.91 to 1.53; Table 2). An independent statistically significant association could not be confirmed in women, who made up 47% of the participants. In analyses stratified by age, the magnitude of the association was only slightly greater in patients ≥65 years old (≥65 years age, adjusted OR per unit increase in WBC=1.32; 95% CI, 1.00 to 1.75; ≥65 years age, adjusted OR=1.29; 95% CI, 0.95 to 1.75; Table 2). An independent statistically significant association could not be confirmed in the <65-year age group, who made up 47% of the participants.

The mean WBC count measured at admission among the progression group was 8.64±2.25×10^9/L compared with a mean WBC count of 7.27±2.23×10^9/L among the no-progression group (P=0.002, Table 3). This difference in mean WBC counts could be explained mainly by the difference in mean neutrophil counts (6.36±2.36×10^9/L in the progression group and 4.63±1.73×10^9/L in the no-progression group, P<0.001) and, to a lesser extent, by the difference in mean monocyte counts (0.42±0.17×10^9/L in the progression group and 0.36±0.13×10^9/L in the no-progression group, P=0.045). The progression group also had a lower mean lymphocyte count (1.70±0.94×10^9/L in the progression group and 2.01±2.23×10^9/L in the no-progression group), although the difference failed to attain statistical significance (P=0.09). In a limited set of patients, convalescent WBC counts measured before (≤1 year) the index event (stroke or TIA) were available. The mean WBC count measured before the index event among the progression group (n=23) was 7.39±2.08×10^9/L, not significantly (P=0.42) higher than the mean WBC count of 6.98±1.64×10^9/L among the no-progression group (n=36). These data suggest that the WBC counts measured on admission and not at prior times were associated with the progression of aortic atheroma.
Complex mobile plaques were noted in 11 of 115 (10%) patients, heteroechogenicity in 34 of 115 (30%) patients, and ulceration in 12 of 115 (10%) patients on the index TEE. Only heteroechogenicity was associated with progression (42% in the progression group versus 21% in the no-progression group, \( P=0.03 \)). These morphological characteristics were not associated with a significantly higher WBC count (\( P=\text{NS} \)).

**Discussion**

Previously, we described aortic arch plaque progression over 12 months to be an independent predictor of cardiovascular events, including stroke and TIA.\(^1\)\(^9\) In that prior report, we used the traditional grading system to define significant aortic plaque progression (≥1 grade over 12 months). Now in this study, using detailed aortic plaque measurements, we were able to define significant progression as an increase in aortic plaque IMT of >0.70 mm. This thickness was the best cutoff in terms of ease of measurement and its ability to predict incident composite vascular events, including stroke and TIA. The admission WBC count was related both to the change in aortic plaque IMT and to conversion to clinically significant plaque progression over a 12-month follow-up period. These associations were independent of other traditional vascular risk factors, including age, sex, hypertension, hypercholesterolemia, smoking, and diabetes mellitus. Although admission WBC count was known to be elevated as an acute-phase reactant after stroke,\(^2\)\(^0\) this is an unlikely explanation for the association between WBC count and aortic plaque progression, as admission WBC counts were performed at similar intervals from the index event in both the progression and the no-progression groups (Table 1). Furthermore, initial stroke severity (measured by National Institutes of Health Stroke Scale on admission) did not influence the association between WBC count and plaque progression. Limited data on convalescent WBC and C-reactive protein values, though concordant with admission WBC counts in their association with plaque progression, did not reach statistical significance.

Results from our study are in agreement with prior studies that investigated the association between WBC count and atherosclerosis. Investigators found WBC count to be independently predictive of subclinical atherosclerosis progression over 2 years in a small sample of Finnish men.\(^4\) Studies of clinical atherosclerotic outcome have found an association between WBC count, coronary artery disease,\(^6\)\(^,\)\(^8\) and stroke.\(^7\)\(^,\)\(^9\) A recent population-based, cross-sectional study found a strong, independent association between WBC count and subclinical aortic arch atherosclerosis measured by TEE.\(^1\)\(^3\)

Our study provides additional evidence that in stroke/TIA patients, elevation in WBC counts may be independently associated with aortic plaque activity measured as progression over 12 months.

There are several studies that have assessed the effect of risk factors on aortic arch atherosclerosis. The available data, though limited by variability in technique for identifying aortic atherosclerosis, include pathologic assessment of lesion size on autopsy,\(^2\)\(^1\)\(^,\)\(^2\)\(^2\) aortic calcification detected by chest x-ray,\(^2\)\(^3\) and atherosclerosis detected on TEE.\(^2\)\(^4\)\(^,\)\(^2\)\(^5\) These studies found an association between age, systolic hypertension, smoking, dyslipidemia, and novel

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**Table 2. ORs for Significant Progression of Aortic Arch Atheroma (≥0.70 mm Over 12 Months) Per Unit Increase in WBC Count, Overall and Stratified by Sex and Age**

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Men</th>
<th>Women</th>
<th>&lt;65 Years</th>
<th>≥65 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>OR</td>
<td>95% CI</td>
<td>OR</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.32</td>
<td>1.09–1.58</td>
<td>1.59</td>
<td>1.16–2.17</td>
<td>1.17</td>
</tr>
<tr>
<td>Adjusted for demographic factors*</td>
<td>1.36</td>
<td>1.12–1.66</td>
<td>1.61</td>
<td>1.18–2.20</td>
<td>1.21</td>
</tr>
<tr>
<td>Adjusted for demographic factors and conventional risk factors†</td>
<td>1.33</td>
<td>1.09–1.61</td>
<td>1.70</td>
<td>1.19–2.42</td>
<td>1.18</td>
</tr>
</tbody>
</table>

*Demographic factors are age and sex in overall analysis, age in the analysis stratified by sex, and sex in the analysis stratified by age.†Conventional risk factors are hypercholesterolemia, hypertension, diabetes mellitus, and current cigarette smoking.

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**Table 3. Hematologic Parameter Comparison Between Patients With and Without Significant Aortic Arch Atheroma Progression (≥0.70 mm Over 12 Months)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Progression (n=45)</th>
<th>No Progression (n=65)</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/dL</td>
<td>13.43±1.67</td>
<td>13.59±2.08</td>
<td>0.69</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>38.93±4.54</td>
<td>39.45±4.33</td>
<td>0.55</td>
</tr>
<tr>
<td>Total WBC count, ×10^9/L</td>
<td>8.64±2.25</td>
<td>7.27±2.23</td>
<td>0.002</td>
</tr>
<tr>
<td>Neutrophil count, ×10^9/L</td>
<td>6.36±2.36</td>
<td>4.63±1.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lymphocyte count, ×10^9/L</td>
<td>1.70±0.94</td>
<td>2.01±0.92</td>
<td>0.09</td>
</tr>
<tr>
<td>Monocyte count, ×10^9/L</td>
<td>0.42±0.17</td>
<td>0.36±0.13</td>
<td>0.045</td>
</tr>
<tr>
<td>Eosinophil count, ×10^9/L</td>
<td>0.19±0.17</td>
<td>0.17±0.11</td>
<td>0.84</td>
</tr>
<tr>
<td>Basophil count, ×10^9/L</td>
<td>0.04±0.05</td>
<td>0.04±0.05</td>
<td>0.67</td>
</tr>
<tr>
<td>Platelets, ×10^9/mL</td>
<td>251.29±70.70</td>
<td>257.56±84.72</td>
<td>0.72</td>
</tr>
</tbody>
</table>
risk factors, including plasma homocysteine, maternal hypercholesterolemia, and fibrinogen, with aortic atheroma.13 Few studies have assessed the effect of risk factors on aortic arch atherosclerosis progression.15,26 None of these prior studies investigated the association between WBC counts and TAA progression.

WBCs have been found in human fatty streaks, even at the earliest stage of the disease process, suggesting that immune processes may play an early role in the development of the atherosclerotic plaque. This is consistent with our finding that the elevated WBC count, supported by increases in neutrophil and monocyte counts, may contribute to the progression of aortic atheroma. Other investigators3,27 have described an association between smoking and WBC counts as well as between smoking and subclinical atherosclerosis.4,28 Smoking itself could be a risk factor for aortic plaque progression, with elevated WBC counts also resulting from smoking. However, in our study, the association between WBC count and aortic plaque progression persisted even after adjustment for smoking. We found that subjects who were male and ≥65 years old had a stronger association between aortic plaque progression and WBC count (Table 2). Because male sex and age are also risk factors for stroke, the finding suggests that an increased risk of atherosclerosis may be one of the mechanisms by which an elevated WBC count is associated with stroke.7

The current study has limitations that merit comment. Although we excluded patients with obvious clinical infection such as pneumonia, we did not prospectively assess the underlying cause(s) of the elevated WBC values. Also, we cannot completely exclude the possibility that aortic atheroma progression led to higher WBC counts. The limitations of TEE prevented us from measuring vessel wall area, an indicator of remodeling associated with atherosclerosis.29 Because TEE is a semi-invasive test, measurements of aortic atheroma at intermediate time points were not performed. Also, we did not assess concomitant inflammatory markers (eg, high-sensitivity C-reactive protein) or biomarkers of hemostasis (eg, fibrinogen) to draw a definite conclusion on the mechanisms by which WBC count may induce aortic plaque progression. Finally, the modest association between admission WBC count and aortic plaque progression, though independent of demographics and conventional risk factors and statistically significant, should be interpreted with caution due to its susceptibility to potential bias, such as those from unmeasured confounding.

In summary, our study supports an independent association between WBC values and the progression of aortic atheroma in stroke/TIA patients; it also reports a standardized method for assessment of significant progression of aortic atheroma that is associated with increased cardiovascular risk. In conclusion, our findings suggest that an increased risk of atherosclerosis may be 1 of the mechanisms by which elevated WBC count is associated with stroke. Our study lays the foundation for future research by providing the means and methodology for investigating possible treatment options for aortic atheroma progression.

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Disclosures
None.

References
15. Salonen R, Salonen JT. White blood cell count and carotid plaque thickness: the Northern Man-


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