Leukocyte Count Predicts Microembolic Doppler Signals During Carotid Stenting
A Link Between Inflammation and Embolization

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Background and Purpose—Protected stenting has emerged as a safe and effective alternative to endarterectomy for the treatment of carotid stenosis in patients at high operative risk. Distal microembolization occurs invariably during carotid stenting. Little is known about the relationship between systemic inflammation and embolization during carotid stenting.

Methods—We examined 43 consecutive patients who underwent carotid stenting with simultaneous transcranial Doppler (TCD) monitoring of the ipsilateral middle cerebral artery. Embolization was quantified by measuring microembolic signals (MES) on TCD. Preprocedure leukocyte counts were related to MES.

Results—In unadjusted analyses, preprocedure leukocyte count was positively correlated with total procedural MES ($r^2 = 0.16$; $P = 0.008$). After considering age, gender, comorbidities, concomitant medical therapies, and the use of emboli prevention devices, increasing leukocyte count ($\beta = 35$ for each 1000/µL increment; $P = 0.018$) remained a significant and independent predictor of embolization (model-adjusted $r^2 = 0.365$; $P = 0.0005$).

Conclusions—Increasing preprocedure leukocyte count independently predicted more frequent MES during carotid stenting. These data suggest that systemic inflammation may influence the degree of procedural embolization. (Stroke. 2005;36:1910-1914.)

Key Words: inflammation ■ leukocytes ■ stents

Materials and Methods

Study Population
We examined 43 consecutive patients who underwent elective carotid artery stenting with simultaneous TCD monitoring of the ipsilateral MCA at the Cleveland Clinic Foundation between April 1998 and June 2001; intraprocedural TCD was routinely available at our institution during this period. Patients with and without symptoms had respective carotid stenoses of $\geq 70\%$ and $\geq 80\%$ by angiography, according to North American Symptomatic Carotid Endarterectomy Trial criteria. Each patient was initially screened by a vascular surgeon, neurologist, and cardiologist, and felt to be at high risk for carotid endarterectomy. All patients underwent carotid stenting under an institutional review board–approved protocol and provided written informed consent before participation.

Leukocyte Count
Leukocyte count was determined using a Sysmex Diagnostics SE-9500 automatic particle counter (Sysmex Corporation) within 24 hours of venipuncture. The mean coefficient of variation for this procedure is $2.5\% \pm 0.18\%$ at our institution. Preprocedure leukocyte count was defined as any result obtained $\leq 30$ days before carotid stenting. If $>1$ measurement was obtained during the 30-day interval, that most proximate to the carotid stent procedure was selected for analyses.
Carotid Stenting Procedure

All patients were examined by a neurologist and underwent carotid ultrasound at baseline. Those with a history of stroke underwent brain computed tomography before the procedure. Carotid stenting was performed as described previously. In brief, patients were pretreated with aspirin (325 mg daily) and an ADP receptor antagonist (either 250 mg ticlopidine twice daily or 75 mg clopidogrel daily). Unfractionated heparin boluses were given to achieve an activated clotting time of ≥250 to 300 seconds. By protocol, platelet glycoprotein (GP) IIb/IIIa inhibitors were used early and distal emboli prevention devices late during the study period. Angiography was performed in ≥2 projections, and intracranial views were obtained. After placing a 0.014-inch shaped guide wire or distal emboli prevention device across the stenosis, balloon predilation, self-expanding stent deployment (stent type determined per protocol), and postdilation were performed under fluoroscopic guidance. Angiography of the target and intracranial vessels was repeated after the final balloon inflation to rule out distal macroembolization or flow abnormality. Brief neurological examinations were performed throughout the procedure, and a detailed evaluation was conducted at the end of the case and the morning after by an independent neurologist. At discharge, patients were prescribed lifelong aspirin therapy, and ADP receptor antagonists were continued for ≥30 additional days. All patients were seen for a follow-up neurological examination at 30 days.

Transcranial Doppler

The detection of MES relies on visual and auditory identification. In the flow spectrum on a fast Fourier transform spectral display, MES are typically transient (0.01 to 0.1 s), high-intensity (>10 dB greater than background) and unidirectional; these signals are also accompanied by a characteristic chirping, whistling, or plopping sound. Experimental studies have demonstrated that platelet, thrombus, and atheroma emboli as small as 200 to 400 μm result in these characteristic MES on TCD.

TCD interrogation was performed according to international consensus group guidelines. MES were recorded during guide wire manipulation, balloon predilation, stenting, and postdilation of the target lesion. All patients underwent continuous TCD monitoring of the ipsilateral MCA during the entire carotid stent procedure. TCD studies were performed with a Pioneer TC 2020 U (Nicolet Biomedical Inc.). Middle cerebral arteries were insonated via the transtemporal window with a 2-MHz monocrystal-focused Doppler transducer (Acoustic). Angiography was performed in 2 projections, and intracranial views were obtained after placing a 0.014-inch shaped guide wire across the stenosis, balloon predilation, self-expanding stent deployment, and postdilation were performed under fluoroscopic guidance. Angiography of the target and intracranial vessels was repeated after the final balloon inflation to rule out distal macroembolization or flow abnormality. Brief neurological examinations were performed throughout the procedure, and a detailed evaluation was conducted at the end of the case and the morning after by an independent neurologist. At discharge, patients were prescribed lifelong aspirin therapy, and ADP receptor antagonists were continued for ≥30 additional days. All patients were seen for a follow-up neurological examination at 30 days.

Table 1. Patient Characteristics in Overall Cohort (n=43)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, mean±SD</td>
<td>70±11</td>
</tr>
<tr>
<td>Male, %</td>
<td>74</td>
</tr>
<tr>
<td>BMI in kg/m², mean±SD</td>
<td>27±4</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>26</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>70</td>
</tr>
<tr>
<td>Hypercholesterolemia, %</td>
<td>65</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>26</td>
</tr>
<tr>
<td>Coronary disease, %</td>
<td>88</td>
</tr>
<tr>
<td>History of ACS, %</td>
<td>60</td>
</tr>
<tr>
<td>History of UA, %</td>
<td>37</td>
</tr>
<tr>
<td>History of MI, %</td>
<td>40</td>
</tr>
<tr>
<td>History of PCI, %</td>
<td>14</td>
</tr>
<tr>
<td>Atrial fibrillation, %</td>
<td>9</td>
</tr>
<tr>
<td>Ejection fraction (% n=32, median (IQR)</td>
<td>50 (28, 55)</td>
</tr>
<tr>
<td>Severe aortic stenosis, %</td>
<td>7</td>
</tr>
<tr>
<td>Renal insufficiency, %</td>
<td>9</td>
</tr>
<tr>
<td>Severe COPD (FEV1 &lt;1.0; %)</td>
<td>14</td>
</tr>
<tr>
<td>Before open heart surgery, %</td>
<td>42</td>
</tr>
<tr>
<td>Before orthotopic heart transplant, %</td>
<td>12</td>
</tr>
<tr>
<td>Symptomatic cerebrovascular disease, %</td>
<td>58</td>
</tr>
<tr>
<td>Previous TIA, %</td>
<td>56</td>
</tr>
<tr>
<td>Previous stroke, %</td>
<td>21</td>
</tr>
<tr>
<td>Amaurosis fugax, %</td>
<td>7</td>
</tr>
<tr>
<td>High-risk anatomy</td>
<td>12</td>
</tr>
<tr>
<td>Contralateral occlusion, %</td>
<td>2</td>
</tr>
<tr>
<td>Previous neck irradiation, %</td>
<td>9</td>
</tr>
<tr>
<td>Previous carotid endarterectomy, %</td>
<td>12</td>
</tr>
<tr>
<td>Previous carotid intervention, %</td>
<td>2</td>
</tr>
</tbody>
</table>

ACS indicates acute coronary syndrome; BMI, body mass index; FEV1, forced expiratory volume during 1 s; MI, myocardial infarction; PCI, percutaneous coronary intervention; UA, unstable angina.

Statistical Analysis

Categorical variables appear as frequencies and percentages, and χ² or Fisher’s exact tests were used where appropriate in univariate analyses of these data. Continuous variables are given as means with SDs when normally distributed or as medians with interquartile ranges (IQRs) when non-Gaussian in distribution; unpaired t tests were used in univariate analyses of the former and Mann–Whitney U tests in the latter. Leukocyte count was examined as a continuous and categorical (ie, by tertile) predictor of embolization during carotid stenting. Total procedural MES were analyzed by each patient characteristic listed in Tables 1 and 2. Candidate univariate predictors (P<0.15) were entered along with age and gender into stepwise multivariable linear regression models to assess the independent effect of leukocyte count on distal cerebral embolization as quantified by TCD-detected MES; non-normally distributed predictor variables were log-transformed as appropriate. The significance level...
Procedural Data
Procedural data appear in Table 2. The primary lesion location in nearly 9 of 10 patients was the internal carotid artery. Among asymptomatic patients, the mean percent stenosis (±SD) was 84±6, whereas among patients with symptomatic disease, the mean was 88±8. Nearly half of the lesions were ulcerated and 1 in 10 were calcified. The Wallstent (Boston Scientific) was deployed in approximately two thirds of patients, with 1 in 6 receiving Smartstents (Cordis) and the remainder Endotex (Endotex), Megalink (Guidant), Palmaz (Cordis), or Precise (Cordis). By protocol, the first 5 patients underwent carotid stenting with neither distal protection nor GP IIb/IIIa inhibitors, the next 31 with GP IIb/IIIa inhibitors only, the following 2 using GP IIb/IIIa inhibitors and distal emboli prevention, and the last 5 using only distal emboli prevention. The mean procedural activated clotting time was 319±43 seconds.

Results

Patient Characteristics
Patient characteristics appear in Table 1. Overall, patients were elderly, mostly male, hypertensive, hyperlipidemic, and the overwhelming majority had concomitant coronary artery disease. Roughly one quarter had diabetes mellitus or smoked. One in 10 had atrial fibrillation. Nearly half underwent carotid stenting before planned open-heart surgery. More than half had a history of transient ischemic attack (TIA), 1 in 5 had a history of stroke, and <10% had amaurosis fugax. Only 1 patient had a contralateral carotid artery occlusion. Approximately 1 in 10 had undergone previous carotid endarterectomy or had previous neck irradiation.

Leukocyte Count
The mean (±SD) leukocyte count×10³ was 7.47±1.96. The first, second, and third tertile ranges were 3.40 to 5.68, 6.66 to 7.62, and 7.88 to 12.50, respectively. The minimum and maximum leukocyte counts were 3.4 and 12.5, respectively.

Distal Embolization
The median (IQR) procedural embolization during wire manipulation, predilation, and stenting/postdilation was 52 (30 to 100), 108 (48 to 146), and 110 (73 to 154), respectively. The median (IQR) total procedural MES was 303 (207 to 402).

Predictors of Embolization
The mean total procedural MES increased with each successive leukocyte tertile (first tertile 259; second tertile 276; third tertile 350) and preprocedure leukocyte count was significantly correlated with the total procedural MES in an unadjusted analysis (r² 0.16; P=0.008; Figure). Simple linear regression analyses relating leukocyte count subpopulations to total procedural MES were performed among patients for whom a complete cell differential was available (n=14). The results of these regression analyses appear in Table 3. Only neutrophil count significantly predicted the total procedural MES.

Other significant positive univariate predictors of MES included atrial fibrillation and chronic obstructive pulmonary disease (COPD). The use of an emboli prevention device was a significant negative univariate predictor of total procedural MES. After considering age, gender, comorbidities, lesion characteristics, concomitant medical therapies including GP IIb/IIIa inhibitors, and distal protection devices, leukocyte count remained significantly associated with the frequency of MES. For every 1000/µL increase in leukocyte count, 35 additional MES were detected. In addition, the use of distal emboli prevention devices and prevalent COPD were negative and positive independent predictors of MES, respectively. Together, these 3 variables accounted for more than one third of the variability in MES occurring during carotid stenting (Table 4). When the cohort was restricted to patients who underwent unprotected carotid stenting (n=36), leukocyte count remained a significant independent predictor of MES (β=0.40; P=0.019). Our results were also unchanged when the analysis was restricted to patients without previous stroke, TIA, or amaurosis fugax (n=18); leukocyte count remained a significant and independent predictor of MES in this cohort (β=0.58, P=0.004).

Clinical Events
Overall, few clinical events occurred during the first 30 days of follow-up (Table 5). During this time, there were 2 deaths,
3 myocardial infarctions, and 2 strokes clustered within 5 patients. The mean leukocyte count was higher among patients with than without these events, but this difference was not statistically significant (7.70 versus 7.44; \( P=0.78 \)). Similarly, the mean total procedural MES were nonsignificantly higher among those with subsequent death, myocardial infarction, or stroke (379 versus 278; \( P=0.12 \)).

## Discussion

In this retrospective observational study, we observed that greater preprocedure leukocyte count was associated with more frequent TCD-detected distal embolization during carotid stenting. The relationship between leukocyte count and procedural embolization was strongest for the neutrophil subpopulation. These observations are novel and may have pathophysiological and therapeutic relevance.

Although inflammation has been associated with atherosclerosis, plaque rupture, and thrombosis in the coronary and cerebral vasculature,\(^6\)\(^{12}\)\(^{13}\) there is little evidence to support a link between inflammation and embolization. In a cross-sectional study by Mazzone et al of 29 patients with unstable angina and negative CK-MB fractions, those with positive troponin T (TnT) at baseline had higher levels of monocyte chemotactic protein-1 (MCP-1) than those with negative TnT.\(^{14}\) Although this association is consistent with an association between inflammation and embolization, these findings suggest but do not confirm that inflammation predisposes to distal embolization.\(^{15}\) Finally, in a multicenter study of 25 patients who developed cholesterol embolization syndrome after a left heart catheterization, preprocedural CRP was the only independent predictor of this adverse outcome.\(^{16}\) To our knowledge, ours is the first study to relate inflammation to embolization during carotid stenting.

The pathophysiologic mechanisms underlying distal microembolization are not completely understood. It appears that platelet aggregation and adhesion are at least in part responsible for this phenomenon, and that agents that inhibit these processes (eg, \( L \)-arginine and \( S \)-nitrosoglutathione) by enhancing NO bioavailability reduce microembolization in the setting of symptomatic carotid stenosis\(^7\) as well as during and after carotid endarterectomy.\(^{19}\) Similar mechanisms may also be involved in balloon-induced distal microembolization.\(^{10}\) Although microembolization of platelet–fibrin thrombus occurs in the setting of carotid stenting, soft acellular amorphous material containing cholesterol clefts, lipid-rich macrophages, fibrous cap connective tissue, and endothelial cells is also commonly found.\(^{20}\) It is plausible that by virtue of its association with more vulnerable lipid-rich plaque and superimposed thrombosis, inflammation might induce greater distal embolization.

The observation that inflammation predicts the degree of procedural embolization may have therapeutic implications as well. Embolization during percutaneous carotid intervention has been reduced by mechanical means with the use of emboli prevention devices.\(^2\)\(^{21}\) Likewise, preliminary data suggest that pharmacological means may be effective in this regard.\(^2\)\(^{17}\)\(^{19}\) It is possible that pretreatment with agents having inherent anti-inflammatory properties such as 3-hydroxy-3-methylglutaryl –coenzyme A reductase inhibitors\(^{22}\) might reduce procedural embolization\(^{23}\) and by doing so limit adverse neurologic outcomes.\(^{24}\) In our study, although not statistically significant, lower levels of distal microembolization were observed among patients who were on preprocedure statin therapy than those who were not (median MES 292 versus 343, respectively; \( P=0.34 \)). Clopidogrel also possesses anti-inflammatory properties in addition to its well-established antiplatelet activity.\(^{25}\) In the Clopidogrel and Aspirin for Reduction of Emboli in Symptomatic Carotid Stenosis (CARESS) trial\(^{25a}\) 107 patients with carotid stenosis and an ipsilateral stroke or TIA within the past 3 months were randomized to clopidogrel or placebo in addition to background low-dose aspirin therapy. Clopidogrel significantly reduced the frequency of spontaneous MES after only 24 hours of therapy. Although it was not possible to differentiate between the antiplatelet and anti-inflammatory effects of clopidogrel in this study, it remains plausible that both contributed to the decrease in spontaneous cerebral embolization that occurred in clopidogrel-treated patients.

There are a number of limitations inherent in our study. Because it was observational, we cannot rule out the possibility that our multivariable analysis failed to adequately adjust for all potential confounders known or unknown. Our analysis was retrospective in design and limited by information available in our carotid stent registry and by chart review. A prospective validation of our findings will be necessary.
The low frequency with which embolic prevention devices were used and the use of glycoprotein IIb/IIIa inhibitors is not representative of contemporary carotid stenting technique at most centers. Nevertheless, these technical issues should not preclude accurate assessment of inflammation, embolization, nor their relationship. The association between inflammation and procedural embolization will need to be re-examined in the embolic prevention era. Because of our small sample size, we had limited ability to examine the role of inflammation in different patient subgroups. Additionally, the small patient cohort limited our ability to relate inflammation or embolization to clinical events. Finally, although leukocyte count and CRP are significantly correlated with each other, leukocyte count was the only inflammatory biomarker consistently available in our study cohort. Future studies should explore additional patient attributes, lesion characteristics, and procedural techniques that may relate to embolization.

Summary

Our observations support the hypothesis that inflammation precedes and increases the propensity toward distal microembolization during carotid stenting. Further studies will be necessary to validate the relationship between leukocyte count and embolization as well as to explore the predictive capacity of other inflammatory biomarkers (eg, CRP). Studies relating agents with anti-inflammatory properties (eg, statins, clopidogrel) to inflammatory biomarkers (eg, CRP). Studies relating agents with anti-inflammatory properties (eg, statins, clopidogrel) to inflationary mediators in unstable angina: correlation with serum troponin T. Heart. 2001;85:571–575.


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