Leukocytosis and Ischemic Vascular Disease Morbidity and Mortality
Is It Time to Intervene?

Barry S. Coller

Abstract—The association between leukocytosis and increased morbidity and mortality of ischemic vascular disease has been observed for more than half a century, and recent studies in >350,000 patients confirm the robustness of the association and the dramatically higher relative and absolute acute and chronic mortality rates in patients with high versus low leukocyte counts. Although there is reason to believe that the association is not causal (that is, that leukocytosis is simply a marker of inflammation), there is also reason to believe that the leukocytosis directly enhances acute thrombosis and chronic atherosclerosis. Leukocytosis also is associated with poor prognosis and vaso-occlusive events in patients with sickle cell disease, and experimental data suggest a direct role for leukocytes in microvascular obstruction. The only way to test whether leukocytes contribute directly to poor outcome in ischemic cardiovascular disease is to assess the effect of modifying leukocyte function or number. Because selective blockade of leukocyte integrin αMβ2 and P-selectin have thus far been disappointing as therapeutic strategies in human cardiovascular and cerebrovascular disease, I discuss the potential risks and benefits of short-term treatment with hydroxyurea to decrease the leukocyte count in select populations of patients at the highest risk of short-term death. (Arterioscler Thromb Vasc Biol. 2005;25:658-670.)

Key Words: leukocytes ■ neutrophils ■ myocardial infarction ■ thrombosis

In the 1949 edition of his classic text, Dr Paul Dudley White wrote (without a reference) that severe and sustained leukocytosis after myocardial infarction was associated with poor prognosis,1 and in 1954, Cole et al reported that >32% of patients with an acute myocardial infarction and a white blood cell (WBC) count of >15,000/μL died within the first 2 months after the infarction, whereas <9% of patients with a WBC count <10,000/μL died within the same period.2 Subsequent studies of the relationship between leukocytosis and coronary artery disease conducted from 1974 to 1996 were summarized in a meta-analysis of 19 prospective studies involving 7229 patients with a weighted mean follow-up of 8 years.3 Comparison of individuals with WBC counts in the highest third with those in the lowest third yielded a coronary heart disease risk ratio of 1.5 (95% CI, 1.4 to 1.6) even though the majority of these studies made adjustments for smoking and other known risk factors (Figure 1). In fact, the risk ratio exceeded 1.0 in each of the 19 studies. Since then, additional studies of WBC count and vascular disease morbidity and mortality involving >350,000 patients treated with more modern therapies have been reported, and in nearly all of these studies, a significant relationship between leukocyte count and vascular disease morbidity and mortality was observed (Table 1).4-27

Strong arguments can be made that despite the robustness of the association, leukocytosis is only a nonspecific marker of other processes that actually cause the increased risk. Thus, it has been known for more than half a century that acute myocardial infarction is commonly accompanied by transient leukocytosis as part of an acute phase reaction.1 Moreover, there is increasing recognition that atherosclerosis has an important inflammatory component28,29 and that cardiovascular risk factors such as smoking and elevated body mass index (BMI) are associated with chronic inflammation.30 Thus, it is reasonable to consider that the elevated WBC count is just a marker of the chronic inflammatory state and that other aspects of inflammation may be the direct cause of the vascular disease.

However, there are a number of potential mechanisms by which leukocytes may contribute directly to acute and chronic ischemic vascular disease, and although there is insufficient space to discuss these mechanisms in detail, they are listed in Table 2 with select references. This review summarizes the recent epidemiologic evidence of an association of WBC count with acute and chronic vascular disease, with a particular focus on data that may bear on the question of a potential causal role of leukocytes in vascular events, and then addresses the question of whether the existing data justify performing studies to assess the safety and efficacy of reducing the leukocyte count medically as part of the therapy of acute or chronic vascular disease.
As suggested by Gurm et al., a very low WBC count may indicate general poor health and thus confer additional mortality risk independent of the coronary artery disease. Inclusion of such a subgroup in the analysis might dilute the association between the WBC count and cardiovascular risk in the remaining population. In the other large study, conducted by Barron et al.,9 patients with leukocyte counts <500/μL were excluded, and this may have reduced the likelihood of identifying a J-shaped relationship. It is possible that subgroups with very low WBC counts and higher mortality may have been missed in other studies analyzing only quartiles of leukocyte count, but some studies did not find this association even when specifically looking for it.20 One likely explanation for this apparent inconsistency suggested by Gurm et al.20 is that the J-shaped curve is more likely to be observed in studies using registry data of unselected patients21,25,31 rather than in clinical trial data20 because patients with low WBC counts and other illnesses are more likely to be excluded from clinical trials.

Data bearing on the mechanism of the association of leukocyte count and short-term morbidity and mortality were provided by Barron et al, who reported on pooled data on 992 patients from the Thrombolysis in Myocardial Infarction (TIMI) 10A and 10B trials of tenecteplase and reteplase treatment of acute myocardial infarction.6 WBC counts were obtained at entry, before drug administration. Higher WBC counts were associated with resistance to thrombolysis, increased thrombus burden, and impaired microvascular perfusion. Strikingly, 30-day mortality was 0% in patients with WBC counts ≤5000/μL and 10.4% in patients with WBC counts >15 000/μL, and a similar difference was noted for the development of congestive heart failure or shock (Figure 3). Careful analysis of the totality of the data led the authors to conclude that an elevated leukocyte count was associated with a poorer response to therapy, not just a larger infarct at presentation. In fact, even after adjusting for admission TIMI flow and myocardial perfusion grades, anterior myocardial infarction location, baseline and maximum creatine kinase (CK) levels, smoking status, and a number of other factors, WBC count still demonstrated a strong trend toward an association with the development of new congestive heart failure or shock (Figure 3). Careful analysis of the totality of the data led the authors to conclude that an elevated leukocyte count was associated with a poorer response to therapy, not just a larger infarct at presentation. In fact, even after adjusting for admission TIMI flow and myocardial perfusion grades, anterior myocardial infarction location, baseline and maximum creatine kinase (CK) levels, smoking status, and a number of other factors, WBC count still demonstrated a strong trend toward an association with the development of new congestive heart failure or shock (Figure 3). Careful analysis of the totality of the data led the authors to conclude that an elevated leukocyte count was associated with a poorer response to therapy, not just a larger infarct at presentation. In fact, even after adjusting for admission TIMI flow and myocardial perfusion grades, anterior myocardial infarction location, baseline and maximum creatine kinase (CK) levels, smoking status, and a number of other factors, WBC count still demonstrated a strong trend toward an association with the development of new congestive heart failure or shock (Figure 3).
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<th>Results</th>
<th>Significant After Adjustment</th>
</tr>
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<tbody>
<tr>
<td>Cooper et al</td>
<td>1999</td>
<td>6642</td>
<td>Entry WBC, neutrophil, and lymphocyte count</td>
<td>Cardiovascular mortality</td>
<td>Stable CHF (LVEF ≤0.35)</td>
<td>Increased rate by WBC and neutrophil counts (P&lt;0.001)</td>
<td>Yes</td>
</tr>
<tr>
<td>Kammersgaard et al</td>
<td>1999</td>
<td>763</td>
<td>Admission WBC count</td>
<td>In-hospital mortality</td>
<td>Acute stroke</td>
<td>Higher mortality with WBC count &gt;9000 μL (25.8%) than WBC ≤9000 μL (15.2%); P=0.0003</td>
<td>No</td>
</tr>
<tr>
<td>Barron et al</td>
<td>2000</td>
<td>975</td>
<td>Admission WBC count</td>
<td>30-day mortality or CHF/shock</td>
<td>AMI</td>
<td>Increased rate by WBC count (P=0.01) (lowest group 0% vs highest group 20.9%)</td>
<td>Yes</td>
</tr>
<tr>
<td>Danesh et al</td>
<td>2000</td>
<td>556 cases 1025 controls</td>
<td>Entry WBC count</td>
<td>Cardiovascular mortality or AMI</td>
<td>Men aged 40–53 in Britain</td>
<td>Increased rate by WBC count; OR, 1.89 (95% CI, 1.43–2.51)</td>
<td>Yes (3 of 4 adjustments)</td>
</tr>
<tr>
<td>Kyne et al</td>
<td>2000</td>
<td>185</td>
<td>Admission WBC count/differential</td>
<td>CHF in 4 days after MI</td>
<td>AMI</td>
<td>Increased rate by neutrophil percentage (OR, 15.2; 95% CI, 6.1–38.1)</td>
<td>Yes</td>
</tr>
<tr>
<td>Barron et al</td>
<td>2001</td>
<td>153 213</td>
<td>Admission WBC count</td>
<td>In-hospital and 30-day mortalities</td>
<td>AMI and age ≥65; WBC &gt;500 and ≤50 000/μL</td>
<td>In-hospital mortality: increased rate by WBC count (lowest quintile 7.7% vs highest 27.3%) 30-day mortality: increased rate by WBC (lowest 10.3% vs highest 32.3%)</td>
<td>Yes</td>
</tr>
<tr>
<td>Brown et al</td>
<td>2001</td>
<td>8914</td>
<td>Baseline WBC count</td>
<td>Coronary heart disease mortality after 17 years</td>
<td>Men and women aged 30–75</td>
<td>Increased mortality by WBC count (age-adjusted RR highest to lowest tertile 2.2, 95% CI 1.8–2.8; lowest 1.9% and highest 3.4%)</td>
<td>Yes</td>
</tr>
<tr>
<td>Cannon et al</td>
<td>2001</td>
<td>7651</td>
<td>Enrollment WBC count</td>
<td>30-day mortality</td>
<td>AMI or ACS</td>
<td>Increased rate by WBC count (lowest 1.4% vs highest 9.3%; P=0.0001)</td>
<td>Yes</td>
</tr>
<tr>
<td>Hajj-Ali et al</td>
<td>2001</td>
<td>1294</td>
<td>6-month post-MI WBC count</td>
<td>Reinfarction or death at 25 months (range 4–49)</td>
<td>AMI</td>
<td>Increased rate by WBC count (P=0.001) (lowest quartile 8.7% vs highest quartile 16.7%)</td>
<td>Yes</td>
</tr>
<tr>
<td>Marchioli et al</td>
<td>2001</td>
<td>11 324</td>
<td>Mean of WBC count at baseline and at 6, 12, 18, 24, 36, and 42 months</td>
<td>4-year mortality</td>
<td>MI in previous 3 months and favorable short term prognosis</td>
<td>Increased rate by WBC count (lowest 6.9% vs highest 17.7%)</td>
<td>Yes</td>
</tr>
<tr>
<td>Noto et al</td>
<td>2001</td>
<td>774</td>
<td>Entry WBC count</td>
<td>8-year stroke</td>
<td>Age &gt;40</td>
<td>OR for WBC count &gt;9200/μL, 2.5 (95% CI, 1.1–5.4; P=0.03)</td>
<td>Yes</td>
</tr>
<tr>
<td>Bickel et al</td>
<td>2002</td>
<td>1240</td>
<td>Pre-Cath WBC count</td>
<td>Cardiac mortality after median of 2.9 years (maximum 4.1)</td>
<td>Stable CAD by angiography</td>
<td>Increased rate by WBC count (P=0.003) (lower 3 quartiles 5.9% vs highest quartile 11.0%)</td>
<td>Yes (2 of 3 models)</td>
</tr>
<tr>
<td>Maekawa et al</td>
<td>2002</td>
<td>149</td>
<td>Serial monocyte counts</td>
<td>Pump failure</td>
<td>AMI</td>
<td>Increased risk by peak monocyte count ≥900 μL; RR 9.83 for pump failure (P=0.0001)</td>
<td>Yes</td>
</tr>
<tr>
<td>Bagger et al</td>
<td>2003</td>
<td>2057</td>
<td>Preoperative WBC count</td>
<td>30-day mortality</td>
<td>Coronary artery bypass surgery</td>
<td>Increased rate by WBC count (P=0.0001; lowest quartile 1.7% vs highest quartile 7.5%)</td>
<td>Yes</td>
</tr>
<tr>
<td>Bhatt et al</td>
<td>2003</td>
<td>10 480</td>
<td>Admission WBC count</td>
<td>6-month mortality</td>
<td>ACS</td>
<td>Increased rate by WBC count (P=0.001; lowest quartile 4% vs highest quartile 8%)</td>
<td>Yes</td>
</tr>
</tbody>
</table>
infarction and leukocyte count was less dramatic in patients who underwent reperfusion or early revascularization treatment than in those who did not receive these therapies. Thus, in the National Registry of Myocardial Infarction 4 (n=115 273), the mortality odds ratio (comparing the highest to the lowest quartile of leukocyte count) was 4.0 for those not undergoing reperfusion therapy (n=83 775), and the absolute in-hospital mortalities were 18.6% and 4.7%, respectively, for the highest and lowest WBC count quartiles, whereas the comparable odds ratio for those undergoing reperfusion therapy with thrombolytic agents or primary percutaneous coronary intervention (PCI; n=30 954) was 2.7 (9.3% versus 3.5%). These data yield absolute differences in mortality between the highest and lowest WBC count quartiles of 13.9% for those not receiving reperfusion therapy and 5.8% for those receiving such therapy.25 In the Platelet Glycoprotein IIb/IIIa in Unstable Angina Receptor Suppression Using Integrilin Therapy (PURSUIT) study (n=10 480),

### TABLE 1. Continued

<table>
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<th>Authors</th>
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<th>Significant After Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dacey et al20</td>
<td>2003</td>
<td>1170</td>
<td>Preoperative WBC count</td>
<td>Coronary artery bypass surgery</td>
<td>Increased risk by WBC count (lowest group 1.4% vs highest group 7.1%)</td>
<td>Yes</td>
</tr>
<tr>
<td>Gurm et al21</td>
<td>2003</td>
<td>7179</td>
<td>Pre-procedure WBC count/differential</td>
<td>PCI</td>
<td>Hazard ratio 1.098 per 1000 μL increase in WBC count (P&lt;0.001; lowest group ~4% vs highest group ~16%)</td>
<td>Yes</td>
</tr>
<tr>
<td>Menon et al21</td>
<td>2003</td>
<td>6530</td>
<td>Admission WBC count</td>
<td>AMI</td>
<td>Increased rate by WBC count (OR, 2.76; 95% CI, 2.23–3.42; lowest group 9% vs highest group 25%)</td>
<td>Yes</td>
</tr>
<tr>
<td>Yen et al22</td>
<td>2003</td>
<td>2279</td>
<td>Admission WBC count</td>
<td>ACS</td>
<td>Increased mortality by quartile (P&lt;0.01; lowest 6.1% vs highest 10.6%)</td>
<td>Yes</td>
</tr>
<tr>
<td>Brown et al102</td>
<td>2004</td>
<td>8459</td>
<td>Baseline WBC count</td>
<td>Cerebrovascular disease mortality after 17 years</td>
<td>Increased mortality by WBC count (age-adjusted RR highest to lowest quartile 2.3; 95% CI, 1.4–4.0; P&lt;0.001)</td>
<td>Yes</td>
</tr>
<tr>
<td>Byrne et al23</td>
<td>2004</td>
<td>732</td>
<td>Initial WBC count</td>
<td>ACS, AMI, urgent PCI, death, or stroke Ischemic stroke, MI, or vascular death over 1.9 year</td>
<td>No association between WBC count and end point</td>
<td>No</td>
</tr>
<tr>
<td>Grau et al24</td>
<td>2004</td>
<td>18 558</td>
<td>Multiple WBCs and Differentials</td>
<td>ACS, AMI, or stable angina Ischemic stroke, MI, peripheral vascular disease</td>
<td>Increased rate by WBC and neutrophil counts (RR highest to lowest quartile 1.39 for WBC count; P&lt;0.001; and 1.53 for neutrophils; P&lt;0.001)</td>
<td>Yes</td>
</tr>
<tr>
<td>Grzybowski et al25</td>
<td>2004</td>
<td>115 273</td>
<td>Admission WBC count</td>
<td>AMI</td>
<td>Increased rate by WBC count, starting ≥5000 μL, with OR highest to lowest quartile 4.09 (95% CI, 3.83–4.75)</td>
<td>Yes</td>
</tr>
<tr>
<td>Grau et al24</td>
<td>2004</td>
<td>18 558</td>
<td>Multiple WBCs and Differentials</td>
<td>Ischemic stroke, MI, or vascular death over 1.9 year</td>
<td>Increased rate by WBC and neutrophil counts (risk ratio, highest to lowest quartile 1.39 for WBC count; P&lt;0.001; and 1.53 for neutrophils; P&lt;0.001)</td>
<td>Yes</td>
</tr>
<tr>
<td>Tong et al26</td>
<td>2004</td>
<td>3776</td>
<td>Initial WBC count</td>
<td>Presence of macrovascular or microvascular disease</td>
<td>Increased prevalence by WBC count (P&lt;0.01 for macrovascular and microvascular disease)</td>
<td>Yes</td>
</tr>
<tr>
<td>Yarnell et al27</td>
<td>2004</td>
<td>4325</td>
<td>Baseline WBC count</td>
<td>Presence of macrovascular or microvascular disease</td>
<td>Increased rate by WBC count (relative odds highest to lowest WBC 2.79; 95% CI, 2.06, 3.77)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

ACS indicates acute coronary syndrome; AMI, acute myocardial infarction; CHF, congestive heart failure; LVEF, left ventricular ejection fraction; OR, odds ratio; MI, myocardial infarction; CAD, coronary artery disease; RR, relative risk; Cath, coronary artery catheterization.
which included patients with acute coronary syndromes, the
6-month mortality odds ratio comparing the highest to lowest
quartiles of leukocyte count was 2.6 (10.5% versus 4.1%
mortality, respectively; \( P < 0.001 \)) in those who did not
undergo PCI or surgical revascularization therapy \( (n = 6791) \)
during their index hospitalization, and 1.0 (4% versus
4%) for those undergoing revascularization, yielding abso-
lute mortality differences of 6.4% and 0%, respectively.18
(Figure 5) Thus, regardless of the mechanism underlying the
association between leukocytosis and mortality after acute
myocardial infarction, it appears that modern reperfusion and
early revascularization strategies mitigate or eliminate the
association between WBC count and mortality during the first
6 months after a myocardial infarction. These data suggest
that patients who are not eligible for, or who do not receive,
reperfusion or revascularization therapy may be a more
appropriate group in which to test the effect of interventions
to lower the WBC count.

TABLE 2. Mechanisms by Which Leukocytosis May Predispose
to Ischemic Vascular Events

<table>
<thead>
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<th>Prothrombotic effects</th>
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<tbody>
<tr>
<td>Monocyte and neutrophil tissue factor, and leukocyte-derived tissue factor-containing microparticles</td>
</tr>
<tr>
<td>Synergism with platelets in providing a catalytic surface for thrombin generation, including receptors for fibrinogen and proteolytic activity that can activate coagulation factor intermediates and platelets</td>
</tr>
<tr>
<td>Platelet–leukocyte aggregates and transcellular metabolism</td>
</tr>
<tr>
<td>Prothrombotic alteration of fibrinogen by post-translational, myeloperoxidase-enhanced nitrating oxidants</td>
</tr>
</tbody>
</table>

Vascular effects

| Rheologic compromise of microvasculature by adhesion, aggregation, platelet recruitment, and obstruction |
| Release of proinflammatory and vasculotoxic factors, reactive oxygen species, proteases, eicosanoids, growth factors, interleukins, and myeloperoxidase |
| Acceleration of atherosclerosis |
| Monocyte infiltration |
| Leukocyte-dependent oxidation of low-density lipoprotein and HDL |
| Platelet–leukocyte aggregates |

Contribution to intimal hyperplasia after vascular injury

WBC Count Association With Perioperative Mortality and Cerebrovascular Accidents

The mortality associated with coronary artery bypass surgery
as a function of preoperative leukocyte count was studied in 2057 patients by Bagger et al.17 When assessed by increasing
quartiles of leukocyte count, the 30-day mortality was 1.7,
2.7, 3.3, and 7.5\% \( (P < 0.0001 \) for trend). Even after exclud-
ing patients with recent myocardial infarction or unstable
angina, or patients with conditions that may have predisposed
them to elevated leukocyte counts, the association remained
significant. In other surgical studies by Dacey et al \( (n = 11270) \) and Albert et al \( (n = 7483) \), preoperative leuko-
cyte counts correlated with in-hospital mortality or perioper-
ative cerebrovascular accidents.

WBC Count As a Risk Factor for Long-Term Ischemic Cardiovascular Disease

In addition to the association of leukocyte count with short-
term morbidity and mortality from vascular disease, there are
impressive data on the association of elevated leukocyte count with long-term vascular disease morbidity and mortality.
In the Caerphilly and Speedwell population studies, men
between the ages of 45 and 63 in the United Kingdom were evaluated and then followed for the next ~10 years. A total of 4325 completed the study, of whom 1005 had a history of vascular disease at the time of entry into the study. At the end of the study, 525 (12.2%) had experienced a myocardial infarction (silent, nonfatal, or fatal). After adjusting for age and investigation site, the relative odds of having a myocardial infarction comparing the highest to lowest quintiles of leukocyte count was 2.79 (95% CI, 2.06 to 3.77). The corresponding relative odds values for having an event for values of total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol (comparing lowest to highest quintiles in the latter) were all lower than that for leukocyte count (2.07 [1.55 to 2.78], 2.72 [1.98 to 3.72], and 2.34 [1.72 to 3.19], respectively. By multivariate analysis, the leukocyte count retained its association with adverse events, and the association was strengthened by adjustment for the imprecision of using only a single leukocyte value (regression dilution bias).

One serious limitation of the studies that used the WBC count at the time of admission for an acute myocardial infarction is the short-term impact of the infarction on WBC count. That is why the Multicenter Diltiazem Postinfarction Trial is of particular interest because patients were enrolled into the study and had their WBC counts analyzed 6 months after their myocardial infarction. Data were obtained on 1294 patients followed for a mean duration of 25 months (range 4 to 49 months). The mean leukocyte count at enrollment in those who went on to experience reinfarction or death was 8100±2400/µL versus 7600±3200/µL for patients who did not experience an event (P<0.001).

In the GISSI-Prevenzione study on myocardial infarction survivors, 11,324 patients with an acute myocardial infarction in the previous 3 months, and with a good short-term prognosis, were studied for a 4-year period, during which there were 1071 deaths. Regression dilution bias was minimized in this study by using the mean of 7 WBC counts obtained at intervals over 42 months (Table 1). In those with leukocyte counts <6000/µL, the mortality was 6.9%, whereas in those with leukocyte counts ≥9000/µL, the mortality was 17.7%, yielding an absolute difference of 10.8%. Investigators used their data to create an assessment risk score and concluded that the leukocyte count was an independent risk factor for death. Relative to having a leukocyte count of <7000/µL, having a count ≥9000/µL was assigned 6 risk score points in men and 8 risk score points in women. For comparison, having left ventricular dysfunction was assigned 7 risk score points in both sexes, having diabetes was assigned 7 points in women and 3 points in men, and having a serum HDL ≤35 mg/dL compared with >55 mg/dL was assigned 6 points in women and 5 points in men.

Grau et al analyzed leukocyte data on 18,558 patients in the Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events (CAPRIE) study, which enrolled patients with a history of ischemic stroke, myocardial infarction, or peripheral arterial disease. Patients were randomized to clopidogrel or aspirin and followed for 1 to 3 years. Baseline leukocyte counts were used unless they were obtained within 28 days after a myocardial infarction or ischemic stroke, in which case, samples obtained 4 weeks after entry were used. Patients were excluded from the study if they had leukocyte counts <3500/µL or ≥25,000/µL. During a mean follow-up period of 1.9 years, 1840 patients (9.9%) experienced ischemic stroke, myocardial infarction, or vascular death. In multivariate analysis, after adjusting for vascular risk factors and diseases, hematocrit, and study treatment, the relative risk of recurrent ischemic events was significantly higher in patients in the highest quartile of leukocyte counts compared with those in the lowest quartile (1.42; 95% CI, 1.25 to 1.63 overall; 1.30, 1.56, and 1.51 for stroke, myocardial infarction, and vascular death). Thus, in addition to supporting an association of WBC count and myocardial infarction and vascular death in a high-risk population, this study established a relationship between leukocyte count and stroke.

Data from the Studies Of Left Ventricular Dysfunction (SOLVD) trials, which enrolled stable patients with left ventricular dysfunction are of interest because the strong association of WBC count with cardiovascular mortality observed in the whole group, even after multivariate analysis (P<0.001), was found to be confined exclusively to the group in which ischemic vascular disease was responsible for the ventricular dysfunction. This observation supports a selective contribution of leukocytosis to the chronic vascular changes or the acute thrombotic phenomena that contribute to ischemic vascular disease.

**Acute Changes in Leukocyte Count Before Onset of an Ischemic Vascular Event**

Separate from the association between chronically elevated leukocyte counts and increased risk of ischemic vascular
events is the issue of whether transient elevations in the WBC count are associated with an increased risk of sustaining an acute event. The design of the CAPRIE study allowed this question to be addressed because leukocyte counts were performed frequently to monitor the potential toxicity of clopidogrel.

In fact, leukocyte counts obtained by chance ≤7 days before the onset of a new vascular event were significantly higher than the baseline counts in these same patients (n = 211; P = 0.004), whereas counts obtained anywhere from 8 to 120 days before a new event (and the last counts obtained in patients who did not sustain a recurrent event) did not differ significantly from the patients’ baseline values. (Figure 6) Although these intriguing data are consistent with the hypothesis that the rise in leukocyte count contributed to the recurrent event, many other interpretations are also possible. Additional clinical observations are also consistent with an association between transient elevations in leukocyte count and acute vascular events because a number of studies have noted an association between recent infection and the onset of myocardial infarction or acute stroke, even though an association with leukocyte count was not specifically studied. 32–34

Granulocyte colony-stimulating factor (CSF) infusions have been well tolerated by individuals donating peripheral stem cells and in some stable patients with myocardial infarctions when given alone or as part of peripheral stem cell infusions. 35–37 However, granulocyte CSF (G-CSF) and granulocyte–macrophage CSF (GM-CSF) treatments have been associated with isolated cases of microvascular and macrovascular venous and arterial thrombosis in patients with malignancies or hematologic disorders. 38–41 and G-CSF treatment has been associated with both progression to myocardial infarction in patients with intractable angina and increased risk of restenosis in patients undergoing PCIs. 36,37,40 The expanding use of stem cell strategies, including those that involve autologous stem cell mobilization with growth factors that can elevate the WBC count, make it important to obtain additional data on the safety of these agents in patients with cardiovascular disease.

Figure 6. Differences in leukocyte counts in 18,293 patients in the CAPRIE study between the baseline value and the last measurement before a recurrent event, or at the end of the study for those not having a recurrent event. Modified with permission from Grau et al. 24

Differences in leukocyte counts in 18,293 patients in the CAPRIE study between the baseline value and the last measurement before a recurrent event, or at the end of the study for those not having a recurrent event. Modified with permission from Grau et al. 24

Figure 7. Univariate association between quartiles of leukocyte count, neutrophil count, monocyte count, and lymphocyte count and risk ratio for annual rate of recurrent ischemic vascular events (ischemic stroke, myocardial infarction, and vascular death) in 18,558 patients in CAPRIE. 24 Risk ratios are expressed relative to lowest quartile.

Evaluation of Ischemic Vascular Event Risk by Type of Leukocyte

Most studies of the association of leukocytosis and ischemic vascular disease recorded a total WBC count without a leukocyte differential analysis, and thus, there are relatively few data on the specific cell type most associated with the increased risk. In the CAPRIE study, elevations in neutrophils and to a lesser extent monocytes showed significant associations with the risk of recurrent ischemic events, but elevations in lymphocyte counts showed, if anything, an inverse relationship to the risk of recurrent events (Figure 7). In the Evaluation of c7E3 for Prevention of Ischemic Complications (EPIC) study, the total WBC count correlated best with 3-year mortality, but the neutrophil count also showed a correlation. 20 The monocyte count was not correlated with outcome, and as in the CAPRIE study, the lymphocyte count demonstrated an inverse relationship. Similarly, in the SOLVD trials, total WBC and neutrophil counts were significantly associated with cardiovascular death, even after multivariate adjustments, and once again, a strong trend was observed for an inverse relationship between the lymphocyte count and cardiovascular mortality. 4

Monocyte counts were monitored daily for 4 days in a study of 149 patients with Q-wave myocardial infarction. 15 In multivariate analysis, a peak monocyte count ≥900/μL was found to be an independent determinant of cardiac pump failure (relative risk, 9.83; P < 0.0001) and cardiac events (death, recurrent myocardial infarction, and readmission for heart failure; relative risk, 6.30; P < 0.0001).

Thus, among the leukocytes, morbidity and mortality is most closely associated with the neutrophil count, but the association with the monocyte count may be underestimated because of greater regression dilution bias (see below). Monocyte production of tissue factor is well established and can be enhanced by activation, providing a direct link to thrombosis; the importance of neutrophils as a source of tissue factor is less certain. 32–44 It is notable that an inverse relationship between mortality and lymphocyte count was observed in all 3 studies, even though the relationship was not as strong as the direct associations involving total WBC count.
and neutrophils. Because lymphocyte counts are depressed by elevated glucocorticoid levels, even during the diurnal cycle, it is possible that the lower lymphocyte counts in patients with a poorer prognosis reflect elevated glucocorticoid levels. In fact, relative lymphocytopenia in patients with chest pain was found to be similar to rapid creatinine kinase-MB in diagnosing a myocardial infarction (sensitivities of 58% and 56% for lymphocytopenia and elevated rapid creatinine kinase-MB, respectively; specificities of 91% and 93%); elevated cortisol levels were, in fact, found in this same group of patients (diagnostic sensitivity of 68% and specificity of 86%).

**Effects of Glucocorticoid and Aspirin Therapy on Leukocyte Counts and Cardiovascular Disease**

Pharmacological doses of glucocorticoids elevate neutrophil counts and depress lymphocyte and monocyte counts, with a net increase in leukocytes most common. Therefore, it is of interest that in a study of >65 000 patients and 80 000 controls, long-term therapy with doses of glucocorticoids exceeding the physiological range was found to be associated with increased risk of cardiovascular events, even after adjustment for known risk factors.

In 4 separate nonrandomized studies of leukocytosis and cardiovascular risk involving a total of >160 000 patients, aspirin use was associated with significantly lower WBC counts ($P=0.04$; $P=0.008$; $P<0.001$; and $P<0.0001$). Because these studies were not randomized, many confounding factors may have influenced the apparent association between aspirin use and lower leukocyte counts. However, if aspirin use actually is associated with lower leukocyte counts, at least a portion of the beneficial effects of aspirin on cardiovascular events may be related to the reduction in leukocyte counts.

**Association of Leukocyte Count With Vascular Complications of Diabetes**

Diabetes, which is known to be associated with increased risk of vascular disease, is also associated with elevated leukocyte counts. Moreover, even within the diabetic population, the extent of leukocytosis is associated with macrovascular and microvascular complications of type 2 diabetes mellitus, as well as endothelial dysfunction. Thus, among 3776 subjects studied in Hong Kong, after adjustment for sex, age, smoking status, disease duration, BMI, mean arterial pressure, waist-to-hip ratio, HbA1c, HDL cholesterol, and low-density lipoprotein cholesterol, patients with higher leukocyte counts remained at higher risk of macrovascular disease (angina, myocardial infarction, stroke, or peripheral vascular disease) and microvascular disease (the combination of retinopathy and albuminuria), with the odds ratio of the patients in the highest-to-lowest quintiles of leukocyte count $>2$ for macrovascular and microvascular disease and with the $P$ value for trend $<0.001$ for both. In another study in type 2 diabetics, leukocyte count was associated with endothelial-dependent and endothelial-independent vasodilation, even after adjusting for other known risk factors, suggesting a potential association between leukocytosis and endothelial dysfunction.

**Relationship Between WBC Count and Fitness, BMI, and Activity Levels**

Exercise and fitness have been demonstrated to decrease the risk of ischemic vascular disease in numerous studies, but the physiological mechanisms responsible for the protective effects have not been clearly defined. Similarly, there is evidence that BMI is associated with increased risk of cardiovascular disease, but it is less clear whether an elevated BMI is an independent risk factor. Therefore, it is of interest that in a study of 4057 nonsmoking men without a previous history of stroke or heart attack, the mean WBC count showed a significant inverse correlation with fitness (low, moderate, and high fitness groups; 6637/µL, 6.258/µL, and 5.653/µL WBC count, respectively; $P<0.001$). Moreover, in accord with previous studies, even after adjustment for age, within each fitness group, the WBC count correlated directly with BMI. Further support for an inverse relationship between WBC count and physical activity, even after adjusting for smoking, sex, cardiovascular disease states, age, race, diabetes, BMI, and hypertension ($P<0.001$), comes from the study by Geffen et al, which was conducted in 5868 men and women $\geq$ 65 years of age. Moreover, in the Atherosclerosis Risk in Communities (ARIC) study of 14 679 participants aged 45 to 64 years, leisure activity, sports activity, and work activity indices correlated inversely with WBC count in whites, and the leisure activity and work activity indices correlated inversely with WBC count in blacks, even after excluding smokers and making several other adjustments. Thus, if leukocytosis contributes directly to ischemic vascular disease, it is possible that at least some of the protective effects of exercise may operate through a reduction in WBC count.

**Possible Underestimation of the Association of Leukocytosis With Cardiovascular Disease Attributable to Variations in Leukocyte Counts and Multivariate Adjustments for Smoking and BMI**

Variabilities in leukocyte determinations and inappropriate multivariate adjustments in the studies reviewed above may have resulted in their underestimating the true association of leukocytosis with increased risk of cardiovascular disease. Thus, because many of the studies used only a single leukocyte determination, short- or medium-term factors such as infection or disease, seasonal or diurnal fluctuations, or variability in the accuracy of leukocyte determinations may have affected the results, resulting in mistakenly categorizing some individuals with lower levels as having higher levels and vice versa. When sufficient data have been available to calculate the impact of such errors (termed regression dilution), they have been reported to result in underestimations of the true associations with cardiovascular disease of as much as 30% to 35% for leukocyte and neutrophil counts and 50% for monocyte counts.
The strong association between cigarette smoking and elevated leukocyte counts has been documented in many studies, and even regular exposure to "second-hand" smoke is associated with increased leukocyte counts. As a result, "adjusting" the association of leukocytosis with ischemic vascular disease for smoking tends to lessen the association between leukocytosis and ischemic vascular events; however, even after this adjustment, the association remains significant in most studies (Table 1). The implicit rationale for using this "adjustment" is the unproved assumption that smoking causes its adverse effects on ischemic vascular disease by mechanisms other than elevating the leukocyte count. If, on the other hand, smoking in fact causes its adverse effects through elevating the leukocyte count, then the effect of smoking should be adjusted for the effects of the elevated leukocyte count and not vice versa. When such an adjustment has been made, much of the effect of smoking is, in fact, eliminated (eg, in the Caerphilly collaborative studies, the unadjusted relative odds for cardiovascular events for current cigarette smokers was 2.27 [95% CI, 1.63 to 3.15], whereas after adjusting for WBC count, it was 1.63 [1.15 to 2.31]). Moreover, the rapid reduction in cardiovascular risk (stroke and myocardial infarction) that occurs after smoking cessation may fit well with the hypothesis that at least some of adverse effects associated with smoking are mediated by leukocytosis.

**Experimental Model and Human Blood Leukodepletion Data on the Role of Leukocytes in Ischemic Vascular Events**

Given the robust epidemiologic data, it is surprising that there have been relatively few studies to assess the effect of leukocyte depletion on experimental ischemic vascular events. Although there is extensive literature on the importance of leukocytes in ischemia/reperfusion injury in different organs, including protection from injury by leukodepletion, adhesion receptor antagonists to αMβ2 and P-selectin that demonstrated efficacy in these models have been disappointing when studied in humans with atherothrombotic ischemic vascular disease. There is intriguing evidence that leukocyte depletion of blood products in humans may be associated with better outcomes in patients undergoing cardiopulmonary bypass surgery, but the studies are not definitive.

**Leukocytes in Sickle Cell Disease**

Sickle cell disease is characterized by a severe hemolytic anemia, but much of the morbidity and mortality of the disorder is attributable to vascular occlusion. Pain crises are thought to be attributable to microvascular obstruction, primarily in postcapillary venules. Macrovacular occlusion, primarily affecting the cerebral circulation in children, also occurs and is attributable to a combination of nonatherosclerotic intimal hyperplasia and acute thrombosis. Studies using modern imaging techniques indicate that nearly half of patients with sickle cell disease have MRI evidence of brain injury before age 10, and nearly two thirds have cerebrovascular disease; of those with brain injury, nearly one third have macrovascular cerebrovascular disease.

The WBC count is elevated in nearly all patients with sickle cell disease, and epidemiologic data indicate that having a higher WBC count is associated with a worse prognosis. In addition, risk of stroke and silent cerebral infarction is greater in at least some patients with higher leukocyte counts. Acute chest syndrome, one of the most serious complications of sickle cell disease, is accompanied by very high leukocyte counts, and there have been 4 separate reports of patients with sickle hemoglobin sustaining the rapid onset of pain crisis, acute chest syndrome, and even death after receiving a myeloid CSF (G-CSF or GM-CSF). Increased expression of leukocyte adhesion molecules has also been associated with more severe disease.

Studies from our laboratory, conducted in collaboration with Dr Paul Frenette and colleagues at Mount Sinai School of Medicine, using intravital microscopy to assess vascular occlusion in postcapillary venules of mice expressing human sickle hemoglobin, demonstrated that leukocytes play a crucial role in the process, with erythrocytes primarily interacting with the WBCs that are adherent to the wall of the venule rather than with the vessel wall itself. Mice lacking the endothelial adhesion molecules that mediate leukocyte rolling and attachment, P-selectin and E-selectin, were protected from vaso-occlusion. Thus, in this experimental model, leukocytes participate directly in microvascular obstruction.

Hydroxyurea was introduced for the therapy of sickle cell disease on the basis of its ability to increase hemoglobin F and ameliorate the frequency of painful crises. However, hydroxyurea also decreases the leukocyte count, and there is evidence that some patients respond to hydroxyurea more rapidly than can be accounted for by changes in hemoglobin F but consistent with the time course of hydroxyurea-induced leukocyte reduction. However, data from the Multicenter Study of Hydroxyurea in Sickle Cell Anemia did not support a major role for leukocyte reduction as a mechanism of the effects of hydroxyurea because there was no evidence that either pretreatment or post-treatment neutrophil counts above or below either 5000/µL or 7000/µL correlated with long-term mortality. It remains possible, however, that hydroxyurea-induced reductions in leukocyte counts contributed to the mortality benefit in more subtle ways.

**Is it Justified to Try to Intervene to Reduce Leukocyte Counts in Patients With Ischemic Vascular Disease?**

Because evidence of an association between elevated leukocyte counts and ischemic vascular events is compelling, and because it is impossible to exclude from current evidence the possibility that leukocytes directly contribute to the risk, it is logical to consider studies to assess the impact of altering leukocyte function or reducing the leukocyte count. Studies targeting leukocyte adhesion via effects on the integrin αMβ2 and P-selectin have not demonstrated positive results in stroke or myocardial infarction, although it is possible that new agents in this class will be more effective. However, it is possible that inhibition of any single leukocyte receptor is inadequate to achieve a therapeutic effect, in which case a...
strategy of lowering the leukocyte count may be more effective. Among the agents available to achieve a reduction in leukocyte count, hydroxyurea has the benefits of rapid onset of action, relatively rapid recovery from its effects when the drug is stopped, relatively little organ toxicity, and an extensive history of its use for long periods of time in patients with myeloproliferative disorders and sickle cell disease.90,92 In general, in sickle cell disease, the toxicity profile has been favorable, with few episodes of symptomatic severe neutropenia or serious anemia.90,92 The mild reduction in platelet count commonly produced by hydroxyurea may, in fact, be an added benefit in reducing the risk of thrombotic events.90 However, there is concern that hydroxyurea is leukemogenic. Data from patients with myeloproliferative disorders suggest that hydroxyurea may increase the risk of leukemia, usually after treatment for 8 years, but there is controversy as to whether these patients’ underlying myeloproliferative disorders place them at higher risk of developing leukemia from hydroxyurea than individuals without such disorders. Although there were no cases of leukemia in 2 studies of hydroxyurea in patients with sickle cell disease, 1 in 122 children treated for an average of 2.8 years (range 0.5 to 8.4 years)92 and the other in 219 adults treated for an average of 7.6 years,90 and there was no increase in acquired DNA mutations as judged by in vitro assays using DNA extracted from peripheral blood mononuclear cells in the latter study,92 there have been 3 reports of patients with sickle cell disease treated with hydroxyurea developing leukemia: 1 after 2 years, the second after 6 years, and the third after 8 years of treatment.98–100

Together, the data summarized in this review make it biologically plausible that reducing the leukocyte count may improve the acute and chronic outcome of ischemic vascular disease. However, because the most attractive drug to accomplish a reduction in leukocyte counts is potentially leukemogenic, it is not certain that treatment would produce more benefit than harm. To maximize the benefit-to-risk ratio, a number of design features could be considered. Thus, restricting a study to patients who are at high risk for short-term mortality after myocardial infarction or acute coronary syndrome, namely, those who are of older age, have contraindications to reperfusion therapy, and have high leukocyte counts, would increase the potential for any single patient obtaining a benefit. Similarly, limiting the therapy to 30 days and again restricting the study to elderly patients would decrease the likelihood that any single patient would experience adverse effects.

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Leukocytosis and Ischemic Vascular Disease Morbidity and Mortality: Is It Time to Intervene?
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