Relationship of Monocyte Count and Peripheral Arterial Disease
Results From the National Health and Nutrition Examination Survey 1999–2002

Khurram Nasir, Eliseo Guallar, Ana Navas-Acien, Michael H. Criqui, João A.C. Lima

Background—Although white blood cell (WBC) count has been consistently associated with cardiovascular end points, little information is available on the independent contribution of specific white blood cell types. The objective of this study is to assess the independent association of WBC types and other inflammatory markers with the presence of reduced ankle-brachial blood pressure index (ABI), a marker of subclinical peripheral arterial disease (PAD).

Methods & Results—Cross-sectional study in 3949 individuals ≥40 years of age without known cardiovascular disease who participated in the 1999 to 2002 National Health and Nutrition Examination Survey (NHANES). PAD was defined as an ABI <0.9 in at least 1 leg. After adjustment for traditional cardiovascular risk factors, the odds ratios of PAD comparing the highest to the lowest quartiles were 2.24 (95% confidence interval 1.24 to 4.04) for monocytes, 1.74 (0.87 to 3.45) for neutrophils, 2.53 (1.62 to 3.96) for C-reactive protein, and 2.68 (1.03 to 6.94) for fibrinogen. When WBC types and inflammatory markers were simultaneously included in the full model, the corresponding odds ratios were 1.91 (95% confidence interval 1.06 to 3.42) for monocytes, 1.15 (0.49 to 2.69) for neutrophils, 1.37 (0.75 to 2.49) for C-reactive protein, and 2.21 (0.88 to 5.57) for fibrinogen.

Conclusions—Monocytes were the only WBC type significantly and independently associated with PAD in a representative sample of the U.S. population after adjustment for other inflammatory markers. These findings reflect the potential role of circulating monocyte counts as markers of atherosclerosis. (Arterioscler Thromb Vasc Biol. 2005;25:0-0.)

Key Words:

Arteriosclerosis, Thrombosis, and Vascular Biology is available at http://www.atvbaha.org DOI: 10.1161/01.ATV.0000175296.02550.e4

Arteriosclerosis is a chronic inflammatory process characterized by early leukocyte recruitment that continues throughout plaque maturation and rupture.1,2 Indeed, white blood cell (WBC) count elevation has been consistently associated with cardiovascular events.1,3 However, WBC count includes several cell types with potentially different pathogenic roles in atherogenesis. Although monocyte-macrophages play a central role in atherogenesis,1,2,4 little attention has been paid to the association of monocyte count with subclinical atherosclerosis. Few studies have assessed the association of specific WBC types with cardiovascular end points,5 and these studies have not evaluated the specific contribution of circulating monocytes with respect to other WBC types and markers of inflammation, such as C-reactive protein (CRP) and fibrinogen.6–8

Peripheral arterial disease (PAD) is strongly associated with increased levels of inflammatory markers, including WBC count, CRP, and fibrinogen.9–11 The objective of this study was to assess the association of circulating blood monocytes and other WBC types with the presence of reduced ankle-brachial blood pressure index (ABI), a highly specific marker of PAD.12–15 We were specifically interested in the independent contribution of each cell type after adjustment for CRP, fibrinogen, and traditional cardiovascular risk factors.

Methods

Since the 1960s, the National Center for Health Statistics has conducted the National Health and Nutrition Examination Surveys (NHANES), a series of cross-sectional surveys designed to provide data representative of the noninstitutionalized civilian U.S. population. In this study we used data from NHANES 1999 to 2002, as ABI measurements were only available in NHANES since 1999. The total population of NHANES 1999 to 2002 was 21 004. Participants 40 years of age and older were asked to participate in the ABI Section of the Lower Extremity Disease examination. Overall 6671 individ-
uals were ≥40 years of age in NHANES 1999 to 2002. Persons were excluded from the examination if they have bilateral amputations or weigh >400 pounds (because of equipment limitations). In addition to these exclusion criteria, some individuals who were eligible for the examination (≥40 years) might not have received the examination due to the following multiple reasons: (1) casts, ulcers, dressings, or other conditions of the participant interfered with testing, (2) participant could not understand the test instructions, (3) participant became ill and the test could not be performed, (4) there was an equipment failure, (5) participant refused, (6) participant came late or left early from the MEC and the LED examination could not be performed, or (7) some other reason. As a result, these eligible persons will have missing data for the ABI variables. Overall 5083 individuals ≥40 years of age had a valid ABI measurement. Individuals with a known history of stroke, myocardial infarction, angina, and congestive heart failure (n=749) were excluded. We also excluded participants with ABI values >1.4 (n=68)14 and those with missing values in variables of interest (n=317), leaving 3949 individuals in the present study.

Ankle Brachial Index
A specific protocol was used to measure ABI in NHANES 1999 to 2002.17 Systolic blood pressure was measured on the right arm (brachial artery) or the left arm if only choice, and both ankles (posterior tibial arteries) with a Doppler device (Parks Mini-Laboratory IV, model 3100, Parks Medical Electronics). Systolic blood pressure was measured twice at each site for participants 40 to 59 years of age and once at each site for participants ≥60 years of age. The left and right ABI measurements were obtained by dividing the ankle systolic blood pressure for each side respectively by the systolic brachial blood pressure, or by using the mean pressures for participants with 2 blood pressure measurements at each site. PAD was defined as an ABI value <0.9 in at least 1 leg.18

WBC Counts, C-Reactive Protein, and Fibrinogen
WBC count was determined on a Coulter Counter Model S-PLUS JR (Coulter Electronics). Target coefficients of variation were <3%. High-sensitivity CRP was measured using latex-enhanced nephelometry on a Behring Nephelometer Analyzer System (Behring Diagnostics, Inc) with NA Latex CRP Kit (Behring Diagnostics, Inc). The intra-assay coefficient of variation was 3.2%. Fibrinogen was determined by a comparison of the clotting time from a sample of the small number of distinct levels, basophil count was grouped in 2 categories (≥0.1 and <0.1 10⁹/L, with 58% and 42% of subjects, respectively), and eosinophil count was grouped in 3 categories (<0.2, 0.2, and ≥0.2 10⁹/L, with 45%, 31%, and 24% of subjects, respectively). Logistic regression was used to estimate the odds ratios for the prevalence of PAD in quartiles 2 to 4 of inflammatory markers compared with the first quartile. Tests for trend across increasing quartiles (or prespecified cutoffs; for eosinophils and basophils) were computed by introducing variables with the median level for each quartile in the regression models.

Three sets of multivariable models were used to examine the association of WBC types, CRP, and fibrinogen in a hierarchical fashion. Model 1 adjusted for age, sex, and race. Model 2 further adjusted for traditional risk factors (smoking status, obesity, hypertension, diabetes, hypercholesterolemia, and GFR). Model 3 adjusted simultaneously for WBC types, CRP, and fibrinogen in addition to the covariates included in Model 2.

Results
There were 220 PAD cases among 3949 study participants (weighted prevalence 3.6%; 95% CI 3.1% to 4.1%). WBC count, CRP, and fibrinogen were higher in PAD cases compared with non-cases (all P<0.001; Table 1). Among WBC subtypes, only monocytes and neutrophils were significantly higher in PAD cases. CRP and fibrinogen were strongly correlated (r=0.54), but their correlation with WBC subtypes was weaker (r≤0.30 for all WBC subtypes; Table 2). Among WBC types, neutrophils were the most strongly correlated with CRP and fibrinogen.
The correlation coefficient of monocytes with neutrophils was 0.45 and similar in males and females.

In age-, sex-, and race-adjusted analyses, monocytes, neutrophils, CRP, and fibrinogen levels were significantly associated with the prevalence of PAD (Table 3, Model 1). These associations persisted, although slightly attenuated, after adjustment for traditional cardiovascular risk factors (Table 3, Model 2). In risk factor adjusted models, the odds ratios comparing the prevalence of PAD in the highest versus the lowest quartiles were 2.24 (95% confidence interval 1.24 to 4.04) for monocytes, 1.74 (0.87 to 3.45) for neutrophils, 2.53 (1.62 to 3.96) for CRP, and 2.68 (1.03 to 6.94) for fibrinogen.

### TABLE 2. Correlation Coefficients Among Inflammatory Markers

<table>
<thead>
<tr>
<th></th>
<th>WBC</th>
<th>Monocytes</th>
<th>Neutrophils</th>
<th>Lymphocytes</th>
<th>Basophils</th>
<th>Eosinophils</th>
<th>CRP</th>
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<tbody>
<tr>
<td>WBC</td>
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<tr>
<td>Monocyte</td>
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<td>Neutrophil</td>
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<td>Lymphocyte</td>
<td>0.59</td>
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<td>0.35</td>
<td>0.23</td>
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<tr>
<td>Basophils</td>
<td>0.35</td>
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<td>0.22</td>
<td>0.28</td>
<td>0.23</td>
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<tr>
<td>Eosinophils</td>
<td>0.31</td>
<td></td>
<td>0.24</td>
<td>0.18</td>
<td>0.23</td>
<td>0.25</td>
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<tr>
<td>CRP</td>
<td>0.31</td>
<td></td>
<td>0.20</td>
<td>0.30</td>
<td>0.13</td>
<td>0.11</td>
<td>0.12</td>
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<tr>
<td>Fibrinogen</td>
<td>0.25</td>
<td></td>
<td>0.15</td>
<td>0.26</td>
<td>0.06*</td>
<td>0.07</td>
<td>0.10</td>
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</tbody>
</table>

WBC indicates White blood cell count; CRP, C-reactive protein.
All variables in log scale except fibrinogen.
All $P<0.001$, except *$P=0.002$.

### TABLE 3. Odd Ratios of Peripheral Arterial Disease by Inflammatory Markers

<table>
<thead>
<tr>
<th>Inflammatory Marker</th>
<th>Quartile 1 Median Level 4.9</th>
<th>Quartile 2 Median Level 6.1</th>
<th>Quartile 3 Median Level 7.3</th>
<th>Quartile 4 Median Level 9.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC, $\times 10^9$/L</td>
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<tr>
<td>Model 1</td>
<td>2.02 (1.03–3.95)</td>
<td>2.16 (1.21–3.85)</td>
<td>3.00 (1.62–5.56)</td>
<td>0.002</td>
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<tr>
<td>Model 2</td>
<td>1.90 (1.01–3.58)</td>
<td>1.94 (1.08–3.45)</td>
<td>2.24 (1.24–4.04)</td>
<td>0.021</td>
</tr>
<tr>
<td>Model 3*</td>
<td>1.92 (1.03–3.59)</td>
<td>1.78 (0.94–3.36)</td>
<td>1.91 (1.06–3.42)</td>
<td>0.075</td>
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<tr>
<td>Monocyte, $\times 10^9$/L</td>
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<tr>
<td>Model 1</td>
<td>2.27 (0.63–2.59)</td>
<td>2.15 (0.62–2.53)</td>
<td>2.09 (0.98–4.48)</td>
<td>0.029</td>
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<tr>
<td>Model 2</td>
<td>1.13 (0.56–2.28)</td>
<td>1.01 (0.48–2.09)</td>
<td>1.52 (0.67–3.45)</td>
<td>0.24</td>
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<tr>
<td>Model 3</td>
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<td>Neutrophils, $\times 10^9$/L</td>
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<td>Model 1</td>
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<td>Model 3</td>
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<td>Lymphocytes, $\times 10^9$/L</td>
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<td>Model 3</td>
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<td>C-reactive protein, mg/L</td>
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<td>Model 3</td>
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<td>Fibrinogen, g/L</td>
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fibrinogen. Basophils or eosinophils were not associated with PAD in either model (data not shown).

When WBC types, CRP, and fibrinogen were introduced in the models in addition to traditional cardiovascular risk factors, monocytes and fibrinogen were still associated with PAD prevalence (Table 3, Model 3). The odds ratios comparing the prevalence of PAD in the highest versus the lowest quartiles were now 1.91 (95% confidence interval 1.06 to 3.42) for monocytes, 1.15 (0.49 to 2.69) for neutrophils, 1.37 (0.75 to 2.49) for CRP, and 2.21 (0.88 to 5.57) for fibrinogen. In subgroup analysis, the association of monocyte count with PAD was similar irrespective of the presence or absence of traditional risk factors and other inflammatory markers (not shown).

**Discussion**

Multiple lines of evidence indicate that inflammation plays a pivotal role in atherogenesis, and that inflammatory markers such as WBC count may aid in the detection of individuals at higher atherosclerotic risk. In this cross-sectional study of apparently healthy men and women, an elevated monocyte count was significantly associated with PAD after adjustment for traditional risk factors. This relationship was not present for other WBC types, and it was independent of CRP and fibrinogen.

WBC is a strong independent risk factor for cardiovascular events, and for the prevalence and progression of subclinical atherosclerosis. A recent meta-analysis concluded that, among WBC types, neutrophils were more strongly associated with future coronary events than monocytes, but none of the studies described in this meta-analysis simultaneously controlled for other WBC types, CRP, or fibrinogen. In our study, neutrophils were more strongly associated with CRP and fibrinogen than monocytes, and adjusting for CRP and fibrinogen virtually eliminated the association of neutrophils with PAD. Inflammation is associated with virtually all stages of vascular disease including atherogenesis, plaque rupture, and end-organ damage secondary to ischemia and/or embolism. It is conceivable that monocyte counts may be more strongly related to early development and progression of atherosclerosis, but once established atherosclerotic disease is present, they do not strongly influence the risk of events. Quantification of the predictive ability of monocyte counts will require fresh evidence from prospective studies with serial measurements of WBC counts, differentials, and other inflammatory markers.

There is extensive evidence for the active contribution of monocytes to atherosclerotic lesion development. The infiltration of circulating monocytes into the blood vessel wall, where they are transformed into lipid-laden foam cells, is one of the earliest events in the development of atherosclerotic lesions. Monocytes are believed to play a critical role not only in initiation of atherosclerosis, but also at multiple stages including promotion of plaque instability and remodeling after a myocardial insult.

The classical immunophenotypic marker for monocytes is CD14, the lipopolysaccharide (LPS) receptor. However, at least 2 distinct subpopulations of monocytes can be distinguished from the expression patterns of membrane surface antigens. About 10% of monocytes coexpress CD16, the low-affinity Fc-gamma receptor III. CD14+/CD16+ monocytes are considered “proinflammatory monocytes” as they efficiently produce tumor necrosis factor-alpha (TNF-α) while producing little antiinflammatory cytokine interleukin-10 (IL-10) in comparison to CD14+/CD16– monocytes. Levels of TNF-α are known to correlate with carotid intima-media thickness. In addition, CD14+/CD16+ monocytes are expanded in hypercholesteremic patients, negatively correlated to HDL cholesterol levels, and associated in a dose-dependent fashion with the expression of the more positively charged apo E4.

In a small case–control study, Schlitt et al found CD14+/CD16+ monocytes to be significantly associated with the prevalence of coronary artery disease, but the retrospective design and the small sample size make it difficult to interpret these findings. Because Schlitt et al measured monocyte levels in cases after occurrence of clinical events, it is impossible to determine in this study whether CD14+/CD16+ levels contributed to the event or were a consequence of it. The evaluation of the role of CD14+/CD16+ monocytes in atherosclerosis progression and cardiovascular outcomes in prospective studies should be a research priority.

In our study, fibrinogen was also independently associated with the prevalence of PAD after taking into account CVD risk factors, WBC differentials, and CRP. The finding is consistent with previous reports demonstrating fibrinogen to be associated with incident CHD events as well subclinical atherosclerosis adjusting for other inflammatory markers. In the Caerphilly study, controlling for CRP did not diminish the effect of fibrinogen for incident CHD indicating it to be a more specific CHD risk factor. In a population sample of adults (n=519, median age 55.5 years, 80% men) without clinically overt atherosclerotic disease, elevated fibrinogen levels was related to carotid IMT independently of a wide range of important confounding variables including CRP.

Baseline fibrinogen levels have also been observed to be associated with higher carotid intimal thickness (IMT) levels in a 5-year follow-up in the Edinburgh Artery Study. Higher fibrinogen levels may potentially promote atherosclerosis by increasing platelet adhesion to the subendothelium as well affecting endothelial function. Our and previous findings support the role of fibrinogen as an independent marker of generalized atherosclerotic lesions in major arterial beds.

The strengths of our study include the rigorous methodology and extensive quality control of NHANES procedures, the use of a nationally representative sample, and the use of a validated and specific measure of PAD. Our findings, however, must be interpreted in the context of certain limitations. Because of the cross-sectional design, we could not determine whether circulating monocytes are directly related to the atherosclerotic process or their recruitment is stimulated by the presence of atherosclerotic lesions. In a small study, elevated monocyte levels independently predicted the progression of carotid stenosis, thus providing support to the former theory. In another study, Muscari et al found that of the WBC differentials, only monocyte count was significantly higher in atherosclerotic than in controls. Our study lacked information on monocyte subtypes as well as the potential role of CD14+/CD16+ monocytes in atherosclerosis.
on the presence of viral or bacterial infections that may be implicated in atherogenesis and may affect monocyte count. Whether an elevated monocyte count has direct vascular or prothrombotic effects, is merely increased secondarily to prevalent atherosclerosis, or is simply a marker of an uncertain environmental or infectious stimulus remains to be determined. Confirmation of these findings in prospective studies is of critical importance. Finally, in our study WBC determination was performed once; factors such as infections, day-to-day variations, as well as variability in WBC determination may have potentially effected our findings by categorizing participants with lower levels as having higher levels and vice versa. It is generally considered that impact of such dilution bias underestimates the true associations with CVD risk as much as 50% for monocyte counts. The association of monocyte count with PAD observed in our study is potentially likely to be underestimated.

Conclusions
This study demonstrates for the first time an independent association between monocyte count and peripheral arterial disease defined as a reduced ankle-brachial blood pressure index in a large population based study. More detailed characterization of the association between different monocyte populations and clinical and subclinical atherosclerotic outcomes is needed to better characterize the role of inflammatory cells in atherosclerosis.

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