Monocyte Count, But Not C-Reactive Protein or Interleukin-6, Is an Independent Risk Marker for Subclinical Carotid Atherosclerosis

Caroline M.L. Chapman, PhD; John P. Beilby, PhD; Brendan M. McQuillan, PhD, FRACP; Peter L. Thompson, MD, FRACP; Joseph Hung, MB, FRACP

Background and Purpose—Systemic inflammatory markers have been shown to predict future cardiovascular events, but whether they are associated with early atherosclerosis is uncertain. We investigated the relationship of inflammatory markers interleukin-6 (IL-6), high-sensitive C-reactive protein (hs-CRP), fibrinogen, monocyte count, and white cell count (WCC) with subclinical carotid atherosclerosis in a healthy community population.

Methods—B-mode carotid ultrasound was performed on 1111 randomly selected male and female subjects aged 27 to 77 years. Serum IL-6, hs-CRP, plasma fibrinogen, monocyte count, and WCC were measured on all subjects, along with conventional cardiovascular risk factors.

Results—Multivariate analysis showed that IL-6 (P<0.0001), fibrinogen (P=0.007), and monocyte count (P=0.001) were associated with carotid plaque formation in the whole population. Monocyte count remained associated independently with carotid plaque formation when adjusted further for conventional risk factors (odds ratio per SD increase in monocyte count 1.4; 95% CI, 1.13 to 1.73; P=0.002). IL-6 (P<0.0001), fibrinogen (P<0.0001), and monocyte count (P=0.04) were also associated with carotid intima-medial thickness (IMT) in the whole population. However, when adjusted further for conventional risk factors, none remained independently predictive of carotid IMT. Further analysis showed an age–monocyte interaction (P=0.03), with monocyte count being an independent predictor of carotid IMT in the older age group only (>53 years; P=0.003).

Conclusion—In a healthy community population, monocyte count is a better independent predictor of common carotid IMT and plaque formation than IL-6, hs-CRP, fibrinogen, and WCC. Monocyte count may represent an inexpensive, easy-to-measure risk marker for subclinical carotid atherosclerosis. (Stroke. 2004;35:1619-1624.)

Key Words: C-reactive protein ■ leukocyte count ■ interleukin-6 ■ carotid arteries ■ atherosclerosis

It is apparent that traditional clinical risk factors for cardiovascular disease do not predict all individuals at risk of developing cardiovascular events. Current evidence suggests that atherosclerosis is, at least in part, an inflammatory process involving the infiltration of circulating monocytes into the vessel wall, where they differentiate into lipid-laden foam cells, the migration of smooth muscle cells, and further recruitment of inflammatory cells, leading to the development of an atherosclerotic plaque. During this process, many inflammatory cytokines (eg, tumor necrosis factor α, interleukin-1 [IL-1], and adhesion molecules) are expressed by macrophages, smooth muscle cells, and the endothelium.

Circulating markers of systemic inflammation, including fibrinogen, high-sensitive C-reactive protein (hs-CRP), IL-6, and white cell count (WCC) have been shown to predict future cardiovascular events. However, it is still unclear whether these markers reflect underlying atherosclerotic burden, an increased tendency for plaque instability and thrombosis, or both. Determining whether levels of inflammatory markers correlate with underlying atherosclerosis in asymptomatic subjects can have important implications for public health practice. High-resolution B-mode carotid ultrasonography has been used as a noninvasive measure of subclinical atherosclerosis in large community-based cohorts. Carotid plaque and intima-medial thickness (IMT) measured in this way have been shown to correlate with standard cardiovascular risk factors, atherosclerosis in other vascular beds, incident myocardial infarction (MI), and strokes.

Previous studies have failed to establish a clear relationship between major inflammatory markers such as hs-CRP and subclinical carotid atherosclerosis in broad-based community
Methods

Subjects

The selection criteria and study design of the community-based Carotid Ultrasound Disease Assessment Study have been detailed previously.20,21 A total of 1111 subjects who were original participants in the 1989 Australian National Heart Foundation Perth Risk Factor Prevalence Survey20 agreed to take part in the study described here. Subjects who had previous carotid artery surgery were excluded. Written informed consent was obtained from all study participants. The study protocol was approved by the Institutional Ethics Committee of the University of Western Australia.

A self-administered questionnaire was used to record a history of smoking, hypertension, hyperlipidemia, diabetes, angina pectoris, MI, stroke, or a family history of premature-onset MI or stroke by age 55 in first-degree relatives.20 Anthropomorphic measurements and the lower of 2 resting blood pressures were recorded.

Biochemical Analysis

A fasting blood sample from each subject was obtained, and measurements were available on 1092 subjects. Serum IL-6 was measured using an ELISA (Quantitative Immunoassay R&D Systems) with an assay range of 0.38 to 10 ng/L. Serum high-sensitive CRP (hs-CRP) was measured by a microparticle turbidity assay with a range of 0.1 to 21.0 mg/L. Plasma fibrinogen was measured by the Clauss method.21 Monocyte count and WCC were obtained using standard techniques. Total cholesterol, high-density lipoprotein (HDL) cholesterol and triglyceride levels were determined enzymatically with a 747 autoanalyzer (Hitachi).

Carotid Ultrasound

Bilateral carotid B-mode ultrasound was performed by 2 trained sonographers using a 7.5-MHz annular phased-array transducer on an Interspec CX 200 ultrasound machine (Apogee) as described previously.21,12 The IMT was defined as the distance between the characteristic echoes from the lumen–intima, and media–adventitia interfaces on the far wall of the distal common carotid artery measured >1 cm in segment length. A thorough search of the distal common carotid, carotid bulb, and internal and external carotid arteries was also made to determine the presence of focal plaque. Plaque was defined as a clearly identified area of focal increased thickness (≥1 mm) of the intima-media layer. Three end-diastolic images were analyzed from the right and left distal common arteries at a site free of any discrete plaque and measurements averaged to give the mean IMT. Repeat measurements of randomly selected scans revealed no significant variation in the IMT measurements.

Statistical Analysis

Outcome variables of the association analyses were IMT and the presence of carotid plaques. The principal explanatory variables were the inflammatory markers IL-6, hs-CRP, fibrinogen, monocyte count, and WCC. Outliers of hs-CRP and IL-6 (n=2) and monocyte count (n=2) were identified using residuals, fitted values, leverage, and normalized residual squared routines available in a statistical program STATA (Stata Corp LP) and were excluded from analysis. IL-6, hs-CRP, monocyte count, WCC, IMT, body mass index (BMI), waisthip ratio (WHR), triglycerides, and HDL were not normally distributed and therefore log 10 transformed before analysis. Geometric means and 95% CIs (1.96×SEM) are given for these variables. Continuous variables were compared using t tests or Mann–Whitney U tests for nonparametric analysis of variables not normally distributed. Categorical variables were compared by χ2 test. Statistical significance was taken as P<0.05.

Spearman rank correlations were used to describe the association of inflammatory markers and conventional risk factors. Forward and backward stepwise multiple logistic regression analyses were used to determine independent predictors of carotid plaque. Model 1 included the inflammatory markers IL-6, hs-CRP, fibrinogen, monocyte count, and WCC. Model 2 included all inflammatory markers together with conventional risk factors. Independent predictors of IMT were determined by generalized linear regression modeling using the models above. Minitab for Windows v12.1 (Minitab Inc) and SPSS version 10.1 for Windows (SPSS Inc) were used in analysis.

Results

Subject Characteristics

The characteristics of the study population have been described previously.11,12 The mean age was 53.3±13 years, and the sex ratio was balanced. The prevalence of standard risk factors was similar between males and females, except that women had significantly lower blood pressure, triglycerides, WHR, and smoking pack years, and had higher HDL cholesterol values than men. A total of 25.6% of the population had ≥1 carotid plaque(s). A previous history of MI or stroke was recorded in 7% of the population.

Inflammatory Markers

Geometric means (95% CI) of IL-6 were 3.65 (3.64 to 3.66) μg/L, hs-CRP 1.76 (1.73 to 1.79) mg/L, and WCC 6.21 (6.20 to 6.22) ×109/L and were similar in males and females. Women had lower monocyte counts (females 0.45 [0.44 to 0.46] ×109/L; males 0.53 [0.52 to 0.54]×109/L; P<0.0001) and higher fibrinogen levels (mean±SD; females 2.79±0.68 g/L, males 2.70±0.64 g/L; P=0.03) than men.

Table 1 shows Spearman rank correlations of the inflammatory markers. IL-6, hs-CRP, and fibrinogen levels were strongly correlated. WCC also correlated with IL-6, hs-CRP, and fibrinogen. However, monocyte counts correlated weakly with IL-6, hs-CRP, and fibrinogen levels. These correlations were similar in males and females.

Table 2 shows the relationship of the inflammatory markers with conventional risk factors in the whole population. hs-CRP, IL-6, and fibrinogen all correlated positively with age, systolic blood pressure, WHR, BMI, and triglycerides, and negatively with HDL in both males and females. Smoking correlated more strongly with IL-6, hs-CRP, and fibrin-
ogen in males compared with females (data not shown) because of a larger proportion of male than female smokers (31% versus 18.3%, respectively). WCC correlated positively with WHR, BMI, triglycerides, and smoking, and negatively with HDL ($P<0.001$) in both males and females. In males, monocyte count correlated positively with age, WHR, triglycerides, smoking (all $P<0.001$), and BMI ($P<0.01$). In females, monocyte counts only correlated with smoking ($P<0.001$) and triglycerides ($P=0.04$).

**Inflammatory Markers and Carotid Plaque**
Univariate analysis showed significant positive correlations of all inflammatory proteins with carotid plaque formation in the whole population (Table 2). Geometric means (95% CI) for inflammatory markers in subjects with plaque versus no plaque were IL-6 $4.23$ (4.01 to 4.46) ng/L versus $3.46$ (3.35 to 3.57) ng/L ($P<0.0001$); hs-CRP $2.13$ (1.88 to 2.41) mg/L versus $1.65$ (1.53 to 1.78) mg/L ($P<0.001$); monocyte count $0.53$ (0.51 to 0.55) $\times 10^9$/L versus $0.48$ (0.47 to 0.49) $\times 10^9$/L ($P<0.0001$); and WCC $6.46$ (6.29 to 6.64) $\times 10^9$/L versus $6.12$ (6.02 to 6.22) $\times 10^9$/L ($P<0.0001$). Mean fibrinogen level ($\pm SD$) for subjects with and without plaque was $2.91\pm0.64$ g/L versus $2.68\pm0.66$ g/L, respectively ($P<0.0001$). Significant differences were equal in males and females.

Table 3 shows the adjusted odds ratios for carotid plaque formation associated with inflammatory markers in the whole population. Analysis of all inflammatory markers together (model 1) shows that increases in IL-6 ($P<0.0001$), fibrinogen ($P=0.007$), and monocyte count per 1 SD change ($P<0.001$) were associated with the presence of carotid plaque. After further adjustment for age, gender, and other conventional risk factors (model 2; Table 3), monocyte count remained an independent risk predictor for the presence of carotid plaque. For each 1 SD increment in monocyte count (0.17 $\times 10^9$/L), the risk of carotid plaque increased by 40% ($P<0.002$). There was no significant interaction of gender with any of the inflammatory markers on the likelihood of plaque. Further subgroup analysis split at the median for each risk factor, and inflammatory marker was performed (see the Figure). This analysis confirms that 1 SD increase in monocyte count was associated with an increased adjusted odds ratio of carotid plaque irrespective of the baseline level of

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<th>TABLE 2. Spearman Rank Correlations of Inflammatory Variables Against Standard Risk Factors, Carotid IMT, and Plaque in the Whole Population</th>
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* $P<0.001$; † $P<0.05$.

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<th>TABLE 3. Logistic Regression Model of the Association of Inflammatory Markers and Carotid Plaque in the Whole Population</th>
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<td>Fibrinogen, g/L</td>
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<td>WCC per SD</td>
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Model 1 simultaneously compares inflammatory markers. Model 2 multivariate analysis of all inflammatory markers adjusted for age, sex, WHR, LDL, smoking (pack years), history of hypertension, MI, and diabetes. SD=1 per SD change.
conventional risk factors for plaque and other inflammatory markers. The only exception is in hypertensive subjects; however, this could be attributable to small numbers (n=266).

**Inflammatory Markers and Carotid IMT**

Univariate analysis showed IL-6, hs-CRP, and fibrinogen all correlated significantly with carotid IMT in the whole population (Table 2). However, monocyte count (P<0.0001) and WCC (P=0.001) were associated with carotid IMT only in males (P>0.05).

Multivariate analysis of inflammatory markers, together in the whole population (model 1; Table 4), showed that IL-6 (P<0.0001), fibrinogen (P<0.0001), and monocyte count per 1 SD change (P=0.04) were all associated with carotid IMT (total r=8.6%). However, after further adjustment for conventional risk factors (model 2; Table 4), none of these inflammatory markers remained significantly associated with carotid IMT (total r=52.4%). However, further analysis showed an age–monocyte interaction (P=0.03), with monocyte count being an independent predictor of carotid IMT in the older age group split by median age of >53 years (P=0.003; model 2; Table 4).

**Discussion**

In this cross-sectional community study, we have shown for the first time that monocyte count among several inflammatory markers (hs-CRP, IL-6, fibrinogen, and WCC) was an independent predictor of subclinical carotid atherosclerosis. For each SD increase in monocyte count (0.17×10^9/L), the risk of having a carotid plaque increased 40%. Monocyte count was also an independent predictor of common carotid IMT in older subjects (>53 years), probably because a thickened IMT is more likely to represent diffuse atherosclerosis than medial hypertrophy in the older age group.

WCC has been shown to be a risk factor for ischemic heart disease. However, evidence linking WCC to prevalent carotid atheroma is weak. In this study, WCC was not a multivariate predictor of carotid plaque or IMT. Atherosclerosis is characterized by the recruitment of monocytes and lymphocytes, but not neutrophils, to the artery wall. Therefore, monocyte count may be a more specific measurement than total WCC of the inflammatory activity in atherosclerosis.

To our knowledge, this is one of the first studies to show that monocyte count within the normal range (per 1 SD change) is an independent predictor of subclinical carotid atherosclerosis in a mainly healthy community population. Another study in a Chinese population showed that among nonsmoking males but not females, monocyte count, after adjustment for age and BMI, was associated with carotid plaque score (P<0.05).

This study shows that monocyte count correlates weakly with IL-6, hs-CRP, and fibrinogen, suggesting that it is a marker for a pathway independent of these acute phase proteins. Monocyte count also correlates weakly with clinical risk factors such as age, systolic blood pressure, BMI, and low-density lipoprotein (LDL). Perhaps this explains why in multivariate analysis, monocyte count was not confounded by adjustment for traditional risk factors, unlike the acute phase reactants. Subgroup analysis also showed that 1 SD increase in monocyte count is associated with an increased odds ratio of carotid plaque independent of the baseline level of most conventional risk factors and other inflammatory markers.

Large-scale prospective studies have shown that baseline levels of hs-CRP are an independent predictor of future cardiovascular events in both men and women. However, the relationship between hs-CRP and subclinical atherosclerosis is less clear. In this study, hs-CRP was not independently predictive of carotid IMT or the presence of plaque. This is in agreement with previous population-based studies that found hs-CRP to be correlated poorly with subclinical atherosclerosis detected by carotid ultrasonography and...
electron beam–computerized tomography for coronary calcium.25,26

However, in the Rotterdam Study of older men and women (≥55 years), CRP was associated independently with both carotid plaque score and common carotid IMT.19 Another recent study13 described a significant association between CRP and carotid stenosis (≥25%) but not common carotid IMT among 3173 men and women, mean age 55 years, enrolled in the Framingham Offspring Study. It should be noted that in the latter study,13 patients underwent ultrasonography 4 years after the CRP measurement, and CRP may have predicted incident rather than prevalent carotid stenosis. The Rotterdam Study of elderly subjects also showed that CRP predicted the progression of atherosclerosis during a 6.5-year follow-up.19 These studies suggest that CRP may be a better predictor of more advanced focal atherosclerosis or atherosclerosis progression in older populations.

IL-6 is a mediator of the acute phase response and a primary determinant of hepatic production of CRP. In a subsample of the Rotterdam Study, IL-6 levels were not found to be associated with either carotid IMT or plaque. In this study, serum IL-6 level was a strong predictor of carotid plaque formation and carotid IMT when all the inflammatory markers were included together, but did not remain a significant predictor after adjustment for conventional risk factors. This was expected because IL-6 levels were correlated strongly with conventional risk factors including age, systolic blood pressure, and WHR, among others. Adipose tissue is also an important source of IL-6 production.27 Thus, adjusting for WHR or BMI may have confounded the relationship of IL-6 with carotid atherosclerosis. This does not exclude the possibility that IL-6 may be an intermediate factor for atherosclerosis.

There are some potential limitations to this study. Levels of inflammatory markers were only measured once; therefore, intraindividual variation in levels of these markers cannot be taken into account. Multivariate analysis included all subjects, although there were minor differences in conventional risk factors and levels of inflammatory markers between males and females. However, sex differences were adjusted for in the multivariate models, and no significant interaction was found between sex and inflammatory markers. Finally, because this is a cross-sectional study, our findings reflect only an association with prevalent and not incident atherosclerosis.

To our knowledge, our study is the first to show monocyte count as an independent risk predictor for early carotid atherosclerosis in a cross-sectional community population. Monocyte count represents an easy-to-measure, inexpensive marker for the presence of carotid plaque and appears to be a better independent predictor than hs-CRP, IL-6, fibrinogen, or WCC for subclinical atherosclerosis. Further studies are needed to evaluate in a prospective manner whether monocyte count can also predict the progression of atherosclerosis and future cardiovascular risk.

Acknowledgments

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