C-Reactive Protein, Interleukin-6, and Soluble Adhesion Molecules as Predictors of Progressive Peripheral Atherosclerosis in the General Population

Edinburgh Artery Study

Ioanna Tzoulaki, MSc; Gordon D. Murray, PhD; Amanda J. Lee, PhD; Ann Rumley, PhD; Gordon D.O. Lowe, MD; F. Gerald R. Fowkes, MBChB, PhD

Background—The relationship between levels of circulating inflammatory markers and risk of progressive atherosclerosis is relatively undetermined. We therefore studied inflammatory markers as predictors of peripheral atherosclerotic progression, measured by the ankle-brachial index (ABI) at 3 consecutive time points over 12 years.

Methods and Results—The Edinburgh Artery Study is a population cohort study of 1592 men and women aged 55 to 74 years. C-reactive protein (CRP), interleukin-6 (IL-6), intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), and E-selectin were measured at baseline. Valid ABI measurements were obtained on 1582, 1081, and 813 participants at baseline and 5-year and 12-year follow-up examinations, respectively. At baseline, a significant trend was found between higher plasma levels of CRP (P ≤ 0.05) and increasing severity of peripheral arterial disease (PAD), after adjustment for baseline cardiovascular risk factors. IL-6 at baseline (P ≤ 0.001) was associated with progressive atherosclerosis at 5 years (ABI change from baseline), and CRP (P ≤ 0.01), IL-6 (P ≤ 0.001), and ICAM-1 (P ≤ 0.01) were associated with changes at 12 years, independently of baseline ABI, cardiovascular risk factors, and baseline cardiovascular disease. Only IL-6 independently predicted ABI change at 5 years (P ≤ 0.01) and 12 years (P ≤ 0.05) in analyses of all inflammatory markers simultaneously and adjusted for baseline ABI, cardiovascular risk factors, and cardiovascular disease at baseline.

Conclusions—These findings suggest that CRP, IL-6, and ICAM-1 are molecular markers associated with atherosclerosis and its progression. IL-6 showed more consistent results and stronger independent predictive value than other inflammatory markers. (Circulation. 2005;112:976-983.)

Key Words: atherosclerosis ■ cell adhesion molecules ■ peripheral vascular disease ■ interleukins ■ C-reactive protein

Inflammation may contribute to atherosclerosis by a variety of mechanisms depending on the stage of the disease.1 Circulating markers of systemic inflammation have been shown to predict future cardiovascular disease (CVD) such as myocardial infarction, stroke, and peripheral arterial disease (PAD).2 These markers of systemic inflammation include C-reactive protein (CRP), proinflammatory cytokines such as interleukin-6 (IL-6), and soluble adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), and E-selectin. CRP has received the most attention3-8 and, along with IL-6,9-11 appears to be a consistent predictor of future cardiovascular events in large prospective studies. Epidemiological data on adhesion molecules predicting risk of CVD are sparse.12,13 Moreover, the role of inflammatory markers in the preclinical stages of atherosclerosis is undetermined. A few studies14-17 have focused on their relationship with measures of atherosclerosis such as carotid intima-media thickness. Only the Rotterdam Study18 has examined the prospective association of CRP with asymptomatic atherosclerotic progression, and no studies have reported on possible similar associations for IL-6 or adhesion molecules. There is also a need for prospective analysis of the relative associations of all these inflammatory markers with lower-extremity atherosclerosis and its progression.

The ankle-brachial index (ABI) is an accurate and reliable marker of subclinical peripheral and generalized atherosclerosis in populations.19,20 In the Edinburgh Artery Study, a

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Methods

The Edinburgh Artery Study began in 1988 as a cross-sectional survey of 809 men and 783 women aged 55 to 74 years. This population, which was almost exclusively of white origin, was selected at random, in 5-year age bands, from 11 general practices serving a range of socioeconomic and geographic areas throughout the city. The response rate was 65%, and follow-up of a sample of nonresponders showed no substantial bias. Details of the study recruitment and examination process have been described. Ethics committee approval was given for this study, and informed consent was obtained from each subject.

Subjects completed a self-administered questionnaire at baseline that contained validated questions regarding personal characteristics, smoking status, intermittent claudication (World Health Organization [WHO] questionnaire), medical history, smoking, diet, alcohol consumption, exercise, and current medication for high blood pressure or diabetes. A similar modified questionnaire was repeated at each of the 5- and 12-year examinations.

Subjects were invited for a comprehensive clinical examination at baseline and at 5 and 12 years after the study commenced. Clinical measurements were conducted by trained research staff during each examination. A 12-lead ECG was taken and coded independently by 2 observers using the Minnesota code. Standing height (without shoes) was measured to the nearest 100 g on digital scales (Soehnle). Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. Systolic and diastolic (phase V) blood pressures were recorded in the right arm only, after 10 minutes of rest with the patient in the supine position, with a Hawksley random zero sphygmomanometer. The femoral, posterior tibial, and dorsalis pedis pulses were palpated in both legs. Ankle systolic pressures were measured first in the right leg and then in the left leg at the posterior tibial artery with the use of a Sound Health Doppler ultrasound probe and a random zero sphygmomanometer with the cuff placed proximal to the malleoli. The pulse was located with the Doppler probe, and the cuff was inflated until the pulse was no longer audible. The cuff was then deflated, and the pressure was noted when the pulse reappeared. If the posterior tibial pulse was not detectable, the dorsalis pedis pulse was used wherever possible. ABI was calculated by dividing the ankle systolic pressure by the brachial systolic pressure. The lower of the 2 indices was used in the analysis as indicative of worse disease. Reproducibility of the ABI was tested before the commencement of the study on 36 subjects who had repeated measurements by 4 observers on 2 separate days. The 95% CIs for variability of the measurement were mean ±16% for 1 measurement of the ABI. Quality control at baseline and at 5- and 12-year examination involved retraining of the observers of each examination, periodic checks for drift, and comparison of ABI measurements of the observers of each examination on the same participant. Three, 5, and 8 subjects at baseline, 5 years, and 12 years, respectively, had values of ABI >1.50 and were excluded because of probable arterial rigidity.

At baseline, a fasting 20-mL sample of venous blood was taken for estimation of biochemical, inflammatory, and hemostatic factors. Tests for serum total cholesterol, HDL cholesterol, and blood glucose were performed on a Cobas Bio analyzer (Roche Products) with standard kits. The total/HDL cholesterol ratio was calculated by dividing the total by the HDL cholesterol value. Diabetic status of the subject was assessed in a number of ways. At baseline examination, a blood sample was taken for measurement of blood glucose, and then each subject not known to be diabetic consumed 75 g of glucose in the form of 335 mL Solripe Gluctoza Health Drink (Strathmore Mineral Water Company). A second blood glucose specimen was taken 2 hours after the oral glucose load. In addition, at baseline, 5-, and 12-year examinations, self-reported diabetic status and use of insulin injections and tablets for diabetes were recorded. High-sensitivity CRP was measured immunologically with a BN ProSpec nephelometer (Dade Behring). Plasma levels of IL-6, ICAM-1, VCAM-1, and E-selectin were measured with high-sensitivity ELISA kits (R&D Systems). Intra-assay and interassay variability coefficients were as follows, respectively: for CRP, 4.7% and 8.3%; for IL-6, 7.4% and 8.8%; for ICAM-1, 4.8% and 7.4%; for VCAM-1, 4.3% and 8.5%; and for E-selectin, 4.8% and 5.7%.

Quality control practices included repeating values outside 95% of the normal range and use of internal quality control plasma with each assay for each marker.

Throughout the 12 years of follow-up, fatal and nonfatal cardiovascular events were recorded and defined with criteria adapted from the American Heart Association. The process of follow-up has been described in detail previously. To identify all deaths occurring in the cohort, each participant’s record was flagged at the UK National Health Service Central Registry so that death certificates would be automatically forwarded to the investigators. If a postmortem examination was not performed, causes of death were verified by consulting hospital or general practitioner records. Intermittent claudication was recorded if either of the following was true: (1) evidence of intermittent claudication by WHO criteria at baseline or at any time during follow-up or (2) verified clinical diagnosis of intermittent claudication by a general practitioner or in the hospital.

Data Analysis

Data were analyzed with the use of the SPSS version 12.0 software package. Pack-years of smoking were calculated as years of cigarette smoking multiplied by the average number of packs smoked per day with the value zero entered for lifelong nonsmokers. The distribution of pack years was skewed, and a square-root transformation was used in all analyses. Distributions of CRP, IL-6, ICAM-1, VCAM-1, and E-selectin were also positively skewed and were logarithmically transformed to approach normality for all analyses. For presentation purposes, means and 95% CIs of transformed variables have been back transformed to geometric means. Ninety-eight subjects with CRP levels >10 mg/L and 11 subjects with IL-6 levels >100 pg/mL were excluded from all analyses because these levels indicate presence of acute inflammatory disease. Physical activity was used as a 4-group categorical variable (no activity, light activity, moderate activity, and strenuous activity) as previously described. Presence of diabetes was defined by either physician recall, treatment for diabetes, or glucose concentration of the 2-hour blood sample ≥11.1 mmol/L. Subjects with CVD at baseline were those who had reported experiencing a myocardial infarction or stroke event.

Associations between logarithmically transformed inflammatory markers were calculated with the Pearson correlation coefficient. Mean levels of inflammatory markers according to PAD status were compared by ANOVA and ANCOVA adjusted for baseline risk factors: age, sex, pack-years of smoking, presence of diabetes, total/HDL cholesterol ratio, BMI, and physical activity. Homogeneity of variance assumption was tested. Symptomatic individuals were defined as those positive in the WHO intermittent claudication questionnaire at baseline, whereas asymptomatic subjects were defined as those without claudication but with ABI ≤0.9 at baseline. We also performed separate linear regression for ABI at baseline with each inflammatory marker as a predictor variable. Analysis was adjusted for age and further adjusted for the aforementioned risk factors and CVD status at baseline.

Change in ABI was calculated as ABI at 5 years or ABI at 12 years minus ABI at baseline. Linear regression was used to test associations between each inflammatory marker and ABI change at 5 and 12 years separately. Analyses were initially adjusted for baseline ABI and age and further adjusted for sex, pack-years of smoking, presence of diabetes, total/HDL cholesterol ratio, BMI, physical activity, and baseline CVD. We then entered all inflammatory markers and baseline risk factors predicting ABI change at 5 and 12 years, respectively, into the model. Tests were made for collinearity, and residual plots were examined to check assumptions of regression.
analysis. We also tested for effect modification between age and sex and inflammatory markers, but the interaction terms were not statistically significant. Finally, we performed separate linear regression on the subgroup \((n=1080)\) that was free of baseline PAD (no symptoms of intermittent claudication or ABI >0.9). Throughout all analyses, a 2-sided probability value \(\leq 0.05\) was taken to denote statistical significance.

**Results**

A total of 1592 subjects were recruited at baseline; 1156 of them participated in the 5-year follow-up examination (131 additional subjects completed the questionnaire only), and 831 participated in the 12-year follow-up (88 others completed the questionnaire only). During the 5 years of follow-up, there were 203 deaths (12.8%) recorded, of which 89 (43.8%) were cardiovascular. Up to the 12-year examination, the total number of deaths was 485 (30.5%), of which 207 (43.8%) were cardiovascular. At baseline, 4.9% of the population was suffering from intermittent claudication and 23% from asymptomatic PAD. In addition, 179 new cases of PAD from asymptomatic PAD. In addition, 179 new cases of PAD were identified by 12 years of follow-up. Of these 179 new cases, 15 (8.4%) had undergone vascular surgery or amputation over the follow-up period.

Characteristics of the baseline population are presented in Table 1.

**TABLE 1. Baseline Population Characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>Mean (SD) or Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>1592</td>
<td>64.8</td>
</tr>
<tr>
<td>Male</td>
<td>809</td>
<td>50.9%</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>1591</td>
<td>25.6 (3.91)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>1573</td>
<td>7.03 (1.33)</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1567</td>
<td>1.44 (0.41)</td>
</tr>
<tr>
<td>Total/HDL cholesterol ratio</td>
<td>1567</td>
<td>5.24 (1.96)</td>
</tr>
<tr>
<td>Pack-years of smoking</td>
<td>1592</td>
<td>23.9 (12.0, 38.6)*</td>
</tr>
</tbody>
</table>

**Diabetes**

- Diabetics                      | 91    | 6%                     |
- Drugs for diabetes†             | 30    | 1.9%                   |

**Blood pressure**

- Systolic blood pressure, mm Hg | 1591  | 144.48 (24.12)         |
- Diastolic blood pressure, mm Hg| 1587  | 77.43 (12.42)          |
- Antihypertensives‡              | 307   | 19.2%                  |

**CVD**

- Myocardial infarction           | 73    | 4.6%                   |
- Stroke                         | 49    | 3.1%                   |

**Physical activity**

- None                           | 158   | 9.9%                   |
- Light                          | 592   | 37.2%                  |
- Moderate                       | 674   | 42.3%                  |
- Strenuous                      | 167   | 10.5%                  |

*Mean (SD) was derived from squared-root distribution and has been back transformed here. Only participants who smoked were included in this calculation.

†People receiving tablets for diabetes or insulin injections.

‡People receiving drugs to treat high blood pressure.

Moreover, CRP and IL-6 as well as ICAM-1 and VCAM-1 were moderately and significantly correlated \((r=0.51\) and \(r=0.38\), respectively; \(P<0.001)\). E-selectin was significantly and positively associated with both ICAM-1 and VCAM-1 \((r=0.36\) and \(r=0.16\), respectively; \(P<0.001)\). Adhesion molecules were all positively and significantly associated with CRP and IL-6, with similar correlation coefficients ranging between 0.10 and 0.30 \((P<0.001)\). Geometric mean values of inflammatory markers according to baseline PAD status are presented in Table 2. A significant trend was found between higher plasma levels of CRP \((P=0.001)\), IL-6 \((P=0.002)\), ICAM-1 \((P=0.01)\), and VCAM-1 \((P=0.03)\) and individuals classified as having worsening PAD (from normal, asymptomatic to symptomatic). This trend remained statistically significant only for CRP \((P=0.02)\) and was borderline significant \((P=0.07)\) for IL-6 after adjustment for baseline risk factors: age, sex, pack-years of smoking, diabetes, total/HDL cholesterol ratio, BMI, and physical activity. Levels of E-selectin did not show any significant trends

**TABLE 2. Levels of Inflammatory Markers According to PAD Status at Baseline**

<table>
<thead>
<tr>
<th>PAD Status*</th>
<th>n</th>
<th>Geometric Mean</th>
<th>Transformed 95% CI</th>
<th>(P) for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP, mg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptomatic</td>
<td>46</td>
<td>2.7</td>
<td>2.2–3.5</td>
<td>(&lt;0.001) 0.02</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>227</td>
<td>2.1</td>
<td>1.9–2.4</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>719</td>
<td>1.5</td>
<td>1.4–1.7</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>992</td>
<td>1.7</td>
<td>1.6–1.8</td>
<td></td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptomatic</td>
<td>45</td>
<td>3.3</td>
<td>2.6–4.2</td>
<td>0.002 0.07</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>239</td>
<td>2.7</td>
<td>2.5–3.1</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>754</td>
<td>2.1</td>
<td>2.1–2.2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1038</td>
<td>2.3</td>
<td>2.2–2.4</td>
<td></td>
</tr>
<tr>
<td>ICAM-1, ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptomatic</td>
<td>57</td>
<td>239.2</td>
<td>225.0–254.2</td>
<td>0.01 0.61</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>269</td>
<td>238.1</td>
<td>230.3–246.0</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>856</td>
<td>217.5</td>
<td>213.5–221.5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1182</td>
<td>222.9</td>
<td>219.5–226.5</td>
<td></td>
</tr>
<tr>
<td>VCAM-1, ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptomatic</td>
<td>57</td>
<td>402.9</td>
<td>373.7–434.3</td>
<td>0.03 0.49</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>269</td>
<td>390.3</td>
<td>380.2–400.6</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>856</td>
<td>376.6</td>
<td>370.8–382.4</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1182</td>
<td>380.9</td>
<td>375.9–385.9</td>
<td></td>
</tr>
<tr>
<td>E-selectin, ng/mL</td>
<td></td>
<td>56</td>
<td>40.6</td>
<td>37.1–44.3</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>269</td>
<td>42.2</td>
<td>40.3–44.2</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>854</td>
<td>39.7</td>
<td>38.6–40.8</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1179</td>
<td>40.3</td>
<td>39.4–41.2</td>
<td></td>
</tr>
</tbody>
</table>

*Symptomatic individuals are those with intermittent claudication as assessed by the WHO intermittent claudication questionnaire. Asymptomatic individuals are those with ABI measurement >0.9 but without intermittent claudication.

†Adjusted for baseline age, sex, pack-years of smoking, BMI, diabetes, total/HDL cholesterol ratio, and physical activity.
(Table 2). On linear regression analyses (after age adjustment) of inflammatory markers as predictor variables for baseline ABI, significant inverse associations were found between CRP (P≤0.001), IL-6 (P≤0.001), and ICAM-1 (P≤0.001) and ABI (data not shown). After adjustment for baseline risk factors (age, sex, pack-years of smoking, diabetes, total/HDL cholesterol ratio, BMI, physical activity) and CVD status, these associations were attenuated, and only IL-6 remained independently associated with baseline ABI, albeit weakly (P=0.05). Significant associations were not present for VCAM-1 and E-selectin in both age-adjusted and risk factor–adjusted analyses.

Table 3 shows the mean (SD) and mean (SD) changes of ABI at the 3 time points (baseline, 5 years, and 12 years). Valid ABI measurements were available for 1582 subjects (99% of participants) at baseline, for 1081 subjects (93.1%) at the 5-year follow-up, and for 813 subjects (97.8%) at the 12-year follow-up. Moreover, 747 subjects had valid ABI measurements at all 3 clinical examinations. As shown in Table 3, mean ABI declined over time to a similar degree in all subjects measured at each time point and in the subgroup of subjects who had an ABI measured at all 3 time points.

The mean ABI change (unadjusted) from baseline to 5 and 12 years of follow-up by tertiles of inflammatory marker levels is demonstrated graphically in the Figure. Subjects with CRP, IL-6, ICAM-1, and VCAM-1 in the top tertile had a greater ABI decline by 5 years than subjects in the bottom tertile. This difference became more prominent after 12 years of follow-up. In fact, the bottom tertiles of CRP and IL-6 did not show any significant change in mean ABI between 5 and 12 years, whereas the middle and top tertiles of CRP and IL-6 showed further decline in mean ABI (mean [SD] ABI change, −0.02 [0.19] and −0.04 [0.20] for top tertiles of CRP and IL-6, respectively). Substantial differences were also observed for ICAM-1. After 12 years, the mean change (SD) in ABI from baseline for subjects in the bottom ICAM-1 tertile was −0.04 (0.18), whereas for those in the top tertile it was −0.11 (0.21). There was no significant relationship of ABI change across E-selectin tertiles.

We then performed separate linear regression analysis for ABI change from baseline to 5 years and from baseline to 12 years with each inflammatory marker as a predictor variable (Table 4). CRP and IL-6 were significant predictors of ABI change at 5 years (CRP, P=0.03; IL-6, P≤0.001) and at 12 years of follow-up (CRP, P=0.002; IL-6, P≤0.001) in analysis adjusted for both age and baseline ABI. Little change in these associations was found after adjustment for baseline ABI and risk factors of age, sex, pack-years of smoking, diabetes, total/HDL cholesterol ratio, BMI, physical activity, and CVD status in the 12-year analysis. However, CRP had only nonsignificant (P=0.08) associations with ABI change at 5 years in risk factor–adjusted analysis. ICAM-1 was significantly inversely associated with ABI change at 5 years (P=0.02) and at 12 years of follow-up (P=0.001) in analysis adjusted for age and baseline ABI. On further adjustments for baseline risk factors, ICAM-1 remained an independent predictor of ABI change at 12 years (P=0.01) only.

Table 4 presents the results of the aforementioned linear regression analyses. To compare the effects of each marker on ABI change, we standardized the regression coefficients to represent mean (95% CI) differences in ABI decline between the top and bottom tertiles of each marker. The magnitude of this difference up to 5 years of follow-up was greatest for IL-6. However, at 12 years, increased levels of CRP, IL-6, and ICAM-1 had effects of similar strength on ABI change. Finally, we examined the simultaneous effect of all inflammatory markers on ABI change at 5 and 12 years using linear regression with all inflammatory markers and baseline risk factors simultaneously entered into the model. In this final analysis, IL-6 was the only significant inflammatory marker that independently predicted ABI change at 5 years (P=0.002) and 12 years (P=0.02), and the difference (95% CI) in ABI decline between subjects in the top and bottom tertiles of IL-6 was −0.017 (−0.120, −0.008) and −0.016 (−0.030, −0.002) at 5 and 12 years, respectively. The aforementioned results were confirmed by repeated-measures analysis of ABI. In this analysis, IL-6, for example, had a significant interaction term with time (baseline, 5 years, or 12 years) in both age-adjusted (P=0.014) and risk factor–adjusted analyses (P=0.024).

Finally, we performed subgroup analysis of the 1080 subjects without baseline PAD (no symptoms of intermittent claudication or ABI >0.9). After 5 and 12 years, in analyses adjusted for baseline ABI, IL-6 (P=0.02) and ICAM-1 (P≤0.03) were significant predictors of ABI change. However, in analyses adjusted for baseline risk factors, IL-6 was the only independent predictor of ABI change at either the 5-year (P=0.01) or 12-year (P=0.03) examination.

<table>
<thead>
<tr>
<th>Subjects With ABI at Each Time Point</th>
<th>Subjects With ABI at All Time Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>n Mean (SD)</td>
<td>n Mean (SD)</td>
</tr>
<tr>
<td>ABI at baseline</td>
<td>1579 1.03 (0.18)</td>
</tr>
<tr>
<td>ABI at 5 y</td>
<td>1076 1.02 (0.17)</td>
</tr>
<tr>
<td>ABI at 12 y</td>
<td>805 1.00 (0.19)</td>
</tr>
<tr>
<td>ABI change at 5 y</td>
<td>1075 −0.04 (0.18)*</td>
</tr>
<tr>
<td>ABI change at 12 y</td>
<td>804 −0.07 (0.18)*</td>
</tr>
</tbody>
</table>

*P for change ≤0.001.
In this prospective study, we have shown that CRP, IL-6, and ICAM-1 were significant predictors of lower-extremity atherosclerotic progression measured by ABI over 12 years of follow-up independently of cardiovascular risk factors. Moreover, IL-6 was a significant and independent predictor of ABI change after 5 years of follow-up. Of all the markers studied, IL-6 was the only one to predict ABI changes at both 5 and 12 years of follow-up independently of other inflammatory markers and of cardiovascular risk factors. All inflammatory markers were moderately and significantly correlated, as previously reported.17

The epidemiological data on adhesion molecules and PAD are very limited, and most reported studies have been either small or cross-sectional.28–32 To our knowledge, this is the first large cohort study to examine, on a community basis, the predictive power of adhesion molecules (E-selectin, ICAM-1, and VCAM-1) for ABI change across 3 time points. Adhesion molecules are thought to contribute to atherosclerotic lesion formation and to plaque rupture. E-selectin mediates the first step in leukocyte adhesion at sites of inflammation, whereas ICAM-1 and VCAM-1 facilitate the firm attachment of leukocytes and their emigration into the arterial wall.33 We did not show any significant association between E-selectin and PAD at baseline and between E-selectin and ABI changes. This is in accordance with previous studies, which did not find any association between E-selectin levels and PAD severity or PAD location.29,31,34–36 We also found no significant associations between VCAM-1 and ABI change. In contrast, ICAM-1 was an independent predictor of ABI change after 12 years of follow-up. A weak trend was found for both markers with PAD severity at baseline, but it was attenuated after adjustment for baseline risk factors. Additionally, ICAM-1 was a significant predictor of ABI change at 5 and 12 years of follow-up in subjects without PAD at baseline (baseline ABI– and age-adjusted analyses). The present study supports previous evidence for a different role between ICAM-1 and VCAM-1 according to the stage of the disease: studies have shown that VCAM-1 is associated with PAD progression in subjects with preexisting disease, but ICAM-1 predicts disease development in healthy populations.28,30,31,32,37,38

IL-6 is one of the most studied cytokines with proinflammatory and proatherogenic activity. It is the main stimulant for hepatic production of CRP and other reactant proteins but also has other important roles leading to increased endothelial cell adhesiveness by upregulating E-selectin, ICAM-1, and VCAM-1 and releasing inflammatory mediators, including IL-6 itself.39 Evidence of an association of CRP and IL-6 with peripheral atheroma is limited. CRP and IL-6 were found to be significantly elevated in some, but not all, case-control studies in selected populations.40–47 Ridker et al48 showed that CRP, along with total/HDL cholesterol ratio, was the strongest predictor of PAD among several biomarkers assessed in healthy individuals in a prospective study. Moreover, in the Rotterdam Study,14 CRP significantly predicted PAD after 6.5 years of follow-up in the general population. In contrast, McDermott et al49 failed to show any significant association between CRP and ABI in subjects without a history of CVD. In another study,49 IL-6, but not CRP, was elevated significantly in patients with subclinical CVD compared with healthy controls.

### Discussion

**TABLE 4. Multiple Regression for ABI Change From Baseline to 5 Years and 12 Years of Follow-up**

<table>
<thead>
<tr>
<th>Standardized Difference (95% CI) in ABI Change Between Top and Bottom Tertiles of Inflammatory Markers*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjusted for baseline ABI and age</td>
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<tr>
<td>CRP</td>
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<td>IL-6</td>
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<td>ICAM-1</td>
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<td>VCAM-1</td>
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<td>E-selectin</td>
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<tr>
<td>Adjusted for baseline ABI and risk factors§</td>
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<tr>
<td>CRP</td>
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<td>IL-6</td>
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<td>E-selectin</td>
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*Regression coefficients have been multiplied by the intertertile range (difference between logarithmically transformed bottom and top tertile). They therefore represent mean differences (95% CI) in ABI change between top and bottom tertile of each inflammatory marker. Intertertile range was 0.41 for CRP, 0.25 for IL-6, 0.09 for ICAM-1, and 0.15 for E-selectin.

†P significant at 0.01 level; ‡P significant at 0.05 level.
§Adjusted for age, sex, pack-years of smoking, diabetes, BMI, total/HDL cholesterol ratio, physical activity, and CVD status.

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For further details, please refer to the original publication.
This is the first study to examine the predictive value of IL-6 in relation to PAD prospectively. We found a significant trend between PAD severity at baseline and levels of IL-6 and CRP. However, the trend remained significant only for CRP after adjustment for cardiovascular risk factors. Furthermore, we showed an independent association between IL-6 with ABI at baseline, ABI change at 5 years, and ABI change at 12 years of follow-up in analyses adjusted for conventional risk factors. These results were also confirmed by longitudinal analysis with the use of ABI as a repeated measurement over

Unadjusted mean (±1 SE) of ABI change from baseline to 5 years and to 12 years of follow-up across tertiles of CRP, IL-6, soluble ICAM-1, soluble VCAM-1, and E-selectin. Bottom tertile (●), middle tertile (○), and top tertile (●) are shown. Cutoff points for tertiles were 1.11 and 2.88 mg/L for CRP, 1.65 and 2.96 pg/mL for IL-6, 197 and 242 ng/mL for ICAM-1, 341 and 410 ng/mL for VCAM-1, and 34 and 48 ng/mL for E-selectin.
3 time points. IL-6 showed a greater effect on ABI change at 5-year follow-up compared with CRP, whereas CRP was an independent predictor of ABI change after 12 years only. On the other hand, IL-6 was also an independent predictor of ABI change at 5 years and at 12 years, when both risk factors and inflammatory markers (CRP, ICAM-1, VCAM-1, and E-selectin) were simultaneously entered as covariates into the model. Finally, IL-6 was the only inflammatory marker that predicted ABI decline after 5 and 12 years of follow-up independently of baseline risk factors in subjects without PAD at baseline.

This independent predictive power of IL-6 over and above other inflammatory markers in relation to peripheral atherosclerotic progression may reflect its vital role both in inflammation and in hemostasis. In addition to upregulating CRP and adhesion molecule production, IL-6 upregulates several hemostatic variables, including fibrinogen, tissue factor, von Willebrand factor, and factor VII. Although our findings suggest a key role for IL-6 in detection of progressive atherosclerosis, CRP merits consideration as a predictive inflammatory marker from a practical viewpoint.

The present study has some limitations. First, the inflammatory markers were measured only once, and intrapersonal variation therefore could not be taken into account. Nevertheless, this would tend to result in an underestimation of the true effect. Second, participants of the 5- and 12-year follow-up examinations were probably healthier at baseline than those who died during the follow-up period. This explains the higher baseline ABI (1.07) of those 747 subjects who attended all 3 clinical examinations. However, the trend in ABI decline during the 12 years between the individual examination attenders and the group who had attended all 3 clinical examinations was similar and suggest that our findings and conclusions are likely to be valid. We did not adjust our analysis for aspirin or statin use at baseline; however, at the time of baseline examination (1987/1988), very few of the Edinburgh population took such drugs for the prevention of CVD. Furthermore, the generalization of our results to other ethnic populations and age groups is unknown.

Finally, in this analysis we studied the predictive power of inflammatory factors on ABI changes. ABI has shown very small changes between tertiles of inflammatory markers, and the clinical importance of our results needs further investigation. On the other hand, the present analysis is suggestive of a relationship between inflammation and progressive atherosclerosis. Despite the prospective design of our study, an observational study cannot establish causal relations, and residual confounding cannot be ruled out. Therefore, the precise pathway through which inflammation influences atherosclerotic progression and the directionality of the reported associations remain unknown. Similarly, nonsignificant associations such as the ones shown by CRP in risk factor–adjusted analysis could result if inflammatory markers exert their effect on ABI via cardiovascular risk factors like diabetes or BMI. If this is true, these variables should not be used as covariates in the model because they are not confounders but intermediary factors.

In summary, we have shown strong independent associations between baseline levels of CRP, IL-6, and ICAM-1 with ABI change measured 12 years after baseline in analyses adjusted for cardiovascular risk factors. IL-6 also predicted ABI change from baseline to 5 years of follow-up independently of conventional risk factors. The 3 markers had comparable effects on ABI change at 12 years, whereas IL-6 had a greater effect than CRP on ABI change at 5 years. Finally, IL-6 predicted the ABI decline after 5 and 12 years of follow-up independently of CRP, ICAM-1, VCAM-1, and E-selectin and of baseline risk factors. Further studies are required to validate these results, to further assess the relative importance of each inflammatory marker on the progression of peripheral atherosclerosis, and to establish whether these associations are of causal significance.

Acknowledgments
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