Interleukin-6 (IL-6) and the Prognosis of Abdominal Aortic Aneurysms

K.G. Jones, FRCS; D.J. Brull, MRCP; L.C. Brown, MSc; M. Sian, MPhil; R.M. Greenhalgh, MD; S.E. Humphries, PhD; J.T. Powell, MD

Background—Abdominal aortic aneurysm is a multifactorial disorder in which inflammation is an important pathophysiological feature. In explant culture, aneurysm biopsies secrete large amounts of interleukin-6 (IL-6), and among aneurysm patients, the circulating concentration of IL-6 appears to be increased.

Methods and Results—We investigated, in 19 patients, whether aneurysm wall was an important source of circulating IL-6. We also tested the hypotheses, in 466 patients with a small aneurysm, that (1) high concentrations of circulating IL-6 signaled rapid aneurysm growth and (2) the −174 G→C polymorphism in the IL-6 promoter predicted survival. For 19 patients with large or inflammatory aneurysms, the concentration of IL-6 was higher in the iliac arteries than the brachial arteries (median difference 26.5 pg/mL, this difference increasing with aneurysm diameter, P=0.01). In 466 patients with small aneurysms, the frequency of the −174 C allele (0.40) was similar to that in a normal healthy population. Patients of GG genotype had lower plasma concentrations of IL-6 than patients of GC and CC genotypes (medians 1.9, 4.8, and 15.6 pg/mL, respectively, Kruskal-Wallis P=0.047). Cardiovascular and all-cause mortalities were lower for patients of GG genotype than for patients of GC and CC genotype: hazard ratios 0.32 (95% CI 0.12 to 0.93), P=0.036, and 0.51 (95% CI 0.25 to 1.00), P=0.05, respectively. There was no association between plasma IL-6 or IL-6 genotype and aneurysm growth.

Conclusions—Aortic aneurysms appear to be an important source of circulating IL-6, the concentration being influenced by genotype. For patients with small aneurysms, the −174 G→C IL-6 genotype predicts future cardiovascular mortality. (Circulation. 2001;103:2260-2265.)

Key Words: aneurysm ■ survival ■ interleukins ■ genetics

A bdominal aortic aneurysm (AAA) is a common cardiovascular disease of increasing incidence. Patients with small AAAs (<5.5 cm in diameter) are at increased risk of cardiovascular death, but prophylactic surgical repair is not recommended. Inflammation appears to be an important component of the pathological process causing aneurysm expansion. Indeed, when biopsies of the aneurysm wall are placed in tissue culture, large amounts of cytokines, including interleukin (IL)-1 and IL-6 are secreted. Elevated circulating concentrations of cytokines have been found in several conditions in which inflammation is an important aspect of the pathology. Patients with AAA appear to have significantly higher serum concentrations of IL-1 and IL-6 than either coronary heart disease patients or control subjects. Furthermore, there has been a suggestion that serum IL-6 concentrations are increased among patients with early aortic dilatation. Circulating cytokines also are being assessed as markers of coronary heart disease, and in healthy men, an elevated concentration of circulating IL-6 is associated with an increased future risk of myocardial infarction. In the first part of the present study, we investigated the possibility that the aneurysm itself was a significant source of circulating IL-6, to explain the increased circulating levels observed in AAA patients.

IL-6 has a pivotal role in stimulating the acute-phase response, which elevates the circulating concentrations of several plasma proteins, including fibrinogen and C-reactive protein (CRP). The circulating concentrations of IL-6 are likely to be influenced by several environmental and genetic factors, including a polymorphic site (−174 G→C) in the IL-6 gene promoter. The familial predisposition to AAA is well established. Therefore, we investigated the extent to which either plasma IL-6 or IL-6 genotype predicted aneurysm growth rate. In AAA patients, most deaths are attributed to a cardiovascular cause. Therefore, after the recent evidence concerning IL-6 as a cardiovascular risk factor, we also wished to investigate whether either plasma IL-6 or IL-6 genotype predicted the survival of AAA patients.

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Methods

Patients
The patients studied for aneurysm growth and survival were randomized to ultrasound surveillance as part of the UK Small Aneurysm Trial. Briefly, patients 60 to 76 years old with asymptomatic AAAs 4.0 to 5.5 cm in diameter were randomized to either early elective surgery or a period of ultrasound surveillance. Patients in the surveillance arm (n = 527) had their aortic diameter measured either every 6 months (AAA 4.0 to 4.9 cm) or every 3 months (AAA 5.0 to 5.5 cm). When aneurysm diameter exceeded 5.5 cm, aneurysm growth rate exceeded 1 cm/y, or the aneurysm became tender, surgery was recommended. All patients were “flagged” with the Office of National Statistics (ONS) to obtain information for deaths and cause of death. Baseline resting ECGs were coded by 2 independent observers. A baseline peripheral venous blood sample was taken to provide cells for the extraction of DNA and plasma for the measurement of cholesterol, cotinine (by radioimmunoassay), fibrinogen, and CRP (both by immunonephelometry) and for storage at −70°C. Stored plasma was used for the measurement of IL-6. Aneurysm growth rates were calculated from linear regression analysis only in patients with IL-6 measurements.

Aneurysm growth was interrupted between 150 and 250 pg/mL. Aneurysm diameter was obtained from immediate preoperative imaging, and patients were placed into 2 groups according to aneurysm wall thickness; < data for 0.2 to 0.5 cm; ≥ data for 1 to 2 cm.

Measurement of IL-6 by ELISA
The polyclonal and monoclonal antibody pair for the IL-6 ELISA, together with the recombinant IL-6 standard, were obtained from R&D, and the assay was amplified with Ampak (Dako): range 3 to 300 pg/mL, coefficient of variation 10%.

IL-6 Genotyping
DNA was prepared from peripheral blood cells and stored in 96-well arrays at −20°C. The DNA was amplified by polymerase chain reaction as previously described. After polymerase chain reaction, genotypes were resolved by use of the restriction endonuclease NdeIII. The digestion product size was determined by electrophoresis on an 8% microtiter array diagonal gel electrophoresis (MADGE). The ~174 G and ~174 C alleles were characterized by 190-bp and 143-bp bands, respectively (the 47-bp cleavage product was not visualized).

Data Analysis
All analyses were performed with STATA software. The distribution of both plasma IL-6 and CRP were highly skewed, and square root (which uses 0 values) and logarithmic transformations, respectively, were used for normalization. For IL-6, the Shapiro-Francia test for normality showed W = 0.47. All other continuous variables (age, initial aneurysm diameter, aneurysm growth rates, and plasma fibrinogen) were normally distributed. The effect of IL-6 and IL-6 genotype on survival was considered in 2 time periods: (1) the surveillance period and (2) the long-term survival until September 2000. For the former analysis, patients were censored at the end of the trial (June 30, 1998) or time of aneurysm rupture and/or surgery.

Univariate estimates of survival were calculated by the Kaplan-Meier method. Cox proportional-hazards models were used to estimate adjusted hazard ratios for the effect of IL-6 genotype on survival.

Results

IL-6 Concentration in Brachial and Iliac Artery Plasma
Plasma IL-6 concentration was measured in samples taken simultaneously in the brachial and iliac arteries from 19 patients. These patients were classified into 2 groups accord-
that of patients with below-median concentrations, $P=0.86$ (data not shown).

**IL-6 Genotype and IL-6 Plasma Concentration**

The $IL-6 -174\, G\rightarrow C$ genotype information was available for 466 of 527 patients. The frequency of the $C$ allele was 0.40, similar to that in a normal healthy population with the genotype distribution in Hardy-Weinberg equilibrium. Table 1 shows the genotype distribution, together with the median IL-6, fibrinogen, and CRP measures. Mean age, sex, smoking status, and initial aneurysm diameter did not differ between the genotypes. Patients of genotype CC had the highest median plasma IL-6 concentration, patients of genotype GC an intermediate plasma IL-6 concentration, and patients of genotype GG the lowest median plasma IL-6 concentration, Kruskal-Wallis $P=0.047$ (Table 1). After normalization of data by square-root transformation, the association between the presence of the $C$ allele and higher plasma IL-6 concentration appeared more significant, ANOVA $P=0.003$ (Table 1). This association was robust to adjustment for age, sex, smoking status, and initial aneurysm diameter. Plasma CRP and fibrinogen concentrations were not significantly different between patients of different genotype (Table 1), and this did not change after adjustment for age, sex, and smoking status. The mean aneurysm growth rates did not vary by genotype (Table 1).

**IL-6 Genotype and Cardiovascular Mortality in the Surveillance Period**

In the surveillance period, the median follow-up was 33 months, and there were 47 deaths (13 from acute myocardial infarction, 7 from stroke, 10 other cardiovascular causes, 9 from cancer, 7 from other causes, and 1 unknown). The median time to surgery was 35 months. There was a significant association between $IL-6$ genotype and cardiovascular mortality, with patients of CC genotype being at highest risk of cardiovascular mortality in the surveillance period and

![Figure 2. Plasma IL-6 concentration and aneurysm growth rate. There was no association between aneurysm growth rate, calculated by linear regression analysis, and plasma IL-6.](http://circ.ahajournals.org/)

### TABLE 1. Baseline Characteristics and IL-6 Genotypes in 466 Patients With AAA

<table>
<thead>
<tr>
<th>IL-6 Genotype ($-174, G\rightarrow C$)</th>
<th>GG</th>
<th>GC</th>
<th>CC</th>
<th>$P$ (Unadjusted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>146</td>
<td>245</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>69.1±4.2</td>
<td>68.7±4.7</td>
<td>68.6±4.7</td>
<td>0.57</td>
</tr>
<tr>
<td>Sex, % male</td>
<td>77</td>
<td>84</td>
<td>90</td>
<td>0.046</td>
</tr>
<tr>
<td>Initial AAA diameter, cm</td>
<td>4.46±0.43</td>
<td>4.53±0.47</td>
<td>4.51±0.46</td>
<td>0.41</td>
</tr>
<tr>
<td>ECG evidence of ischemia, n</td>
<td>88</td>
<td>151</td>
<td>43</td>
<td>0.36</td>
</tr>
<tr>
<td>Smoking, n</td>
<td>45</td>
<td>74</td>
<td>32</td>
<td>0.37</td>
</tr>
<tr>
<td>Current</td>
<td>92</td>
<td>157</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>9</td>
<td>15</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>9</td>
<td>15</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>IL-6, pg/mL, median (IQR)</td>
<td>1.9 (0–106)</td>
<td>4.8 (0–126)</td>
<td>15.6 (0.5–215)</td>
<td>0.047</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>4.53±1.36</td>
<td>4.31±1.38</td>
<td>4.46±1.41</td>
<td>0.29</td>
</tr>
<tr>
<td>ln(CRP), ng/mL</td>
<td>4.1±0.9</td>
<td>4.2±0.9</td>
<td>4.1±1.0</td>
<td>0.28</td>
</tr>
<tr>
<td>AAA growth, cm/y</td>
<td>0.38</td>
<td>0.36</td>
<td>0.36</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Values for continuous variables are reported as mean±SD with comparisons by ANOVA, except for median IL-6, for which the Kruskal-Wallis $P$ value is given. Categorical variables were compared by $\chi^2$ test. The number of missing values for each analyte is given in square brackets.
patients of GG genotype at lowest risk (Table 2). The unadjusted hazard ratio was 0.32 (95% CI 0.12 to 0.95), \( P = 0.036 \), and after adjustment for age, sex, aneurysm diameter, and smoking, the hazard ratio was 0.32 (95% CI 0.13 to 0.94), \( P = 0.032 \). For all-cause mortality, the unadjusted hazard ratio was 0.51 (95% CI 0.25 to 1.00), \( P = 0.05 \), and after adjustment for age, sex, aneurysm diameter, and smoking, the hazard ratio was 0.52 (95% CI 0.24 to 1.01), \( P = 0.05 \). The Kaplan-Meier curves for cardiovascular mortality according to IL-6 genotype are shown in Figure 3.

**ECG Findings, Angina, Ankle Pressures, and IL-6 Genotype**

To test whether the association between IL-6 genotype and cardiovascular mortality was indirect, we investigated the association between IL-6 genotype and baseline resting ECG findings, angina as determined by the Rose questionnaire,\(^{14}\) and ankle/brachial systolic pressure index (ABPI, mean of right and left leg). We also performed subgroup survival analysis in those without a history of angina or myocardial infarction (\( n = 403 \)). The distribution of genotypes according to ECG status is shown in Table 3. The frequency of the \( -174 \) C allele was 0.42 for patients with no ECG evidence of ischemia, 0.39 for patients with possible ischemia, and 0.49 for patients with probable ischemia, \( \chi^2 P = 0.2 \). Among those with no evidence of ischemia, cardiovascular mortality was lowest for the GG genotype, hazard ratio 0.52 (95% CI 0.2 to 1.04), \( P = 0.09 \). The frequency of the \(-174\) C allele for patients with and without a history of angina was 0.45 and 0.42, respectively, \( \chi^2 P = 0.3 \). In patients without history of angina or myocardial infarction, those of GG genotype were at lowest risk of cardiovascular mortality, hazard ratio 0.54 (95% CI 0.32 to 0.91), \( P = 0.02 \). The frequency of the \(-174\) C allele was 0.40 for patients with a baseline ABPI of \( \geq 0.9 \) (\( n = 307 \)), compared with 0.45 for patients with ABPI <0.9 (\( n = 154 \)), \( \chi^2 P = 0.1 \).

**IL-6 Genotype and Long-Term Survival**

In total, there were 194 deaths in the period to September 2000; the median time to death or censoring was 72 months (IQR 55 to 87 months). The median time to aneurysm repair was 37 months. There was a nonsignificant trend for patients of CC genotype to have the highest all-cause and cardiovascular mortality (Figure 4). Although the Kaplan-Meier curves remain separated at 84 months, the separation is most marked in the first 3 to 4 years. After 72 months, the survival was 71%, 66%, and 62% in patients of GG, GC, and GG genotype, respectively, unadjusted hazard ratio 0.86 (95% CI

### TABLE 2. Three-Year Survival During the Surveillance Period Before Aneurysm Rupture and/or Surgery

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients, n (Patient-Years of Follow-Up)</th>
<th>Total Deaths, n (3-Year Survival, %)</th>
<th>Hazard Ratio (95% CI)</th>
<th>Adjusted Hazard Ratio* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG</td>
<td>146 (403)</td>
<td>9 (94)</td>
<td>0.51 (0.25–1.00)</td>
</tr>
<tr>
<td></td>
<td>GC</td>
<td>245 (672)</td>
<td>29 (92)</td>
<td>( P = 0.05 )</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>75 (208)</td>
<td>9 (86)</td>
<td>( P = 0.03 )</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>146 (403)</td>
<td>4 (98)</td>
<td>0.58 (0.34–0.98)</td>
</tr>
<tr>
<td></td>
<td>GC</td>
<td>245 (672)</td>
<td>19 (94)</td>
<td>( P = 0.04 )</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>75 (208)</td>
<td>7 (90)</td>
<td>( P = 0.03 )</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, smoking, and aneurysm diameter.

### TABLE 3. Baseline ECG Findings and IL-6 Genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No Evidence of Ischemia, n</th>
<th>Possible Evidence of Ischemia, n</th>
<th>Probable Evidence of Ischemia, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>88</td>
<td>46</td>
<td>12</td>
</tr>
<tr>
<td>GC</td>
<td>151</td>
<td>62</td>
<td>30</td>
</tr>
<tr>
<td>CC</td>
<td>43</td>
<td>16</td>
<td>11</td>
</tr>
</tbody>
</table>

\( \chi^2 = 4.38, P = 0.4 \).
secreted from the AAA could stimulate the hepatic acute-
phase response more directly than peripheral sources of IL-6.
Furthermore, in a much larger study than previously, we also failed to observe an association between plasma IL-6 concentration and aneurysm growth. Therefore, in contrast to previous studies, we suggest that the effect of IL-6 secretion from the aneurysm on distant targets is more important than its effect on aneurysm formation.

Patients with AAA have established cardiovascular disease, and ~67% of these patients will die of a cardiovascular cause. The survival of these patients is lower than the age- and sex-matched population both before and after aneurysm surgery. Therefore, if the AAA is an important source of plasma IL-6 and plasma IL-6 predicts future myocardial infarction, we might have expected to observe an association between plasma IL-6 and cardiovascular mortality. No such association was observed for the 231 patients in whom IL-6 concentration was measured in peripheral venous plasma. This was perhaps too small a population in which to observe association, particularly when the biological activity of IL-6 is not determined by immunoassay and IL-6 concentration is affected by diurnal variation, low-grade infection, environmental and genetic factors, and the concentration of circulating soluble IL-6 receptor. For these latter reasons, IL-6 genotype may provide a more robust marker of cardiovascular risk.

The association between IL-6 genotype and mortality, particularly cardiovascular mortality, was strongest during the surveillance period before aneurysm repair. The group of patients with the CC genotype, associated with the highest plasma IL-6 concentrations, had the highest mortality risk. These data from a group of fit patients were gathered prospectively and were of high quality, with no patients lost to follow-up. The association between IL-6 genotype and survival remained after adjustment for factors known to influence patient survival, eg, age and aneurysm diameter. A potential weakness of our study is the possibility that the mortality risk associated with IL-6 genotype resulted from an association of genotype with preexisting coronary artery disease. There was no evidence of a confounding association, however, between IL-6 genotype and ischemic changes on baseline ECG, history of angina, or ABI, a marker of generalized atherosclerosis.

During the course of the UK Small Aneurysm Trial, >60% of the surveillance patients eventually underwent aneurysm repair, with median time to surgery 2.9 years. Many of the remaining surveillance patients may have undergone aneurysm exclusion after the trial closed, but these later events are not recorded, and follow-up information relates to mortality only. The overall survival curve (Figure 4) clearly shows the early survival disadvantage for patients of CC genotype. Although the survival curves remain separated, in the longer term this disadvantage becomes nonsignificant.

Our results showed a clear association of plasma IL-6 concentration and mortality in the surveillance period with IL-6 genotype. Intriguingly, the association between the −174 C allele and higher levels of plasma IL-6 is opposite to that previously reported in healthy subjects. It also is in contrast to in vitro data in HeLa cells, which showed that compared with the −174 G construct, the −174 C construct showed lower basal and stimulated (lipopolysaccharide or...
IL-1) expression of reporter gene. Several explanations for this apparent discrepancy can be proposed. The relative activity of −174 alleles may be reversed in physiologically relevant cells, such as endothelial cells and macrophages, that secrete IL-6 and express IL-6 receptors. Some recent data focusing on the importance of haplotype support this hypothesis and show that −174 C constructs support higher reporter gene expression in endothelial and macrophage cell lines. In addition, the important determinant of plasma IL-6 concentration may not be simply the peak value of expression after an inflammatory stimulus but rather the time taken for activity to return to basal levels after stimulation. Thus, for AAA patients, the constant chronic stimulation appears to result in higher IL-6 expression from the −174 C allele than from the −174 G allele. Furthermore, the overall control of IL-6 expression is extremely complex, partly because of the autocrine effects of IL-6 and its interaction with soluble IL-6 receptors. Therefore, in subjects with extensive atherosclerosis (most AAA patients), the association between IL-6 genotype and plasma IL-6 may be the converse of that observed in a healthy, younger population. Perhaps surprisingly, we did not detect any association between IL-6 genotype and fibrinogen or CRP. The strong effect of smoking on plasma fibrinogen and CRP, coupled with the poor reporting of smoking status, may explain why there was no association between these analytes and IL-6. Moreover, recent evidence indicates that in smaller samples, association of risk with fibrinogen may depend on the particular assay used.

The secretion of IL-6 from an abdominal aortic aneurysm is likely to have important systemic effects. Exclusion of the aneurysm from the circulation is likely to diminish these effects and explain why, in AAA patients, the association between IL-6 genotype and survival is strongest in the surveillance period.

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