Plasma Concentrations of Interleukin-6 and Abdominal Aortic Diameter Among Subjects Without Aortic Dilatation

Luis E.P. Rohde, Luis H. Arroyo, Nader Rifai, Mark A. Creager, Peter Libby, Paul M. Ridker, Richard T. Lee

Abstract—Previous studies suggest that cytokine-induced tissue inflammation may participate in the pathogenesis of abdominal aortic aneurysms. Serum inflammatory markers may reflect arterial inflammation in asymptomatic phases of the aneurysmal disease. We studied 120 outpatients (62 men; age, 65±9 years) by ultrasound evaluation of the abdominal aorta to evaluate the association of circulating levels of interleukin-6 (IL-6) with abdominal aortic diameter in subjects with normal aortic size. Aortic diameter was measured at the infrarenal level and indexed for body surface area. Seven patients with abdominal aortic dilatation (indexed aortic diameter, >1.3 cm/m²) were also identified. Plasma concentrations of IL-6, serum amyloid A (SAA), C-reactive protein (CRP), total homocysteine, and lipids were measured. Among the 113 subjects without aortic dilatation, indexed aortic diameter was positively associated with serum levels of IL-6 (P<0.01), SAA (P<0.01), and total homocysteine (P=0.01). IL-6 levels increased in a stepwise fashion among dichotomized groups of aortic size (low and high aortic diameters) and peaked in patients with aortic dilatation (2.3±1.2 versus 2.7±0.9 versus 3.2±0.9 pg/mL, respectively; P for trend=0.039). None of the serum lipid measurements correlated with abdominal aortic diameter. Although CRP levels were associated with SAA levels (r=0.60; P<0.001), associations between CRP and aortic diameter were nonsignificant. In multivariate analysis, levels of IL-6 (P=0.02), SAA (P=0.001), and total homocysteine (P<0.001) were independent correlates of indexed aortic diameter. In conclusion, circulating levels of IL-6, SAA, and total homocysteine may reflect processes involved in the early phases of abdominal aortic aneurysm formation, before dilatation of the abdominal aorta is established. These data support a role for chronic inflammation in the progression of asymptomatic aortic disease. (Arterioscler Thromb Vasc Biol. 1999;19:1695-1699.)

Key Words: atherosclerosis ■ inflammation ■ aortic aneurysm ■ homocysteine

Increasing evidence suggests that elevated levels of acute-phase proteins and their determinants occur in acute coronary syndromes,1,2 and may also predict future cardiovascular events.3–6 Interleukin-6 (IL-6) is an inflammatory cytokine that seems to play a pivotal role in the acute-phase response and elevated levels of IL-6 have been associated with worse prognosis in unstable angina.7 In addition, circulating levels of IL-6 are elevated in patients with abdominal aortic aneurysms (AAAs), compared with controls and patients with coronary heart disease.8 These findings support several observations that indicate that tissue inflammation, possibly mediated by IL-6, participates in the pathogenesis of AAAs. These immunoinflammatory processes are hypothesized to operate in early phases of the aneurysmal disease, even before true dilatation develops. We therefore tested the hypothesis that plasma levels of IL-6 correlate with abdominal aortic diameter in clinically stable subjects without aortic dilatation.

Methods

Patients

Outpatients older than 50 years of age referred to the Noninvasive Cardiac Laboratory of the Brigham and Women’s Hospital for a transthoracic echocardiogram were invited to participate in this study. Patients with a clinical history of active infectious or systemic inflammatory disease or patients taking oral or parenteral corticosteroids were excluded. Overall, 120 subjects agreed to participate in the study. Among these subjects, 7 patients were identified with abdominal aortic dilatation (4 patients with incidental dilatation and 3 patients with previously known abdominal aortic aneurysms [AAAs]). Abdominal aortic dilatation was defined as abdominal aortic diameter >1.3 cm/m² based on data from a cohort of subjects without cardiovascular disease.10 Before the imaging procedures, individual data concerning atherosclerosis risk factors, prior cardiovascular history, and other comorbidities were obtained. The protocol was reviewed and approved by the Human Research Committee of the Brigham and Women’s Hospital and informed written consent was obtained from all patients.

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Aorta Imaging
Two experienced ultrasonographers, using commercially available equipment (Hewlett-Packard Sonos 2500, Hewlett-Packard Medical Products) and a 2.7/3.5-MHz transducer, performed the ultrasound evaluations. The patients were examined in the supine flexed position, to relax the abdominal wall. The abdominal aorta was imaged from the xyphoid process to the periumbilical level, including the aortic bifurcation whenever possible. Longitudinal and lateral views of the abdominal segments at the maximum 2-dimensional diameter were recorded on standard S-VHS videotape for off-line analysis. The best images in each location were digitized and blindly evaluated with a custom written software program. Luminal size of the abdominal aorta was measured at the periumbilical level, corresponding to the infrarenal segments. The echo-free lumen of the vessel was measured between the inner trailing edge of the anterior wall and the inner leading edge of the posterior wall, at the peak of the electrocardiographic R wave to avoid pulsatile cycle variations. Aortic diameter indexed to body surface area was used for statistical analysis. Intraobserver variability and interobserver variability were calculated in a subset of 30 patients and were <5% and <10%, respectively.

Blood Measurements
EDTA-anticoagulated blood was obtained by using a 19-gauge butterfly needle and immediately centrifuged for 20 minutes at 2500 rpm. Aliquots were stored at −70°C. IL-6 was measured in duplicate by the sandwich ELISA technique (Immunotech). Serum amyloid A (SAA) was measured by a nephelometric method (Dade Behring) on the BNII analyzer that possesses a sensitivity down to 0.8 mg/mL. (SAA) was measured by a nephelometric method (Dade Behring) on the BNII analyzer that possesses a sensitivity down to 0.8 mg/mL. C-reactive protein (CRP), total homocysteine, total cholesterol, LDL and HDL cholesterol, Lp(a), and apoB were also assayed, as described elsewhere.4,11–13

Statistical Analysis
Skewed variables underwent logarithmic transformation, resulting in a near normal distribution. The associations between indexed aortic diameters and serum levels of IL-6, SAA, CRP, total homocysteine, and lipids were evaluated by Pearson correlation coefficients. In addition, subjects without aortic dilatation were dichotomized into 2 groups according to low (<0.84 cm/m²) or high (≥0.84 cm/m²) indexed aortic diameters, representing the median of the sample. The significance of any differences in values on the categorized aortic diameter groups (low and high) and in patients with abdominal aortic dilatation was computed by using 1-way analysis of variance. The best images in each location were digitized and blindly evaluated with a custom written software program. Luminal size of the abdominal aorta was measured at the periumbilical level, corresponding to the infrarenal segments. The echo-free lumen of the vessel was measured between the inner trailing edge of the anterior wall and the inner leading edge of the posterior wall, at the peak of the electrocardiographic R wave to avoid pulsatile cycle variations. Aortic diameter indexed to body surface area was used for statistical analysis. Intraobserver variability and interobserver variability were calculated in a subset of 30 patients and were <5% and <10%, respectively.

Results

Patient Characteristics
Approximately half of the 120 subjects had a history of hypertension or hypercholesterolemia, and 66% were past or present smokers (Table 1). Previous myocardial infarction had occurred in 24 (20%) patients and 20 (16%) had a clinical history consistent with angina pectoris. Reasons for ordering the echocardiogram included left ventricular function evaluation (22%), valvular assessment (21%), coronary artery disease (12%), preoperative evaluation (6%), arrhythmia (5%), or other (34%).

IL-6 levels were not normally distributed and underwent logarithmic transformation, resulting in a near normal distribution. Mean and median IL-6 levels for this group were 18±0.6 and 8.3 (10.2) pg/mL, respectively. The lipid profile and other serum measurements were described in Table 1. Indexed abdominal aortic diameter among patients with aortic dilatation (n=7) and without aortic dilatation (n=113) was 2.1±0.6 and 0.86±0.1 cm²/m², respectively (P<0.01). No severe abdominal aortic occlusive disease was identified within this cohort.

Association Between Aortic Diameters and Serum Measurements
Among the 113 participants without aortic dilatation, indexed aortic diameter was positively associated with serum levels of IL-6 (P<0.01), SAA (P<0.01), and total homocysteine (P=0.01) (Table 2). Adjustment for age did not substantially alter these correlations. Of note, none of the serum lipid measurements [total, LDL and HDL cholesterol, Lp(a) and apoB] correlated with indexed aortic diameter. Although CRP levels were strongly associated with SAA levels (r=0.60; P<0.001), associations between CRP levels and indexed aortic diameters were also nonsignificant.

Figure 1 displays the linear regression line and 95% confidence interval between circulating levels of IL-6 and

| TABLE 1. Baseline Characteristics of Study Population |
|---------------------------------|------------------|
| Characteristics | Values* |
| Subjects | 120 |
| Age (y) | 65±9 |
| Sex (male/female) | 63 (52)/57 (48) |
| Past or present smoking | 81 (66) |
| Diabetes | 18 (15) |
| Hypertension | 67 (56) |
| Hyperlipidemia | 55 (46) |
| Positive family history of IHD | 32 (27) |
| Previous angina pectoris | 20 (16) |
| Previous myocardial infarction | 23 (19) |
| Serum measurements | |
| IL-6 (pg/mL) | 18±0.20 (15.4) |
| SAA (mg/dL) | 2.5±3.8 (3.0) |
| CRP (mg/dL) | 0.8±1.4 (0.4) |
| Total homocysteine (μmol/L) | 11.1±4.8 (10.2) |
| Total cholesterol (mg/dL) | 200±44 |
| HDL (mg/dL) | 46±13 |
| LDL (mg/dL) | 118±33 |
| Lp(a) (mg/dL) | 29±29 |
| apoB (mg/dL) | 114±30 |

IHD indicates ischemic heart disease; SAA, serum amyloid A; CRP, C-reactive protein.

*Expressed as n (%) or mean±SD (median).

| TABLE 2. Pearson Correlation Coefficients Between Indexed Distal Aortic Diameter and Serum Measurements Among Subjects With Normal Aortic Size (Indexed Distal Aortic Diameter, <1.3 cm²) |
|---------------------------------|------------------|
| Serum Measurement | Unadjusted | Age Adjusted |
| | r | P | r | P |
| IL-6 (pg/mL) | 0.285 | 0.002 | 0.283 | 0.003 |
| SAA (mg/dL) | 0.274 | 0.004 | 0.271 | 0.004 |
| CRP (mg/dL) | 0.03 | 0.76 | 0.03 | 0.75 |
| Total homocysteine (μmol/L) | 0.241 | 0.01 | 0.242 | 0.01 |

r represents Pearson correlation coefficient; P represents level of significance.
indexed abdominal aortic diameter among patients without aortic dilatation (Pearson coefficient = 0.28; \( P = 0.002 \)). In addition, log IL-6 values increased in a stepwise fashion among groups of aortic size (low and high aortic dimension as defined in Methods) and peaked in patients with aortic dilatation (2.3 ± 1.2 versus 2.7 ± 0.9 versus 3.2 ± 0.9 pg/mL, respectively, \( P \) for trend = 0.039; Figure 2).

**Adjusted Associations**

In multivariate regression analysis adjusted for age, hypertension, diabetes, smoking, history of myocardial infarction or angina, and lipid and nonlipid serum measurements, the only significant correlates of indexed aortic diameter were plasma concentrations of IL-6 (\( P = 0.02 \)), SAA (\( P = 0.001 \)), and total homocysteine (\( P < 0.001 \)). These variables explained 26% of the variance in indexed aortic diameter (Table 3).

**Discussion**

These data indicate that plasma concentration of IL-6, SAA, and total homocysteine increase in a stepwise fashion with abdominal aortic dimension among subjects with normal aortic size. With regard to IL-6 and SAA, these findings suggest that inflammatory cytokines and acute-phase reactants may play a role in early phases of AAA pathogenesis, even before frank dilation of the abdominal aorta is established. Our data support the notion that chronic inflammation participates in the pathogenesis of asymptomatic aortic disease. With regard to total homocysteine levels, these data suggest a role of this amino acid in the pathogenesis of aortic disease as well.

Previous studies have evaluated levels of circulating inflammatory markers in different atherosclerotic syndromes. Recent data from the Physicians’ Health Study, for example, indicate that baseline levels of CRP predict future risk of developing myocardial infarction, stroke, and symptomatic peripheral arterial disease. Levels of IL-6 and SAA are increased among patients with unstable angina and identify patients with a worse short-term prognosis. In addition, Szekeanecz et al demonstrated that tissue culture supernatants from human atherosclerotic AAAs produce significantly more IL-6 and interferon-\( \gamma \) than supernatants from normal aortic tissue. In a similar manner, Juvonen et al recently demonstrated that levels of circulating IL-6, IL-2, and tumor necrosis factor-\( \alpha \) are elevated in patients with AAA, compared with controls, although a substantial overlap in individual levels was observed between groups. IL-6 may derive from infiltrative white blood cells or from intrinsic vascular wall cells, such as smooth muscle cells. There is a growing body of evidence that suggests that cytokine-induced tissue inflammation plays an important role in the pathogenesis and progression of AAA. For example, a range of inflammatory cytokines including tumor necrosis factor-\( \alpha \) and IL-6 can upregulate matrix metalloproteinases by macrophages. Such enzymes have been shown to be present in human aortic aneurysm walls, and can degrade specific components of the extracellular matrix. McMillan et al explored the relation between extracellular remodeling with aortic diameter and elegantly demonstrated that messenger RNA transcript levels of type IV collagenase differ according to abdominal aortic dimension. Recently, Allaire et al demonstrated that decreased levels of matrix metalloproteinases induced by overexpression of plasminogen activator inhibitor-1 can prevent aneurysm development and rupture. Our data thus extend previous findings, as they address the hypothesis that elevated inflammatory markers correlate with aortic diameter in a cohort of stable outpatients without fully developed aortic dilatation. Furthermore, no other clinical characteristic or serum measurement, except for total homocysteine levels, was associated with abdominal aortic dimension in this sample. The positive association between plasma concentration of total homocysteine with abdominal aortic diameter concurs with a growing body of evidence that suggests that...
levels of total homocysteine are associated with increased risk of arterial occlusions and correlate with the extent of atherosclerotic disease.\textsuperscript{22} In this regard, Malinow et al\textsuperscript{23} demonstrated an increased prevalence of hyperhomocysteinemia among patients with peripheral arterial occlusive disease, compared with elderly controls. Recently, Robinson et al\textsuperscript{24} have also demonstrated that elevated homocysteine concentrations were associated with an increased risk of vascular disease independent of several traditional cardiovascular risk factors. Further, case reports suggest an association between hyperhomocysteinemia and multiple aneurysms.\textsuperscript{25} The mechanisms by which homocysteine could cause vascular damage and ultimately reflect vascular dimensions are unclear. Several in vitro studies suggest that the presence of reduced forms of homocysteine may induce endothelial injury,\textsuperscript{26,27} inhibit endothelial anticoagulant mechanisms,\textsuperscript{28} and modify composition of LDLS.\textsuperscript{29}

The reasons for the lack of association between CRP levels and aortic size are unclear, as the clinical information provided by CRP and SAA are expected to be similar.\textsuperscript{1} In this cohort, CRP may be insufficiently specific to uncover association between initial inflammatory processes and vascular remodeling. Differences in clearance of CRP and SAA could partially explain these differences.\textsuperscript{30} In addition, we cannot exclude that the associations were not detected because of the small sample size.

Some limitations of our study merit consideration. Greater aortic luminal dimension may not necessarily indicate future dilatation. However, ultrasound characteristics that predict progression of atherosclerotic lesions and vascular dilatation have not been established. Indeed, few prospective studies have evaluated independent risk factors for AAA.\textsuperscript{31} The coexistence of larger luminal dimensions and extraaortic atherosclerosis may have confounded our findings. This possibility seems unlikely because multivariate analysis showed that traditional risk factors and other cardiac comorbidities did not substantially affect the observed associations. Our sample represents a selected population of patients with or at risk for cardiovascular disease. We cannot ensure that mild or subclinical viral or bacterial infection may have increased circulating levels of IL-6 in this cohort of stable outpatients, although every attempt was made to avoid this scenario. Even so, we believe that this would probably not affect the correlations described in these data, and if so, would most likely reduce the associations between inflammatory markers and aortic dimensions.

In summary, our data indicate that IL-6, SAA, and total homocysteine levels are associated with abdominal aortic dimension even in subjects without aortic dilatation. Although the relation between atherosclerosis and AAA is debatable, our findings suggest that some of the inflammatory markers commonly associated with atherothrombotic syndromes\textsuperscript{1-8} may also be involved in early phases of aneurysmal disease. Further studies are necessary to address whether increased circulating levels of these markers can predict progression of dilatation and may identify subgroups of patients in whom more careful follow-up should be planned.

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