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Relationship of Interleukin-6 and C-Reactive Protein to the Prothrombotic State in Chronic Atrial Fibrillation

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OBJECTIVES We sought to test the hypothesis that there is a relationship between inflammation and the prothrombotic state in atrial fibrillation (AF).

BACKGROUND Atrial fibrillation is associated with a prothrombotic or hypercoagulable state, which may contribute to an increased risk of stroke and thromboembolism. Inflammation may be involved in the pathogenesis of AF, but the role of inflammation in the pathophysiology of the prothrombotic state of AF has not been studied in detail, despite evidence of a link between inflammation and arterial atherothrombotic disorders.

METHODS We measured plasma indexes of inflammation (C-reactive protein [CRP] and interleukin-6 [IL-6]) and the prothrombotic state, including markers of platelet activation (soluble P-selectin), endothelial damage/dysfunction (von Willebrand factor), the coagulation cascade (tissue factor [TF], fibrinogen), and indexes of blood rheology (plasma viscosity, plasma fibrinogen, and hematocrit) in 106 patients with chronic AF and 41 healthy control subjects included in a cross-sectional analysis.

RESULTS Compared with controls, AF patients had higher levels of IL-6 ($p = 0.034$), CRP ($p = 0.003$), TF ($p = 0.019$), and plasma viscosity ($p = 0.045$). Plasma IL-6 levels were higher among AF patients at "high" risk of stroke ($p = 0.003$). After adjusting for potential confounding clinical variables (e.g., vascular disease), AF remained significantly associated with a raised logarithmic transformation (log) of TF ($p = 0.04$), but the relationships between AF and log IL-6, log CRP, and plasma viscosity became nonsignificant. Among AF patients, log TF ($p < 0.001$) and high stroke risk ($p = 0.003$) were independent associates of log IL-6 (adjusted $r^2 = 0.443$), whereas log fibrinogen ($p < 0.001$) and plasma viscosity ($p = 0.04$) were independent associates of log CRP (adjusted $r^2 = 0.259$).

CONCLUSIONS Increased plasma IL-6, CRP, and plasma viscosity support the case for the existence of an inflammatory state among "typical" populations with chronic AF. These indexes of inflammation are related to indexes of the prothrombotic state and may be related to the clinical variables of the patients (underlying vascular disease and co-morbidities), rather than simply to the presence of AF itself. (J Am Coll Cardiol 2004;43:2075–82) © 2004 by the American College of Cardiology Foundation

Atrial fibrillation (AF) is a major cause of morbidity and mortality from stroke and thromboembolism (1), usually due to embolization of thrombus formed within the fibrillating left atrium and its appendage (2). Atrial fibrillation is associated with a prothrombotic or hypercoagulable state (3), with evidence of abnormal hemostasis, endothelial damage/dysfunction, and platelet activation (4–24). Furthermore, certain indexes of the prothrombotic state have been associated with an increased risk of left atrial appendage thrombus (25) and spontaneous echo contrast (26), suggesting an increased risk of thromboembolism (27), and may even predict future stroke and cardiovascular morbidity/mortality in those with AF (28). The high levels of these markers in AF may be due to AF itself (6) or the presence of additional cardiovascular co-morbidities (29,30), but the pathophysiologic mechanisms underlying the prothrombotic state in AF remain poorly understood.

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Inflammatory mechanisms, including C-reactive protein (CRP) and interleukin-6 (IL-6), are suspected to play a role in arterial thrombogenesis (for example, in myocardial infarction [31–33]), but their association with the prothrombotic state of AF has not been studied in detail, despite reports of increased CRP levels in AF (34). To test the hypothesis that inflammation might be associated with the prothrombotic state of AF, we measured plasma indexes of inflammation (CRP and IL-6), platelet activation (soluble P-selectin [sP-sel]), endothelial damage/dysfunction (von Willebrand factor [vWF]), the coagulation cascade (tissue factor [TF], fibrinogen), and indexes of blood rheology (plasma viscosity, plasma fibrinogen, and hematocrit) in 106 patients with chronic AF and 41 healthy control subjects included in a cross-sectional analysis.

METHODS

Patients. We studied 106 outpatients (67 men; mean [±SD] age 69 ± 10 years) with chronic, permanent nonvalvular AF who were attending our specialist AF clinic. Chronic AF was confirmed by electrocardiography on at least two separate occasions (≥6 weeks apart). We excluded

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Abbreviations and Acronyms

AF = atrial fibrillation
CRP = C-reactive protein
IL-6 = interleukin-6
sP-sel = soluble P-selectin
TF = tissue factor
vWF = von Willebrand factor

any patients with hematologic, renal, or hepatic impairment; inflammatory, neoplastic disorders; and those who had a recent (<3 months) myocardial infarction or stroke, or acute AF precipitated by thyrotoxicosis or any acute infection. Patients who consumed regular nonsteroidal anti-inflammatory drugs, corticosteroids, or hormone replacement therapy were also excluded, as were post-cardioversion patients. A "high" and "low to moderate" risk of stroke was defined according to prospectively validated risk stratification criteria (35). "Lone" AF was defined as AF in the absence of risk factors, with a normal electrocardiogram (apart from the AF), chest X-ray, and echocardiography. Blood results in patients with AF were compared with 41 age- and gender-comparable healthy control subjects (25 men; mean [±SD] age 67 ± 10 years), which consisted of normal subjects recruited from healthy hospital staff, relatives of the patients, and those attending the hospital for routine senile cataract surgery. Control subjects were normotensive and in sinus rhythm, with no clinical evidence of disease, on careful history, examination, and routine laboratory tests. Written, informed consent was obtained from all participants in the study, and ethical approval for the study was obtained from the West Birmingham Research and Ethics Committee.

Blood sampling and laboratory analysis. Blood samples were drawn atraumatically and without stasis into EDTA and trisodium citrate (0.011 mol/l) tubes. The citrated plasma was stored at −70°C until batch analyses. Platelet-poor plasma fractions were obtained by centrifugation at 4°C for 20 min at 3,000 rpm within 1 h of collection. Aliquots were stored at −70°C to allow batch analysis. Measurements of IL-6, sP-sel, vWF, and TF were performed using ELISA with reagents from R&D Systems (Abingdon, United Kingdom) (for IL-6, sP-sel, and TF) and Dako-Patts (Ely, United Kingdom) (for vWF). Intra-assay coefficients of variation for all ELISAs were <5%; interassay variances were <10%. Plasma CRP was measured by a high-sensitivity latex particle turbidimetric assay (Wako, Neuss, Germany). The lower limit of sensitivity of this method was 0.01 mg/dl, and the intra- and interassay coefficients of variation were 2.2% and 5.0%, respectively. Fibrinogen was measured using a modified Clauss technique, using a coagulometer and thrombin from Pacific Haemostasis (Huntersville, North Carolina).

The EDTA samples were processed within 1 h of collection. Hematocrit was measured using an automated blood count machine (Advia 120, Bayer Diagnostics, Newbury, United Kingdom). Plasma viscosity was measured using a Coulter viscometer II (Coulter Electronics Ltd., Luton, United Kingdom). None of the blood samples from the participants included in this study have been previously used for research studies from our department.

Power calculations and statistical analysis. We hypothesized that patients with AF would have levels of TF approximately 0.4 of a standard deviation higher than those of subjects in sinus rhythm (5). To achieve this with a 1-beta power of 0.80 and $p < 0.05$, 40 subjects per group are required. In view of our measurement of multiple indexes and to minimize the risk of a type II error, we recruited far in excess of this number of AF patients.

Continuous data are expressed as the mean value ± SD or median value (interquartile range). Differences between patients and controls were evaluated using the two-sample *t* test, Mann-Whitney *U* test, and chi-square test, as appropriate. Linear regression analysis was performed for each marker associated with AF on *univariate* analysis (nonparametrically distributed variables examined after logarithmic transformation [log]) to adjust for possible confounding by clinical variables associated with AF. Correlations were evaluated using Spearman's rank correlation method, in view of the skewed distributions of CRP, IL-6, fibrinogen, sP-sel, and TF. Stepwise linear regression analyses were then performed (nonparametrically distributed variables examined after log transformation), entering all variables significantly associated with the marker on univariate comparison, to identify independent associates of each marker in AF. A value of $p < 0.05$ was considered as statistically significant.

RESULTS

As expected, there were significant differences between patients and controls in terms of therapy and additional cardiovascular co-morbidities commonly found among AF patients (Table 1). Of the AF patients, 55 (52%) were at high risk of future stroke, with the remaining 51 (48%) at low to moderate risk. Only 28 (26%) of the 106 patients with AF could be classified as lone AF.

Compared with controls, AF patients had higher levels of IL-6 ($p = 0.034$), CRP ($p = 0.003$), TF ($p = 0.019$), and plasma viscosity ($p = 0.045$) (Table 1). After adjusting for potential confounding clinical variables, AF remained significantly associated with raised log TF ($p = 0.04$), but the relationships between AF and log CRP, log IL-6, and plasma viscosity became nonsignificant (Table 2). There were no significant relationships between antithrombotic therapy use (aspirin, warfarin) and our research indexes on univariate analysis (all $p > 0.1$, data not shown).

Lone AF patients versus controls. In addition to the expected differences in therapy, systolic and diastolic blood pressure was lower among lone AF patients than among controls. Plasma TF was higher among lone AF patients ($p = 0.047$), but no other statistically significant relationships were seen (all $p > 0.05$) (Table 3).

Table 1. Clinical Characteristics and Plasma Markers of Inflammation and the Prothrombotic State in Patients With Atrial Fibrillation and Healthy Control Subjects

	AF Patients (n = 106)	Healthy Controls (n = 41)	p Value
Age (yrs)	69 ± 10	67 ± 10	0.28
Male	67 (63%)	25 (61%)	0.80
Hypertension	56 (53%)	0	<0.001
Heart failure	23 (22%)	0	0.001
Diabetes	16 (15%)	0	0.008
Previous stroke	18 (17%)	0	0.005
Ischemic heart disease	29 (27%)	0	<0.001
Cigarette smokers	23 (22%)	4 (10%)	0.10
Systolic blood pressure (mm Hg)	138 ± 19	138 ± 13	0.91
Diastolic blood pressure (mm Hg)	81 ± 11	81 ± 7	0.70
Cholesterol (mmol/l)	5.2 ± 1.1	5.5 ± 0.9	0.25
Antithrombotic therapy			
None	15 (14%)	41 (100%)	<0.001
Aspirin	12 (11%)	0	
Warfarin	79 (75%)	0	
Hematocrit (%)	43 ± 5	43 ± 5	0.43
Plasma viscosity (mPa)	1.73 ± 0.13	1.68 ± 0.10	0.045
von Willebrand factor (IU/dl)	132 ± 26	125 ± 21	0.14
Fibrinogen (g/l)	2.57 (2.25–3.28)	2.68 (2.19–3.34)	0.61
Tissue factor (pg/ml)	115 (85–176)	95 (75–120)	0.019
Soluble P-selectin (ng/ml)	54 (41–65)	50 (38–65)	0.70
Interleukin-6 (pg/ml)	24 (3–77)	3 (3–62)	0.034
C-reactive protein (mg/dl)	0.27 (0.11–0.50)	0.13 (0.07–0.24)	0.003

Data are expressed as the mean value ± SD, median value (interquartile range), or number (%) of subjects.
AF = atrial fibrillation.

High- versus low to moderate risk in AF patients. By definition, high-risk AF patients were older and less likely to be male than those at low to moderate risk, and this high-risk group included all patients with a previous stroke or heart failure (Table 4). These high-risk patients had higher plasma IL-6 levels ($p = 0.003$) (Fig. 1), but lower hematocrit ($p = 0.006$), than those at low to moderate risk (Table 4).

Correlations and multivariate analyses among AF patients. Log TF ($p < 0.001$) and high stroke risk ($p = 0.003$) were independent associates of log IL-6 (adjusted $r^2 = 0.443$). The correlation between IL-6 and TF is illustrated in Figure 2. Log fibrinogen ($p < 0.001$) and plasma viscosity ($p = 0.04$) were independent associates of log CRP (adjusted $r^2 = 0.259$). The correlations between CRP and fibrinogen and vWF are shown in Figure 3, although vWF was not an *independent* associate of CRP. We found no significant correlations between plasma levels of IL-6 and CRP among either AF patients ($r = 0.125$, $p = 0.107$) or controls ($r = 0.246$, $p = 0.065$).

Log IL-6 ($p < 0.001$) and hematocrit ($p = 0.014$) were independent associates of log TF (adjusted $r^2 = 0.385$). Plasma viscosity ($p = 0.002$) was the only independent associate of vWF (adjusted $r^2 = 0.135$). Log CRP ($p < 0.001$) was the only independent associate of log fibrinogen (adjusted $r^2 = 0.223$). Log TF ($p < 0.001$), vWF ($p = 0.038$), and plasma viscosity ($p = 0.025$) were independent associates of log sP-sel (adjusted $r^2 = 0.279$). Heart failure ($p = 0.038$), log CRP ($p = 0.022$), and vWF ($p = 0.009$) were independent associates of plasma viscosity (adjusted $r^2 = 0.240$). Current smoking ($p = 0.012$) and high risk ($p = 0.004$) were independent associates of hematocrit (adjusted $r^2 = 0.126$).

All the independent associations previously listed were positive associations, except for the relationships between hematocrit and TF and hematocrit and high risk, which were inverse associations. Other correlations were not statistically significant (data not shown).

DISCUSSION

We found increased plasma levels of IL-6 and CRP and raised plasma viscosity among AF patients compared with a matched healthy control population, suggesting the presence of inflammation among our cases, although the rela-

Table 2. Effect of Atrial Fibrillation on Levels of Plasma CRP, IL-6, TF and Plasma Viscosity: Linear Regression Analyses

	Expected Change in Log CRP (μg/dl)	Expected Change in Log IL-6 (pg/ml)	Expected Change in Log TF (pg/ml)	Expected Change in Plasma Viscosity (mPa)
Atrial fibrillation	0.23 (0.04–0.41), p = 0.02	0.30 (0.03–0.57), p = 0.03	0.12 (0.02–0.21), p = 0.02	0.05 (0.00–0.10), p < 0.05
Atrial fibrillation, adjusted*	0.15 (−0.22–0.39), p = 0.57	0.41 (−0.02–0.83), p = 0.06	0.17 (0.01–0.33), p = 0.04	0.00 (−0.07–0.08), p = 0.84

*Adjusted for hypertension, diabetes mellitus, previous stroke, heart failure, ischemic heart disease, and antithrombotic therapy use.
Log CRP = logarithmic transformation of plasma C-reactive protein (CRP); Log IL-6 = logarithmic transformation of plasma interleukin-6 (IL-6); Log TF = logarithmic transformation of tissue factor (TF).

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Table 3. Clinical Characteristics and Plasma Markers of Inflammation and the Prothrombotic State: Lone Atrial Fibrillation Patients Versus Healthy Control Subjects in Sinus Rhythm

	Lone AF Patients (n = 28)	Healthy Controls (n = 41)	p Value
Age (yrs)	68 ± 13	67 ± 10	0.827
Male	16 (57%)	25 (61%)	0.750
Cigarette smokers	6 (21%)	4 (10%)	0.190
Systolic blood pressure (mm Hg)	129 ± 15	138 ± 13	0.011
Diastolic blood pressure (mm Hg)	76 ± 8	81 ± 7	0.011
Antithrombotic therapy			
None	5 (18%)	41 (100%)	<0.001
Aspirin	5 (18%)	0	
Warfarin	18 (64%)	0	
Hematocrit (%)	44 ± 4	43 ± 5	0.817
Plasma viscosity (mPa)	1.73 ± 0.12	1.68 ± 0.10	0.121
Cholesterol (mmol/l)	5.8 ± 1.0	5.5 ± 0.9	0.259
von Willebrand factor (IU/dl)	132 ± 24	125 ± 21	0.212
Fibrinogen (g/l)	2.60 (2.18–3.50)	2.68 (2.19–3.34)	0.953
Soluble P-selectin (ng/ml)	59 (40–73)	50 (38–65)	0.465
Tissue factor (pg/ml)	108 (91–140)	95 (75–120)	0.047
Interleukin-6 (pg/ml)	18 (3–66)	3 (3–62)	0.157
C-reactive protein (mg/dl)	0.28 (0.08–0.45)	0.13 (0.07–0.24)	0.056

Data are expressed as the mean value ± SD, median value (interquartile range), or number (%) of subjects.
AF = atrial fibrillation.

tionships between inflammatory markers and AF became nonsignificant after adjusting for potential confounding clinical variables. However, plasma IL-6 levels were significantly higher among AF patients at high risk of stroke. No correlations were found between plasma levels of IL-6 and CRP among AF patients, but correlations were found between both of these inflammatory markers and certain prothrombotic plasma markers. Notably, we found raised plasma levels of TF in AF even after adjustment for confounders and among lone AF patients, and TF levels strongly correlated with IL-6 levels among patients.

Intravascular thrombogenesis is responsible for much of the mortality and morbidity of cardiovascular disease, but the mechanisms behind thrombogenesis remain poorly un-

Table 4. Clinical Characteristics and Plasma Markers of Inflammation and the Prothrombotic State in Atrial Fibrillation: Relationship to Stroke Risk

	High Risk (n = 55)	Low to Moderate Risk (n = 51)	p Value
Age (yrs)	71 ± 10	67 ± 10	0.042
Male	27 (49%)	40 (78%)	0.002
Hypertension	32 (58%)	24 (47%)	0.252
Heart failure	23 (42%)	0	<0.001
Diabetes	7 (13%)	9 (18%)	0.480
Previous stroke	18 (33%)	0	<0.001
Ischemic heart disease	17 (31%)	12 (24%)	0.394
Cigarette smokers	13 (24%)	10 (20%)	0.615
Systolic blood pressure (mm Hg)	141 ± 21	134 ± 17	0.100
Diastolic blood pressure (mm Hg)	80 ± 12	82 ± 11	0.469
Antithrombotic therapy			0.794
None	9 (16%)	6 (12%)	
Aspirin	6 (11%)	6 (12%)	
Warfarin	40 (73%)	39 (76%)	
Cholesterol (mmol/l)	5.2 ± 1.2	5.3 ± 1.1	0.584
Hematocrit (%)	41 ± 5	44 ± 4	0.006
Plasma viscosity (mPa)	1.75 ± 0.12	1.71 ± 0.13	0.250
von Willebrand factor (IU/dl)	136 ± 25	127 ± 26	0.066
Fibrinogen (g/l)	2.69 (2.32–3.26)	2.53 (2.22–3.34)	0.447
Tissue factor (pg/ml)	135 (90–210)	100 (85–135)	0.105
Soluble P-selectin (ng/ml)	55 (45–65)	53 (36–68)	0.436
Interleukin-6 (pg/ml)	37 (14–112)	7 (3–51)	0.003
C-reactive protein (mg/dl)	0.29 (0.10–0.56)	0.25 (0.10–0.43)	0.326

Data are expressed as the mean value ± SD, median value (interquartile range), or number (%) of subjects.

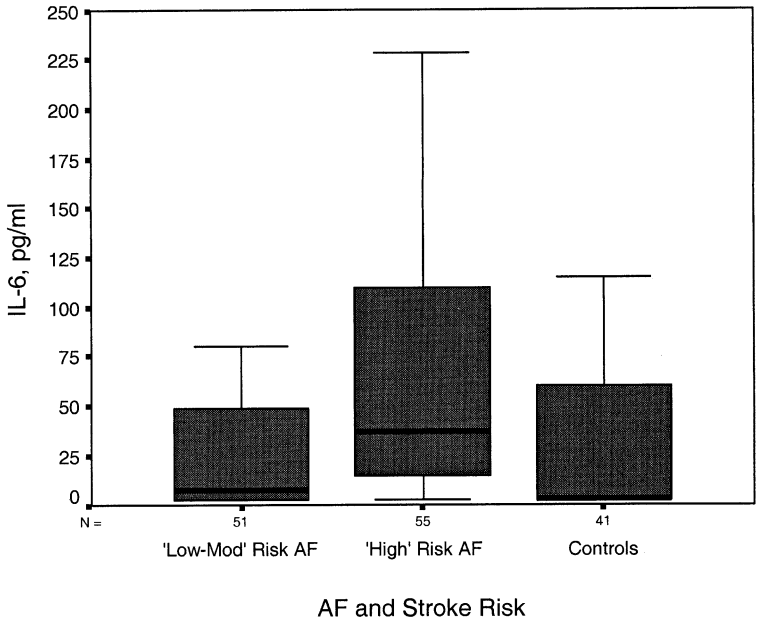

Figure 1. Relationship between plasma interleukin-6 (IL-6) and stroke risk stratification in atrial fibrillation (AF).

derstood. Recently, increasing attention has focused on possible links between inflammatory and thrombotic processes, particularly in the setting of coronary atherothrombotic occlusion (31–33). Despite evidence that both IL-6 and CRP may predict such events (33), there have been few studies examining the possible role of inflammatory mediators in the prothrombotic state of AF.

The hypothesis that inflammation may play a role in the pathogenesis of AF was initially suggested by the temporal relationship between elevation in circulating IL-6 and CRP after cardiac surgery and the onset of postoperative arrhythmias (36). Inflammatory infiltrates, necrosis, and fibrosis have also been demonstrated in atrial tissue from patients with lone AF (37), although intra-atrial inflammatory

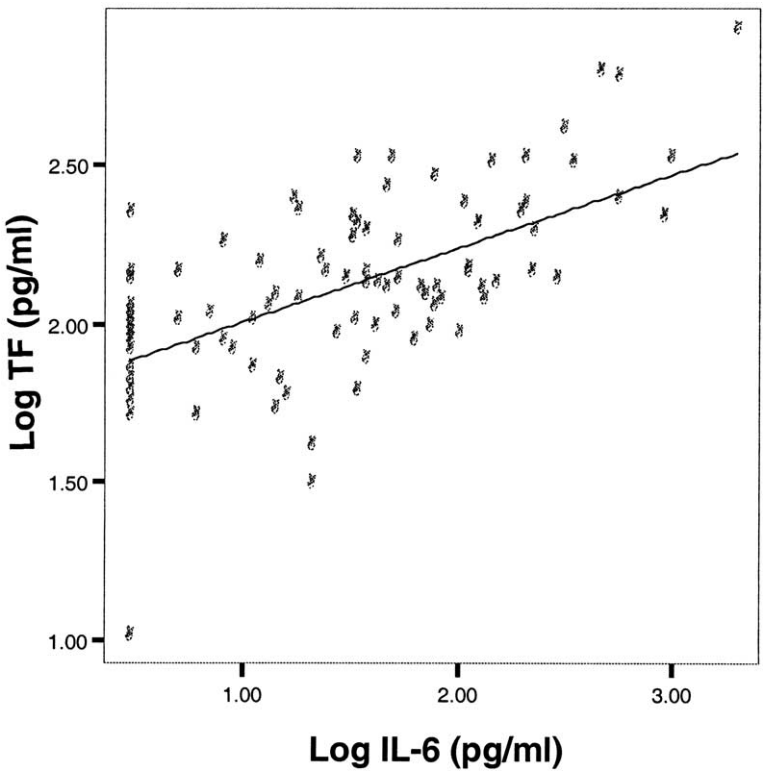

Figure 2. Correlation between plasma levels of interleukin-6 (IL-6) and TF in atrial fibrillation (AF) patients. Log IL-6 = logarithmic transformation of plasma IL-6; Log TF = logarithmic transformation of tissue factor.

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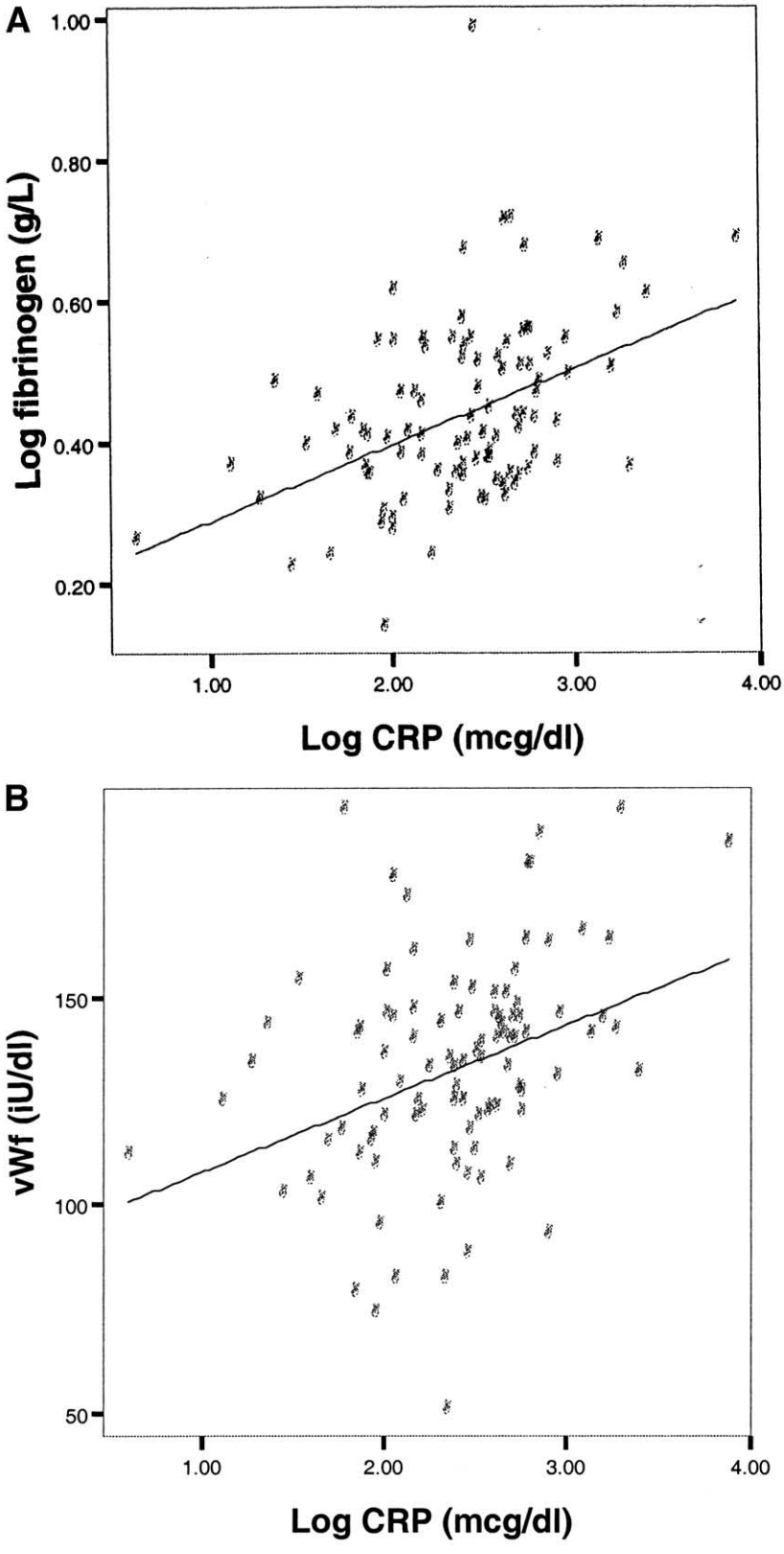

Figure 3. **(A)** Correlation between plasma levels of C-reactive protein (CRP) and fibrinogen in atrial fibrillation (AF) patients. Spearman correlation coefficient: $r = 0.415$, $p < 0.001$. **(B)** Correlation between plasma levels of CRP and von Willebrand factor (vWF) in AF patients. Spearman correlation coefficient: $r = 0.380$, $p < 0.001$. Log CRP = logarithmic transformation of plasma CRP. Log fibrinogen = logarithmic transformation of fibrinogen.

cytokines are not apparent in chronic AF complicating structural heart disease (38). Raised circulating inflammatory indexes in AF may therefore not necessarily reflect *local* molecular changes in the atrial wall, but still might have implications for a prothrombotic state. In support of an inflammatory hypothesis, two studies have found raised circulating levels of CRP in AF (34,39), leading the authors of the larger study to suggest that CRP might be important in the prothrombotic state in AF via stimulation of TF production from monocytes (34,40). However, raised levels of IL-6 and CRP in AF may simply be secondary to underlying atherosclerosis, as the relationships between the inflammatory markers and AF in the present study became nonsignificant after adjusting for potential confounding clinical variables (mostly classic atherosclerosis risk factors and/or associates).

We were surprised to find no significant correlation between IL-6 and CRP among our AF patients, despite raised levels of both markers. Although factors such as multiple drug therapy might be partly responsible for suppression of the relationship between these markers in the AF group, we also found no such correlation among the control group. To validate our assay results, we had concurrently analyzed plasma samples from patients admitted to our hospital with acute sepsis/infection, among whom we found the anticipated (strong) correlation between IL-6 and CRP ($r = 0.760$, $p < 0.001$; data not shown). Therefore, it is possible that while IL-6 may be involved in an increase in CRP in *acute inflammation*, it may play a lesser role in determining CRP levels in *low-grade, chronic inflammatory states* (such as our AF cohort) and in the "normal" population, with perhaps a greater influence of other factors/cytokines on CRP in these situations.

Atrial fibrillation has previously been shown to be associated with evidence of a prothrombotic state (3). Certain of these markers have also been demonstrated to predict the presence of left atrial thrombus (25) or spontaneous echo contrast (26), or to predict future cardiovascular mortality/morbidity (including stroke) in AF (28). In our current study, TF was the only prothrombotic marker significantly raised in AF patients. Tissue factor is responsible for the initiation of the coagulation cascade, via activation of factor VII to factor VIIa, eventually leading to formation of a fibrin-rich thrombus. Furthermore, TF is expressed by both the vascular endothelium and circulating monocytes, although the primary source of TF in the plasma is unclear (41). In an earlier study (5), we found that raised plasma levels of TF among AF patients strongly correlated with plasma levels of vascular endothelial growth factor, perhaps supporting an endothelial origin of TF, whereas the present study finding of a strong correlation with the inflammatory cytokine IL-6 may support cytokine-driven TF production from monocytes (42). However, despite the hypothesis by Chung et al. (34), no relationship was seen between CRP and TF in this study, although CRP did significantly correlate with fibrinogen and plasma viscosity (plus vWF and sP-sel on univariate analysis; data not shown), supporting a relationship between CRP and the prothrombotic state in AF.

Study limitations. Our study is limited by the cross-sectional design, and we are thus unable to examine temporal relationships between inflammatory and prothrombotic indexes, or the effect of these indexes on clinical outcome. Furthermore, the individuals in our study had established chronic AF, and alteration of antithrombotic therapy for the purposes of the study or the introduction of warfarin for several weeks among healthy controls would not have been ethical. However, we have attempted to minimize the risk of confounding by adjustment for antithrombotic therapy in the case-control comparison, and no significant relationships to our research indexes were found. Indeed, antithrombotic therapy in AF patients has previously been shown to have no significant effect on plasma levels of IL-6 (43), fibrinogen (14), vWF, or sP-sel (14,29), whereas evidence for the effects of antithrombotic therapy on other inflammatory and prothrombotic indexes remains inconclusive (44–47). Another limitation of our study is that the design does not allow us to establish or refute an *independent* effect of AF itself or of associated vascular disease on our markers, although this was not the primary aim of our study. A study of a much larger AF cohort with relatively even proportions of each risk factor, plus a second control group in sinus rhythm but with risk factors, would be needed to conclusively determine the independent contribution of AF.

Accepting these limitations, our finding of elevated plasma levels of CRP, IL-6, and TF among a typical population of AF patients, with significant correlations between inflammatory and prothrombotic indexes, is consistent with a potential role for inflammation in the prothrombotic state of AF. However, the inflammatory state in AF may be related to the clinical variables of the patients (e.g., underlying vascular disease and co-morbidities), rather than simply to the presence of AF itself. Indeed, cytokines other than IL-6 may influence the inflammatory state in AF and deserve investigation.

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