Arterial Stiffness Is Related to Systemic Inflammation in Essential Hypertension

Azra Mahmud, John Feely

Abstract—The acute phase–reactant high-sensitivity C-reactive protein, a marker of vascular inflammation and an atherosclerotic risk factor, is related to arterial stiffness in healthy subjects and in systemic vasculitis. To explore the relationship between markers of inflammation, interleukin-6 (IL-6), tumor necrosis factor–α (TNF–α), and high-sensitivity C-reactive protein with arterial stiffness, we studied untreated patients (n=78; 56% male; 47±1 years of age; mean±SEM) with essential hypertension. After overnight fast, augmentation index and pulse wave velocity were assessed noninvasively and related to plasma levels of inflammatory markers measured by ELISA. Pulse wave velocity was significantly related to plasma high-sensitivity C-reactive protein (r=0.31; P<0.001), TNF–α, (r=0.30; P<0.001) and IL-6 (r=0.21; P<0.05). There was also a relationship between heart rate–corrected augmentation index to high-sensitivity C-reactive protein (r=0.37; P<0.001), IL-6 (r=0.24; P<0.05), and TNF–α (r=0.19, P=0.06). High-sensitivity C-reactive protein was an independent predictor of pulse wave velocity and augmentation index in a multiple stepwise regression model. High-sensitivity C-reactive protein, a marker of systemic inflammation, is independently related to pulse wave velocity, a marker of aortic stiffness, and augmentation index, a manifestation of wave reflection, in essential hypertension. (Hypertension. 2005;46:1118-1122.)

Key Words: hypertension, arterial A arteries A atherosclerosis A elasticity A endothelium

Aortic stiffness, measured by carotid femoral pulse wave velocity (PWV) has been shown to be a strong independent predictor of cardiovascular morbidity in hypertension, type II diabetes, and of all-cause mortality in patients with hypertension and end-stage renal disease. The augmentation index (AIx), a composite of PWV, arterial wave reflection, and left ventricular ejection, is also an independent factor associated with poor survival in end-stage renal disease and the extent of angiographic coronary artery disease (CAD) in men <60 years of age.

In addition to these functional hemodynamics measures, biochemical markers, particularly of vascular inflammation such as high-sensitivity C-reactive protein (hs-CRP), have been shown to be predictive of cardiovascular events. In a cross-sectional study, hs-CRP levels were found to be elevated in subjects with hypertension, and increased hs-CRP levels in normotensive subjects also predicted the future development of hypertension. In the hypertensive population, hs-CRP is an independent predictor of progression of atherosclerosis that is superior to either pulse pressure (PP) or systolic blood pressure (BP). In healthy subjects, changes in arterial structure, assessed by carotid intima-media thickness and presence of plaques, and in arterial stiffness, are not the compounding factors in the relationship between hs-CRP and PP. The primary proinflammatory cytokines TNF–α and interleukin-6 (IL-6) are the main inducers for the hepatic synthesis of hs-CRP. A recent study found that hs-CRP and IL-6 are independent predictors of increased risk of CAD in men and women, whereas tumor necrosis factor–α (TNF–α) predicted risk only in women. IL-6 and TNF–α are also independent risk factors for high BP in apparently healthy subjects.

Although there are some inconsistencies, a number of recent studies have suggested that in a healthy population, there may be a significant relationship between hs-CRP and measures of arterial stiffness. Yasmin et al found hs-CRP to be related to PWV but not to AIx. In contrast, Kampus et al found hs-CRP to be independently and significantly associated with AIx but not with the timing of the reflected pressure wave (TR). However, in inflammatory conditions, the relationship may be stronger. In patients with systemic vasculitis, in which hs-CRP levels are markedly elevated, they were positively correlated with PWV and AIx.

Because there is no current information on the possible contribution of systemic inflammation in patients with essential hypertension to arterial stiffness, we looked at the relationship between hs-CRP, TNF–α and IL-6, and PWV and AIx in a cross-sectional study of patients with essential hypertension.

Methods

Patient Protocol
We recruited 78 never-treated white healthy hypertensive subjects with diagnosis based on 3 outpatient measures of BP >140/
90 mm Hg and confirmed by ambulatory BP (135/80 mm Hg) monitoring. The mean (±SD) age was 47±1 years, and 56% were male. Patients with a history or clinical evidence of recent infection, malignancies, chronic degenerative diseases, CAD (including a normal ECG), peripheral vascular disease, cerebrovascular disease, endocrine pathologies, and on medications such as anti-inflammatory, vasoactive agents, steroids, or vitamins were excluded. The patients were studied supine, having fasted and abstained from smoking, alcohol, and caffeinated beverages in the 12 hours before the study. The patients gave informed consent, the study had institutional ethics committee permission, and the procedures followed were in accordance with institutional guidelines and the principles of the Declaration of Helsinki.

Measurements
Height, weight, waist, and hip of each patient were recorded. Venous samples were drawn into EDTA tubes, centrifuged (4°C; 2500 rpm) for 15 minutes and supernatant stored at −80°C. The ELISA technique was used to measure levels of hs-CRP (Immundiagnostik Ag). The sensitivity of the assay was 0.05±0.007. The cytokines IL-6 and TNF-α (Quantikine HS; R & D Systems Ltd.) were measured by ELISA in duplicates. The TNF-α and IL-6 assays detected concentrations down to 0.32 and 0.11 pg/mL, respectively. Intra-assay variability was <9% for the 3 assays. In addition, the levels of serum cholesterol, LDL, HDL, triglycerides, and glucose were measured.

Blood Pressure
Brachial BP was measured using an automated oscillometric device (Omron Model HEM 705-CP; Omron Corporation) in the right arm, with patients lying in the supine position by a trained observer. Three BP readings were taken at 1-minute intervals, and the mean was used for data analysis. Peripheral PP was calculated as the difference between brachial systolic and diastolic BP.

PWV Measurement
Carotid-femoral PWV was measured in the supine position using the automatic device (Compilior; Artech Medical) that measured the time delay between the rapid upstroke of the carotid and femoral artery pulse waves. The distance between the 2 arterial points was measured on the surface of the body using a tape measure. PWV was calculated as the distance traveled by the arterial pulse wave (meters) divided by the time delay between the 2 arterial points (seconds), thus expressed as meters per second. The mean of ≥12 successive readings to cover a complete respiratory cycle was used in the analysis.

Pulse Wave Analysis
Applanation tonometry was used to record radial artery pressure waveform continuously, and mean values of ≥2 screens of pulse waves of good quality were used for analysis. On the basis of the collected data, an averaged radial pressure waveform was generated and a corresponding aortic pressure waveform and BP calculated by the validated transfer function (SphygmoCor version 7.1; AtCor Medical). The aortic pressure waveform was used to calculate the AIx (difference in height between the first and second systolic peaks expressed as a percentage of PP). Because heart rate is a major confounder of AIx, the software also generates an AIx, corrected to a heart rate of 75 bpm (AIxN). The T0 was calculated as the time between the foot of the central waveform and the first systolic shoulder (inflection point).

Statistical Analysis
Data were analyzed using JMP version 5.0 (SAS for Windows). Results are expressed as mean±SD for continuous variables and percentages for categorical data, with P<0.05 considered significant. Analysis of normality was performed with the Shapiro–Wilk W test. Non-normally distributed data were logarithmically (Log10) transformed before analysis. The relationship between inflammatory markers, PWV, AIx, and other parameters was analyzed using nonparametric methods (Spearman ρ correlations). To study whether there was an independent relationship between PWV or AIx and the cytokines, stepwise regression analysis was used. In the first model, we took PWV as the dependent variable, and age, mean arterial pressure (MAP), gender, body mass index, waist/hip ratio, heart rate, smoking status, hs-CRP, plasma glucose, and lipoproteins as independent variables. In the second model, with the same independent variables, AIx was analyzed as the dependent variable. Results are expressed as mean±SD, with P<0.05 considered significant.

Results
Baseline patient characteristics are given in Table 1. We did not find any gender difference in plasma levels of hs-CRP, IL-6, and TNF-α. Smokers had significantly higher plasma levels of hs-CRP (0.62±0.14 versus 0.26±0.04 mg/L; P<0.01) and IL-6 (2.5±0.6 versus 1.4±0.1 pg/mL; P<0.01) than nonsmokers, although TNF-α levels were not significantly different between the 2 groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Smokers [n (%)]</th>
<th>20 (28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>170±9</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>87±18</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>30±5</td>
<td></td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.91±0.1</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5±1</td>
<td></td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.9±0.1</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.7±1</td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.6±1</td>
<td></td>
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<tr>
<td>Brachial systolic BP</td>
<td>152±20</td>
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<tr>
<td>Brachial diastolic BP</td>
<td>91±10</td>
<td></td>
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<tr>
<td>MAP (mm Hg)</td>
<td>113±14</td>
<td></td>
</tr>
<tr>
<td>Heart rate (min⁻¹)</td>
<td>70±12</td>
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</tr>
<tr>
<td>Aortic systolic BP</td>
<td>140±20</td>
<td></td>
</tr>
<tr>
<td>Aortic diastolic BP</td>
<td>92±11</td>
<td></td>
</tr>
<tr>
<td>Aortic PP (mm Hg)</td>
<td>61±14</td>
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Values are expressed as mean±SD unless otherwise indicated.

Relationship Between PWV and Inflammatory Markers
PWV was significantly and positively related to brachial systolic BP (r=0.64; P<0.0001), age (r=0.59; P<0.0001), brachial diastolic BP (r=0.46; P<0.0001), MAP (r=0.58; P<0.0001), AIx (r=0.32; P<0.0001), AIxN (r=0.47; P<0.0001), and plasma glucose (r=0.25; P<0.05), and negatively to T0 (r=−0.27; P<0.05). There was a significant positive relationship between PWV and log hs-CRP (r=0.31; P<0.001), log TNF-α (r=0.30; P<0.001), and log IL-6 (r=0.21; P<0.05). When corrected for age, MAP, and gender, the relationship between PWV and log hs-CRP (r=0.57; P<0.0001) and log IL-6 (r=0.37; P<0.0001) was

**TABLE 1. Baseline Characteristics of the Patient Population (n=78)**

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<tr>
<td>Age (years)</td>
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<td></td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>43/35</td>
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<tr>
<td>Height (cm)</td>
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<td>Total cholesterol (mmol/L)</td>
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stronger but weaker with log TNF-α \((r=0.20; P<0.05; \text{Figure 1})\). In a stepwise regression model, using PWV as the dependent variable, only age, MAP, and hs-CRP were the independent predictors of PWV \((R^2=0.55; P<0.0001; \text{Table 2})\).

### Relationship Between AIx and Inflammatory Markers

Plasma hs-CRP levels were significantly related to IL-6 \((r=0.26; P<0.01)\), but there was no correlation between hs-CRP and TNF-α. There was a weak correlation between IL-6 and TNF-α \((r=0.20; P=0.06)\).

### Discussion

This is the first study to show in patients with essential hypertension that circulating levels of not only hs-CRP but also the inflammatory cytokines IL-6 and TNF-α are related to PWV and wave reflection. Previous studies in healthy subjects have shown a positive relationship between either PWV or AIx with hs-CRP but not with both.\(^{11,12}\) However, PWV and AIx measure different arterial properties; PWV is a classic marker of aortic stiffness, whereas AIx is far more complex and is composite of the magnitude of arterial wave reflection, PWV, and pattern of left ventricular ejection. Also, the determinants of these indices differ. As shown here, AIx is influenced to a greater extent by the level of heart rate, BP, gender, age, and height, whereas PWV is predominantly determined by age and BP. This stresses the value of using PWV and AIx as complementary measures in assessing arterial stiffness and ventricular–vascular interactions rather than using them as alternatives.
We also noted a positive relationship between heart rate and hs-CRP. Elevated heart rate is associated with a greater risk of developing hypertension and a predictor of cardiovascular morbidity and mortality. Heart rate influences AIx, and Gatzka et al have shown in a large cohort of elderly patients with hypertension that the AIx is confounded by heart rate through its effect on ventricular ejection time rather than any underlying change in time to wave reflection. Therefore, we also studied the relationship between hs-CRP and AIx, a heart rate–corrected estimate of AIx that correlates very strongly with hs-CRP and IL-6. However, hs-CRP was an independent determinant of AIx in the stepwise regression model when adjusted for heart rate among other variables, highlighting the independent role of hs-CRP in mediating changes in AIx.

hs-CRP is a prototype downstream marker of inflammation. Several mechanisms may explain the role of hs-CRP in mediating increased arterial stiffness in hypertension. hs-CRP is associated with insulin resistance and diabetes mellitus, both of which are related to arterial stiffness. However, although plasma glucose levels were significantly related to PWV and aortic PP in our patient group, there was no relationship between glucose, hs-CRP, IL-6, and TNF-α. Furthermore, association between PWV and AIx with hs-CRP was independent of the cardiovascular risk factors in the multiple regression model.

The fundamental determinant of arterial stiffness is the constant elastin and collagen turnover in the extracellular matrix of the vascular wall. Enzymes such as matrix metalloproteinases are key players in the degradation of collagen in the vessel wall, and their activity has been shown to be related to arterial stiffness. Determinants of large artery stiffness in addition to structural arrangements include functional regulators such as transmural pressure and mediators of vascular tone. Acute systemic inflammation is associated with endothelial dysfunction, an early feature of vascular abnormality maintaining endothelial dysfunction and the extent of endothelial dysfunction and CRP concentration may independently predict cardiovascular death. Normalization of CRP levels over time are associated with a significant improvement in endothelium-dependent forearm blood flow responses. Increased arterial stiffness has been noted in the offspring of hypertensive parents before an elevation in BP, raising the possibility that early vascular inflammation and associated reduced NO bioavailability may lead to increased stiffness, which may contribute to the development of hypertension, particularly systolic hypertension.

Whether the vascular inflammation promotes the cycle of arterial stiffening and hypertension or the high BP stiffens the arteries to initiate a cascade that results in vascular inflammation and increased arterial stiffness is not clear from these data. However, because hs-CRP levels may be increased before the onset of hypertension, it is more likely that vascular inflammation contributes to the development of stiffness rather than hypertension producing inflammation. Although we did not have a normotensive group, we found no significant relationship between the extent of BP, either peripheral or central, and the markers of inflammation. Furthermore, although hs-CRP is now considered to be biologically active, contributing to the progression of atherosclerosis and vascular inflammation, we find the relationship also extends to IL-6 and TNF-α.

Levels of TNF-α, a primarily proinflammatory cytokine, and IL-6, a messenger cytokine that leads to systemic enhancement of inflammation including hepatic production of hs-CRP, were all positively correlated with PWV, a classic index of large artery stiffness, and AIx. These represent not only large artery stiffness but, very importantly, the vascular smooth muscle tone in the peripheral medium-sized muscular arteries, emphasizing the systemic nature of the inflammatory response encompassing biochemical and hemodynamic parameters. hs-CRP in its turn also influences vascular vulnerability to the inflammatory response and decreased production of the endogenous vasodilator NO. Inhibition of basal NO synthesis increases aortic AIx, and velocity in vivo. We found a significant relationship between levels of hs-CRP and IL-6 in our study, which may reflect the role of IL-6 in inducing CRP synthesis in the liver. Along with hs-CRP, the other inflammatory marker to independently predict PWV and AIx was TNF-α but not IL-6. Because we found no relationship between hs-CRP and TNF-α, the suggestion is that the latter may also be an independent modulator of arterial mechanics. In the Rotterdam Study, levels of hs-CRP were linearly associated with carotid femoral PWV and predicted progression of atherosclerosis measured at various sites including the aorta, carotid, and lower limb. Not only are hs-CRP and PWV independent predictors of cardiovascular morbidity and mortality, but they may also predict disease progression.

Perspectives

Whether the combined effect of raised hs-CRP and increased arterial stiffness is additive in a hypertensive population is unclear. However, we do believe that raised hs-CRP may be biologically significant. We have already alluded to its role in maintaining endothelial dysfunction. It is considered that angiotensin II may be an important mediator of the inflammatory response and increased arterial stiffness in hypertension. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers reduce AIx and PWV; ACE inhibitors and angiotensin receptor blockers may also reduce hs-CRP. Furthermore, the effect of ACE inhibition after ischemic stroke in reducing cardiovascular risk may in part be mediated by reduced hs-CRP as well as BP lowering. In addition, statins and rosiglitazone reduce hs-CRP and arterial stiffness. Patients who have low hs-CRP after statin therapy have better clinical outcomes than those with higher levels, regardless of resultant LDL cholesterol level. Reduction of hs-CRP may therefore present a new therapeutic approach to reducing the major risk to the hypertensive population associated with increased arterial stiffness.

References
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Hypertension. 2005;46:1118-1122; originally published online October 10, 2005;
doi: 10.1161/01.HYP.0000185463.27209.b0

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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