Serum C-Reactive Protein Level Is Associated With Abdominal Aortic Aneurysm Size and May Be Produced by Aneurysmal Tissue

Tryfon Vainas, MD; Tim Lubbers, MSc; Frank R.M. Stassen, PhD; Selma B. Herngreen, BSc; Marja P. van Dieijen-Visser, MD, PhD; Cathrien A. Bruggeman, PhD; Peter J.E.H.M. Kitslaar, MD, PhD; Geert Willem H. Schurink, MD, PhD

Background—Abdominal aortic aneurysms (AAA) are characterized by extensive transmural inflammation and C-reactive protein (CRP) has emerged as an independent risk factor for the development of cardiovascular disease. Therefore, we evaluated a possible association between serum CRP and aneurysm dimension in patients with asymptomatic AAA. Furthermore, the possibility of CRP production by aneurysmal tissue has been examined.

Methods and Results—Serum CRP was determined highly sensitive (hsCRP) and aneurysmal size was measured in 39 patients with AAA. The presence of CRP mRNA was assessed in the aneurysmal tissue of 16 patients. Mean (SD) hsCRP was 3.23 (2.96) mg/L. After log-transformation, hsCRP correlated significantly with aneurysmal size ($r=0.477$, $P=0.002$). When the patients were divided into 3 equally sized groups according to hsCRP level, aortic diameter increased from lowest to upper hsCRP-tertile (49 mm, 61 mm, and 67 mm, respectively; $P<0.05$ for 3rd versus 1st tertile). This association persisted after correction for risk factors. CRP mRNA was found in 25% of aneurysmal aortic tissues.

Conclusions—This is the first report showing that serum hsCRP is associated with aneurysmal size and that—in at least some patients—CRP may be produced by aneurysmal tissue. These data underscore the inflammatory nature of AAA formation, suggesting that serum hsCRP may serve as a marker of AAA disease and that CRP produced in vascular tissue might contribute to aneurysm formation. (Circulation. 2003;107:1103-1105.)

Key Words: aorta ■ aneurysm ■ inflammation

The degenerative form of the abdominal aortic aneurysm (AAA) is a medial disease characterized by dilatational remodeling with degradation of extracellular matrix components and medial thinning. Inflammation appears to be an important component of aneurysm formation as illustrated by the extensive medial/adventitial inflammatory cell infiltrations. Accordingly, increased expression of proinflammatory cytokines can be found in aneurysmal tissue, and circulating levels of inflammatory cytokines are elevated in patients with AAA. The serum concentration of several cytokines is associated with aneurysm diameter (interleukin-8), AAA symptomatology (tumor necrosis factor-$\alpha$), and increased AAA expansion rate (interferon-$\gamma$).

C-reactive protein (CRP) has recently emerged as a strong independent risk factor for atherosclerosis and atherosclerosis-related complications in apparently healthy individuals and patients with cardiovascular disease. In 1987, Powell and colleagues showed that, among patients undergoing elective aortic reconstruction, serum CRP was elevated in AAA-patients compared with patients with obstructive disease. Although subsequently elevated serum CRP has been reported in patients with symptomatic or ruptured AAA, this early observation of elevated CRP level in patients with asymptomatic AAA has not been verified. Therefore, in the present study, serum CRP was, for the first time, measured highly sensitive in patients with asymptomatic AAA. The association between serum CRP and AAA dimension, and the possibility of local CRP-production by aneurysmal tissue were also explored.

Methods

Patients with AAA (n=39), admitted at the surgical clinic of the University Hospital of Maastricht for vascular reconstruction, were included in this study. Patients with ruptured/symptomatic AAAs, recent infections, active inflammatory disorders, and/or serum CRP>10 mg/L were excluded. The study was approved by the institutional medical ethical committee and all patients gave written informed consent.
Determination of Serum CRP

Venous blood samples drawn on admission were immediately centrifuged for 10 minutes at 1200 rpm and at 4°C. Serum was stored at −20°C until analysis. CRP was determined highly sensitive (hsCRP) with the IMMULITE CRP method (Diagnostic Product Corporation). This assay provides a detection limit of 0.10 mg/L and has been approved by the Food and Drug Administration for clinical use in the United States.

AAA Dimension

Computer assisted tomography was used to visualize the aorta and to determine the maximal aneurysm diameter. Because of logistical reasons, echo-doppler ultrasonography was used to determine aneurysm dimensions in 5 of the 39 patients. hsCRP-level and aneurysm size did not differ between patients who had tomography or ultrasonography (data not shown).

CRP RT-PCR

Aneurysm tissue was snap frozen in liquid nitrogen after explantation and stored at −80°C until analysis. Total RNA from ~0.1 g of each tissue sample (n=16) was isolated according to the manufacturer’s instructions with TRIzol Reagent (Life Technologies). After treatment with DNase I in the presence of RNA guard (both Amersham Pharmacia Biotech), the RNA concentration was determined measuring the optical density at 260 nm. One microliter of serum was reverse transcribed into cDNA with oligo dT (RACE-1, Amersham Pharmacia Biotech), the RNA concentration was determined measuring the optical density at 260 nm. One microliter of RNA was reverse transcribed into cDNA with oligo dT (RACE-1, Amersham Pharmacia Biotech) and Superscript II RNase H⁻ (Invitrogen). For every RNA isolate, a RT-PCR reaction was also performed in the absence of reverse transcriptase to demonstrate the specific amplification of mRNA instead of genomic DNA. For amplification of CRP, the following primer pair (amplifying a 441 bp sequence) was used: forward, 5′-TCGTATGCCACCAAGAGACA-A G A CA-3′; reverse, 5′-AACACTTCGCCTTGCACTTCATACT-3′. Two microliters cDNA were transferred to an amplification mixture containing HotStar Taq DNA polymerase (Qiagen) and amplified for 40 cycles. Amplification products were separated on a 1% ethidium bromide stained agarose gel. Resulting bands were imaged with a FluorChem 8000 analyzer (Alpha Innotech Corporation). To control for the identity of the bands, PCR product was (1) sequenced and (2) treated with the endonuclease ApaI splitting the amplified CRP sequence in 2 fragments of 381 bp and 60 bp, respectively. The cDNA of the housekeeping gene cyclophilin (primer pair: forward, 5′-ATGTTCAACCCACCGTGTCTCG-3′; reverse, 5′-CGTGTGAGTCACCCACCTGACACA-3′) was amplified in parallel in every run to control for RNA integrity.

Statistical Analysis

Pearson’s r was computed to explore the correlation between aneurysm size and log-transformed serum hsCRP. ANOVA was used to further study the univariate association between serum hsCRP and aneurysm dimension and to assess the independent effect of sex, age, smoking, hypertension, dyslipidemia, diabetes, and the extent of vascular disease on aneurysm size. Parameters that associated significantly with aneurysm size in the univariate analyses were entered as potential confounders in a multivariate ANOVA to study the relation between aneurysm size and serum hsCRP.

Results

A total of 39 patients (5 women) aged 71 (±5) years with an atherosclerotic risk profile (hypertension, 74%; diabetes, 15%; dyslipidemia, 74%; smoking, 77%) were included in this study. Seventy-seven percent of the patients had extensive vascular disease affecting at least 2 vascular territories (coronary, cerebral, and/or aortofemoral).

CRP in Aneurysmal Disease

The average (SD), 25th, 50th, and 75th percentile of serum hsCRP was 3.23 (2.96), 1.03, 1.71, and 4.99 mg/L, respectively.

CRP mRNA was detected in 4 of 16 tissue samples (25%), whereas cyclophilin was detected in all samples (Figure).

Aneurysm Dimension and Serum CRP

The average (SD) maximal aneurysmal dimension was 59 (15) mm. Aneurysm size correlated with (log-transformed) serum hsCRP (r=0.477, P<0.002). When patients were divided in 3 equally-sized groups according to hsCRP level (hsCRP<1.13, 1.13≤hsCRP<4.15, and hsCRP≥4.15), aortic diameter increased from lowest to upper tertile (49 mm, 61 mm, and 67 mm, respectively; P<0.05 for 3rd versus 1st tertile; Table). From the risk factors for cardiovascular disease, only sex and diabetes showed a trend toward association with aneurysm size. When these confounders were
entered in a multivariate model, the association between hsCRP and aneurysm size persisted. The corrected (for sex and diabetes) aneurysm diameter (mean [95%-CI], mm) of patients in the upper hsCRP tertile (73 [63 to 83], \( P = 0.003 \)) was significantly elevated, and that of the patients in the middle tertile (60 [51 to 68], \( P = 0.083 \)) tended to be elevated compared with corrected aneurysm size of patients in the lowest hsCRP tertile (49 [40 to 58], Table).

**Discussion**

Moderately elevated serum hsCRP has been reported in patients with stenotic atherosclerotic disease and is associated with an increased risk of developing cardiovascular events.\(^7\)\(^8\) However, scarce information exists about serum hsCRP in patients with aneurysmal disease. Powell et al showed that patients with asymptomatic AAA had increased serum CRP compared with patients with obstructive disease.\(^9\) The high mean serum CRP level (56±10 mg/L) that was reported may reflect the suboptimal performance of the CRP method used compared with modern highly sensitive assays, or may suggest that patients with acute inflammatory conditions were included in that analysis. A recent study showed that patients with symptomatic and ruptured aneurysms had elevated serum CRP compared with patients with asymptomatic AAAs, but failed to verify the elevated serum CRP in asymptomatic AAA.\(^10\) The CRP assay used by these investigators lacked the sensitivity (detection limit = 5 mg/L) to be used in the assessment of CRP in cardiovascular disease.\(^11\) In the present study, for the first time, serum CRP has been measured highly sensitive in patients with asymptomatic AAAs. Even though patients with symptomatic/ruptured AAA, active inflammatory/infectious disorders, or hsCRP >10 mg/L were excluded, the mean serum hsCRP was above the range for supposedly healthy individuals\(^7\) and was elevated, compared with serum hsCRP of a healthy population measured in our laboratory.\(^12\)

Intriguingly, serum hsCRP of asymptomatic AAA patients showed a strong association with aneurysm dimension in the present study. Despite the fact that hsCRP has been proven to be an independent risk factor for cardiovascular complications, it remains unclear whether serum hsCRP would be a useful marker for the prediction of aneurysm growth and rupture in patients with AAA.

It is believed that moderately elevated serum hsCRP (<10 mg/L) results from chronic hepatic stimulation. However, it has been shown that CRP is produced in coronary plaques,\(^13\) in Alzheimer’s disease brain tissue,\(^14\) and in myocardial infarcts.\(^15\) In the present study, we were able to show that, in some patients, CRP was produced in aneurysmal tissue as well. These findings corroborate the notion that CRP upregulation is a generalized reaction to several types of tissue injury. Macrophages and smooth muscle cells might be the producers of “vascular” CRP.\(^13\) Quantitative analysis of CRP mRNA and protein in normal and in AAA tissue should give more insight into the up-regulation of CRP production during aneurysm formation.

In conclusion, our data suggest that serum hsCRP is associated with aneurysm size in patients with asymptomatic AAA and that, in some cases, aneurysmal tissue is capable of producing CRP. Future studies should evaluate the usefulness of serum hsCRP as a marker of disease progression and elucidate the role of locally produced CRP in AAA formation.

**References**

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