C-Reactive Protein Is Increased in Patients With Degenerative Aortic Valvular Stenosis

Alberto Galante, MD,*† Antonio Pietroiusti, MD,* Marina Vellini, MD,* Paola Piccolo, MD,* Gianfederico Possati, MD,‡ Michele De Bonis, MD,‡ Rita L. Grillo, BSc,§ Carla Fontana, MD,§| Cartesio Favalli, MD|| Rome and Velletri, Italy

OBJECTIVES The goal of this study was to assess the presence of systemic inflammation in degenerative aortic valvular stenosis.

BACKGROUND Local inflammatory changes, resembling those observed in atherosclerosis, have been recently reported in degenerative aortic valvular stenosis. It is presently unknown whether systemic signs of inflammation, similar to those observed in atherosclerosis, may be present in this disorder.

METHODS C-reactive protein (CRP) was measured by enzyme immunoassay in 141 subjects: 62 with trileaflet degenerative valvular aortic stenosis and 79 volunteers with similar demographic and clinical characteristics. IgG antibodies against Helicobacter pylori (enzyme-linked immunosorbant assay) and Chlamydia pneumoniae (microimmunofluorescence assay) were also measured.

RESULTS C-reactive protein levels (mg/dl, mean ± SD) were 0.848 ± 1.42 in patients and 0.394 ± 0.50 in controls (p = 0.0001, Mann-Whitney U test). Seroprevalence of H. pylori was 68.7% in patients and 79.7% in controls (p = NS), whereas seroprevalence of C. pneumoniae infection was higher in patients than it was in controls (59.7% vs. 33%, p = 0.003; chi-square test). After adjustment for various covariates in multiple logistic regression, the odds ratio for degenerative aortic stenosis was 3.41 for C. pneumoniae infection (95% confidence intervals [CI]: 1.60 to 7.30) and 2.76 for CRP (95% CI: 1.08 to 7.05). There was no significant difference in patients or controls in CRP levels according to the serostatus for C. pneumoniae.

CONCLUSIONS Systemic signs of inflammation, similar to those found in atherosclerosis, are present in patients with degenerative aortic valvular stenosis. They do not seem to be linked to C. pneumoniae or H. pylori infection. (J Am Coll Cardiol 2001;38:1078–82) © 2001 by the American College of Cardiology

It has recently been found that so-called chronic degenerative valvular aortic stenosis is an active process and that histologic valve changes are predominantly inflammatory and, in part, similar to those found in atherosclerotic plaques (1). Furthermore, some classic risk factors for atherosclerosis seem to predispose to valvular aortic stenosis (2,3).

Since it is now firmly established that chronic systemic inflammation may play an important role in the development of atherosclerosis (4), it is possible that this condition may also play a role in the development or progression of degenerative valvular aortic stenosis. This hypothesis has never been explored.

In this report, we compared the levels of C-reactive protein (CRP), the most sensitive marker of systemic inflammation, between patients with chronic degenerative valvular disease and volunteers without this disease.

Furthermore, we evaluated whether Chlamydia pneumoniae and Helicobacter pylori, two microorganisms that are able to induce chronic inflammatory changes (5–13) and have been linked to coronary artery disease (5–7,14–21), may be associated with alterations of CRP in patients with degenerative valvular aortic stenosis.

METHODS The study sample included consecutive patients with degenerative trileaflet valvular aortic stenosis and absence of atherosclerotic lesions admitted to the Department of Cardiac Surgery of the Catholic University of Rome for elective valvular substitution from January 1998 to December 1999. The absence of atherosclerosis was assessed by normal angiography of coronary vessels, by less than 25% stenosis of the carotid tree (Doppler ultrasonography) and by normal physical examination of the lower limb arteries. A diagnosis of degenerative valvular disease was made on the basis of clinical criteria (no history of rheumatic disease) and the echocardiographic finding of thickening and increased echogenicity of the cusps (excluding the free edges) with reduced systolic opening.

The degree of calcification of the aortic valve was assessed on the basis of recently proposed echocardiographic criteria.
The confirmation of calcification of the valvular leaflets of the aortic valve at surgery was an additional criterion. Aortic jet velocity (m/s) and aortic valve area (cm²) were measured at echocardiography and taken as parameters of aortic stenosis severity. Patients with echocardiographic findings suggestive of congenital valve stenosis (identification of two cusps in systole and systolic cusp doming, highly asymmetric thickening or both) or rheumatic valve stenosis (commisural fusion and mitral valve involvement) were excluded.

At the time of hospitalization, key demographic and clinical characteristics were collected including age, gender, presence of traditional risk factors for atherosclerosis (hypertension, diabetes, obesity, smoking, hyperlipidemia, positive family history). For the purposes of this study, patients were defined as: hypertensive if they had a diastolic pressure higher than 90 mm Hg and a systolic pressure higher than 140 mm Hg or if they were being treated for at least one year for this disorder, diabetic if they had fasting levels of glucose higher than 126 mg/dl in two distinct instances or if they were being treated for at least one year with hypoglycemic drugs, smokers if they reported a daily habit of 10 cigarettes/day or more for at least one year during the last 10 years, hyperlipidemic if they had a total cholesterol level higher than 220 mg/dl or if they were being treated for at least one year with lipid lowering drugs. Body mass index (BMI) (kg/m²) was taken as a measure of obesity.

A control population of volunteers over 40 years of age, relatives of patients hospitalized at the Department of Internal Medicine of Tor Vergata University of Rome for noncardiac, noninfectious diseases was enrolled during the same time period as patient enrollment. The same clinical and instrumental evaluations performed in patients were made in controls, except for coronary angiography. A negative clinical history for cardiac disease was a prerequisite for inclusion in the study. Furthermore, all control subjects performed a 12-lead electrocardiogram and echocardiography; those with normal findings were admitted to the study. Both patients and controls were living in the same geographic area (the Italian district of Lazio).

Informed consent was obtained by all patients and controls for blood draws for the determination of CRP, and IgG antibodies against *H. pylori* and *C. pneumoniae*. The study protocol was approved by the ethical committee of our institutions.

In the patient group, blood draws were performed during the first 24 h of hospitalization, and all subjects were in stable clinical condition. In the control group, blood was taken within one week from the time that consent to participate in the study was given.

To minimize external influence on CRP determination, additional exclusion criteria, for patients and controls, were a history of cancer or the development, during the month preceding the inclusion in the study, of any active inflammatory or hematologic disorder.

All samples were kept at −80°C and were analyzed simultaneously by technicians who were blinded to subject group.

**Determination of CRP.** C-reactive protein was assessed by rate nephelometry (Behring NA latex CRP, Behring Institute, Scoppito, l’Aquila, Italy) and, in samples with less than 0.25 mg of C-reactive protein per deciliter, enzyme immunoassay (Imx, Abbott Laboratories, North Chicago, Illinois), calibrated with the World Health Organization’s International Reference Standard for CRP Immunoassay (23); the range of value detected by the assay is 0.005 to 3 mg per deciliter (24).

**Serology testing.** The frozen sera of the patients and controls were simultaneously investigated for IgG antibodies to *H. pylori* by an enzyme linked immunosorbent assay (Pyloriset, ORION Diagnostica, Espoo, Finland) by one of the authors who was blinded to the disease status. Titers ≥ 300 were regarded as positive. Detection of IgG antibodies to *C. pneumoniae* was done by a microimmunofluorescence assay obtained from Labsystem OY (Helsinki, Finland). IgG titer ≥1:32 and <1:512 were considered as evidence of preexisting infection.

**Statistical analysis.** When not otherwise stated, data are presented as mean ± SD. A two-tailed value of p ≤ 0.05 was considered statistically significant.

To evaluate whether the two groups were comparable for the recorded clinical parameters (age, gender, smoking habit, diabetes, hypertension, family history of coronary artery disease, hyperlipidemia and BMI), a univariate analysis was performed. Student t test was used to compare continuous variables, and chi-square was used to compare categorical variables.

Since CRP is not normally distributed, a nonparametric test (Mann-Whitney U test) was used when comparing this variable between patients and controls.

To evaluate whether CRP levels may be related to the severity of the aortic valve stenosis, a tertile analysis of CRP levels in relation to the aortic jet velocity and aortic valve surface area was performed by means of one way analysis of variance.

The seroprevalence of *C. pneumoniae* and *H. pylori* was compared between patients and controls using the chi-square test. Unconditional logistic regression was used to assess the independent association of CRP or of a positive antibody titer with degenerative valvular aortic stenosis, while simultaneously controlling for age (years), gender, BMI (kg/m²) (≥28 vs. <28), smoking (smokers vs. non-smokers), history of hypertension, hyperlipidemia, diabetes and familial history of coronary artery disease.
CRP values were compared between infected and noninfected subjects in cases and controls separately with the Mann-Whitney U test. All computations were made with MINITAB software release 13 (MINITAB INC, State College, Pennsylvania).

RESULTS

Sixty-eight patients (group 1) and 92 healthy controls (group 2) were considered during the study period. Six subjects of group 1 and 13 subjects of group 2 were excluded from the analysis of the data for the following reasons: history of rheumatic fever elicited after blood drawing (six subjects, group 1), abnormal electrocardiogram (eight patients, group 2) and abnormal echocardiographic findings (five subjects, group 2). Data, therefore, refer to 62 patients of group 1 and 79 patients of group 2.

The primary indication for valve replacement was heart failure in 38 (61%), syncope or near syncope in 18 (29%), angina in 4 (6%) and severe asymptomatic aortic stenosis in 2 (4%).

Factors considered for univariate analysis are shown in Table 1. None of the considered factors showed significant differences between the two groups. C-reactive protein levels (mg/dl) were 0.848 ± 0.50 in group 1 vs. 0.394 ± 0.50 in group 2 (p = 0.0001). Table 2 shows the prevalence of infection with C. pneumoniae and H. pylori in the two groups. Seroprevalence of infection with C. pneumoniae was 59.7% in patients and 33% in controls (p = 0.003), whereas the prevalence of H. pylori was not significantly different between the two groups, being 68.7% and 79.7%, respectively.

Aortic jet velocity values (m/s) of patients belonging to different tertiles of CRP levels were as follows: lower tertile: 4.86 ± 0.79; intermediate tertile: 5.18 ± 0.47; higher tertile: 5.03 ± 0.61 (p = NS); the corresponding values of aortic-valve surface area (cm²) were: 0.62 ± 0.14, 0.58 ± 0.12 and 0.59 ± 0.14, respectively (p = NS). Aortic valve surface area was not available in seven patients.

The CRP level (mg/dl) was 1.0 ± 1.7 in patients with moderate valve calcification and 0.71 ± 1.04 in those with severe calcification (p = NS).

Tables 3 and 4 present the results of multivariable logistic regression analyses in which the association between C. pneumoniae and CRP and aortic degenerative valvular stenosis was adjusted for age and gender and a variety of potential confounders. The odds ratio (OR) for the disease given a seropositivity for C. pneumoniae was 3.45 (95% confidence interval [CI]: 1.67 to 7.12) in the first model and 3.41 (95% CI: 1.60 to 7.30) in the fully adjusted model; the ORs for the disease according to CRP levels were 2.62 (95% CI: 1.06 to 6.49) and 2.76 (95% CI: 1.08 to 7.05), respectively. Table 5 shows measures of central tendency for CRP in patients and controls according to C. pneumoniae IgG serostatus. None of the comparisons was significantly different between the two groups.

DISCUSSION

Main findings. Our data indicate that a systemic inflammatory response, evaluated by CRP, is present in patients with severe degenerative trileaflet aortic stenosis requiring surgical repair. We also evaluated whether H. pylori and C. pneumoniae may be associated with the inflammatory response since these two agents have been extensively studied in this regard in patients with atherosclerosis (2–18). In this study, we did not detect any difference in the prevalence of H. pylori infection between patients and controls, whereas the prevalence of IgG antibodies against C. pneumoniae was increased in patients with degenerative valvular aortic stenosis. However, no difference in CRP levels was found between patients with and without C. pneumoniae infection.

Pathophysiologic and clinical implications. The presence of chronic systemic inflammation in patients with degenerative aortic valvular stenosis adds support to the hypothesis that a common pathophysiologic pathway is present in this condition and in atherosclerosis.

A similarity between the inflammatory infiltrate at the level of the aortic plaque and that found at the level of the CAD injury site was noted. The inflammatory response, evaluated by CRP, is present in patients with severe degenerative aortic stenosis. Moreover, H. pylori infection may be associated with the inflammatory response, as suggested by the presence of IgG antibodies against C. pneumoniae in patients with atherosclerosis (2–18). In this study, we did not detect any difference in the prevalence of H. pylori infection between patients and controls, whereas the prevalence of IgG antibodies against C. pneumoniae was increased in patients with degenerative valvular aortic stenosis. However, no difference in CRP levels was found between patients with and without C. pneumoniae infection.
Table 4. Odds Ratios for CRP in Aortic Valvular Stenosis After Adjustment for Possible Confounding Factors

<table>
<thead>
<tr>
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<th>Odds Ratio</th>
<th>Odds Ratio</th>
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<tbody>
<tr>
<td>CRP</td>
<td>2.62 (1.06–6.49)*</td>
<td>2.76 (1.08–7.05)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>0.99 (0.96–1.03)</td>
<td>0.99 (0.95–1.03)</td>
</tr>
<tr>
<td>Gender</td>
<td>1.21 (0.61–2.41)</td>
<td>0.97 (0.45–2.08)</td>
</tr>
<tr>
<td>Smoking status</td>
<td>1.36 (0.52–3.54)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>2.43 (0.93–6.30)</td>
<td></td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>1.19 (0.24–5.87)</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.66 (0.32–8.60)</td>
<td></td>
</tr>
<tr>
<td>BMI &gt; 25</td>
<td>1.14 (0.51–2.54)</td>
<td></td>
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<tr>
<td>Family history of CHD</td>
<td>1.55 (0.31–7.72)</td>
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*Values in parentheses are 95% confidence intervals.

BMI = body mass index; CHD = coronary heart disease; CRP = C-reactive protein.

diseased valve has been shown in recent years (1). Our findings show that an association with a systemic inflammatory status is shared by these two disorders.

This fact suggests that, on the basis of the host susceptibility, the presence of the same risk factor (chronic inflammation) may induce a different disease. Alternatively, it is possible that different disorders may be associated at a certain point of their natural history with a systemic inflammatory reaction.

Elevated CRP levels, whether preceding the development of degenerative valvular aortic stenosis or established at some time during the course of the disease, may have a detrimental influence on the natural history of the disease by inducing local activation of complement and subsequent amplification of local inflammation and cellular damage (25).

From a clinical point of view, it is important to note that the deleterious effects of long-term CRP activation may theoretically be halted by aspecific long-term anti-inflammatory therapy (26) or by a specific treatment against the factor(s) responsible for its increase. Although the two putative factors considered in the present study (H. pylori and C. pneumoniae) did not show an association with increased CRP levels, our findings, showing an increased seropositivity of C. pneumoniae in patients with degenerative valvular aortic stenosis, are in accordance with recent data showing a high frequency of this agent in aortic valves (27–29). Our data, therefore, support the performance of trials of antibiotic therapy against C. pneumoniae in order to evaluate whether the elimination of this organism may modify the natural history of the disease.

Our findings in the context of available literature. No data are available in the literature on the possible correlation between CRP levels and valvular degenerative aortic stenosis or on the relationship between seroprevalence of H. pylori infection and this disorder.

Three studies (27,28,30) evaluated evidence of previous infection with C. pneumoniae by means of serum IgG antibodies, and seroprevalence ranged from 65% to 86%. Although the populations considered in these studies were heterogeneous in comparison with our patients (patients with rheumatic disease, bicuspid valves and concomitant atherosclerosis were included), from one of these reports (27) it was possible to extrapolate data for patients with characteristics similar to ours; the prevalence of seropositivity to C. pneumoniae was 71% in this subgroup, which is slightly higher than the 59% found in this study, giving support to the hypothesis that there is a link between this organism and degenerative aortic valvular stenosis.

However, our data seem to exclude that the association between C. pneumoniae and degenerative valvular aortic stenosis may be mediated by chronic systemic inflammation induced by the organism, since there was no difference in the levels of CRP between infected and noninfected patients. Thus, the possible causal role, if any, of C. pneumoniae in the pathogenesis and evolution of degenerative aortic valvular stenosis is probably confined to a local action at the level of valvular tissue.

The mechanism(s) responsible for the observed increase in CRP in patients with degenerative aortic valvular stenosis remain to be elucidated.

Study limitations. The cross-sectional nature of the study does not allow us to recognize whether the detected increase in CRP levels in degenerative valvular aortic stenosis plays a causal role in the development of the disease or is induced by the disease itself or by associated conditions. Furthermore, our analysis was restricted to patients with an advanced stage of the disease, requiring surgical therapy. We feel that this fact may at least, in part, explain why we were not able to detect a relationship between CRP levels and the severity of aortic stenosis. For example, previous studies have reported that patients with moderate-to-severe aortic calcification or those with high jet velocity had a poorer prognosis than patients without these characteristics (22,31). Both these studies, however, enrolled only asymptomatic patients, and aortic valve replacement represented the end point for an adverse outcome. In this study, all patients required surgical therapy, had moderate-to-severe aortic calcification and had a high jet velocity. Thus, the lack of patients with mild disease in our series probably did not allow us to evaluate the possible relationship between systemic inflammation and the severity of aortic valve stenosis.

Should the presence of chronic systemic inflammation be

Table 5. CRP in Patients and Controls According to Chlamydia pneumoniae Infection

<table>
<thead>
<tr>
<th></th>
<th>C. P. +</th>
<th>C. P. −</th>
<th>Odds Ratio</th>
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<tbody>
<tr>
<td>Patients (n = 62)</td>
<td>n = 37</td>
<td>n = 25</td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.5</td>
<td>0.5</td>
<td>0.83 (0.30–2.30)*</td>
</tr>
<tr>
<td>Controls (n = 79)</td>
<td>n = 26</td>
<td>n = 53</td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.2</td>
<td>0.2</td>
<td>0.38 (0.14–1.02)</td>
</tr>
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</table>

*Values in parentheses are 95% confidence intervals. For calculating odds ratio and confidence intervals, the population was dichotomized into those with high and those with low concentration of CRP, based on the median values.

C. P. + = C. pneumoniae positive patients; C. P. − = C. pneumoniae negative patients; CRP = C-reactive protein.
limited to the final stages of the disease, the potential usefulness of anti-inflammatory treatment would be greatly reduced.

Finally, although quite relevant from a statistical point of view, some overlapping of CRP levels was observed between patients and controls. This finding seems to suggest that chronic systemic inflammation cannot be regarded as a universal phenomenon in degenerative valvular aortic stenosis.

Conclusions. To our knowledge, this study shows for the first time that signs of chronic systemic inflammation, assessed by means of CRP, are present in patients with degenerative aortic valvular stenosis. This fact may influence the natural history of the disease and may have therapeutic implications. C. pneumoniae, although linked to this disorder, is probably not responsible for this finding.

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Reprint requests and correspondence: Dr. Alberto Galante, Dipartimento di Medicina Interna, Università Tor Vergata, Via di Tor Vergata 135, 00133 Rome, Italy. E-mail: galante@med.uniroma2.it.

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