Systemic Inflammatory Response in Cardiac Allograft Vasculopathy
High-Sensitive C-Reactive Protein Is Associated With Progressive Luminal Obstruction

Klaus Pethig, MD; Bernd Heublein, MD; Ingo Kutschka, MD; Axel Haverich, MD

Background—Response to immunologic and nonimmunologic injury has been reported to initiate the development of cardiac allograft vasculopathy (CAVD). Although histopathologic examinations reveal signs of focal inflammation, little is known about the systemic inflammatory response in this accelerated coronary syndrome.

Methods and Results—Therefore, we investigated high-sensitive C-reactive protein (CRP) in a large cohort of heart transplant (HTX) recipients (n=102, 90 male, mean age 45.2±11.5 years, 6.1±3.3 years after HTX) in correlation with a progression of luminal obstruction as assessed by serial coronary angiography (defined as an increase of focal stenosis ≥30% or detection of a new lesion) after a mean interval of 1.8±1.0 years. Patients with signs of an acute rejection or infection were excluded. In the entire group, CRP levels ranged from 0.2 to 12.7 mg/L (mean 2.6±2.7 mg/L). Patients with progressive CAVD (n=35) presented with significantly higher levels of CRP (4.1±3.3 mg/L) than did those with a nonprogressive course (n=67) (1.8±1.9 mg/L, P=0.001). These observations were independent of the initial indication for HTX (atherosclerotic disorder versus cardiomyopathy, P=0.18) and the severity of CAVD at baseline examination (P=0.12).

Conclusions—Progressive cardiac allograft vasculopathy is accompanied by a systemic inflammatory reaction, which gives further insight into the pathogenesis of this coronary syndrome and may well serve as an indicator for patients at risk. (Circulation. 2000;102[suppl III]:III-233-III-236.)

Key Words: transplantation ■ coronary disease ■ inflammation

Chronic inflammation is increasingly recognized as a major hallmark of atherosclerotic disease. Over recent years, several studies have demonstrated that not only focal but also systemic inflammatory reactions can be detected in a variety of cardiovascular disorders. C-reactive protein (CRP) is one of the most sensitive markers of systemic inflammation. It is produced by hepatocytes and is believed to reflect and amplify the overall cytokine activation in the organism. Recent publications underlined the clinical association between CRP and outcome in stable and unstable coronary syndromes as well as in peripheral vascular disease.

Beyond native coronary artery disease, cardiac allograft vasculopathy (CAVD) represents another unique form of an accelerated coronary syndrome. Injury of the vascular endothelium in the transplanted heart, initiated by a variety of factors like rejection, infection, and cholesterol, has been reported to result in intimal hyperplasia. However, although local inflammation could be demonstrated by histopathologic examinations, little is known about the systemic inflammatory status in heart transplant (HTX) recipients and its correlation with the progression of CAVD. Therefore, to characterize the inflammatory status, we analyzed high-sensitive CRP levels in a large cohort of HTX recipients and correlated them with the severity and progression of CAVD by serial angiography.

Methods

Within a period of 12 months, all HTX recipients undergoing routine surveillance angiography at the local HTX program (written informed consent) were screened for the present study. Individuals with clinical signs of an infection or acute rejection (diagnosed either by echocardiography or biopsy) were excluded (n=6). A total of 102 patients (mean age 45.3±11.5 years, 6.1±3.3 years after HTX, 90 male and 12 female) were chosen for further analysis. The underlying disease was ischemic heart disease in 32 patients (31.4%), dilated cardiomyopathy in 65 (63.7%), and miscellaneous disorders in 5 (4.9%).

Data involving high-sensitive CRP, leukocyte count, cholesterol, fibrinogen, homocysteine, and cytomegalovirus and Chlamydia pneumoniae serology were retrieved from the main transplantation database at the time of heart catheterization.

Coronary angiography was performed on a routine basis (annually within the first 4 years after HTX, biannually later) by use of the Judkins technique. Coronary arteries were visualized in multiple and standardized projections under maximal vasodilation with nitroglycerin (0.1 to 0.2 μg). The amount of luminal obstruction (visual...
estimation) was graded as described previously: 0, no visible wall irregularities; 1, luminal obstruction of <30% in one of the major coronary arteries; 2, luminal obstruction of 30% to 50%; 3, luminal obstruction of >50%; and 4, vascular occlusion. Progression was defined as an increase in focal luminal obstruction of ≥30% or the development of a new lesion since the last examination.

Statistics
Statistical analysis was performed by using a computer-assisted software package (SPSS, version 8.0). Continuous variables are presented as mean ± SD. For comparison of CRP values between the different stages of disease, an ANOVA was performed. Bivariate comparison of inflammatory, infectious, and metabolic risk factors was based on the Student t test; comparison of underlying disease was performed by χ² test. Multivariate analysis between these risk factors was based on a logistic regression (backward). A value of P < 0.05 was considered to be statistically significant.

Results
Luminal Obstruction/Progression of Disease
In HTX recipients, coronary angiography revealed no signs of CAVD in 36 patients (35.3%), but luminal obstruction was observed as follows: grade 1 in 29 patients (28.4%), grade 2 in 11 patients (10.8%), grade 3 in 16 patients (15.7%), and grade 4 in 10 patients (9.8%) (Figure 1). A progression of stenosis compared with the results of the last catheterization (1.8 ± 1.0 years before) was present in 35 patients (34.3% of the cohort).

CRP in Correlation With CAVD
Mean values for CRP were found to be 2.6 ± 2.7 mg/L (range 0.2 to 12.5 mg/L). However, there was a trend toward higher concentrations in advanced stages of CAVD, without reaching statistical significance. CRP levels for the different grades were as follows: grade 0, to 1.9 ± 2.3 mg/L, grade 1, to 2.4 ± 2.1 mg/L, grade 2, to 3.0 ± 2.9 mg/L, grade 3, to 3.4 ± 4.2 mg/L, and grade 4, to 3.6 ± 2.6 mg/L (Figure 2).

Independent from the severity of CAVD, significant differences were found in patients with progressive changes compared with the last examination. CRP values in the “progressive” group were 4.1 ± 3.3 versus 1.8 ± 1.9 mg/L in the nonprogressive patients (P = 0.001, Figure 3). Mean latency between the 2 examination time points did not differ significantly between the progressive and the nonprogressive group (1.8 ± 1.1 versus 1.7 ± 0.9 years).

Inflammatory, Infectious, and Metabolic Risk Factor Profile
Beyond CRP, leukocyte count and fibrinogen were analyzed as acute-phase indicators, cholesterol and homocysteine were analyzed as metabolic risk factors, and cytomegalovirus and Chlamydia pneumoniae serology were analyzed to assess possible infectious influences. By use of a bivariate analysis, only CRP and the leukocyte count demonstrated a statistical significance in progressive versus nonprogressive changes. By use of a multivariate approach, only CRP could be identified as an independent marker of progression (values are detailed in the Table).

Discussion
Inflammation is an important feature in cardiovascular disease. It is associated with cytokine activation and the proliferation and activation of macrophages and endothelial and smooth muscle cells. Stimulated by findings in native coronary artery disease, recent interest was focused on allograft vasculopathy, one of the most rapidly progressing coronary syndromes in humans. A multifactorial endothelial injury, based on alloantigen-dependent and nonimmunologic factors, has been reported to be involved in pathogenesis. Focal inflammation is a frequent finding; however, signs of systemic inflammation are not characterized in detail. Therefore, results obtained by the present study might be of interest under pathophysiological as well as clinical aspects. These data confirmed that CAVD is associated with a systemic

Figure 1. Severity of CAVD graded from 0 to 4 (see Methods) as assessed by angiography at enrollment in study.

Figure 2. Mean values for high-sensitive CRP detailed for different CAVD grades. Note trend to higher CRP values in advanced stages of disease. These differences did not reach statistical significance.

Figure 3. Scatterplot of CRP values in progressive vs nonprogressive forms of CAVD, graphically printed as mean ± SEM. Although there is a distinct overlap, significantly higher values are seen in progressive forms (P = 0.001).
Inflammatory, Infectious, and Metabolic Risk Factor Profile in Progressive vs Nonprogressive CAVD

<table>
<thead>
<tr>
<th>Variable</th>
<th>Progressive</th>
<th>Nonprogressive</th>
<th>Bivariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-sensitive CRP, mg/dL</td>
<td>4.1±3.4</td>
<td>1.8±1.9</td>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>Leukocyte count, ×10⁹/L</td>
<td>8.4±2.4</td>
<td>7.1±2.1</td>
<td>0.01</td>
<td>0.83</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>6.2±1.3</td>
<td>6.0±1.2</td>
<td>0.44</td>
<td>0.63</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>3.6±0.8</td>
<td>3.5±1.2</td>
<td>0.70</td>
<td>0.15</td>
</tr>
<tr>
<td>Homocysteine, μmol/L</td>
<td>17.1±7.1</td>
<td>17.0±7.3</td>
<td>0.77</td>
<td>0.47</td>
</tr>
<tr>
<td>CMV-IgG, mean titer</td>
<td>16 987±17 464</td>
<td>14 975±13 619</td>
<td>0.57</td>
<td>0.89</td>
</tr>
<tr>
<td>Chlamydia-IgG, mean titer</td>
<td>137±100</td>
<td>131±104</td>
<td>0.81</td>
<td>0.80</td>
</tr>
<tr>
<td>Underlying disease (CAD), n/N</td>
<td>13/32</td>
<td>22/70</td>
<td>0.20</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Values are mean±SD. CMV indicates cytomegalovirus; CAD, coronary artery disease.

Inflammatory response. Although only a positive trend was found between the severity of CAVD and high-sensitive CRP (Figure 2), patients with active progressing forms (compared with the last angiographic examination 1.8 years before) presented with significantly higher CRP levels as well as a higher leukocyte count than did those with a nonprogressive course. Fibrinogen as another indicator of a acute-phase reaction fails as a marker of progressive disease.

Systemic Inflammation and CAVD

Only few studies have addressed this important issue so far. Interest has been concentrated predominantly on a possible association between CRP and infection, rejection, and graft dysfunction.¹¹,¹² One study, published by Fyfe et al in 1997, is focused on a comparable question of systemic inflammatory response in CAVD. The authors found significantly elevated levels of serum amyloid A, another acute-phase protein, in HTX recipients suffering from CAVD. More recently, further observations regarding CRP were presented.¹⁴ Eisenberg et al demonstrated that the high-sensitive CRP might be a useful and prospective marker for survival after HTX.

CRP: Indicator or Cause of Disease?
The background of the observed elevation of CRP in association with active disease processes remains speculative. In native atherosclerosis, the most probable association between circulating CRP levels and pathogenesis is a reflection of systemic inflammation, related to an active atherosclerotic process. This hypothesis is supported by observations that other acute-phase indicators, such as fibrinogen,¹⁵ interleukin-6,¹⁶ and serum amyloid A,⁴ and the leukocyte count¹⁷ show a similar pattern of activation.³ However, a causal relationship between elevated CRP levels and the development of symptomatic coronary artery disease cannot be excluded. The presence of CRP deposits in the diseased human vascular wall (focally associated with inflammatory cellular infiltration and lipid-rich cores),¹⁸ a stimulation of the production of tissue factor by CRP,¹⁹ and the interactions with complement²⁰ and LDLs²¹ may indicate a direct involvement of CRP in the atherosclerotic process.

Despite some common features between late forms of CAVD and native coronary artery disease, a continuing immunologic response, due to alloantigen recognition of the graft, represents a potential basis for a pronounced inflammatory reaction. Further studies are required to clarify these associations.

Clinical Implications of CRP Measurements

The clinical aspects of these findings should be discussed in relation to possible procedural and therapeutic implications. First, elevated levels of high-sensitive CRP might serve as an indicator for individual patients at risk for progressive forms of CAVD. Although limited by a certain overlap of values, a risk-adapted modification of scheduled surveillance angiographies could be the consequence. Second, therapeutic protocols should be reassessed under the aspect of an anti-inflammatory activity. Data from native coronary artery disease suggest that statins as well as aspirin may reduce the systemic inflammatory activity, which might further support the use of these drugs in HTX recipients. In the transplant setting, the role of immunosuppressive drugs in association with systemic inflammation must be defined and might represent an option in suppressing systemic inflammation.

Study Limitations

First, the diagnostic approach might be criticized. Characterization of CAVD was performed by a semi quantitative angiographic grading. As is known from intravascular ultrasound studies, these results can be misleading in terms of a possible underestimation of intimal hyperplasia as well as an inability to identify the presence and type of remodeling processes.²⁴,²⁵ Second, although large for a single-center study, the size of the cohort may be regarded as small in epidemiological terms. Multicenter evaluations, eg, within national or international registries, are needed to clarify associations between inflammatory markers and the development of CAVD. In such a context, multiple measurements over distinct periods of time should be used to avoid possible environmental influences of single-step evaluations.

Conclusions

The data obtained by the present study provide considerable evidence that CAVD is associated with a systemic inflammatory status in HTX recipients, suggesting a potential patho-
physiological link between inflammation and the development of this accelerated coronary syndrome. Further investigation is required to clarify the possible role of a risk indicator under clinical aspects.

References

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Circulation. 2000;102:Iii-233-Iii-236
doi: 10.1161/01.CIR.102.suppl_3.III-233

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/102/suppl_3/Iii-233

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