Cytomegalovirus Infection With Interleukin-6 Response Predicts Cardiac Mortality in Patients With Coronary Artery Disease

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Background—Prospective data relating previous exposure to cytomegalovirus (CMV) to the risk of cardiac mortality are controversial. We investigated the effect of previous exposure to CMV infection on the risk of future cardiac disease–related death in relation to an underlying inflammatory response.

Methods and Results—Coronary angiography was performed in 1134 subjects, and 989 patients with documented coronary artery disease were studied prospectively. CMV-IgG titers and interleukin (IL)-6 levels were measured before angiography. Increasing titers of CMV correlated with the elevation of IL-6 levels (P<0.001) after adjustment for possible confounders. All patients were followed up for a median of 3.1 years (maximum 4.3 years). During follow-up, 96 patients died, 70 of cardiac disease. Overall, CMV seropositivity was not related to cardiac mortality after adjustment for confounding variables (P=0.19). In contrast, in patients with elevated IL-6 levels (≥11.9 pg/mL, median level), CMV seropositivity was independently associated with a 3.2-fold (95% CI 1.4 to 7.3, P=0.007) increase in risk of future cardiac death, whereas in individuals without IL-6 elevation, previous CMV infection had no effect on cardiac mortality.

Conclusions—CMV seropositivity in patients with an inflammatory response is independently associated with future cardiac mortality, whereas this association is lost in patients who do not demonstrate an inflammatory response. These data support the hypothesis that the atherosclerotic effects of CMV are mediated through an underlying inflammatory response. (Circulation. 2001;103:2915-2921.)

Key Words: viruses ■ risk factors ■ ischemia ■ thrombosis ■ survival

A mplification of experimental evidence indicates that cytomegalovirus (CMV) infection contributes to the development of atherosclerosis and restenosis.1 The seroepidemiological evidence, however, appears weaker. Some studies have found an association between CMV seropositivity and accelerated atherosclerosis,2,3 but others were not able to confirm these results.4,5

Increasing evidence supports the hypothesis that atherosclerosis is based on a chronic inflammatory process.6 The pleiotropic cytokine interleukin (IL)-6, secreted by a number of different cells, such as macrophages, lymphocytes, and endothelial cells in response to proinflammatory cytokines and to infectious stimuli, is a key mediator of the acute-phase response, including hepatocellular C-reactive protein (CRP) production.7 Elevated CRP and IL-6 levels have been found in patients with unstable angina8 and are associated with future cardiovascular events in initially healthy individuals.9,10

It is not entirely clear which stimuli are responsible for this systemic inflammatory process; infectious agents have been proposed to be in part responsible.

CMV infection has been shown to upregulate IL-6 gene expression11 and cytokine production itself.12 Mediated by this systemic inflammatory process, CMV infection might contribute to the atherosclerotic and atherothrombotic processes.13,14

On the basis of this experimental experience and the hypothesis that the inflammatory host response might provide the link between infection and acceleration of atherosclerosis,15 we investigated the prognostic value of previous exposure to CMV with special emphasis on the underlying inflammatory response as reflected by IL-6 measurements.

Methods

Study Population

Between November 1996 and July 1998, we examined 1134 adult patients of both sexes who had been admitted to the second medical clinic of the University Clinic Mainz for diagnostic angiography because of symptoms of suspected coronary artery disease (CAD). Patients were considered to suffer from CAD if a diameter stenosis...
Follow-up period. Hazard risks of cardiac mortality are presented as infarction, current drug use, and CABG or PTCA during the follow-up period. The Kaplan-Meier method was applied, including the classic risk factors, the extent of coronary artery disease, and septic complications. Hazard risks of cardiac mortality were calculated for patients with a history of cholesterol levels > 240 mg/dL.

A total of 983 of 989 patients (99.4%) were followed up at a median of 3.1 years (maximum 4.3 years). Patients either presented in our clinic (84.3%) or were interviewed by phone by trained medical staff. Follow-up information was obtained about cardiac death (n = 70), non–cardiac disease–related death (n = 26), and nonfatal myocardial infarction (n = 58). Information about the cause of death or any clinical events was obtained from the hospital or the general practitioners’ charts. Of the 26 deaths due to noncardiovascular causes, 18 were due to cancer and 8 to other noncardiac causes.

In general, study patients were of German nationality and lived in the Rhein-Main area. The study was approved by the ethics committee of the University of Mainz. Participation was voluntary, and each study subject gave written informed consent.

Laboratory Methods
In all study subjects, blood was drawn under standardized conditions after an overnight fasting period. The blood sample was collected before coronary angiography was performed. Samples were immediately placed on ice, and within 30 minutes, blood was centrifuged at 4000g for 10 minutes and frozen at −80°C until analysis.

Each individual was tested for specific anti-CMV IgG antibody by use of a quantitative in vitro ELISA according to the manufacturer’s instructions (CMV IgG ELISA, EUROIMMUN). Inactivated cell lysates of MRC-5 cells infected with the AD169 strain of CMVs provided the source of CMV antigens. Serum IL-6 was measured by the ELISA technique (EASIA, Biosource Europe). The detection range is 0 to 1540 pg/mL. The within-run coefficients of variation were 4.7% (mean 75.6 pg/mL) and 5.6% (mean 205.4 pg/mL), respectively. The between-run coefficients of variation were 2.2% (mean 70.7 pg/mL) and 7.7% (194.9 pg/mL), respectively. CRP was determined by a highly sensitive, latex particle–enhanced immunosassay (detection range of 0 to 20 mg/L); the between-day imprecision CVs of this assay (n = 21) were 2.14% and 1.44% at mean levels of 1.90 and 4.33 mg/L, respectively (Roche Diagnostics GmbH). Fibrinogen was determined by the derived method.

Lipid serum levels were measured immediately by routine methods (cholesterol, Roche Diagnostics GmbH; HDL cholesterol, Rolf Greiner Biochemica; LDL, calculated according to the Friedewald formula; triglycerides, Roche Diagnostics GmbH).

Statistical Considerations
Categorical variables were analyzed according to χ² analysis, and continuous variables by t tests and Mann-Whitney U tests. To investigate the effect of CMV seropositivity on IL-6 levels, we used logarithmically (log) transformed variables and the Kruskal-Wallis test for univariate analysis and skewed distributions. To indicate the independent predictive value of CMV titers on the log-transformed IL-6 level, a linear regression analysis was carried out. To investigate the association between infectious serology and survival, we used the Kaplan-Meier method. For adjusted survival analysis, Cox regression was applied, including the classic risk factors, the extent of CAD (number of diseased vessels), history of previous myocardial infarction, current drug use, and CABG or PTCA during the follow-up period. Hazard risks of cardiac mortality are presented as risk ratios with 95% CIs. Values of P ≤ 0.05 were considered to be significant. All computations were carried out with the SAS V.6.12 program.

Results

Study Population
The baseline characteristics of the 70 patients who died of cardiac causes and the 913 patients in whom this was not the case are summarized in Table 1. As expected, the patients who died of heart disease were older and more likely to have a higher prevalence of diabetes and lower levels of HDL cholesterol and tended to have more severe vessel disease. Statin drugs and β-blockers were used more often in patients who did not experience fatal cardiac events.

Markers of Inflammation and Death From Cardiac Causes
Concentrations of IL-6 correlated with levels of CRP (r = 0.44, P < 0.001) and to a lesser extent with fibrinogen (r = 0.29, P < 0.001). IL-6 revealed higher levels of acute coronary syndrome in patients with unstable angina than in those with stable angina (15.1 versus 11.4 pg/mL, P = 0.036). After control for age, sex, classic risk factors, and statin drug intake, this association did not achieve significance.

As outlined in Table 1, baseline levels of CRP and especially IL-6 were highly significantly elevated in patients who suffered future cardiac death, whereas levels of fibrinogen were only moderately associated with future cardiac death.

CMV Seropositivity and Long-Term Prognosis
Table 2 demonstrates that the risk of cardiac mortality increased with the elevation of CMV titers (univariate P = 0.01). In a fully adjusted Cox regression model, the predictive power of CMV seropositivity was weakened and lost significance (P = 0.19). Furthermore, no significant association between CMV seropositivity and the combined end point of death of cardiac causes and nonfatal myocardial infarction (n = 128) could be demonstrated (Table 2). Interestingly, if the predictive value between CMV seropositivity and future fatal cardiac event is evaluated according to sex, the overall positive result is driven by this association primarily in women (women: univariate P = 0.009; men: univariate P = 0.2).

Independent Influence of CMV Seropositivity on Serum Levels of IL-6
In linear regression analysis, confounders of serum concentration of IL-6 were age, HDL cholesterol levels, CMV seropositivity, and statin drug intake. Patients receiving statin medication had significantly lower IL-6 (median values: 10.3 versus 13.5 pg/mL, P = 0.001) and CRP (median values: 3.9 versus 5.6 mg/L, P < 0.001) concentrations than patients not receiving a statin drug. Other medication did not influence either inflammatory marker.

Table 3 demonstrates levels of IL-6 with respect to increasing CMV titers (n = 942). Median values of IL-6 increased from 10.4 pg/mL in patients with a CMV titer < 20 relative units (RU) to 13.5 pg/mL in patients with a CMV titer > 100 RU. In a multivariate linear logistic regression model,
increasing CMV titers retained independent statistical significance for determining log-transformed IL-6 levels ($P=0.001$). With analysis stratified into patients receiving and not receiving statin medication, the inflammatory response to CMV infection is observed predominantly in patients not receiving statin medication ($P$ fully adjusted <0.001).

### CMV Seropositivity and Long-Term Prognosis According to the Inflammatory Response

Figure 1 demonstrates the effect of CMV seropositivity on cardiac mortality according to the inflammatory response, which was defined by an IL-6 level greater than the median ($\geq 11.9$ pg/mL). In patients who did not demonstrate an inflammatory response, CMV seropositivity had no effect on
future cardiac death. In contrast, seropositivity to CMV had a predictive value for future cardiac death in patients with elevated IL-6 levels (Table 4). After full adjustment, patients with CMV seropositivity and inflammatory response had a 3.2-fold increase in the risk of future cardiac death (95% CI 1.4 to 7.3, \(P = 0.007\)). Similar results could be found concerning the association between CMV seropositivity with inflammatory response and the combined end point of death of cardiac causes and nonfatal myocardial infarction (Table 4).

If CMV seropositivity and the dichotomized IL-6 variable were put into the fully adjusted Cox regression model, the interaction term of the 2 variables revealed an independent, 4.1-fold increase in the risk of future cardiac mortality (95% CI 1.3 to 13.1, \(P = 0.02\)).

To assess whether the effect of baseline CMV seropositivity and elevated IL-6 levels on death of cardiac causes varied over time, we stratified analysis by time of follow-up (Figure 2). Risk was found to increase slightly over time.

If the inflammatory response was defined by elevated CRP levels (upper CRP tertile \(>9.6\) mg/L) (Figure 1), similar results were obtained concerning CMV seropositivity and future fatal cardiac event in patients with elevated CRP levels (3.1-fold increase in risk, 95% CI 1.2 to 8.1 in the fully adjusted model).

Discussion

In this prospective study of a large, angiographically defined patient cohort, we found a trend for an antibody titer-dependent association between previous CMV infection and future cardiac death. This trend was driven primarily by women, which is in accordance with a proposed sex-based difference in host response to CMV infection. After adjustment for traditional risk factors, clinical features, and therapeutic strategies, CMV seropositivity lost its significant predictive value (hazard risk ratio 1.2, 95% CI 0.9 to 1.7). We could demonstrate, however, that an independent correlation between CMV antibody titers and the concentration of IL-6 retained significance even after adjustment for most possible confounders (\(P = 0.001\)). Finally and most importantly, we found that in patients who showed an inflammatory response, as demonstrated by elevated IL-6 levels, CMV seropositivity remained a strong and independent predictor for cardiac death after full adjustment (hazard risk ratio 3.2, 95% CI 0.9 to 1.7). The results are consistent with our hypothesis that CMV infection leading to inflammatory activity in the host independently contributes to the risk of future fatal cardiac events.

Inflammation represents an important feature of CAD. In accordance with experimental data, recent findings demonstrated an association between elevated IL-6 levels and overall mortality or future myocardial infarction in initially healthy individuals. The triggers of IL-6 elevation are not entirely clear. Levels of IL-6 increase with infection, and infection in particular with Chlamydia pneumoniae and CMV might accelerate atherosclerosis. The inflammatory response to infectious stimuli may contribute to the atherosclerotic process.

### Table 2. Risk Ratios of Cardiac Events to Antibody Titers of CMV

<table>
<thead>
<tr>
<th>Antibody Titer, RU</th>
<th>Death of Cardiac Causes (n=70)</th>
<th>Univariate (P)</th>
<th>(P) Adjusted for Multiple Covariates</th>
<th>Risk Ratio Adjusted for Multiple Covariates</th>
<th>(P) Fully Adjusted†</th>
<th>Risk Ratio Fully Adjusted†</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>14/282 (5.0%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20–100</td>
<td>20/142 (4.2%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;100</td>
<td>50/559 (8.9%)</td>
<td>0.01</td>
<td>0.09</td>
<td>1.3 (0.96–1.8)</td>
<td>0.19</td>
<td>1.2 (0.90–1.7)</td>
</tr>
<tr>
<td>Death of Cardiac Causes and MI (n=128)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>31/282 (11.0%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20–100</td>
<td>17/139 (12.0%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;100</td>
<td>80/559 (14.3%)</td>
<td>0.37</td>
<td>0.44</td>
<td>1.1 (0.90–1.3)</td>
<td>0.384</td>
<td>1.1 (0.90–1.4)</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, body mass index, HDL cholesterol, and history of hypertension, diabetes, cigarette smoking, and family history of CAD.
†Additionally adjusted for CABG, PTCA, number of diseased vessels, unstable angina, history of myocardial infarction, and medical therapy.

### Table 3. Correlation of CMV Antibody Titers With IL-6 Levels

<table>
<thead>
<tr>
<th>CMV, RU</th>
<th>IL-6, pg/mL, all patients (n=942)</th>
<th>IL-6, pg/mL, statin (−) (n=633)</th>
<th>IL-6, pg/mL, statin (+) (n=309)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RU&lt;20</td>
<td>10.4 (4.6/20)</td>
<td>10.5 (5.2/23.1)</td>
<td>10.2 (4.0/17.0)</td>
</tr>
<tr>
<td>RU 20–100</td>
<td>11.0 (5.2/19.8)</td>
<td>12.9 (5.1/23.1)</td>
<td>9.2 (5.6/13.4)</td>
</tr>
<tr>
<td>RU&gt;100</td>
<td>13.5 (6.7/28.3)</td>
<td>15.0 (7.0/31.8)</td>
<td>11.2 (6.2/24.1)</td>
</tr>
</tbody>
</table>

Data presented are median values (25th/75th quartiles).

*Kruskal-Wallis test; †linear logistic regression model with adjustment for age, sex, body mass index, HDL cholesterol, unstable angina, and history of cigarette smoking, diabetes, and hypertension. Log-transformed variable of IL-6 was used in the linear regression model.
As a marker for subsequent inflammatory response to CMV infection, we chose the proinflammatory cytokine IL-6, for the following reasons: first, IL-6 has experimentally been shown to be directly elevated by CMV infection in endothelial cells,12 and in addition, IL-6 gene expression is upregulated by CMV infection.11 Second, IL-6 is the main candidate that circulates in the blood and links systemic inflammation17 with local vessel-wall pathology.18 Finally, we could confirm the independent influence of CMV infection on IL-6 level in our patient cohort. Interestingly, this inflammatory response was influenced by statin intake. This observation is in line with recent studies that demonstrated an influence of statin intake on CRP plasma concentration19 as well as an inhibitory effect on C pneumoniae–induced cytokine release.20

In contrast to the consistent data regarding inflammatory markers, seroepidemiological evidence for the association between CMV seropositivity and accelerated atherosclerosis is sparse, with conflicting results from several case-control studies. Whereas some cross-sectional studies revealed an association between CMV seropositivity and accelerated atherosclerosis2,3 (for nonadjusted data), others were not able to confirm these results.4

As a possible explanation for these heterogeneous results, a different inflammatory response of the host to CMV infection was recently proposed.21 In a cross-sectional study with 238 individuals, Zhu et al15 showed that mainly individuals with an inflammatory response to CMV infection as demonstrated by an elevation in levels of CRP are susceptible to the atherogenic effects of CMV. Patients with CMV seropositivity and elevated CRP levels (>0.5 mg/dL) had the highest prevalence of CAD (OR 4.3), compared with the subgroup of patients with CMV seropositivity but without elevation of CRP (OR 1.3). Similar data were prospectively shown by Muhlestein et al,22 who demonstrated an independent association between CMV seropositivity and all-cause mortality in 985 individuals. Interestingly, this association was found predominantly in patients with elevated CRP levels.

In line with this argument, our study revealed for the first time the strong and independent predictive value of CMV seropositivity for future mortality of cardiac causes or fatal and nonfatal cardiac events in patients with an underlying inflammatory response. Our data therefore support the hypothesis that the propensity of CMV to lead to accelerated atherosclerosis and plaque instability depends on the host’s inflammatory reaction.

Other prospective studies also found an association between CMV seropositivity and accelerated subclinical atherosclerosis,23 restenosis,24 or overall mortality.22 In contrast, some important prospective studies4,5,25 did not find an association between CMV seropositivity and future cardiovascular events. These disparate results may be attributed to several factors, as follows.

Each of these studies was restricted to a highly homogeneous, apparently healthy population. Despite the advantage of such highly selected populations (eg, elimination of various confounders), these populations may not necessarily represent other populations, in which the inflammatory response to CMV infection may be different. In contrast, our study population was much more heterogeneous, and even more importantly, all individuals already suffered from angiographically documented CAD. If infectious agents may accelerate and exacerbate existing atheroma rather than initiate it, one might miss the relevant population if evaluating only healthy individuals or patients with stable angina. Finally, we documented that high CMV titers in association with an inflammatory response, indicated by cytokine elevation, lead to an increase in fatal cardiovascular events during

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**Figure 1.** Kaplan-Meier curves for survival according to absence (dotted line) or presence (solid line) of CMV seropositivity in patients with and without IL-6 or CRP elevation.
the follow-up period, whereas this association was not seen in CMV seropositivity alone without inflammatory response.

Some limitations of this study also should be considered. Although this study is prospective in design, we cannot definitively prove that CMV plays a causative role in atherogenesis. Treatment studies are difficult to perform for CMV infection, however, and do not take into account the complex, possibly indirect atherogenic pathomechanism. Furthermore, IL-6 is subject to diurnal variations, and its serum half-life is <6 hours. Blood samples were obtained in the morning, however, so these effects should be minor. Finally, although CMV is known to trigger the elevation of IL-6, there might be other types of immune response by which CMV infection could contribute to atherosclerosis.

In conclusion, seropositivity to CMV independently correlates with an elevation in IL-6 levels. Furthermore, CMV infection is predictive for cardiac mortality in patients with elevated IL-6 levels. Thus, these results are consistent with our hypothesis that CMV infection leading to an inflammatory activity in the host independently contributes to the risk of future cardiac events.

Appendix

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References


Table 4. Risk Ratios of Cardiac Events According to CMV Seropositivity Dependent on IL-6 Level

<table>
<thead>
<tr>
<th>IL-6 negative*</th>
<th>Negative</th>
<th>12/228 (5.3%)</th>
<th>0.6 (0.3–1.5)</th>
<th>NS</th>
<th>0.6 (0.3–1.5)</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td></td>
<td>10/237 (4.2%)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IL-6 positive*</th>
<th>Negative</th>
<th>7/181 (3.9%)</th>
<th>3.2 (1.4–7.3)</th>
<th>0.005</th>
<th>3.2 (1.4–7.3)</th>
<th>0.007</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td></td>
<td>37/291 (12.7%)</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IL-6 negative*</th>
<th>Negative</th>
<th>30/228 (13.2%)</th>
<th>0.5 (0.3–1.0)</th>
<th>NS</th>
<th>0.6 (0.3–1.1)</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td></td>
<td>21/237 (8.9%)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IL-6 positive*</th>
<th>Negative</th>
<th>17/181 (9.4%)</th>
<th>2.0 (1.3–3.4)</th>
<th>0.02</th>
<th>2.0 (1.3–3.6)</th>
<th>0.02</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td></td>
<td>54/291 (18.6%)</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*IL-6 negative*: median value (<11.9 pg/mL); IL-6 positive*: median value (>11.9 pg/mL).

†Adjusted as described in Table 2.


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Circulation. 2001;103:2915-2921
doi: 10.1161/01.CIR.103.24.2915
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:
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