Previous Cytomegalovirus Infection and Restenosis After Coronary Stent Placement

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Background—Reactivated cytomegalovirus may promote neointima formation after percutaneous coronary interventions by facilitating cell cycle progression through inhibition of the eukariotic tumor suppressor protein p53. This prospective study sought to investigate the effect of previous cytomegalovirus infection on restenosis after coronary stenting.

Methods and Results—In 551 consecutive patients with successful stent placement, we determined cytomegalovirus IgG titers. Primary and secondary end points were the rate of angiographic restenosis at 6 months and the rate of target vessel reintervention at 1 year, respectively. Three hundred forty patients (62%) had a positive cytomegalovirus IgG titer. We obtained angiographic follow-up in 82% of all patients. Angiographic restenosis rate was 28.7% in patients with positive cytomegalovirus titers and 34.6% in patients with negative titers (P=0.18). Between the groups with and without positive cytomegalovirus titers, there were no significant differences in late lumen loss (1.16±0.90 mm and 1.23±0.86 mm, respectively, P=0.44). Target vessel reintervention was performed in 16.8% of the patients with positive cytomegalovirus titers and in 17.5% of those without (P=0.82). Even after correction for potential confounding variables by multivariate analysis, positive cytomegalovirus titers did not manifest as a predictor of angiographic restenosis (adjusted odds ratio [95% confidence interval], 0.78 [0.52 to 1.19]).

Conclusions—Previous cytomegalovirus infection does not carry an increased risk of restenosis after stenting.

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Key Words: viruses ■ stents ■ angioplasty ■ restenosis ■ infection

In 1996, Zhou et al1 reported that prior cytomegalovirus (CMV) infection increased the risk of restenosis after directional atherectomy. As the underlying mechanism, this group proposed reactivation of latent CMV within the coronary plaque by mechanical trauma.1–3 Reactivation induces expression of CMV immediate/early gene products. Among these CMV proteins, IE2 to 84 inactivates the eukariotic protein p53, a suppresser of cell cycle progression,2 and thereby facilitates proliferation of vascular smooth muscle cells after percutaneous coronary interventions. In addition, reactivation of CMV can promote inflammatory and thrombotic responses in endothelial cells and smooth muscle cells that are involved in the initiation of neointima formation.4–8

The concept that CMV seropositivity carries an increased risk of restenosis has recently been challenged. Manegold et al9 reported almost identical restenosis rates after plain balloon angioplasty in patients with and those without positive CMV titers. As a potential explanation for the discrepancy to the study by Zhou et al,1 Manegold et al9 proposed that the contribution of smooth muscle cell proliferation to restenosis may be larger after directional atherectomy than after balloon angioplasty alone. This concept is supported by recent histological documentation of increased neointimal smooth muscle cell proliferation in restenotic tissue after directional atherectomy in comparison with restenotic tissue after balloon angioplasty.10

Bertrand and Bauters11 suggested that in-stent restenosis would be an excellent model to test the impact of prior CMV on neointima formation. After stenting, neointima proliferation accounts for >90% of the late lumen loss.12 We therefore assessed the impact of previous CMV infection on outcome after stenting in a consecutive series of patients. Our finding of increased 30-day risk of thrombotic adverse cardiac events in patients with previous CMV infection has been communicated briefly beforehand because of the clinical impact of these often life-threatening events.13 Here we report the results relating to the primary objective of our prospective study, which is the effect of previous CMV infection on restenosis after coronary stenting.

Methods

Patient Selection and Study Protocol

Patients undergoing coronary stent placement were eligible for the study. All patients gave informed consent, and the study was approved by our institutional ethics committee.
Stent placement was performed as described earlier. During the study period, stent placement was the preferred catheter treatment for coronary disease except for in-stent restenotic lesions. In patients with acute myocardial infarction, small target vessels (reference diameter <2.8 mm), or thrombus-containing lesions, we administered the glycoprotein IIb/IIIa receptor blocker abciximab. In addition, all patients received ticlopidine for 4 weeks and aspirin indefinitely. We obtained blood samples after successful stent placement and stored plasma aliquots at −70°C for later determination of CMV IgG-titers by ELISA (Enzygnost, Dade Behring). Titer <1:230 were considered negative; lower dilutions of samples increase the likelihood of false-positives. According to the manufacturer, the sensitivity and specificity of the test system are 99.3% and 98.2%, respectively.

For the detection of circulating CMV DNA, peripheral blood was analyzed with a TaqMan polymerase chain reaction (PCR) system. After preparation of DNA from disodium ethylene-diamine-tetra-acetic-acid anticoagulated whole blood with a commercially available kit (Qiagen), CMV sequences were amplified with the primers HIVPO1 (5’-TCA TCT ACG GGG ACA CGG AC-3’) and HIVPO2 (5’-TGC GCA CCA GAT CCA CG-3’). The 2-step thermocycling procedure consisted of 45 cycles of denaturation at 95°C for 15 seconds and annealing and extension at 60°C for 60 seconds. The PCR product was hybridized to a CMV-specific, FAM-labeled probe (5[prime]-CCA CTT TGC CGA TGT AAC GTT TCT TGC A-3’). Serving as a positive control, plasmid DNA containing the CMV target sequence was used in separate reactions on each TaqMan assay plate. The assay was validated in various patient groups, including heart transplant recipients, in whom it revealed a proportion of patients with detectable CMV DNA in the circulating blood in the expected range (13%).

Follow-up angiography was performed at 6 months or earlier if the patient had recurrent symptoms or signs of ischemia. Patients who had undergone angiography at <4 months after recruitment without meeting the criteria for a clinical end point were encouraged to undergo repeat angiography at 6 months. In addition, trained medical personnel interviewed the patients over the phone for clinical follow-up at 30 days and at 1 year.

Quantitative Angiography and Definitions
Quantitative analysis of angiographic images was performed as described previously. We obtained minimal luminal diameter (MLD), reference diameter, percent diameter stenosis, and diameter of the maximally inflated balloon from the analysis system (MEDIS Medical Imaging Systems). Acute gain was calculated as the difference between poststenting and predilation MLD, late loss as the difference between poststenting MLD and MLD at follow-up, net gain as the difference between MLD at follow-up and predilation MLD, and loss index was calculated as the ratio of late loss to acute gain. Angiographic restenosis was defined as diameter stenosis ≥50%.

Study End Points
The primary end point was the proportion of patients with angiographic restenosis. The secondary end point was the proportion of patients with target vessel revascularization during 1-year follow-up as measure of clinical restenosis. Target vessel revascularization was defined as coronary artery bypass surgery or repeat balloon angioplasty involving the stented vessel. It was performed for symptoms or signs of ischemia in the presence of angiographic restenosis. In addition, we assessed clinical outcome by the composite rate of death or nonfatal Q-wave myocardial infarction during 1-year follow-up. End point adjudication and collection of clinical, procedural, and angiographic data were completed before blinded determination of CMV titers.

Statistical Analysis
According to published data, calculation of sample size was based on the assumption of an average angiographic restenosis rate of 31.4% and of a rate of seropositivity of 65%. In the study on restenosis after directional atherectomy, the lower boundary of the 95% confidence interval for the odds ratio of restenosis rate in seropositive patients to that in seronegative patients was 1.91. We therefore designed the study to detect an odds ratio of 1.8 at a level of significance of α<0.05 and a power of β=80%, which for the given assumptions corresponds to a restenosis rate of 23% in seronegatives and of 36% in seropositives. These power calculations yielded a total sample size of 450 patients. To account for losses to follow-up angiography, we intended to include 550 patients.

Discrete variables are reported as counts (percentages) and continuous variables as mean±SD or number of patients (percentage). Data are expressed as mean±SD or number of patients (percentage).

Results
Baseline Characteristics
The study included 551 consecutive patients with successful coronary stent placement. A positive CMV titer was present in 62% of the study population, and in none of the patients did we detect CMV DNA in the circulating blood. Between the 2 groups defined by CMV titer, most of the baseline characteristics were evenly distributed, and there were no significant differences in any of the procedural or angiographic baseline variables (Tables 1 and 2; Figure 1). Nevertheless, the group with positive CMV titers was significantly older, comprised significantly more women, and tended to have a higher rate of hypertension and lower rate of acute infarction at presentation than the group with negative CMV titers (Table 1).

Angiographic Follow-Up
We obtained follow-up angiography in 451 patients, comprising 80% of the patients with positive CMV and 85% of the patients with negative titers (P=0.19). The binary restenosis

| TABLE 1. Baseline Demographic and Clinical Characteristics of the Study Cohort |
|---------------------------------------------|------------------|
| CMV-Positive (n=340) | CMV-Negative (n=211) |
| Age, y | 65.7±11.3 | 63.3±11.8 | 0.02 |
| Women | 95 (27.9) | 35 (16.6) | 0.002 |
| Active smoker | 115 (33.8) | 72 (34.1) | 0.94 |
| Hypercholesterolemia | 209 (61.5) | 124 (58.8) | 0.53 |
| Arterial hypertension | 230 (67.6) | 126 (59.7) | 0.06 |
| Diabetes mellitus | 55 (16.2) | 34 (16.1) | 0.98 |
| Multivessel disease | 246 (72.3) | 154 (73.0) | 0.87 |
| Previous balloon angioplasty | 49 (14.4) | 32 (15.2) | 0.81 |
| Previous coronary bypass operation | 26 (7.6) | 10 (4.7) | 0.18 |
| Previous myocardial infarction | 78 (22.9) | 43 (20.4) | 0.48 |
| Reduced left ventricular function | 139 (40.9) | 76 (36) | 0.26 |
| Acute myocardial infarction | 52 (15.3) | 45 (21.3) | 0.07 |
| Unstable angina | 113 (33.2) | 63 (29.9) | 0.41 |

Data are expressed as mean±SD or number of patients (percentage).
CMV-Positive (n=340) CMV-Negative (n=211) P

<table>
<thead>
<tr>
<th>Characteristic</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex lesions</td>
<td>288 (84.7)</td>
</tr>
<tr>
<td>Chronic occlusions</td>
<td>28 (8.2)</td>
</tr>
<tr>
<td>Lesion in venous bypass graft</td>
<td>18 (5.3)</td>
</tr>
<tr>
<td>Restenotic lesion</td>
<td>46 (13.5)</td>
</tr>
<tr>
<td>Vessel size, mm</td>
<td>3.09±0.52</td>
</tr>
</tbody>
</table>

**Before stenting**

- MLD, mm: 0.45±0.36 vs. 0.46±0.41, 0.74
- Diameter stenosis, %: 85.4±11.3 vs. 85.3±11.8, 0.97

**Procedural variables**

- Balloon-to-vessel ratio: 1.09±0.11 vs. 1.09±0.12, 0.69
- Inflation pressure, atm: 14.4±2.7 vs. 14.4±2.5, 0.88
- No. of stents: 1.75±1.07 vs. 1.72±1.27, 0.77
- Stent type: 0.55
- Jo-Stent: 97 (28.5) vs. 71 (33.6), 0.44
- InFlow: 97 (28.5) vs. 64 (30.3), 0.54
- Multilink: 97 (28.5) vs. 51 (24.2), 0.52
- Palmaz-Schatz: 36 (10.6) vs. 17 (8.1), 0.52
- Other: 13 (3.8) vs. 8 (3.8), 0.52
- Stented length, mm: 23.6±14.0 vs. 22.7±14.0, 0.47
- Perinterventional abciximab: 99 (29.1) vs. 65 (30.8), 0.67
- Multilink intervention: 67 (19.7) vs. 52 (24.6), 0.17
- After stenting
  - MLD, mm: 3.04±0.54 vs. 3.03±0.51, 0.76
  - Diameter stenosis, %: 5.3±8.9 vs. 4.8±6.1, 0.40

Data are expressed as mean±SD or number of patients (percentage). The rate of CMV-positive patients was 28.7% (95% confidence interval, 23.3% to 34.1%) in patients with positive CMV titers and 34.6% (95% confidence interval, 27.7% to 41.6%) in patients with negative CMV titers (odds ratio [95% confidence interval], 0.76 [0.51 to 1.14]; P=0.18). Even after correction for potentially confounding variables (age, sex, hypertension, acute myocardial infarction) by multivariate analysis, seropositivity for CMV was not predictive of angiographic restenosis (adjusted odds ratio, 0.78 [0.52 to 1.19]), nor were absolute titers (P=0.67). Patients with restenosis had similar CMV titers as patients without restenosis (median, 1/2650 versus 1/4100; P=0.29). Likewise, patients with CMV titers in the highest quintile (>1/11 000) of seropositive patients had a restenosis rate of 31.3%, which was not significantly different from that in seronegative patients (P=0.57). The lack of significant effect of seropositivity for CMV on angiographic restenosis was prevalent in various subgroups (Figure 2).

Other angiographic parameters of neointima formation, including late loss, loss index, net gain, and percent diameter stenosis at follow-up showed no significant differences between patients with and those without positive CMV titers (Table 3 and Figure 1). Neither seropositivity for CMV nor the height of titer correlated significantly with late loss on univariate (P=0.48) or multivariate analysis (P=0.38).

**Clinical Follow-Up**

One-year clinical follow-up was completed in all patients. Corresponding to the angiographic findings, we did not find significant differences in clinical restenosis rate between the two groups. The rate of target vessel reintervention was 16.8% (95% confidence interval, 12.8% to 20.7%) in patients with positive CMV titers and 17.5% (95% confidence interval, 12.4% to 22.7%) in patients with negative titers (odds ratio [95% confidence interval], 0.95 [0.60 to 1.49]; P=0.82).

During 1-year follow-up, 8 (2.4%) patients with positive CMV titers and 6 (2.8%) patients with negative CMV titers died (P=0.72). In addition, 6 (1.8%) Q-wave myocardial infarctions occurred in the group with positive CMV titers and 2 (0.9%) in the group with negative CMV titers (P=0.44).

**Discussion**

Our study does not suggest that previous CMV infection affects restenosis after coronary stent placement. Both clinical and angiographic restenosis rates were not higher in patients with previous CMV infection than in patients with-
Previous CMV Infection and Neointima Formation
To our knowledge, this is the largest study on the relation between previous CMV infection and restenosis after percutaneous coronary interventions. Our study was sufficiently powered to detect a clinically relevant effect of CMV infection on restenosis after stenting. It was even large enough to identify a difference smaller than the lower boundary of the 95% confidence interval of the odds ratio for restenosis in the earlier study that described a positive effect of CMV infection on restenosis after directional atherectomy.1 By trend, the point estimates of all indexes of restenosis were more favorable after CMV infection. Our conclusion that previous CMV infection does not induce a clinically relevant increase in restenosis after stenting, therefore, must be considered robust.

Our inability to demonstrate an effect of previous CMV infection on restenosis cannot be attributed to confounding variables. Most of the demographic, angiographic, or procedural covariables were evenly distributed between the groups with and without previous CMV infection. Nevertheless, there were significant inhomogeneities in age and sex between the groups defined by CMV titer and the prevalences of hypertension as well as presentation with myocardial infarction as shown by the current study. By multivariate analyses, we corrected for these inhomogeneities. These analyses confirmed that restenosis after stenting was independent of previous CMV infection.

We assessed the relation between neointima formation and CMV infection in the clinical setting that is most apt for this question.11 Neointima formation accounts for >90% of the angiographically detectable late lumen loss after stenting.17 Our findings, therefore, decline the hypothesis that previous CMV infection promotes neointima formation.

This inference is in conflict with one earlier study that assessed restenosis after directional atherectomy in 75 patients. The larger deep vessel injury after directional atherectomy as compared with stenting may serve as an explanation for the discrepancy between our study and the earlier study.1 A larger injury may be associated with a larger CMV reactivation. Hence, extensive vessel injury after atherectomy could still be a special, perhaps the only condition in which CMV-related mechanisms take effect on neointima formation. In addition, it must be considered that our study comprised a broader spectrum of patients. Contrary to the study by Zhou et al, it included small-vessel disease, venous bypass graft lesions, restenotic lesions, and infarct-related lesions. Our subgroup analyses (Figure 2) reveal that these lesion characteristics cannot account for the outcome of our study. Likewise, they do not support an interference by abiciximab, which had not been administered in the earlier study.

Mechanistic Considerations
Our inability to show an effect of previous CMV infection on neointima formation may be surprising in the light of earlier experimental studies.2,18,19 There is ample evidence that CMV infection inhibits apoptosis and facilitates cell cycle progression in cultured cells.2,18,19 It must be considered, however, that in the intact organism, additional mechanisms come into play. In the rat model, it has been shown that CMV infection induces endothelial proliferation.20 Accordingly, CMV infection may accelerate reendothelialization of the stented segment and thereby inhibit the inflammatory responses involved in neointima formation. In addition, CMV infection induces upregulation of the FAS-receptor, which is potentiated by locally released interferon-γ.21 By this mechanism, infection with CMV could render vascular smooth muscle cells more prone to T-lymphocyte–induced apoptosis.22 Under certain circumstances, CMV infection may even directly induce apoptosis and growth suppression, as shown for hematopoietic progenitors.22

Limitations of the Study
Intravital diagnosis of vascular infection is not feasible in patients, and the frequency of CMV DNA in the circulating blood of immunoocompetent patients is too low to be relevant to restenosis. As in previous studies,1,9 we therefore had to rely on antibodies titers to detect previous CMV infection. Although a more direct assay of vascular infection might have been desirable scientifically, from a clinical perspective only a relation between titers and restenosis would be relevant to decision-making. The antibody test that we used has proved a reliable tool to identify patients with previous CMV infection.9

Implications
In a previous publication,13 we reported an increased risk of early thrombotic events after coronary stent placement in patients with previous CMV infection. In the long run, however, the risk of serious adverse cardiac events is very similar in patients with or without previous CMV infection, as shown by the current study. In particular, we can now exclude additional hazards from increased neointima formation. In conjunction with the study on CMV infection and restenosis after plain balloon angioplasty9 and another large scale epidemiological survey,23 our current study strongly suggests that the vascular pro-proliferative effects of previous CMV infection are very limited, if at all present.

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