Statin Therapy Interacts With Cytomegalovirus Seropositivity and High C-Reactive Protein in Reducing Mortality Among Patients With Angiographically Significant Coronary Disease

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Background—Seropositivity to cytomegalovirus (CMV) and elevated C-reactive protein (CRP) may jointly predict increased mortality rates in patients with coronary artery disease (CAD). Therapy with statins reduces lipid levels but may also have other beneficial (eg, antiinflammatory) effects. This study prospectively evaluated the effect of statins on CMV- and CRP-associated death among patients with significant, angiographically defined CAD.

Methods and Results—We monitored 2315 patients with angiographically significant CAD (stenosis ≥70%) for an average of 2.4 years (maximum, 5.8 years). Anti-CMV IgG antibody levels and CRP concentrations were measured at baseline, and statin prescription was recorded. As previously reported, mortality rate was higher for CMV seropositivity (+) with high CRP (hazard ratio [HR], 2.0) and lower for statins (HR, 0.50). Compared with CMV(−)/low CRP (mortality rate, 5% with statin versus 4% without statin), the protective effect of statin therapy was markedly greater for CMV(+)/low CRP (mortality rate, 2% versus 7%; HR, 0.44; 95% CI, 0.16 to 1.3), CMV negative (−)/high CRP (mortality rate, 1% versus 8%; HR, 0.16), and CMV(+)/high CRP (mortality rate, 6% versus 17%; HR, 0.42; 95% CI, 0.25 to 0.70). After adjustment, interactions were found for statin therapy with CMV(+)/low CRP (P for interaction=0.065), CMV(−)/high CRP (P for interaction=0.051), and CMV(+)/high CRP (P for interaction=0.024).

Conclusions—The survival benefit of statins interacted with CMV seropositivity and high CRP to significantly reduce mortality rates among patients with CAD. This finding supports the hypothesis that statins have beneficial, “lipid-independent,” antiinflammatory effects. The mechanism of statin benefit associated with CMV seropositivity remains to be determined. (Circulation. 2003;107:258-263.)

Key Words: infection ▪ inflammation ▪ drugs ▪ survival ▪ risk factors

Inflammation may play a role in the initiation and progression of atherosclerosis.1–2 The inflammatory marker C-reactive protein (CRP) has been known to be elevated in patients with acute myocardial infarction (MI) for a considerable time3 and has been shown to predict risk of recurrent events.4–7 Despite the evidence of its association with coronary artery disease (CAD), the source of the CRP elevation in CAD has not been explained.

Although infections are not the only cause of inflammation, infections are a potential source of chronically elevated CRP. A variety of bacteria and viruses have been associated with CAD.8,9 One of these, cytomegalovirus (CMV), is a herpes-class respiratory virus that has been associated with coronary restenosis, transplant-induced atherosclerosis, and death in patients with CAD.10–12 Furthermore, an interactive increase in risk has been reported for the combination of CMV seropositivity and elevated CRP.11,12 It is unknown whether this CMV-related increase in risk is due to chronic CMV infection or is a marker of some adverse host-response to prior infection, but it does suggest potential new directions for therapy development.

Although anti-infective treatment trials are underway targeting Chlamydia pneumoniae, it is not yet clear whether such an approach will be successful in reducing events.13 Furthermore, antivirals remain in development. Anti-infective therapy for CAD treatment is thus not a clinically indicated reality14; however, other agents may provide benefit.

Statins are an available treatment for CAD that are safe and effective in lowering lipid levels and in reducing both
primary and secondary events.\textsuperscript{15,16} Although statins certainly provide clinical benefit through their lipid effects, some studies propose that statins may also have beneficial “lipid-independent” properties. In some bench studies, statins have been shown to possess immune modifying\textsuperscript{17,18} and anti-inflammatory effects.\textsuperscript{17,19,20} Recent clinical studies show a differential effect of statins on CRP-associated risk, supporting this anti-inflammatory hypothesis.\textsuperscript{6,21}

Although statin therapy appears to reduce CRP-related events, no study has evaluated the effect of statins on infection-related events. The purpose of the present study was to prospectively evaluate the effect of statin therapy on CMV-related and CRP-related death among patients with significant, angiographically defined CAD.

**Methods**

**Study Population**

Study patients were drawn from the cardiac catheterization registry of the Intermountain Heart Collaborative Study, a population of patients undergoing coronary arteriography at the LDS Hospital (Salt Lake City, Utah). Patients were of unrestricted age and sex and gave written informed consent for a blood draw at the time of angiography for use in confidential studies approved by the hospital’s institutional review board. Of 3970 consecutive patients enrolled between 1994 and 1999, 2703 were found to have clinically significant CAD, as defined by one or more \( \geq 70\% \) stenosis in \( \geq 1 \) coronary artery or a primary branch. These 2703 were considered for inclusion in this study.

**Follow-Up and Patient Outcomes**

Patients were followed up until death or 1999. Deaths were determined by telephone survey, hospital records, and health department records and were verified through Social Security death records. Patients not listed as deceased in any registry were considered to be alive.

**Variables Examined**

Determination of statin prescription, CRP concentrations, and CMV titers has been described.\textsuperscript{12,21} Briefly, enzyme-linked immunosorbent assay measured anti-cytomegalovirus IgG antibodies (Wampole Laboratories). Seropositivity was assigned by product specifications. CRP testing was performed with a regular sensitivity assay (Abbott Diagnostics), and CRP was categorized as high or low, on the basis of a previously determined break-point (1.2 mg/dL).\textsuperscript{12}

Prescription of statins at hospital discharge was determined from the LDS Hospital informatics system. Statin use before hospitalization was unknown, and compliance with therapy was not determined. Statin status was available for 2588 patients, of whom 2315 also had a CMV test and a CRP level, and these 2315 comprised the study cohort.

Other study variables included age, sex, diabetes, hypertension, hyperlipidemia, smoking, family history of early CAD, kidney failure, presenting diagnosis, number of diseased coronary vessels (as determined by angiography), and clinical interventions, as previously described.\textsuperscript{12,21} Furthermore, congestive heart failure (CHF), prior MI, prior cerebrovascular accident (CVA), discharge prescription of a \( \beta \)-blocker, and discharge prescription of an ACE inhibitor were included.

Briefly, physician-reported diabetes included patients with a fasting blood sugar \( \geq 126 \) mg/dL or use of an antihypertensive medication. Hyperlipidemia was physician-reported for total cholesterol \( \geq 180 \) mg/dL, LDL \( \geq 130 \) mg/dL, or use of cholesterol-lowering medication. Family history was self-reported when a first-order relative had cardiovascular death, MI, or coronary revascularization before age 65 years. Smoking included active or previous (>10 pack-years) tobacco use. Presentation included stable angina (exertional symptoms only), unstable angina (progressive symptoms or symptoms at rest), or MI (CK-MB \( >6 \) mg/dL and CK-MB index \( >3\% \)). Treatment included medical therapy (only), percutaneous coronary interventions (PCI) (angioplasty, atherectomy, and/or stent), and CABG. Comorbid kidney failure, CHF, prior MI, and prior CVA were determined from physician report. Prescription of \( \beta \)-blockers and ACE inhibitors was determined in the same way as statins.

**Statistical Considerations**

The \( \chi^2 \) test and Student’s \( t \) test were used appropriately to examine univariate associations with death. In situations with small numbers of events, Fisher’s exact test was used. Univariate Cox regression (SPSS, version 10.0) was also used to confirm these initial findings.

Multiple variable Cox regression was used to determine hazard ratios (HR) and 95% confidence intervals corrected for confounding. All covariates were included in regression modeling, and significant \( (P<0.05) \), near-significant \( (P<0.10) \), or confounding variables were entered; probability values are 2-tailed. Interaction analysis evaluated the multiplicative statistical interactions of statins with CMV and CRP, and the interactions were modeled as statins \( \times \) seropositive (+) CMV (low CRP), statins \( \times \) high CRP (seronegative (−) to CMV), and statins \( \times \) CMV (+)/high CRP.

**Results**

**Study Population**

The population characteristics by statin status are listed in Table 1; Table 2 shows them based on CMV and CRP. Overall, death was confirmed in 216 patients (9.3%). The average patient follow-up was 2.4 years (maximum, 5.8 years). Statins were prescribed at discharge to 28% of patients, CMV seropositivity was found in 76% of patients, and 59% of patients had a high CRP.

Stratification by CMV and CRP revealed that 11% of patients were CMV(−) with low CRP, 30% were CMV(+) but had low CRP, 13% were CMV(−) with high CRP, and 46% were CMV(+) and had high CRP.

**Associations With Death**

In multiple variable Cox regression, statins significantly reduced the mortality rate (Figure 1) from 12% for no statin to 4% for statin recipients (HR, 0.50; CI, 0.32 to 0.78; \( P=0.002 \)). Mortality rate was 14% for CMV(+)/high CRP patients (HR, 2.0; CI, 1.1 to 3.7; \( P=0.03 \)) compared with 4% for CMV(−)/low CRP; however, both CMV(+)/low CRP (mortality rate, 5%; HR, 0.87; CI, 0.44 to 1.7; \( P=0.69 \)) and CMV(+)/low CRP (mortality rate, 6%; HR, 1.1; CI, 0.52 to 2.4; \( P=0.77 \)) were not different from the event rate in the CMV(−)/low CRP group (Figure 2). Similar findings have been shown previously in subsets of this population.\textsuperscript{12,21}

Other multivariable predictors of death included age (HR, 1.6 per decade; CI, 1.4 to 1.8), diabetes (HR, 1.6; CI, 1.2 to 2.2), hyperlipidemia (HR, 0.73; CI, 0.55 to 0.97), CHF (HR, 2.4; CI, 1.8 to 3.2), prior CVA (HR, 2.0; CI, 0.91 to 4.3), presentation with unstable angina (HR, 1.4; CI, 1.03 to 2.0) or acute MI (HR, 1.5; CI, 1.1 to 2.1), 2-vessel (HR, 1.6; CI, 1.1 to 2.4) or 3-vessel disease (HR, 2.0; CI, 1.4 to 2.9), PCI (HR, 0.75; CI, 0.52 to 1.08), and CABG (HR, 0.52; CI, 0.36 to 0.73). \( \beta \)-Blockers and ACE inhibitors were not significant predictors of death in this general CAD population and did not confound the effect of statins.
Interaction of Statins With CMV and CRP

The effect of statins on death is demonstrated by Kaplan-Meier survival curves for the CMV/CRP strata in Figure 3. Although no difference was found between statins and no statins for patients with CMV(−)/low CRP, patients received profound benefit from statins when either CMV was positive or CRP was high.

The mortality trend among patients not receiving statins (P for trend <0.001, Wald score=38.42) was highly significant, but not for those receiving statins (P for trend=0.18, Wald score=1.79). Furthermore, compared with the protective effect of statins in CMV(−)/low CRP patients (mortality rate, 5% versus 4%; P=0.44; HR, 1.7; CI, 0.44 to 6.5), the statin benefit was markedly greater in each other stratum (Figure 4). Patients with CMV(+) (low CRP) had mortality rates of 2% (statins) compared with 7% without statins (HR, 0.44; 95% CI, 0.16 to 1.3), and the interaction was near significant (P interaction=0.065). For patients with high CRP [CMV(−)], mortality rate was 1% compared with 8% (HR, 0.16; 95% CI, 0.02 to 1.2), and the interaction was near significant (P interaction=0.051). Patients with CMV(+) high CRP had mortality rates of 6% compared with 17% (HR, 0.42; 95% CI, 0.25 to 0.70) and a significant interaction existed (P interaction=0.024).

Discussion

Inflammation and Infection in CAD

Inflammation, marked by CRP, is a strong predictor of the initiation and progression of atherosclerosis.1–7 and CRP may be an important component for clinical risk evaluations. Because infection is a cause of inflammation, it may contribute to chronically increased CRP.
Several pathogens have been associated with CAD, especially the bacterium *Chlamydia pneumoniae*, but also including several viruses such as CMV. It is not known if infection-related risk is due to chronic infection, is a marker of some autoimmune disorder, or is an epidemiologic surrogate for a previously nondescribed factor. Because of the wealth of evidence for *C pneumoniae*, though, trials of anti-infective treatment among patients with CAD have been undertaken. These studies focus on antibiotics that cannot affect viral infections such as CMV; additionally, event reductions in these studies have not been as profound as was initially hoped. Because CMV is a virus and antivirals are in their infancy, treating CAD by elimination of CMV is not currently possible.

**Statins and CAD**

Beyond anti-infective therapies, other medications may provide a means to treat infection-associated effects in CAD. Statins are an effective and available treatment for CAD that reduces plasma lipid levels and reduces events.

**Figure 1.** Kaplan-Meier survival curve showing effect of statins on death. As we first reported, the statin benefit is seen very soon after posthospitalization therapy initiation.

**Figure 2.** Kaplan-Meier survival curve for death, based on CMV and CRP.

**Figure 3.** Kaplan-Meier survival curves for statins in CMV/CRP groups. Although the statin effect was not significant among (A) CMV negative (−)/low CRP patients, patients with (B) CMV positive (+)/low CRP, (C) CMV(−)/high CRP, and (D) CMV(+) high CRP all had clinically important survival benefits.

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Statins, CMV, CRP, and Risk

In the present study, patients had a significantly greater relative reduction in mortality rates from statin therapy if they were seropositive to CMV and/or had high CRP. That is, the effect of statins among patients who were CMV-seropositive or had high CRP was more than the additive effect of having higher absolute mortality rates in the CMV and CRP strata, and in fact was a multiplicative effect, wherein statins produced not only a greater absolute reduction but also a significantly greater relative reduction in the CMV-seropositive and high CRP groups. Furthermore, the effects of statins on CMV-associated risk in CAD were independent of their effects on CRP-related risk, with the interactive statin benefit being seen in all patients who were CMV-seropositive, who had a high CRP, or who had both conditions (of note, a similar result has been observed for LDL and CRP). To our knowledge, this is the first prospective study reporting a beneficial effect from statin therapy on infection-associated cardiovascular outcomes.

The mechanisms whereby statins might reduce CRP- or CMV-associated events are not demonstrated by the present study. For CMV, although a direct antiviral effect of statins is not out of the realm of possibility, it is unlikely in our view. The potential immune-altering, antiinflammatory, or antioxidant effects of statins described above are more likely explanations. Perhaps statins act on a final common oxidant/inflammatory pathway through which smoldering CMV infection, oxidized LDL and other lipid moieties, and other common risk factors act.

Study Limitations

This study was a prospective but observational evaluation of mortality rates. Treatment was not randomized, but statin type and dose were left to the physician’s discretion. Only statin prescription at discharge was known. Drop-in and drop-out rates were not determined, although data from a subset of these patients suggests the persistence of statin use over 3 years. It should be noted that both drop-in to treatment by patients not prescribed a statin at discharge and drop-out from treatment by those who did receive a prescription would necessarily only bias the study outcome toward the null hypothesis.

The statin benefits in the present study could be confounded by, or due in part to, selection biases. We adjusted the effect of statins for many potential confounders, including CRP, concomitant medications, and clinical treatment. Despite these adjustments, the effect of statins was little altered and remained independently significant. Furthermore, because CMV serological status and CRP concentration were unknown and not used to prescribe statin therapy, they did not differ on the basis of statin use over 3 years. It should be noted that both drop-in to treatment by patients not prescribed a statin at discharge and drop-out from treatment by those who did receive a prescription would necessarily only bias the study outcome toward the null hypothesis.

The use of all-cause death may be seen as a limitation. For a cause of death to be assigned, however, definitions of the cause must be constructed, and these are usually defined according to mechanistic constructs that rely on adjudication of a patient’s symptoms and for which correct classification does not always occur. In contrast, all-cause death is a definite, indisputable end point. All-cause death does not rely on adjudication of symptoms. Additionally, all-cause death is more reliable because mechanisms of death that are unrelated to the study hypothesis—if truly unrelated—should be spread randomly among the categories of any given variable. Finally, although use of cause of death may be a time-honored tradition, we have only been able to determine cause of death among ~60% of the patients; however, among those for whom cause is known, ~80% of deaths are cardiovascular.
A paradoxical negative association with death of a clinical diagnosis of hyperlipidemia has been observed consistently in our database. A diagnosis of hyperlipidemia may be, in part, a surrogate for more aggressive treatment with statins after discharge and other medical or preventive therapies. In our study, the benefit of statins remained even after correction for hyperlipidemia and other standard risk factors.

Conclusions
Statins interacted with CMV and CRP to reduce mortality rates among patients with significant, angiographically defined CAD. This is the first study to report a benefit of statin therapy on infection-associated clinical events: Statins reduced the increased mortality rates associated with CMV seropositivity. This benefit was independent of CRP but also reduced CRP-associated mortality rates. These results support the concept that statins exert non-lipid-lowering effects on CAD risk, including antiinflammatory properties. Uniquely, they also suggest that these effects extend beyond those associated with CRP. Additional studies will be required to verify this observation and provide a mechanistic basis. Meanwhile, this study supports an aggressive approach to statin use among the CAD population, especially among those with inflammatory and infectious risk markers.

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References
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