Higher Immunoglobulin G Antibody Levels Against Cytomegalovirus Are Associated With Incident Ischemic Heart Disease in the Population-Based EPIC-Norfolk Cohort

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Background. Cytomegalovirus (CMV) is associated with ischemic heart disease (IHD) among organ transplant recipients. The relationship between the levels of antibody for CMV with IHD in immunocompetent individuals is uncertain.

Methods. We measured baseline CMV immunoglobulin G (IgG) levels in 12,574 participants without IHD from the population-based EPIC-Norfolk cohort, aged 40–70 years old at recruitment in 1993–1997. Underlying causes of death or hospitalization until 31 March 2008 were abstracted from death certificates and a database of hospital admissions, respectively.

Results. Of the participants, 58% were seropositive for CMV. After a mean follow-up of 12 years (standard deviation, 2.2 years), 1,356 first-time IHD events occurred. After adjustment for classic IHD risk factors, belonging to the highest antibody group was associated with an increased risk of incident IHD, compared with seronegativity (hazard ratio, 1.22; 95% confidence interval, 1.05–1.42). After additional adjustment for measures of social class, inflammation, and possible confounders, this association was unchanged (hazard ratio, 1.21; 95% confidence interval, 1.04–1.41).

Conclusions. This is the first population study to show that CMV IgG antibody levels are related to incident IHD compared to seronegativity. Studies correlating CMV antibody levels with direct measurements of active infection will be necessary.

Cytomegalovirus (CMV) is a herpes virus that, after primary infection, persists in a latent state for the duration of the life of the host. CMV has been thought to be causally related to accelerated atherosclerosis of various solid organ transplants [1]. CMV has also been associated with the development of ischemic heart disease (IHD) among recipients of heart transplants [2–4].

The association between CMV and IHD in immunocompetent persons is not established [5]. Although several prospective studies have shown possible associations between CMV and measures of coronary artery atherosclerosis, very few have studied well defined acute myocardial infarction (MI) outcomes. Instead, they have either examined mixed vascular outcomes that only partly included classic IHD [6–17] or coronary restenosis after medical interventions in individuals with established IHD [18–20]. The associations of cardiovascular risk factors with the vascular anatomy are not uniform [21], however, and these studies might have missed a clinically important association with CMV. Other prospective studies have reported on the association between previous infection with CMV...
and fatal and nonfatal MI [22–28], but with conflicting results. Moreover, 4 of these studies were trials [22, 23, 25, 28] and 2 were limited in their inclusion criteria [26, 27]; only 1 of the studies was population based [24] but was limited to participants >65 years old. One study has shown an association between high CMV antibody levels and IHD but did not have seronegativity as the reference category [29]. Furthermore, very few studies have attempted to correct for confounding by socioeconomic status, although this may be important for IHD [30], given that CMV infection is socially patterned [31]. As a result, the role of CMV in the etiology of native primary IHD in the general population remains unclear.

CMV is known to increase experimental atherosclerosis and to modulate vascular-wall activity [32, 33] and has been shown to cause allograft vasculopathy in the context of organ transplantation [34]. High CMV immunoglobulin G (IgG) levels are thought to represent more frequent CMV reactivation, although such correlation has not been conclusively shown. In the current study we examined whether seropositivity for CMV and the level of CMV IgG antibody are associated with IHD among participants of a population-based cohort study and whether the associations are confounded by socioeconomic status.

METHODS

Cohort Characteristics

We studied this association in the EPIC-Norfolk study, a UK population-based cohort [35], which recruited 25,639 men and women, aged 40–79 years at baseline, between 1993 and 1997. All volunteers gave written informed consent and the study was approved by the Norfolk Research Ethics Committee. At the time of the study, 19,437 participants had stored available serum samples; from these, we randomly selected 13,090 participants. Because the aim was to study the risk of incident primary IHD and to avoid including individuals with clinically significant IHD, we excluded 425 participants who had reported a history of MI at baseline, leaving 12,665 participants, all white, eligible for CMV antibody measurements.

Questionnaires and Biochemical and Hematological Analyses

Anthropometric measurements were taken, and a health and lifestyle questionnaire that included questions on housing, occupational social class, educational level, use of tobacco, and physical activity was completed at baseline. A validated 4-point ordered categorical physical activity index was used [35]. The participants were asked whether they had ever had a diagnosis of a “heart attack (myocardial infarction)” or “diabetes” and whether any first-degree family member had ever had a “heart attack (myocardial infarction).” Biochemical and hematological parameters were measured using standard assays [35].

IHD Ascertainment

Mortality data and hospital admission data for all EPIC-Norfolk participants were available up to 31 March 2008. All participants have been flagged for death certification at the Office of National Statistics (United Kingdom), with vital status ascertained for the whole cohort. Coding of death certificates was executed by trained nosologists according to the International Classification of Diseases (ICD), versions 9 or 10. All deaths with ICD-9 codes were recoded into corresponding ICD-10 codes. Cause-specific hospital admission was determined via ENCORE, the hospital admissions database kept by the East Norfolk Health Commission, using individuals’ unique National Health Service numbers. IHD deaths were defined as any death where the underlying cause of death was angina pectoris, MI and its complications or other acute IHD (codes 410–414 [ICD 9] or I20–I25 [ICD 10]); IHD hospital admissions were similarly defined. Self-reported MIs did not qualify as incident disease. Death certificates and ENCORE show high accuracy in correctly identifying underlying causes of death and hospitalization, as previously shown in EPIC-Norfolk [36]. The primary outcome was any first IHD event during follow-up, and the end point was defined as either hospital admission or death because of IHD. Follow-up was censored at the earliest day of IHD hospital admission, the date of death, or 31 March 2008.

CMV Measurements

CMV-specific IgG measurements were performed by the Cambridge University Hospitals Virology Laboratory, using an indirect chemiluminescence immunoassay (Liaison; Diasorin), with baseline blood samples only. The amount of isoluminol-antibody conjugate is measured by a photomultiplier in relative light units (RLUs). The machine, using an internal algorithm, converts RLUs to antibody levels. The coefficient of variation for the assay is <8%, and the assay compares favorably to other CMV IgG assays for confirmation of past CMV infections [37]. Samples were shipped to the laboratory in randomly ordered boxes, with no identifiers other than a barcode. A sample was defined as being negative, equivocal, or positive for CMV IgG antibody according to the clinical antibody cutoffs for the assay (<0.4, 0.4–0.6, and >0.6 IU/mL, respectively). Ninety-one participants had equivocal test results after measurement and were excluded. Data from 12,574 participants (5,551 men and 7,023 women) were included in this analysis.

Statistical Analyses

RLUs were standardized by calculating the difference from the mean and dividing by the standard deviation (SD) within the day of measurement, to account for any differences in assay performance from day to day. Standardized RLUs among
participants with positive tests only were grouped into thirds of the distribution.

We summarized baseline characteristics within the cohort using means and SDs for continuous variables with an approximately normal distribution, medians and interquartile ranges for continuous variables with a skewed distribution (CMV antibody level, Townsend deprivation index [38], alcohol consumption, and C-reactive protein [CRP], high-density lipoprotein [HDL], and triglyceride levels), and percentages for binary variables. Among participants seropositive for CMV IgG, a P value for linear trend in the baseline characteristic across thirds of the distribution was calculated using linear regression (continuous variables) or logistic regression (binary variables), with adjustment for age and sex. Continuous variables with skewed distributions were log transformed. Similar methods were used to test the difference between participants with positive and negative test results.

We used Cox proportional hazards regression models to estimate age- and sex-adjusted hazard ratios (HRs) of incident IHD, comparing seropositive with seronegative individuals, and also comparing seropositive participants in 3 categories of RLU values. The assumption of proportional hazards was assessed by inspecting Kaplan-Meier plots of the survivor function within each group.

Within the Cox model we tested for interactions between the exposure of interest (either seropositive vs seronegative or by thirds across the distribution of RLU values) and sex, age at time of entry to the study (continuous), socioeconomic status (manual vs nonmanual employment), physical activity, body mass index (continuous), CRP level (continuous), lipid levels (high-density lipoprotein and total cholesterol; continuous), history of prevalent diabetes mellitus and family history of IHD at baseline. No interactions were identified. We fit multivariable models, adjusting for various potential confounders. Because of low data missingness, we fit the models at the maximum number of participants for whom covariate information was available.

Covariates were chosen as possible confounders based on a priori hypotheses and whether they were IHD risk factors that were associated with CMV seropositivity in our study. All statistical analyses were performed using Stata/SE 12.0 software (StataCorp).

**RESULTS**

**CMV Seropositivity**

Fifty-eight percent of the participants were seropositive for CMV. Significantly more women than men were seropositive men (60% vs 56%, respectively; P < .001), and CMV seropositivity increased with age. Higher CMV IgG antibody levels were associated with older age and female sex. After adjustment for age and sex, seropositivity for CMV was associated

<table>
<thead>
<tr>
<th>Table 1. Baseline Demographic, Lifestyle, and Comorbidity Characteristics of Participants in the EPIC-Norfolk Cohort CMV Study (N = 12 574), Grouped by CMV Antibody Status</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Seronegative for CMV Antibodies (n = 5225)</strong></td>
</tr>
<tr>
<td>CMV IgG antibody, median (IQR), IU/mL</td>
</tr>
<tr>
<td>Age at study entry, mean (SD), y</td>
</tr>
<tr>
<td>Women, %</td>
</tr>
<tr>
<td>Education, A level and above, %</td>
</tr>
<tr>
<td>Social class, nonmanual employment, %</td>
</tr>
<tr>
<td>Townsend deprivation index, median (IQR)</td>
</tr>
<tr>
<td>Ever smokers, %</td>
</tr>
<tr>
<td>Alcohol consumption, median (IQR), units/wk</td>
</tr>
<tr>
<td>Physical activity, moderately or very active, %</td>
</tr>
<tr>
<td>Family history of IHD, %</td>
</tr>
<tr>
<td>Prevalent diabetes mellitus, %</td>
</tr>
</tbody>
</table>

Abbreviations: CMV, cytomegalovirus; IgG, immunoglobulin G; IHD, ischemic heart disease; IQR, interquartile range; SD, standard deviation.

a participants with positive tests only were grouped into thirds of the standardized RLU distribution as described in the methods.

b Age- and sex-adjusted P values for trend among antibody groups within seropositive participants.

c Age- and sex-adjusted P values for comparisons between participants who were seropositive for CMV IgG antibodies and those who were seronegative.

d “A level” corresponds to 12 years of school education in the United Kingdom.

e The Townsend deprivation index is described elsewhere [38].
with a personal history of smoking, manual employment, lower academic attainment and deprivation, higher body mass index, lower HDL levels, and higher triglyceride levels (Tables 1 and 2). After adjustment for age and sex, there was an association between higher CMV IgG antibody levels and higher CRP, higher total cholesterol, lower HDL, and higher triglyceride levels (Tables 1 and 2). After adjustment for classic IHD risk factors, belonging to the highest antibody group, compared with seronegativity, was associated with an increased risk of incident IHD (HR, 1.22; 95% CI, 1.05–1.41) (Table 3). The association with high antibody levels also raises the possibility that recent or active CMV reactivations might be mainly associated with IHD rather than all CMV infections. Indeed, CMV infection could be one of the drivers of inflammation, de

\[ H_R = \frac{1.22}{1.05} \]

This is the first population-based study examining IHD in association with CMV seropositivity as well as CMV IgG antibody levels, in a comparison with seronegative individuals. The data suggest that increasing antibody levels of CMV may be associated with incident IHD. Higher CMV IgG antibody levels as well as CMV DNA in the urine are more frequently found among older individuals [39] suggesting longer exposure to subclinical CMV reactivation. This observed dose response, added to previous observations of the association between CMV and cardiovascular diseases, underlines the relevance of CMV infection for native primary coronary artery atherosclerosis. The association with high antibody levels also raises the possibility that recent or active CMV reactivations might be mainly associated with IHD rather than all CMV infections. Indeed, CMV infection could be one of the drivers for inflammation [23], previously associated although possibly not causally related to IHD. Adjustment for inflammation, as measured by CRP, did not affect the HRs, suggesting that the observed association is more likely a CMV-specific effect, rather than solely an inflammatory effect.

**DISCUSSION**

The prospective studies in the past that examined the association between CMV seropositivity and incident MI and found it to be null did not examine the associations with antihypertensives, statins, or glucose-control medications.

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**Table 2. Baseline Anthropometric and Biochemical Characteristics of Participants in the EPIC-Norfolk Cohort CMV Study (N = 12,574), Grouped by CMV Antibody Status**

<table>
<thead>
<tr>
<th></th>
<th>Seronegative for CMV Antibodies (n = 5,227)</th>
<th>Low Antibody Group (n = 2,459)</th>
<th>Middle Antibody Group (n = 2,449)</th>
<th>High Antibody Group (n = 2,439)</th>
<th>( p^a )</th>
<th>( p^b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV IgG antibody, median (IQR), IU/mL</td>
<td>0.2 (0.2–0.2)</td>
<td>3.1 (1.9–4.1)</td>
<td>6.9 (5.7–8.4)</td>
<td>14.1 (10.7–20.8)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Body mass index, mean (SD), kg/m(^2)</td>
<td>26.0 (3.7)</td>
<td>26.4 (3.7)</td>
<td>26.3 (3.9)</td>
<td>26.5 (4.1)</td>
<td>.51</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Waist-to-hip ratio, mean (SD)</td>
<td>0.85 (0.1)</td>
<td>0.86 (0.1)</td>
<td>0.85 (0.1)</td>
<td>0.85 (0.1)</td>
<td>.51</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>C-reactive protein, median (IQR), mg/L</td>
<td>1.4 (0.7–3)</td>
<td>1.6 (0.8–3.2)</td>
<td>1.6 (0.8–3.4)</td>
<td>1.8 (0.9–3.6)</td>
<td>.04</td>
<td>.12</td>
</tr>
<tr>
<td>Cholesterol, mean (SD), mmol/L</td>
<td>6.1 (1.1)</td>
<td>6.2 (1.2)</td>
<td>6.2 (1.2)</td>
<td>6.2 (1.4)</td>
<td>.001</td>
<td>.85</td>
</tr>
<tr>
<td>HDL, median (IQR), mmol/L</td>
<td>1.4 (1.1–1.7)</td>
<td>1.4 (1.1–1.6)</td>
<td>1.4 (1.1–1.7)</td>
<td>1.4 (1.1–1.7)</td>
<td>.002</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>LDL, mean (SD), mmol/L</td>
<td>3.9 (1.0)</td>
<td>3.9 (1.0)</td>
<td>3.9 (1.0)</td>
<td>4.0 (1.0)</td>
<td>.07</td>
<td>.73</td>
</tr>
<tr>
<td>Triglycerides, median (IQR), mmol/L</td>
<td>1.5 (1–2.2)</td>
<td>1.5 (1–2.2)</td>
<td>1.5 (1–2.3)</td>
<td>1.5 (1–2.2)</td>
<td>.61</td>
<td>.002</td>
</tr>
<tr>
<td>SBP, mean (SD), mm Hg</td>
<td>134 (18)</td>
<td>135 (18)</td>
<td>136 (18)</td>
<td>137 (19)</td>
<td>.05</td>
<td>.59</td>
</tr>
<tr>
<td>DBP, mean (SD), mm Hg</td>
<td>82 (11)</td>
<td>82 (11)</td>
<td>83 (11)</td>
<td>83 (11)</td>
<td>.12</td>
<td>.99</td>
</tr>
</tbody>
</table>

Abbreviations: CMV, cytomegalovirus; DBP, diastolic blood pressure; HDL, high-density lipoprotein; IgG, immunoglobulin G; IQR, interquartile range; LDL, low-density lipoprotein; SBP, systolic blood pressure; SD, standard deviation.

\( ^a \) Age- and sex-adjusted \( P \) values for comparison between participants who were seropositive for CMV IgG antibodies and those who were seronegative.

\( ^b \) Age- and sex-adjusted \( P \) values for trend among antibody groups within seropositive participants.

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1900 • JID 2012:206 (15 December) • Gkrania-Klotsas et al
CMV antibody levels. Studies that did find associations between CMV antibody levels and IHD did not have seronegativity as the reference category, did not adjust for potential confounding factors [29], or examined postangioplasty coronary restenosis [40] or composite outcomes [17]. As a result, the observed positive associations could have been due to confounding, did not support a possible CMV infection dose effect, and had limited clinical applicability for IHD alone, because CMV-driven atherosclerosis might not be as important for all vascular territories. Our study shows for the first time that high CMV antibody levels, compared with seronegativity, are associated with IHD in a population-based cohort study and that the association is independent of a number of possible confounding factors.

Deaths from cardiovascular diseases represent a third of global deaths every year [41] and are predicted to remain the single most important cause of death, well into 2030. It is estimated that approximately a quarter of the patients with a first MI do not have any of the established risk factors other than age and sex [42]. The prevalence of CMV infection in most countries of the world is 70%–90% and is highest in the developing countries [43]. After the recent successes in reducing incident IHD by controlling hyperlipidemia and smoking [44], the relative importance of possible common risk factors with small effects increases. This is especially true for the developing world, where dramatic rises in the incidence of IHD are expected [45]. Further studies of the association between CMV and human atherosclerosis are needed.

Measurement error may have affected our results, although the assay used has good performance characteristics [46]. Error may have also been introduced by inaccuracies in death certificate information and hospital admission diagnostic codes although it is unlikely that such error would have been differential with regard to CMV IgG status.

Residual confounding might have also affected our results. Although it is postulated that higher CMV IgG antibody levels represent more frequent or intense subclinical CMV reactivation from latency, this has not been conclusively shown. It is possible that CMV IgG antibody levels are elevated through some unknown mechanism and that the underlying cause is also associated with the risk of incident IHD. This unknown cause is less likely to be contemporary social deprivation, because

Table 3. Hazard Ratios for Ischemic Heart Disease

<table>
<thead>
<tr>
<th>Model and Covariates Includeda</th>
<th>Participants, No.</th>
<th>All Seropositive for CMV IgG Antibodies</th>
<th>Low Antibody Group</th>
<th>Middle Antibody Group</th>
<th>High Antibody Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1: age, sex</td>
<td>12 574</td>
<td>1.12 (.99–1.25)</td>
<td>1.05 (.90–1.21)</td>
<td>1.07 (.92–1.24)</td>
<td>1.25 (1.08–1.44)</td>
</tr>
<tr>
<td>Model 2: age, sex, smoking, SBP, DBP, LDL, HDL, triglycerides, prevalent diabetes, family history</td>
<td>11 858</td>
<td>1.09 (.97–1.23)</td>
<td>1.02 (.87–1.20)</td>
<td>1.04 (.90–1.22)</td>
<td>1.22 (1.05–1.42)</td>
</tr>
<tr>
<td>Model 3: age, sex, smoking, SBP, DBP, LDL, HDL, triglycerides, prevalent diabetes, family history, educational level, occupation, and Townsend index</td>
<td>11 608</td>
<td>1.09 (.97–1.22)</td>
<td>1.02 (.87–1.19)</td>
<td>1.04 (.90–1.22)</td>
<td>1.21 (1.04–1.41)</td>
</tr>
<tr>
<td>Model 4: age, sex, smoking, SBP, DBP, LDL, HDL, triglycerides, prevalent diabetes, family history, educational level, occupation, Townsend index, and use of antihypertensives, statins, or glucose-control medications</td>
<td>11 602</td>
<td>1.10 (.97–1.23)</td>
<td>1.03 (.88–1.21)</td>
<td>1.04 (.90–1.22)</td>
<td>1.22 (1.04–1.41)</td>
</tr>
<tr>
<td>Model 5: age, sex, smoking, SBP, DBP, LDL, HDL, triglycerides, prevalent diabetes, family history, educational level, occupation, Townsend index, use of antihypertensives, statins, or glucose-control medications, alcohol use, body mass index</td>
<td>11 584</td>
<td>1.10 (.97–1.23)</td>
<td>1.03 (.88–1.21)</td>
<td>1.05 (.90–1.23)</td>
<td>1.21 (1.04–1.41)</td>
</tr>
<tr>
<td>Model 6: age, sex, smoking, SBP, DBP, LDL, HDL, triglycerides, prevalent diabetes, family history, educational level, occupation, Townsend index, use of antihypertensives, statins, or glucose-control medications, alcohol use, body mass index, C-reactive protein</td>
<td>11 022</td>
<td>1.08 (.95–1.22)</td>
<td>1.01 (.86–1.19)</td>
<td>1.02 (.87–1.20)</td>
<td>1.21 (1.04–1.41)</td>
</tr>
</tbody>
</table>

The reference group includes participants seronegative for CMV IgG antibodies, from the EPIC-Norfolk cohort CMV study. Participants with positive tests only were grouped into thirds of the standardized RLU distribution as described in the methods.

Abbreviations: CMV, cytomegalovirus; DBP, diastolic blood pressure; HDL, high-density lipoprotein; IgG, immunoglobulin G; LDL, low-density lipoprotein; SBP, systolic blood pressure.

a Age indicates age at recruitment to the study; family history, any history of a first-degree family member having had a myocardial infarction or “heart attack”; smoking, lifetime history of smoking; Townsend index, Townsend deprivation index [38].
after adjustment for multiple variables associated with social status in our analysis, the observed HRs did not change. A longitudinal study prospectively correlating the frequency and intensity of CMV reactivation with CMV IgG levels would be necessary to further elucidate the observed association.

In conclusion, this is the first population cohort study to show a significant association between high CMV antibody levels and IHD, in a comparison with seronegative individuals. We show that the association with IHD is not explained by various measures of social deprivation and persists after adjustment for various IHD risk factors and possible confounders. Studies attempting to correlate the levels of IgG CMV antibody with longitudinal measurements of viral load or other direct measurements of CMV reactivation will be necessary to further explore the observed associations.

Notes

Acknowledgments. All authors contributed to the study conception and design and the analysis and interpretation of data and approved the final version of the article to be published.

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