

Circulation

JOURNAL OF THE AMERICAN HEART ASSOCIATION



Prospective Study of Pathogen Burden and Risk of Myocardial Infarction or Death

Jianhui Zhu, F. Javier Nieto, Benjamin D. Horne, Jeffrey L. Anderson, Joseph B. Muhlestein and Stephen E. Epstein

Circulation 2001;103:45-51

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75214

Copyright © 2001 American Heart Association. All rights reserved. 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/cgi/content/full/103/1/45>

Subscriptions: Information about subscribing to *Circulation* is online at
<http://circ.ahajournals.org/subscriptions/>

Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, a division of Wolters Kluwer Health, 351 West Camden Street, Baltimore, MD 21202-2436. Phone: 410-528-4050. Fax: 410-528-8550. E-mail:
journalpermissions@lww.com

Reprints: Information about reprints can be found online at
<http://www.lww.com/reprints>

Prospective Study of Pathogen Burden and Risk of Myocardial Infarction or Death

Jianhui Zhu, MD, PhD; F. Javier Nieto, MD, PhD; Benjamin D. Horne, MPH; Jeffrey L. Anderson, MD; Joseph B. Muhlestein, MD; Stephen E. Epstein, MD

Background—We previously demonstrated that the risk of coronary artery disease (CAD) increased in relation to the number of pathogens (the “pathogen burden”) in a cross-sectional study. In the present prospective study with a different patient cohort, we evaluated the effect of pathogen burden on the risk of myocardial infarction (MI) or death among CAD patients.

Methods and Results—IgG antibodies to cytomegalovirus (CMV), hepatitis A virus (HAV), herpes simplex virus type 1 (HSV1), HSV type 2 (HSV2), *Chlamydia pneumoniae* and *Helicobacter pylori*, and C-reactive protein (CRP) levels were tested in baseline blood samples from 890 patients who had significant CAD on angiography. The mean follow-up period was 3 years. The baseline prevalence of antibodies directed against CMV, HAV, HSV1, or HSV2, but not *C pneumoniae* and *H pylori*, was significantly higher among patients who subsequently developed MI or death than among control subjects. After adjustment for traditional risk factors, number of diseased vessels, and clinical presentation, relative hazards (95% confidence limits) for MI or death were 2.0 (1.4 to 3.2) for CMV, 1.6 (1.1 to 2.3) for HAV, and 1.5 (1.0 to 2.2) for HSV2. Increasing pathogen burden was significantly associated with increasing risk of MI or death in a dose-response fashion. Adjusted relative hazards of MI or death associated with pathogen burden were significant among individuals with low or high CRP levels.

Conclusions—The results suggest that infection plays an important role in incident MI or death and that the risk posed by infection is independently related to the pathogen burden. (*Circulation*. 2001;103:45-51.)

Key Words: pathogens ■ antibodies ■ coronary disease ■ myocardial infarction

Despite accumulating evidence that infection predisposes to the development of atherosclerosis,¹⁻¹⁰ several controversial areas remain¹¹⁻¹⁴; these areas include the continued reports of seroepidemiological studies that fail to show an association between infection and atherosclerosis and the question of which (if any) of the several pathogens associated with atherosclerosis, including cytomegalovirus (CMV), *Chlamydia pneumoniae*, *Helicobacter pylori*, and herpes simplex virus (HSV), pose a true risk.

We reasoned that if infection is in fact causally related to coronary artery disease (CAD), it would be unlikely that a single agent plays a unique role. Rather, we considered that multiple infectious agents contribute to atherosclerosis and hypothesized that the risk of cardiovascular disease posed by infection is related to the number of pathogens to which an individual has been exposed (the “pathogen burden”).¹⁵ We evaluated that hypothesis in a group of individuals under evaluation for CAD and found that CAD risk increased significantly in relation to pathogen burden.¹⁶ We also demonstrated for the first time that exposure to hepatitis A virus (HAV), as demonstrated by IgG anti-HAV antibodies, was significantly associated with increased CAD risk.¹⁶

Although these results were intriguing, the study was cross-sectional in design. To determine the validity of the original observation, in the present study we examined prospectively, and in an entirely different population, the hypothesis that the risk of myocardial infarction (MI) or death among CAD patients relates to pathogen burden and that HAV is one of the pathogens that contributes to this risk.

Methods

Patients

The patient cohort consisted of 890 individuals undergoing coronary angiography at LDS Hospital, Salt Lake City, Utah. Each patient gave informed consent; the study was approved by the hospital institutional review board. Blood was drawn at angiography, and patient follow-up ranged from 1.8 to 4.3 years (mean 3 years).

Clinical Evaluation

All patients underwent a clinical examination. A patient was defined as having CAD if there was angiographic evidence of atherosclerosis ($\geq 70\%$ stenosis of ≥ 1 major coronary artery). Myocardial infarction (MI) reported during study follow-up was confirmed if medical record review demonstrated symptoms consistent with MI and the presence of either diagnostic ECG changes or cardiac enzymes. It

Received June 12, 2000; revision received August 21, 2000; accepted August 21, 2000.

From the Cardiovascular Research Institute (J.Z., S.E.E.), MedStar Research Institute, Washington Hospital Center, Washington, DC; Department of Epidemiology (F.J.N.), The Johns Hopkins University School of Public Health, Baltimore, Md; and LDS Hospital and University of Utah (B.D.H., J.L.A., J.B.M.), Salt Lake City.

Correspondence to Dr Stephen E. Epstein, CRI, Washington Hospital Center, 110 Irving St, NW, Suite 4B-1, Washington, DC 20010.

© 2001 American Heart Association, Inc.

Circulation is available at <http://www.circulationaha.org>

was not possible to confirm a cardiovascular basis for all deaths. Therefore, the total number of deaths, uncensored relative to presumed cause, was used in our analyses.

Risk Factors

Risk factors considered in this study included age, male sex, cigarette smoking, diabetes, hyperlipidemia, hypertension, family history of coronary heart disease, levels of C-reactive protein (CRP), and seropositivity status to CMV, *C pneumoniae*, *H pylori*, HAV, HSV1, and HSV2. A history of past and current smoking was obtained. A patient who stopped smoking >20 years ago and who was <30 years of age when smoking was stopped was considered not to have smoking as a risk factor. A patient was considered to have diabetes if he or she was taking insulin or oral hypoglycemic agents or had previously received such treatment and was currently using dietary modification to control the condition. A patient was considered to have hyperlipidemia if he or she had a serum cholesterol value of >240 mg/dL (6.2 mmol/L) or was receiving antihyperlipidemic treatment. A patient was considered to have hypertension if he or she had received the diagnosis or was being treated with antihypertensive medications or dietary modification.

Infectious Serology

Serum obtained from patients was frozen at -80°C , and aliquots were thawed only when specific tests were performed. Commercially available ELISA kits were used to determine serum IgG antibodies to CMV, HSV1, and HSV2 (Wampole), to *H pylori* (Meridian Diagnostics), to HAV (ETI-AB-HAVK; DiaSorin Inc), and to *C pneumoniae* (Savyon Diagnostics). (The latter IgG ELISA had $\approx 95\%$ sensitivity compared with the microimmunofluorescence assay.)

CRP Level

Serum CRP was measured with a fluorescence polarization immunoassay (TDxFLEx analyzer; Abbott Labs); 95% of healthy individuals ($n=202$) had a CRP level of ≤ 0.5 mg/dL and 98% had a level of ≤ 1.0 mg/dL (Abbott Laboratories Diagnostic Division, list No. 9550, January 1996). The between-run coefficient of variation of this assay ($n=31$) was 4.3% and 2.2% at a mean level of 1.10 and 2.94 mg/dL, respectively.

Statistical Analysis

Categorical data were analyzed by the χ^2 test. All statistical tests were 2-sided. Covariates that were considered were age, sex, smoking, diabetes, hyperlipidemia, hypertension, family history, and seropositivity status to CMV, *C pneumoniae*, *H pylori*, HAV, HSV1, and HSV2, as well as the aggregate number of seropositive tests. All covariates were examined as predictors of MI or death in univariate analyses. A multivariate Cox regression analysis of these covariates as predictors of increased incidence of MI or death was made with an SAS software system (SAS Institute) for PC Windows. Relative hazard was used as a measure of risk of incident events in patients with a given risk factor compared with those without that factor or as a multiplicative factor for each unit increase in age or pathogen burden. The presence of statistically significant linear trends of increasing relative hazards with increasing pathogen burden was assessed by entering pathogen burden as a single ordinal covariate in the Cox regression and using the standard Wald statistic to test the statistical significance of the corresponding regression coefficient.

Results

Patient Characteristics

A total of 890 study subjects were followed for a mean of 3 years after CAD diagnosis. Men constituted 77% of the cohort. Most participants were Utah residents, a population that ethnically is primarily of northern European descent. Ages ranged from 34 to 95 years (mean age 65 years). Baseline clinical characteristics and prevalence of IgG anti-

TABLE 1. Characteristics of Study Subjects

	All Subjects (n=890)	MI or Death During Follow-UP	
		No (n=723)	Yes (n=167)
Age, y	65.3	64.7	69.5‡
Male sex, %	77.1	78.0	73.1
Diabetes, %	19.7	18.8	23.4
Smoking, %	26.8	27.5	24.0
Hyperlipidemia, %	47.5	49.2	40.1*
Hypertension, %	52.7	52.8	52.1
Family history of CHD, %	33.5	34.3	29.9
CRP, mg/dL	2.34	2.23	2.81*
CMV Ab+, %	75.2	72.3	87.4‡
HAV Ab+, %	53.4	49.6	69.5‡
<i>H pylori</i> Ab+, %	56.0	54.5	62.3
<i>C pneumoniae</i> Ab+, %	81.2	80.8	83.2
HSV1 Ab+, %	86.0	84.5	92.2‡
HSV2 Ab+, %	72.6	70.8	80.2*
Pathogen burden, %			
0	0.9	1.1	0.0
1	3.1	3.7	0.6
2	7.2	8.2	3.0
3	16.2	18.0	8.4
4	24.6	24.9	23.4
5	28.1	25.7	38.3
6	19.9	18.4	26.4§

Data presented are mean or percent of patients. CHD indicates coronary heart disease; Ab, antibody; and +, positive.

* $P<0.05$, † $P<0.01$, ‡ $P<0.001$, § $P<0.0001$.

||Percent of positive antibodies.

bodies against the pathogens in these subjects are presented in Table 1. Only 8 (0.9%) patients tested negative for all 6 pathogens, whereas 427 (48%) were positive for 5 or all 6 pathogens.

During follow-up, 76 (9%) of 890 patients developed MI, 104 (12%) died, and 167 (19%) experienced an MI or death. Table 1 shows the distribution of risk factors, baseline prevalence of infection, and pathogen burden according to eventual outcome (MI or death). In these univariate analyses, seropositivity prevalence for infectious pathogens, especially CMV and HAV, was significantly higher in patients with events than in those without events. Pathogen burden was also significantly higher in patients who experienced incident MI or death (of whom 65% were positive for 5 or all 6 pathogens).

Multivariate Analyses of Anti-Pathogen IgG Antibodies and MI or Death

Cox multivariate regression analysis revealed significant relative hazards of MI or death associated with baseline seropositivity to CMV, HAV, and HSV2 (Table 2). In contrast, significant relative hazards for seropositivity to *H pylori* were found for MI but not for the combined end point. No significant associations were found for seropositivity to *C pneumoniae* in either individual or combined end points.

TABLE 2. Proportional Hazards Regression Analysis of Incident MI or Death in Relation to Baseline Antipathogen Antibodies and Risk Factors

	Incident Events During Follow-Up		
	MI, RH (95% CL)	Death, RH (95% CL)	MI or Death, RH (95% CL)
Age, 10 y	1.19 (0.94–1.51)	2.02 (1.59–2.55)	1.57 (1.32–1.86)
Male sex	1.13 (0.64–1.99)	0.83 (0.53–1.31)	0.89 (0.62–1.28)
Diabetes	1.13 (0.62–2.05)	1.91 (1.23–2.97)	1.46 (1.01–2.11)
Smoking	1.18 (0.70–1.90)	0.91 (0.55–1.51)	1.09 (0.75–1.59)
Hyperlipidemia	1.48 (0.91–2.41)	0.56 (0.36–0.86)	0.82 (0.59–1.14)
Hypertension	0.87 (0.53–1.41)	0.95 (0.63–1.44)	0.99 (0.72–1.38)
Family history of CHD	0.76 (0.45–1.29)	1.21 (0.78–1.89)	1.03 (0.73–1.45)
CRP			
>Median	1.23 (0.77–1.97)	1.43 (0.94–2.17)	1.32 (0.95–1.82)
1 mg/dL*	1.03 (0.94–1.12)	1.11 (1.04–1.18)	1.08 (1.03–1.14)
CMV Ab+	2.33 (1.18–4.61)	1.63 (0.90–2.94)	2.01 (1.26–3.21)
HAV Ab+	1.66 (1.00–2.74)	1.49 (0.95–2.33)	1.61 (1.14–2.27)
<i>H pylori</i> Ab+	1.98 (1.18–3.31)	0.76 (0.51–1.14)	1.12 (0.81–1.54)
<i>C pneumoniae</i> Ab+	1.47 (0.76–2.85)	0.97 (0.51–1.64)	1.07 (0.70–1.63)
HSV1 Ab+	2.20 (0.87–5.56)	1.23 (0.61–2.47)	1.57 (0.88–2.80)
HSV2 Ab+	1.60 (0.88–2.90)	1.45 (0.86–2.37)	1.51 (1.02–2.23)

RH (95% CL) indicates relative hazards with 95% confidence limits; CHD, coronary heart disease; Ab, antibody; and +, positive.

Values were derived from proportional hazards regression analysis (adjusted covariates: age, sex, number of vessels, presentation, diabetes, smoking, hyperlipidemia, hypertension, renal failure, and family history of CHD).

*As a continuous variable.

Multiple Infections (Pathogen Burden) and MI or Death

We defined pathogen burden using seropositivity to all 6 pathogens tested: CMV, HAV, *C pneumoniae*, *H pylori*, HSV1, or HSV2. As shown in Table 3, a graded dose-response relationship of increasing relative hazards with increasing pathogen burden was observed. After multivariate adjustment, those positive to 5 or 6 pathogens were >6 times more likely to have incident MI or death than were those with 0 or only 1 seropositive result. Because of the limited sample size in the reference group (36 subjects), the 95% confidence limit (CL) values were large, although a highly significant dose-response trend test was observed even after multivariate analyses ($P=0.0005$). In alternative analyses, we used the 0 to 2 pathogens as reference group (total of 100 subjects); the relative hazards for individuals with 5 or 6 pathogens (Table 3, last column) was >3 in multivariate analyses, now with narrower 95% CL values that did not overlap 1.

Additional analyses were conducted that defined pathogen burden on the basis of 4 pathogens: CMV, HAV, HSV1, or HSV2. These 4 were chosen for the multivariate analysis because each was significantly associated with the combined end point in the univariate analysis. The Figure shows the MI- or death-free survival curves according to pathogen burden defined according to positivity to the 4 pathogens. There is a dose-response effect (P for trend <0.05). The corresponding Cox regression analyses (Table 3, bottom) showed significantly elevated relative hazards in both univariate and mul-

tivariate analyses, with a graded dose-response type of relationship, and a particularly strong association present with high levels of pathogen burden. The adjusted relative hazard of MI or death in a comparison of the seropositivity of 4 pathogens with none was 7.6 (95% CL 1.04, 55.7). Furthermore, as demonstrated in the Cox regression analyses (Table 4), an increased risk of either of the individual end points (MI or death) and of the combined end point (MI or death) was significantly related to the number of pathogens with which an individual is infected.

Because CMV and HAV are the pathogens most strongly associated with MI or death in this study, we repeated the analyses using the seropositive versus seronegative status of all 6 pathogens but excluded the results of either CMV or HAV. The relation between increasing pathogen burden with increasing frequency of the risk of MI or death remained significant (data not shown).

Pathogen Burden and CRP Levels

In our study population, the mean CRP level was 2.34 mg/dL and the median level was 1.4 mg/dL. Mean CRP levels were 1.76, 2.21, 2.28, 2.22, 2.42, and 2.61 mg/dL in patients exposed to 0 to 1, 2, 3, 4, 5, or 6 pathogens (P for trend 0.05).

To determine whether the association between pathogen burden and MI or death is modified by CRP, we analyzed 2 subgroups of patients: all individuals with CRP below its mean (or median) value, and those at or above this value (Table 5). We found stronger associations among those with

TABLE 3. Proportional Hazards Regression Analysis of Incident MI or Death in Relation to Baseline Pathogen Burden (Number of Positive IgG Antibodies)

No. of Positive IgG Antibodies	Cases/Total, n	Relative Hazard (95% CL)		
		Unadjusted	Partially Adjusted*	Fully Adjusted†
Pathogen burden defined using 6 infectious agents				
0	0/8
1	1/28	1.00‡	1.00‡	1.00‡
2	5/64	2.68 (0.31–22.9)	2.40 (0.28–20.6)	2.43 (0.28–20.9)
3	14/144	3.45 (0.45–26.1)	2.97 (0.39–22.6)	2.98 (0.39–22.8)
4	39/219	6.53 (0.90–47.5)	4.95 (0.68–36.2)	4.85 (0.66–35.6)
5	64/250	9.57 (1.33–69.0)	6.68 (0.92–48.6)	6.47 (0.89–47.2)
6	44/177	9.55 (1.32–69.3)	6.42 (0.88–47.1)	6.26 (0.85–46.1)
Trend test (P value)		0.0001	0.0003	0.0005
Pathogen burden defined using 4 infectious agents				
0	1/40	1.00	1.00	1.00
1	6/70	3.43 (0.41–28.5)	2.96 (0.36–24.7)	3.17 (0.38–26.6)
2	20/171	4.74 (0.64–35.3)	3.61 (0.48–27.0)	3.64 (0.48–27.4)
3	56/293	8.01 (1.11–57.9)	6.07 (0.84–44.0)	6.09 (0.83–44.6)
4	84/316	11.64 (1.62–83.6)	7.61 (1.05–55.3)	7.61 (1.04–55.7)
Trend test (P value)		0.0001	0.0001	0.0001

*Adjusted for age, sex, number of affected vessels, and presentation.

†Adjusted for the partially adjusted factors plus diabetes, smoking, hyperlipidemia, hypertension, family history of coronary heart disease, and renal failure.

‡Categories 0 and 1 were combined as reference category because there were no cases among the 8 individuals with 0 infections.

§Categories 0, 1, and 2 were combined as reference category.

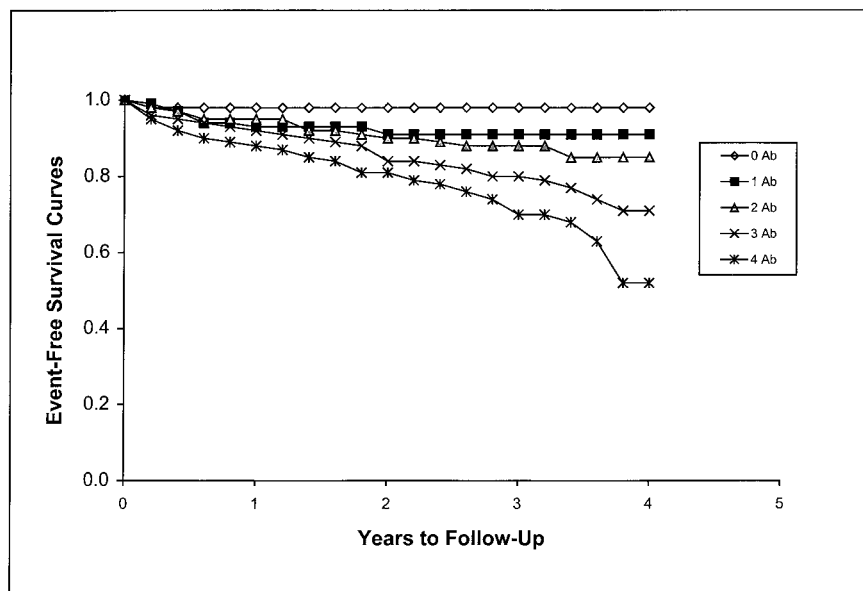
||Excluding *C pneumoniae* and *H pylori*.

lower CRP values, although a significant trend was present in both CRP strata levels. These results provide further evidence that suggests pathogen burden significantly predicts the combined end point of MI or death independent of CRP.

Discussion

In our previous cross-sectional study,¹⁶ we examined the relation between pathogen burden and the presence of angio-

graphically documented CAD. We selected for study a panel of 6 pathogens because they shared 2 common characteristics: all were obligate intracellular pathogens, and all established persistent antibodies targeted to the pathogen. Five of the pathogens (*CMV*, *C pneumoniae*, *H pylori*, *HSV1*, and *HSV2*) are known to establish life-long latent or persistent infections, whereas 1 pathogen (*HAV*) is not currently recognized as establishing a persistent infection despite the



MI- or death-free survival curves according to pathogen burden (number of positive IgG antibodies). Pathogen burden was based on seropositivity to 4 pathogens: *CMV*, *HAV*, *HSV1*, and *HSV2*. Ab indicates antibody.

TABLE 4. Pathogen Burden* and Incident Events in 890 Study Subjects

	No. of IgG Antibodies					P Trend
	0	1	2	3	4	
MI						
Cases, n (%)	0/40 (0.0)	3/70 (4.3)	13/171 (7.6)	20/293 (6.8)	40/316 (12.7)	
RH (95% CL), unadjusted	1.0‡		2.70 (0.77–9.49)	2.43 (0.72–8.17)	4.70 (1.45–15.2)	0.001
RH (95% CL), multivariate†	1.0‡		2.93 (0.82–10.4)	2.50 (0.73–8.58)	5.00 (1.48–16.8)	0.002
Death						
Cases, n (%)	1/40 (2.5)	3/70 (4.3)	11/171 (4.2)	37/293 (12.6)	52/316 (16.5)	
RH (95% CL), unadjusted	1.0	1.69 (0.18–16.3)	2.54 (0.33–19.7)	5.15 (0.71–37.6)	6.72 (0.93–48.6)	0.0001
RH (95% CL), multivariate	1.0	1.19 (0.12–11.6)	1.24 (0.16–9.78)	2.55 (0.34–19.0)	2.57 (0.34–19.1)	0.02
MI or death						
Cases, n (%)	1/40 (2.5)	6/70 (8.6)	20/171 (11.7)	56/293 (19.1)	84/316 (26.6)	
RH (95% CL), unadjusted	1.0	3.43 (0.41–28.5)	4.74 (0.64–35.3)	8.01 (1.11–57.9)	11.64 (1.62–83.6)	0.0001
RH (95% CL), multivariate	1.0	3.17 (0.38–26.6)	3.64 (0.48–27.4)	6.09 (0.83–44.6)	7.61 (1.04–55.7)	0.0001

RH indicates relative hazards.

*Defined as the number of baseline positive IgG antibodies (CMV, HAV, HSV1, HSV2).

†Cox regression analysis adjusted for all of the covariates listed in Table 2.

‡The RHs of the combination of the 0 and 1 categories (number of IgG antibodies) for MI are shown because there were no cases in the reference category (zero positive antibodies).

life-long persistence of anti-HAV antibodies. The results of that study demonstrated that increasing pathogen burden was associated with increasing risk of CAD and, for the first time, that HAV was one of the pathogens that contributes to atherosclerosis.

The results of the present investigation confirm and extend those findings. This study is prospective in design and involves an entirely different patient cohort. We determined, for the first time, whether pathogen burden predicts MI or death in a cohort with angiographically documented CAD. The results demonstrated that a graded dose-response relationship of increasing relative hazards for MI or death was observed with increasing pathogen burden (Table 3). The statistical association became even more robust when the 4 pathogens (CMV, HAV, HSV1, and HSV2) were considered (Table 3, Figure). Furthermore, in addition to the combined end point, pathogen burden also significantly predicted an increased risk of both individual end points (MI or death) (Table 4).

TABLE 5. Adjusted Relative Hazards* of Incident MI or Death Associated With Pathogen Burden,† Stratified by CRP Levels

No. of IgG Antibodies	CRP Level‡			
	<Mean	≥Mean	<Median	≥Median
0–1	1.0	1.0	1.0	1.0
2	2.14	0.65	2.72	0.98
3	3.24	1.63	4.43	1.80
4	4.72	1.33	6.21	1.88
P trend	0.0001	NS	0.001	0.047

*Adjusted covariates include age, sex, number of vessels, and clinical presentation.

†Defined as the number of baseline positive IgG antibodies (CMV, HAV, HSV1, HSV2).

‡Mean CRP=2.34 mg/dL, median=1.4 mg/dL.

Although the primary thrust of this investigation was to determine whether pathogen burden predicted cardiovascular events, the results related to the individual pathogens are of interest. We found that the baseline prevalence of antibodies directed against CMV, HAV, HSV1, or HSV2, but not *C pneumoniae* and *H pylori*, was significantly higher among patients who subsequently developed MI or death (Table 1). After multivariate adjustment, CMV, HAV, and HSV2 demonstrated positive relative hazards for MI or death (Table 2). It is possible that different populations will exhibit different relationships among the pathogens and CAD events. However, the results do point out that it cannot be taken for granted that *C pneumoniae* seropositivity will be an important risk factor for future events in a patient population with known CAD, an assumption implicit in the numerous prospective trials that are under way in which the effects of macrolide antibiotics on future event rate are being assessed.¹⁷

Our results also suggest that prior HAV infection influences the course of atherosclerosis. In our initial study, HAV was selected as one of the pathogens to be studied because, like the others, it is an obligate intracellular pathogen and anti-HAV antibodies persist throughout the life of the host. The basis for such selection criteria was derived from our belief that infection predisposes to CAD, at least in part through immune responses and perhaps infection-induced autoimmune responses that could involve molecular mimicry.¹⁸ We thought that a persistent antibody response could reflect a process compatible with immunopathology and autoimmune disease. It is unknown whether the persistent antibody response to HAV represents persistent infection with HAV, reflects consistent exposure to cross-reactive self-antigens, or reflects intermittent exposure of the host to other pathogens that contain antigens that are cross-reactive with HAV antigens. Regardless of the precise mechanism, the

fact that HAV is associated with both CAD and cardiovascular events suggests that other pathogens that share these characteristics may also contribute to the atherosclerotic process.

Importantly, we found that increasing pathogen burden is associated with increasing CRP levels. An elevated CRP level, believed to reflect underlying inflammation, is a risk factor for cardiovascular events.^{19–21} Therefore, our results suggest that one of the mechanisms by which pathogen burden contributes to cardiovascular events is through the inflammatory response elicited by infection with multiple pathogens. However, in stratified analyses of the relation between pathogen burden and MI or death on the basis of the median CRP value, we found stronger associations among those with lower CRP values (although a significant trend was present in both strata of CRP levels). These results provide further evidence that suggest pathogen burden significantly predicts the combined end point of MI or death independent of CRP.

Of interest, we found in our particular cohort, which consists of patients with angiographically documented CAD, that some of the risk factors traditionally associated with CAD did not predict risk of incident events. This may be in part due to the fact that once CAD is diagnosed, it is likely that treatable risk factors will be aggressively treated, thereby moderating any future risk they may cause. This could explain the fact that smoking, hyperlipidemia, and hypertension were not identified as risk factors for incident events in the present study. Importantly, in this context, we found that pathogen burden is not an inconsequential risk factor. The predictive values of CMV, HAV, and HSV infection on MI or death were of comparable or greater magnitude than those of the major CAD risk factors as traditionally defined (Table 2). As an example of the relative risk posed by infection, according to the point estimates of the adjusted Cox regression coefficients, each of these pathogens has an independent association with MI or death equal in magnitude to that of an age increase of 15.6, 10.6, and 9.2 years, respectively.

Despite the strong associations between pathogen burden and MI or death, it is possible that pathogen burden does not causally relate to the precipitation of such events. It may, for example, be a surrogate marker of other factors, not considered in the present investigation, that are the true causal factors that contribute to the identified association. The likelihood that pathogen burden is not just a surrogate marker for some other factor or factors is suggested by the extremely high risk posed (Table 3) and the many mechanistic studies that demonstrate pathogens evoke cellular changes that in themselves could account for the development of atherogenesis and for the precipitation of the acute complications of atherosclerosis.^{22–24}

One potential confounding influence not evaluated in the present study that could influence our conclusions is the impact of socioeconomic status (SES). Unfortunately, we cannot address this issue definitively because information related to SES was not obtained as part of the database. However, several considerations make this possibility unlikely. First, most of the patients in this investigation lived in Utah, which has a very homogeneous population that is

ethnically primarily of northern European descent. Moreover, all study participants had some degree of access to health care, as evidenced by the fact that all of them had, by definition, diagnostic arteriography at study inception; hence, extremes of SES probably play no role in outcome. Also, arguing against an important confounding influence of SES is the disparity in the associations we observed between the individual pathogens and outcome events. Although we found strong associations between events and seropositivity to HAV and CMV, we found no association between outcome events and seropositivity to *H pylori* or *C pneumoniae*. The frequency of each of these latter infections is strongly influenced by low SES. It therefore would be likely that if low SES was the confounder that influenced outcome, each of these pathogens would have been found to be associated with outcome, but this possibility appears unlikely.

In conclusion, our investigation provides further evidence that infection plays an important role in cardiovascular disease. Given the study results that the risk of incident MI or death occurring in CAD patients is independently related to the pathogen burden, it may seem somewhat paradoxical that the traditional incidence of CAD has been very low in areas of the world in which infection with multiple pathogens occurs at a very high rate. However, any effect of infection on the incidence of CAD must be considered in the context of coexisting CAD risk factors. In this regard, CAD is reaching epidemic proportions in rapidly developing Third World countries,²⁵ as the populations adopt Western diets and a high-stress lifestyle typically associated with a high incidence of CAD. Thus, the impact of infection on the incidence of CAD and its complications in such populations will probably become all too evident during the next several decades.

References

1. Adam E, Probstfield JL, Burek J, et al. High levels of cytomegalovirus antibody in patients requiring vascular surgery for atherosclerosis. *Lancet*. 1987;2:291–293.
2. Zhou YF, Leon MB, Waclawiw MA, et al. Association between prior cytomegalovirus infection and the risk of restenosis after coronary atherectomy. *N Engl J Med*. 1996;335:624–630.
3. Nieto FJ, Adam E, Sorlie P, et al. Cohort study of cytomegalovirus infection as a risk factor for carotid intimal-medial thickening, a measure of subclinical atherosclerosis. *Circulation*. 1996;94:922–927.
4. Saikku P, Leinonen M, Mattila K, et al. Serological evidence of an association of a novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. *Lancet*. 1988;2:983–986.
5. Thom DM, Grayston JT, Siscovitch DS, et al. Association of prior infection with *Chlamydia pneumoniae* and angiographically demonstrated coronary artery disease. *JAMA*. 1992;268:68–72.
6. Saikku P, Leinonen M, Tenkanen L, et al. Chronic *Chlamydia pneumoniae* infection as a risk factor for coronary heart disease in the Helsinki Heart Study. *Ann Intern Med*. 1992;16:273–278.
7. Muhlestein JB, Hammond EH, Carlquist JF, et al. Increased incidence of *Chlamydia* species within the coronary arteries of patients with symptomatic atherosclerotic versus other forms of cardiovascular disease. *J Am Coll Cardiol*. 1996;27:1555–1561.
8. Mendall MA, Goggin PM, Molineaux N, et al. Relation of *Helicobacter pylori* infection and coronary heart disease. *Br Heart J*. 1994;71:437–439.
9. Pasceri V, Cammarota G, Patti G, et al. Association of virulent *Helicobacter pylori* strains with ischemic heart disease. *Circulation*. 1998;97:1675–1679.
10. Sorlie PD, Adam E, Melnick SL, et al. Cytomegalovirus/herpesvirus and carotid atherosclerosis: the ARIC study. *J Med Virol*. 1994;42:33–37.

11. Havlik RJ, Blackwelder WC, Kaslow R, et al. Unlikely association between clinically apparent herpesvirus infection and coronary incidence at older ages. *Arteriosclerosis*. 1989;9:877–880.
12. Adler SP, Hur JK, Wang JB, et al. Prior infection with cytomegalovirus is not a major risk factor for angiographically demonstrated coronary artery atherosclerosis. *J Infect Dis*. 1998;177:209–212.
13. Weiss SW, Roblin PM, Gaydos CA, et al. Failure to detect *Chlamydia pneumoniae* in coronary atheromas of patients undergoing atherectomy. *J Infect Dis*. 1996;173:957–963.
14. Ridker PM, Hennekens CH, Buring JE, et al. Baseline IgG antibody titers to *Chlamydia pneumoniae*, *Helicobacter pylori*, herpes simplex virus, and cytomegalovirus and the risk for cardiovascular disease in women. *Ann Intern Med*. 1999;131:573–577.
15. Nieto FJ. Infections and atherosclerosis: new clues from an old hypothesis? *Am J Epidemiol*. 1998;148:937–948.
16. Zhu J, Quyyumi AA, Norman JE, et al. Effects of total pathogen burden on coronary artery disease risk and C-reactive protein levels. *Am J Cardiol*. 2000;85:140–146.
17. Jackson LA, Stewart DK, Wang SP, et al. Safety and effect on anti-*Chlamydia pneumoniae* antibody titers of a 1 month course of daily azithromycin in adults with coronary artery disease. *J Antimicrob Chemother*. 1999;44:411–414.
18. Epstein SE, Zhou YF, Zhu J. Infection and atherosclerosis: emerging mechanistic paradigms. *Circulation*. 1999;100:e20–e28.
19. Ridker PM, Cushman M, Stampfer MJ, et al. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med*. 1997;336:973–979.
20. de Beer FC, Hind CR, Fox KM, et al. Measurement of serum C-reactive protein concentration in myocardial ischemia and infarction. *Br Heart J*. 1982;47:239–243.
21. Liuzzo G, Biasucci LM, Gallimore JR, et al. The prognostic value of C-reactive protein and serum amyloid A protein in severe unstable angina. *N Engl J Med*. 1994;331:417–424.
22. Hajjar DP, Pomerantz KB, Falcone DJ, et al. Herpes simplex virus infection in human arterial cells: implications in arteriosclerosis. *J Clin Invest*. 1987;80:1317–1321.
23. Jacob HS, Visser M, Key NS, et al. Herpes virus infection of endothelium: new insights into atherosclerosis. *Trans Am Clin Climatol Assoc*. 1992;103:95–104.
24. Speir E, Modali R, Huang ES, et al. Potential role of human cytomegalovirus and p53 interaction in coronary restenosis. *Science*. 1994;256:391–394.
25. Reddy S, Yusuf S. Emerging epidemic of cardiovascular disease in developing countries. *Circulation*. 1998;97:596–601.