Presence of anti-viral and anti-parasitic antibodies and cardiovascular mortality: Insights from NHANES III ☆

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Although inflammatory changes associated with infections have been postulated to play a key role in the pathogenesis of atherosclerosis [1], studies evaluating the association of antibodies against various pathogens and cardiovascular (CV) outcomes have met with contradictory results [2–10]. Prior data on the role of infections in CV outcome is widely heterogeneous with most studies focusing on only certain viral infections, having a limited follow-up time, limited study subjects, and being restricted to only certain cohorts [2–10].

In order to assess the relationship between the presence of anti-viral and anti-parasitic antibodies and CV mortality in a cross section of the US population, we performed a retrospective observational cohort study using prospectively collected data from the National Health and Nutrition Examination Survey-III (NHANES III, 1988–1994).

NHANES III, conducted by the National Center for Health Statistics, includes data from oral surveys and general health examinations. It was designed to assess the demographic, socio-economic, dietary, and overall health status of a nationally representative sample in noninstitutionalized patients from all 50 states. NHANES III consisted of 19,215 participants more than 18 years of age [11].

The public data set of the National Health and Nutrition Examination Survey III (NHANES III) between years 1988 and 1994 was used for the analysis. We included patients more than 18 years of age with data on positive antibodies against hepatitis A (HAV), hepatitis B (HBV), hepatitis C (HCV), hepatitis E (HEV), herpes simplex virus 1 and 2 (HSV 1 and 2), toxoplasma, varicella, cytomegalovirus (CMV), human herpes virus 8 (HHV-8), and *Toxocara* ($n = 7{,}876$) (Appendix A).

The primary outcome considered was CV mortality (composite of death due to ischemic heart disease, stroke or atherosclerosis). The NHANES III–linked mortality public-use file was used to obtain the mortality status. It provided mortality data through December 31, 2006. The National Death Index was used as the primary determinant of mortality for eligible NHANES III participants. Underlying causes of death were provided by death certificate data contained in the same mortality files and was classified according to the *International Classification of Diseases, Injuries and Causes of Death, Tenth Revision* guidelines (ICD-10) [11].

NHANES III had a complex nonrandom multistage stratified sample design. All analyses were performed using the designated weighting, which was specified in the NHANES III data set to minimize bias. The total NHANES III pseudo-stratum was used as our stratum variable. The total NHANES III pseudo-primary sampling unit was used as our survey-sampling unit, and the total mobile examination center final weight as our sampling unit weight.

For categorical variables, chi-square analysis was used to evaluate group differences. For continuous variables, in order to evaluate group differences, one-way ANOVA was used if the variable was normally distributed, and the Kruskal–Wallis test was used if the distribution was not normal. Cox proportional hazard regression modeling was used to calculate hazard ratio (HR) of CV mortality for individual infections as well as the combined infection burden variable. The aforementioned infections were combined to create a single infection burden variable, which was split into quartiles. Other variables entered in the regression model were age, gender, race, body mass index, hypertension, smoking, coronary artery disease or CAD equivalent (stroke or peripheral vascular disease), congestive heart failure, diabetes mellitus, family history of CAD, hypercholesterolemia, glomerular filtration rate, C-reactive protein (CRP), and social class (educational level, annual family income, and type of health insurance). All statistical analyses were performed using STATA SE 11.1 (STATA Corp. LP, College Station, Texas). A 2-sided *p*-value of 0.05 was considered statistically significant.

The mean age was 41.6 ± 17.4 years, 48.2% were males, 30.5% were African Americans, 27.5% were smokers, and 23.1% had hypertension. The mean body mass index (BMI) was 27.9 ± 5.8 kg/m^2 and the mean CRP was 0.44 ± 0.66 mg/L. The highest quartile (4) of infection burden (titers positive for 6–10 infections) was present in 1,521 (19.3%) of subjects. Subjects in the fourth quartile of infection burden were older, more likely males, hypertensive, and diabetics. These subjects were also less likely to have a higher family income, less likely to have a higher education levels, and less likely to have a private insurance (Table 1).

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Table 1
Baseline characteristics of the study population ($n = 7,898$).

	Overall population	Quartiles of infection (no. of observations)				*p*-value
	$n = 7,898$	1 (2,673)	2 (1,980)	3 (1,715)	4(1,530)	
Mean age	41.6 ± 17.4	34.7 ± 13.3	41.5 ± 17.0	45.8 ± 18.1	49.16 ± 18.69	0.0001
Mean BMI ($n = 7,880$)	26.9 ± 5.8	26.4 ± 5.8	27.0 ± 5.7	27.2 ± 5.6	27. 29 ± 5.85	0.0001
Mean CRP ($n = 7,894$)	0.44 ± 0.66	0.38 ± 0.53	0.44 ± 0.67	0.46 ± 0.68	0.51 ± 0.81	0.0001
Mean GFR ($n = 7,864$)	79.0 ± 16.8	80.5 ± 15.1	79.7 ± 16.7	77.8 ± 17.5	77.1 ± 18.6	0.0001
Female (%)	4,091	1,308 (48.9%)	1,047 (52.8%)	914 (53.2%)	822 (53.7%)	0.004
Race (%)						<0.001
White (%)	3,029	1,450 (54.2%)	695 (35.1%)	530 (30.9%)	354 (23.1%)	
Hispanic (%)	2,215	455 (17.0%)	646 (32.6%)	575 (33.5%)	539 (35.2%)	
Black (%)	2,413	721 (26.9%)	574 (28.9%)	534 (31.1%)	584 (38.1%)	
Other (%)	241	47 (1.7%)	65 (3.2%)	76 (4.4%)	53 (3.4%)	
Hypertension (%)	1,831	469 (17.5%)	455 (22.9%)	457 (26.6%)	450 (29.4%)	<0.001
Current smoking (%)	2,176	727 (27.2%)	537 (27.1%)	464 (27.0%)	448 (29.2%)	0.4
CHF (%)	166	14 (0.5%)	36 (1.8%)	50 (2.9%)	66 (4.3%)	<0.001
Diabetes mellitus (%)	617	115 (4.3%)	142(7.1%)	180(10.5%)	180 (11.7%)	<0.001
Family h/o CAD (%)	618	192(7.1%)	171 (8.6%)	143 (8.3%)	112 (7.3%)	0.2
CHD equivalent (%)	856	176 (6.5%)	201 (10.1%)	225 (13.1%)	254 (16.6%)	<0.001
Hypercholesterolemia (%)	2,852	782 (29.2%)	686 (34.6%)	730 (42.5%)	654 (42.7%)	<0.001
Annual family income ($n = 7,138$)						<0.001
<$ 20,000 (%)	3,138	790 (31.4%)	773 (43.2%)	786 (51.4%)	789 (60.2%)	
$20,000–50,000 (%)	2,940	1,152 (45.8%)	749 (41.9%)	596 (38.9%)	443 (33.8%)	
>$ 50,000 (%)	1,060	571 (22.7%)	264 (14.7%)	147 (9.6%)	78 (5.9%)	
Educational level ($n = 7,857$)						<0.001
Less than high school (%)	2,787	392 (14.7%)	712 (36.1%)	805 (47.1%)	878 (58.1%)	
High school (%)	2,597	1,029 (38.5%)	655 (33.2%)	532 (31.1%)	381 (25.2%)	
More than high school (%)	2,473	1,246 (46.7%)	604 (30.6%)	371 (21.7%)	252 (16.6%)	
Health insurance ($n = 7,404$)						<0.001
Government insurance (%)	1,842	336 (13.1%)	436 (23.2%)	525 (32.9%)	545 (39.3%)	
Private insurance (%)	4,167	1,850 (72.5%)	1,065 (56.8%)	740 (46.4%)	512 (36.9%)	
No insurance (%)	1,395	364 (14.2%)	373 (19.9%)	329 (20.6%)	329 (23.7%)	

Body mass index was defined as weight in kilograms divided by the square of the height in meters. Hypertension was defined as either self reported history of high blood pressure/hypertension or participants are taking medication for high blood pressure or during physical examination participants had a mean systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥90 mmHg after 3 measurements. Hypercholesterolemia was defined as either participant self-reported history of hypercholesterolemia or taking medication or low-density lipoprotein (LDL) is not in the range of target goal according to ATP3 guidelines. Coronary artery disease was defined as either participant self-reported history of heart attack, history of chest pain suggestive of typical angina or presence of electrocardiographic changes suggestive of probable myocardial infarction. Diabetes was defined as self-reported history of diabetes, use of diabetes medications or subjects with elevated glycohemoglobin levels (≥6.5%). Family history of heart attack was defined as participants having any of the first-degree relatives have been diagnosed or died because of heart attack before the age of 50 years. Positive antibodies against hepatitis A, hepatitis B, hepatitis C, hepatitis E, herpes simplex virus 1 and 2, toxoplasma, varicella, cytomegalovirus (CMV), human herpes virus 8, and Toxocara were combined to create a single infection burden variable. The variable was split into quartiles.

Table 2
Multivariable predictors of CV mortality.

Variable	Hazards ratio	95% Confidence interval	*p*-value
Infection quartile (Quartile 1 as referent)			
2	0.70	0.43–1.13	0.1
3	1.21	0.74–1.98	0.4
4	0.72	0.43–1.19	0.1
Age	1.11	1.08–1.13	<0.001
Female gender	0.61	0.42–0.90	0.01
CRP	1.32	1.16–1.49	<0.001
Hypertension	1.47	1.05–2.06	0.02
Current smoking	1.99	1.33–2.96	0.001
Diabetes mellitus	1.86	1.38–2.51	<0.001
CAD equivalent	2.17	1.58–2.98	<0.001
*Annual family income (<**$** 20.000 as referent)*			
$20–50,000	0.87	0.60–1.25	0.4
>$50,0000	0.66	0.37–1.19	0.1
Education level (less than high school = referent)			
High school	1.11	0.74–1.67	0.6
More than high school	1.08	0.71–1.62	0.7
Health insurance (government insurance = referent)			
Private insurance	0.71	0.39–1.30	0.2
No insurance	1.49	0.44–5.00	0.5

Multivariable model was controlled for age, gender, race, body mass index, hypertension, smoking, serum cholesterol, glomerular filtration rate, potassium, left ventricular mass index, C-reactive protein (CRP), and social class (education, income, and insurance). Positive antibodies against hepatitis A, hepatitis B, hepatitis C, hepatitis E, herpes simplex virus 1 and 2, toxoplasma, varicella, cytomegalovirus (CMV), human herpes virus 8, and Toxocara were combined to create a single infection burden variable. The variable was split into quartiles. Quartile 1 (positive titers for 0–3/10 infections), quartile 2 (positive titers for 4/10 infections), quartile 3 (positive titers for 5/10 infections), and quartile 4 (positive titers for 6–10/10 infections).