No Association between Hepatitis C Virus Seropositivity and Acute Myocardial Infarction

Christine M. Arcari,1 Kenrad E. Nelson,2 Dale M. Netski,1 F. Javier Nieto,1 and Charlotte A. Gaydos2,3
1University of Wisconsin School of Medicine and Public Health, Madison; and 2Johns Hopkins University Bloomberg School of Public Health and 3Johns Hopkins University School of Medicine, Baltimore, Maryland

Recent studies have linked hepatitis C virus (HCV) infection with carotid atherosclerosis. We investigated the association between HCV seropositivity and acute myocardial infarction using a well-established cohort of young men in the US military and found no evidence to support this association.

Coronary heart disease is the leading cause of death among both men and women in the United States [1]. Although established risk factors account for a significant portion of the incidence of coronary heart disease [2], infection has been hypothesized to be a contributing risk factor [3]. Various potential causative mechanisms, which have either a direct or an indirect effect, have been proposed to explain the association between infectious agents and coronary heart disease [4].

Recently, there have been reports of an association between hepatitis C virus (HCV) seropositivity and coronary artery disease and between HCV core protein and carotid atherosclerosis [5–7]. Using a well-established cohort of young men in the US military, we investigated the association between HCV seropositivity and acute myocardial infarction.

Methods. The study population was drawn from a previously described case-control sample of men aged 30–50 years who were on active duty in the US Army during the period of 1991–2000 [8]. In brief, case patients were individuals who had been hospitalized for a first myocardial infarction (defined as International Classification of Disease, Ninth Revision, [ICD-9] code 410) during the period of 1991–2000; case patients had no prior history of hospitalization for cardiovascular disease (ICD-9 codes 410–429), and a serum sample had been available for ≥1 year before the onset of myocardial infarction. Control subjects were men with no history of hospitalization for cardiovascular disease (ICD-9 codes 410–429) before the date of the case event and were individually matched to case patients by age (±1 year), race/ethnicity (“white, non-Hispanic,” “black, non-Hispanic,” and “other”), and availability of a serum specimen that had been collected within 60 days of collection of the case patient’s specimen.

Information on health risk factors was obtained from the health risk appraisal, which included self-reported information on past and current smoking habits, work stress, and diabetes, as well as measurements for height, weight, blood pressure, and serum cholesterol level [9]. The health risk appraisal is completed by military personnel at the time of entry into the military, every 2 years thereafter, and at every 5-year physical examination. The health risk appraisal closest in date to the serum specimen was selected for analysis.

Serum samples were obtained from the Department of Defense serum repository, which was created to store all serum samples that remain from the mandatory HIV testing program. Serum specimens were tested for the presence of HCV-specific antibodies using the commercially available Ortho ELISA, version 3.0 (Ortho Clinical Diagnostics), in accordance with manufacturer’s instructions. Samples that had an optical density reading 0.1 above or below the assay cutoff were repeated. Chlamydia pneumoniae IgG and IgA antibodies were measured by microimmunofluorescence assay [10]; a high titer was defined as ≥1:256 for C. pneumoniae IgG and as ≥1:64 for C. pneumoniae IgA on the basis of previous literature and expert opinion [11, 12].

Statistical analyses were performed using SAS software, version 8.01 (SAS Institute). The 2-sample t test and the χ2 test were used to examine differences in demographic characteristics between case patients and control subjects and to assess potential confounding variables. Multivariate logistic regression was conducted to study the relationship between case-control status, infection status, and demographic characteristics and cardiovascular risk factors [13]. Variable selection for final models was based on the likelihood ratio test. Because HCV test results were not available for the entire study population, unconditional regression analyses were conducted to maximize statistical power; however, the matching variables (age and race) were included in all final models to partially account for matching and to control for residual confounding. The study was reviewed and approved by the institutional review boards at
Johns Hopkins University (Baltimore, MD) and the Walter Reed Army Institute of Research (Silver Spring, MD).

**Results.** HCV test results and demographic data were available for 292 case patients (97.3%) and 290 control subjects (96.7%). By design, there were no differences between case patients and control subjects with respect to age (mean age, 40.2 years) and race/ethnicity (61.0% were white), and there were no significant differences with respect to military rank (69.8% were Senior Enlisted/Officer overall); however, case patients tended to be less educated (63.0% reported having only a high school education, compared with 51.0% of control subjects; $P < .01$) and were more likely to be married (93.8%) than were control subjects (86.6%; $P < .01$) (table 1).

Overall, 52 participants tested positive for anti-HCV antibodies, a prevalence of 8.9% (table 1). A significant increased prevalence was observed among black, non-Hispanic subjects (13.2%), compared with white, non-Hispanic subjects (6.8%) and with subjects from other ethnic groups (9.4%; $P < .05$). There was no significant difference in the prevalence of HCV infection between case patients (7.6%) and control subjects (9.8%; $P = .44$). No association was found between HCV positivity and acute myocardial infarction (relative risk [RR], 0.91; 95% CI, 0.52–1.61). This estimate remained essentially unchanged after adjustment for age, race, education, and marital status (adjusted RR, 0.94; 95% CI, 0.52–1.68) (table 2).

As reported in detail elsewhere [8], a larger proportion of study participants were found to have a high titer of *C. pneumoniae* IgG (19.9%) and *C. pneumoniae* IgA (20.3%). A high titer of *C. pneumoniae* IgA was significantly associated with acute myocardial infarction (RR, 1.61; 95% CI, 1.04–2.49), although the association weakened slightly after adjustment for age, race, education, and marital status. The association between a high *C. pneumoniae* IgG titer and acute myocardial infarction had borderline statistical significance (RR, 1.59; 95% CI, 0.97–2.63).

Information on established risk factors for acute myocardial infarction was available for a smaller subset of the study population (159 case patients [54.4%] and 246 control subjects [84.8%]). Increased risk of acute myocardial infarction was associated with current smoking (RR, 4.26; 95% CI, 2.77–6.54), hypercholesterolemia (RR, 3.06; 95% CI, 1.90–4.91), and presence of work stress (RR, 1.84; 95% CI, 1.19–2.87). In contrast, hypertension and overweight status were not associated with an increased risk of myocardial infarction in this sample. These apparently paradoxical findings can be explained by the limited range of these exposures, because individuals who do not meet the height and weight standards designed by the military and/or who have severe hypertension are dismissed from active duty and, thus, were ineligible to participate in the study.

**Discussion.** The results of this study do not indicate any relationship between HCV seropositivity and acute myocardial infarction and do not support previous reports in the literature.

---

**Table 1. Demographic characteristics, by case patient and control subject status, and prevalence of antibodies to hepatitis C virus (HCV).**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%) of subjects</th>
<th>Prevalence of HCV infection per 100 subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case patients</td>
<td>Control subjects</td>
</tr>
<tr>
<td>All subjects</td>
<td>292</td>
<td>290</td>
</tr>
<tr>
<td>Age, years$^b$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30.0–39.9</td>
<td>137 (46.9)</td>
<td>136 (46.9)</td>
</tr>
<tr>
<td>40.0–50.0</td>
<td>155 (53.1)</td>
<td>154 (53.1)</td>
</tr>
<tr>
<td>Race$^b$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, non-Hispanic</td>
<td>177 (60.6)</td>
<td>178 (61.4)</td>
</tr>
<tr>
<td>Black, non-Hispanic</td>
<td>88 (30.1)</td>
<td>86 (29.7)</td>
</tr>
<tr>
<td>Other</td>
<td>27 (9.25)</td>
<td>26 (8.9)</td>
</tr>
<tr>
<td>Military rank</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Junior enlisted</td>
<td>94 (32.2)</td>
<td>82 (28.3)</td>
</tr>
<tr>
<td>Senior enlisted/office</td>
<td>198 (67.8)</td>
<td>208 (71.7)</td>
</tr>
<tr>
<td>Education level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school</td>
<td>184 (63.0)</td>
<td>148 (51.0)</td>
</tr>
<tr>
<td>College or higher</td>
<td>108 (37.0)</td>
<td>142 (49.0)</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>274 (93.8)</td>
<td>251 (86.6)</td>
</tr>
<tr>
<td>Single/other</td>
<td>18 (6.2)</td>
<td>39 (13.4)</td>
</tr>
</tbody>
</table>

**NOTE.** NA, not applicable.

$^a$ The 2-sample $t$ test was used for continuous variables, and $\chi^2$ was used for categorical variables.

$^b$ Matching criteria in the original study population.
of this association. Ishizaka et al. [7] reported an independent association between HCV seropositivity and the presence of carotid artery plaque (OR, 2.21; 95% CI, 1.80–2.72) and thickening of intima media (OR, 4.18; 95% CI, 3.39–5.14). A second study by Ishizaka et al. [6] investigated the role of HCV core protein and carotid atherosclerosis and found a positive association between HCV core protein and carotid plaque (OR, 5.51; 95% CI, 2.38–12.76); however, only 25 subjects (1.3%) tested positive for HCV core protein. Vassalle et al. [5] reported that HCV seropositivity represented an independent predictor of coronary artery disease after adjusting for confounding risk factors (OR, 4.2; 95% CI, 1.4–13.0).

The previously reported associations observed in all 3 studies [5–7] between markers of HCV infection and coronary artery disease were stronger than the associations shown for all other established risk factors presented, including male sex, increasing age, hypertension, cholesterol, and smoking status. It seems unlikely that an association between HCV infection and coronary artery disease would be stronger than associations with established risk factors.

In the studies by Ishizaka et al. [6, 7], the study populations appeared to be healthy individuals who were enrolled during a general health screening. The study by Vassalle et al. [5] used hospital-based case patients and control subjects. Details were not provided on subject selection; this limits the interpretation and generalizability of results. A wide range of ages among the study populations may further complicate analysis, particularly in the study by Vassalle and colleagues, in which control subjects appeared to be younger than case patients. Infections may only play a role in coronary heart disease during particular stages of the natural history of atherosclerosis. All 3 studies were unable to establish the temporal sequence of infection and coronary artery disease. Previous publications have not discussed the putative biological basis for an association between HCV infection and coronary heart disease.

The results of the present study agree with a study conducted by Kiechl et al. [14], who showed no association between chronic active hepatitis B virus and/or HCV infection and carotid plaque. Furthermore, the C. pneumoniae IgG and IgA data in the present study are consistent with those in the published literature [11].

Although samples with an optical density reading of 0.1 above or below the assay cutoff value were repeated, a limitation of our study was that confirmatory HCV testing was not performed; it
is possible that some of the low positive values are false-positive results. Because low positive values (signal-to-cutoff ratio, <3.8) were evenly distributed between case patients and control subjects, they would not bias the results of any association between HCV infection and acute myocardial infarction.

The prevalence of antibody to HCV in the general US population is estimated to be 1.8% [15]. A large study of the general civilian population of the United States found that the prevalence of HCV infection among persons who had served in the military (1.7%) was similar to the prevalence among persons who had not served in the military (2.2%) [15]. Hyams et al. [16] performed a study of 10,000 randomly selected, active-duty personnel and reported a prevalence of HCV infection of 0.5% overall, with a prevalence of 1.2% among persons aged ≥30 years. Among users of the Veterans Administration (VA) health care system, a high prevalence of HCV infection (4%–35%) has been reported [17]. However, VA hospital users have been shown to be demographically very different from the general US population and from veterans who do not use the VA system [17]. A high prevalence of HCV infection (8.9%) was observed in this study population of active-duty US military personnel. However, even if we assume that a signal-to-cutoff ratio <3.8 generally represents false-positive results, the prevalence of HCV infection in the study population would be 4.0%, with the highest prevalence occurring in non-Hispanic black subjects; this finding would be similar to those in the study by Hyams et al. [16]. Although there is a high prevalence of HCV infection in the study population, this translated to only 52 HCV-positive participants, and the 95% CIs around the estimate are wide. It should also be noted that the study population is not reflective of the characteristics of most people with acute myocardial infarction in the United States. Active duty military personnel tend to be in overall good health, and the age range is much lower than the average age of a patient with acute myocardial infarction. It is possible that some of the control subjects in our study may have had asymptomatic coronary heart disease or will experience a future hospitalization for acute myocardial infarction. The resulting misclassification would result in conservative RR estimates (i.e., closer to the null hypothesis) but would not explain an RR <1.0 if, in fact, HCV infection was a risk factor.

In summary, the present study conducted with active-duty military personnel who had a high prevalence of HCV infection does not support a relationship between HCV infection and coronary heart disease.

Acknowledgments

We are grateful to Col. Patrick Kelley, Col. Mark Rubertone, and Col. Margot Krauss from the Department of Defense for their invaluable assistance in accessing data and specimens. We thank Dr. David Thomas for the use of his laboratory for HCV testing and Melissa Theodore, Billie Jo Wood, Justin Hardick, Jeff Holden, and Anthony Quinn from the Johns Hopkins International Chlamydia Research Laboratory for technical assistance.

Financial support. American Heart Association (AHA 024010N).

Potential conflicts of interest. All authors: no conflicts.

References