Smoking Is a Potential Confounder of the *Chlamydia pneumoniae*–Coronary Artery Disease Association

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Two recent studies, which did not adequately control for smoking status, found associations between *Chlamydia pneumoniae* serological titers and various manifestations of coronary artery disease (CAD). The validity of *C. pneumoniae*–CAD associations found in case–control studies has been criticized on the basis that smoking, known to be associated with CAD and hypothesized to be associated with *C. pneumoniae* seroreactivity via an increased prevalence of respiratory infection in smokers, could be an uncontrolled confounder in these studies. We investigated associations between current smoking status and *C. pneumoniae* serological titers in a cohort of 365 outpatients (mean age, 34 years) with respiratory illness. Current smokers were significantly ($p=0.04$) more likely than nonsmokers to have *C. pneumoniae* titers $\geq 1:128$, and there was a significant ($p<0.05$) "dose–response" association between titer category and smoking, which persisted after controlling for age and sex in a logistic-regression model. These results support the hypothesis that smoking may be a confounder of the association of *C. pneumoniae* antibody titer and smoking-associated diseases such as CAD. Future studies into these associations should control for cigarette use. (*Arteriosclerosis and Thrombosis* 1992;12:945–947)

KEY WORDS • *Chlamydia pneumoniae* • smoking • coronary artery disease • antibody titers

*C*hlamydia pneumoniae (strain *TWAR*), a recently discovered respiratory pathogen,[1] is an important cause of acute respiratory infections, including bronchitis and pneumonia, in all age groups.[2] Additionally, long-term exposure to this pathogen has been hypothesized to cause immunopathologically mediated diseases, such as adult-onset asthma[3] and some cases of sarcoidosis.[4]

Both acute and chronic *C. pneumoniae* infections have recently been associated with various diseases of the heart. *C. pneumoniae* can cause endocarditis,[5,6] indicating that this organism is capable of acute colonization of the endocardium. Quantitative associations have been reported between *C. pneumoniae* serological titers and chronic coronary heart disease, acute myocardial infarction,[7] and angiographically demonstrated coronary artery disease (CAD),[8] raising the possibility that chronic *C. pneumoniae* infection may be an additional risk factor for CAD. One of these studies[7] has been criticized for not reporting data on smoking, which could have been a confounder of the *C. pneumoniae*–CAD association.[9] The other study[8] could not report smoking data because they were not available. The hypothesis that smoking may be a confounder of the *C. pneumoniae*–CAD association is based on the specula-

tion that because smokers have higher rates of respiratory illness than nonsmokers, smokers will have higher rates of *C. pneumoniae* infection. The purpose of this article was to compare the prevalence of *C. pneumoniae* seropositive status in currently smoking versus non-smoking adults with acute respiratory illness.

Methods

This article reports data from a cohort of 365 middle-class, white outpatients with respiratory illness who were prospectively enrolled from four primary care (family practice) clinics between September 1, 1988 and January 31, 1991. Patient smoking status (current smoker versus current nonsmoker), as well as sera for microimmunofluorescence testing for *C. pneumoniae*, was obtained at the time of study enrollment. Sera were obtained for more than 82% of the patients during the convalescent phase. *C. pneumoniae* seropositive status was defined as either an acute or a convalescent serological titer $\geq 1:16$.

Additionally, *C. pneumoniae* titer category was defined for each patient as either $<1:16$ (seronegative), 1:16, 1:32, 1:64, or $\geq 1:128$ based on either the higher of the acute or convalescent titer or the acute titer if a convalescent titer was not available. Further details of the study population, data collection methods, and serological techniques have been published elsewhere.[3]

Statistical Methods

Fisher's exact test was used to analyze 2×2 tables. Logistic regression was performed by using the GLIM program.[10] Two-sided probability values ≤ 0.05 are reported as significant.

TABLE 1. Current Smoking and *Chlamydia pneumoniae* Seropositive Status

	Smokers (n=109)	Nonsmokers (n=252)	p*
C. pneumoniae seropositive (%)	66.1	56.0	0.08
C. pneumoniae titer ≥1:128 (%)	11.9	5.6	0.04

*Fisher's exact test.

Results

Smoking status was recorded for 361 (98.9%) of 365 patients in the study cohort. Of these 361 who make up the current study group, 109 (30.2%) were current smokers and 252 (69.8%) were nonsmokers at the time of study enrollment. Average age of the study group was 34.1 years (SD, 14.1 years). The study group comprised 151 males (41.8%) and 210 females (58.2%), of whom 41 males (27.2%) and 68 females (32.4%) were current smokers (p=NS).

Data in Table 1 demonstrate that overall, *C. pneumoniae* seropositive status in the study group was marginally associated with current smoking (p=0.08) and that high *C. pneumoniae* titers (≥1:128) were significantly associated with current smoking (p=0.04). Furthermore, there was a significant ($p<0.05$) quantitative linear relation between *C. pneumoniae* titer category and current smoking status, after controlling for age and sex by logistic regression (Table 2).

Discussion

Saikku et al[7] have reported associations of *C. pneumoniae* serological titers with both acute myocardial infarction and chronic coronary heart disease. The significance of these associations, which were not controlled for smoking, have been questioned.[9] In a reply to the criticism that smoking was a likely confounder of the *C. pneumoniae*-CAD associations reported in their original article, Saikku et al[11] presented univariate data showing no association between *C. pneumoniae* seropositive status and smoking. They did not, however, control their analysis for age or sex, nor did they report data on dose-response associations between smoking and titer level. A recent case-control study reported a significant association of angiographically proven CAD with *C. pneumoniae* immunoglobulin G antibody seropositive status after controlling for age and sex but not for smoking.[8] In this article we have reported a significant quantitative association of current smoking with *C. pneumoniae* serological titer. The reason for this association is uncertain, but it could be due to a greater

TABLE 2. Current Smoking Status and *Chlamydia pneumoniae* Antibody Titer Category

Titer category	Smoking* (%)	Odds ratio (95% confidence interval)†
<16	37/148 (25)	1.0 (referent)
16	21/70 (30)	1.3 (0.66–2.4)
32	24/72 (33.3)	1.4 (0.77–2.7)
64	14/43 (32.6)	1.5 (0.69–3.1)
≥128	13/28 (46.4)	2.4 (1.03–5.8)

*Curent smokers/total patients in category.
†From logistic regression, adjusted for age and sex (test for trend in the odds ratio, $p<0.05$).

frequency of respiratory infections among smokers, as hypothesized.[9,12] Whatever the reason for the smoking–*C. pneumoniae* antibody association found in this study, the results favor the assertion that smoking could be a confounder of the *C. pneumoniae*-CAD association and strongly suggest that future studies of *C. pneumoniae* infection and CAD should control for smoking.

Significant limitations of our report are that quantitative smoking data (pack-years) were not obtained, nor were those who never smoked distinguished from past smokers in our study. Future studies should include these measures of smoking as well as documentation of current smoking status. Because misclassification of exposure generally results in attenuation of relative risks, the association of smoking with *C. pneumoniae* infection might have been stronger if smoking had been measured more accurately in our study. Lack of detailed quantitative information concerning past smoking does not detract from the conclusion that smoking may be a confounder of *C. pneumoniae*-CAD associations.

Thom et al[8] reported an estimated relative risk for CAD of 1.5 for *C. pneumoniae* titers between 1:16 and 1:32 and an estimated relative risk for CAD of 2.0 for titers of 1:64 and greater. Our study found associations of similar or slightly greater magnitude between smoking and comparable levels of *C. pneumoniae* titer (Table 2). A mathematical property of the relative risk is that "... spurious associations due to confounding are always weaker than the underlying genuine associations when strength of association is measured by relative risk" (Breslow and Day,[13] p 69). It is therefore mathematically possible that controlling for smoking could have eliminated the associations reported by Thom et al,[8] despite the seemingly weak association between smoking and *C. pneumoniae* infection found in our study.

It is important to note that a causal link between *C. pneumoniae* infection and CAD might still exist even if the statistical association between *C. pneumoniae* serological titer and CAD in case-control studies is attenuated or even eliminated by control of confounding due to smoking. This is possible if smoking and *C. pneumoniae* infection are associated with CAD in a causal chain of events (e.g., smoking→*C. pneumoniae* infection→CAD). In such proposed analyses, it would be important to determine whether smoking and *C. pneumoniae* titer category are statistically independent and whether there are any interactions between these two variables. The potential importance to public health, should the *C. pneumoniae*-CAD association prove causal, is of sufficient magnitude that biological studies proposed by Thom et al[8] should be performed to answer such questions as: 1) Is *C. pneumoniae* infection merely incidental in smokers? or 2) Is smoking associated with CAD indirectly via promotion of *C. pneumoniae* infection?

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