Short Communication

Association of Chlamydia pneumoniae Immunoglobulin A Seropositivity and Risk of Lung Cancer

Lisa A. Jackson,1 San-Pin Wang, Valle Nazar-Stewart,2 J. Thomas Grayston, and Thomas L. Vaughan

Departments of Epidemiology [L. A. J., J. T. G., T. L. V.] and Pathobiology [S-P. W., J. T. G.], School of Public Health and Community Medicine, University of Washington; Center for Health Studies [L. A. J.], Group Health Cooperative of Puget Sound; and Program in Epidemiology [V. N-S., T. L. V.], Fred Hutchinson Cancer Research Center, Seattle, Washington 98101.

Abstract

Chlamydia pneumoniae is a common respiratory pathogen that has also been associated with risk for chronic diseases, including atherosclerotic cardiovascular disease. Two recent studies have reported an association between serological evidence of past infection with the organism and lung cancer. To further evaluate this association, we conducted a case-control study among a subgroup of white male smokers identified for a population-based case-control study of lung cancer in western Washington between 1993 and 1995. Serum specimens obtained at study enrollment from 143 cases and 147 controls were tested for C. pneumoniae IgG, IgM, and IgA antibodies. In multivariate analysis controlling for smoking variables and educational status, IgA antibody titer ≥16 was independently associated with risk of lung cancer among subjects <60 years of age [odds ratio (OR), 2.67; 95% confidence interval (CI), 1.21–5.89] but not among older subjects (OR, 0.69; 95% CI, 0.34–1.43). Among subjects <60 years of age, there was suggestive evidence of a stronger association among current smokers (OR 4.31; 95% CI, 1.36–13.68) than former smokers (OR 1.50; 95% CI, 0.48–4.75; P for interaction term, 0.26).

Additional studies, including prospective serological evaluations, are needed to further assess the possible significance of this association.

Introduction

Chlamydia pneumoniae is an obligate intracellular bacterium that is a well-documented cause of acute respiratory infections, including sinusitis, bronchitis, and pneumonia. Exposure to the organism is common, as evidenced by IgG seropositivity rates of >50% among adults, and many of these infections are clinically inapparent (1). C. pneumoniae has been associated with atherosclerotic cardiovascular disease (1, 2), and its detection in atherosclerotic plaque tissue (1, 2), as well as in specimens from lung, liver, and spleen (3), indicates that it can persist chronically in the lung and other tissues after initial respiratory inoculation.

A recent study conducted among participants in the ATBC study first suggested a potential association between infection with C. pneumoniae and risk of lung cancer (4). The ATBC study was a randomized trial of α-tocopherol and β-carotene supplementation for prevention of lung cancer among male smokers 50–69 years of age in southwestern Finland. In a nested case-control study, 52% of cases compared with 45% of controls met a serological criterion that included detection of anti-C. pneumoniae IgA antibodies and immune complexes in specimens obtained at enrollment and at year 3 of follow-up (but prior to the diagnosis of lung cancer for cases). After controlling for age, years of smoking, and number of cigarettes per day, cases were significantly more likely to meet the serological criterion than controls (OR, 1.6; 95% CI, 1.0–2.3). The association was evident only among persons 50–59 years (OR, 2.9; 95% CI, 1.5–5.4) and not among the older age group (OR, 0.9; 95% CI, 0.5–1.6).

A second study from Sweden compared the results of serological testing of specimens obtained at the time of bronchoscopic diagnosis of lung cancer for cases with that of controls from healthy adults and persons with CHD (5). In analyses that did not control for smoking status or age, male lung cancer patients were more likely to have IgG titers ≥512 than male CHD controls, and both male and female lung cancer patients were more likely to have IgA titers ≥64 than sex-matched CHD controls.

The findings from these two studies are consistent with a hypothesis that chronic inflammation, resulting from persistent C. pneumoniae infection, may be an etiological factor in the occurrence of lung cancer among smokers. To further evaluate this question, we tested specimens obtained from a western Washington population-based case-control study of males with lung cancer for serum IgG, IgA, and IgM antibodies for C. pneumoniae.

Materials and Methods

Study Population. This report used a subset of subjects from a larger case-control study that was designed to examine the risk of lung cancer among workers in the wood industry. Cases were prospectively identified by the Cancer Surveillance System of the Fred Hutchinson Cancer Research Center, a population-based registry covering 13 counties of western Washington that operates as part of the Surveillance, Epidemiology, and End Results program of the National Cancer Institute. Eligible

---

Received 1/11/00; revised 8/25/00; accepted 9/11/00.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom requests for reprints should be addressed, at Center for Health Studies, 1730 Minor Avenue, Suite 1600, Seattle, WA 98101. Phone: (206) 442-5216; Fax: (206) 287-4677; E-mail: lajack@u.washington.edu.

2 Present address: Center for Research on Occupational and Environmental Toxicology, Oregon Health Sciences University, Portland, OR 97201.

---

The abbreviations used are: ATBC study, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; OR, odds ratio; CI, confidence interval; CHD, coronary heart disease.
cases included males 20–74 years who were diagnosed with any histological type of lung cancer between 5/1/93 and 7/31/96 and who resided in an 11-county area that excluded the most populous counties surrounding the cities of Seattle and Tacoma. Of 929 eligible cases identified, telephone interviews were successfully completed for 821 (88.4%). To maintain comparability with controls, 37 cases without a telephone at the time of diagnosis were excluded, yielding 784 cases to be analyzed.

Male controls were identified by random digit dialing from the same geographical area over the same time period and frequency matched to cases by 5-year age groups (6). Overall, 8129 residential phone numbers were identified, of which 7870 (96.8%) were successfully screened for an eligible control. A total of 1047 eligible controls were identified and approached for interview, of which 883 (84.3%) were successfully interviewed. Seven controls without a residential telephone 1 year before the interview were excluded, leaving 876 for analyses.

Trained interviewers conducted structured telephone interviews with cases and controls that inquired about demographics, history of exposure to tobacco products, and occupational and residential histories. All questions referred to the time period before the reference date, which was 1 year before the diagnosis for cases and 1 year before ascertainment for controls. If a subject were deceased before the interview could be arranged, or too ill, a proxy respondent (usually the wife) was interviewed. This occurred for 45.0% of case and 3.3% of controls. If a subject were deceased before the interview could be arranged, or too ill, a proxy respondent (usually the wife) was interviewed. This occurred for 45.0% of case and 3.3% of controls. If a subject were deceased before the interview could be arranged, or too ill, a proxy respondent (usually the wife) was interviewed. This occurred for 45.0% of case and 3.3% of controls. If a subject were deceased before the interview could be arranged, or too ill, a proxy respondent (usually the wife) was interviewed. This occurred for 45.0% of case and 3.3% of controls.

Clinical information including histological type and stage at diagnosis was determined from medical records. Subjects eligible for the blood collection phase of the study, which are the focus of this report, included those who resided in one of the six largest counties closest to the Cancer Center. The interview response rates for cases and controls in these counties were virtually identical to those of the entire population.

### Table 1 Characteristics of cases and controls

<table>
<thead>
<tr>
<th></th>
<th>Cases (n = 143)</th>
<th>Controls (n = 147)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, yr (95% CI)</td>
<td>59.8 (58.3–61.3)</td>
<td>59.4 (57.8–60.9)</td>
</tr>
<tr>
<td>Current smoker (n, %)</td>
<td>83 (59)</td>
<td>52 (35)</td>
</tr>
<tr>
<td>Mean pack-years (95% CI)</td>
<td>52.9 (48.4–57.3)</td>
<td>34.8 (30.3–39.4)</td>
</tr>
<tr>
<td>Education (n, %)</td>
<td>Less than high school 32 (22)</td>
<td>15 (10)</td>
</tr>
<tr>
<td></td>
<td>High school</td>
<td>60 (42)</td>
</tr>
<tr>
<td></td>
<td>More than high school</td>
<td>50 (35)</td>
</tr>
</tbody>
</table>

### Table 2 Prevalence of IgA seropositivity (titer $\geq 16$) by age group and case status

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N % IgA $\geq 16$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>143</td>
<td>147</td>
<td>38 &amp; 0.15</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35–49</td>
<td>21</td>
<td>23</td>
<td>0.22</td>
</tr>
<tr>
<td>50–59</td>
<td>43</td>
<td>45</td>
<td>0.025</td>
</tr>
<tr>
<td>60–74</td>
<td>79</td>
<td>79</td>
<td>0.75</td>
</tr>
</tbody>
</table>

* Comparing IgA titer $\geq 16$ with negative titers.

### Results

Selected demographic and clinical characteristics of the cases and controls are given in Table 1. An IgG titer of $\geq 16$ was detected in equal proportions, 80%, of cases and controls. IgM antibodies were not detected in any sample. IgA titers $\geq 16$ were detected in 47% of cases and 38% of controls overall (Table 2). Among this age-group, all cases and controls of 60 years of age and an age range random sample of those 60 years of age and older were selected to yield 148 cases and 148 controls. Of those, six subjects were excluded because adequate serum samples were not available for testing, leaving 143 cases and 147 controls in the study population.

### Serological Testing

The microimmunofluorescence test (7) was used to detect *C. pneumoniae*-specific IgG, IgM, and IgA antibodies. Titters are expressed as reciprocals of serum dilution. All assays were performed by a single observer who was unaware of the case or control status of the specimens. Seropositivity to IgG, IgA, or IgM was defined *a priori* as a titer $\geq 16$. In the risk factor analyses, subjects with IgA titters $\geq 16$ were compared with those with undetectable titers (<8); subjects with titters of 8 were excluded.

### Statistical Analysis

Fisher’s exact test was used to compare differences in proportions of categorical variables; student’s t-test was used to compare differences in means of continuous variables. To examine the relationship between IgA seropositivity to *C. pneumoniae* and lung cancer, an unconditional logistic regression model was used to calculate the odds of an IgA titer $\geq 16$ after stratification by age (<60 or $\geq$60 years) with adjustment for smoking status (current or former), pack-years (<40 or $\geq$40), and educational level (less than high school, high school, or more than high school).

### Results

Selected demographic and clinical characteristics of the cases and controls are given in Table 1. An IgG titer of $\geq 16$ was detected in equal proportions, 80%, of cases and controls. IgM antibodies were not detected in any sample. IgA titers $\geq 16$ were detected in 47% of cases and 38% of controls overall (Table 2). Among this age-group, all cases and controls of 60 years of age and an age range random sample of those 60 years of age and older were selected to yield 148 cases and 148 controls. Of those, six subjects were excluded because adequate serum samples were not available for testing, leaving 143 cases and 147 controls in the study population.

### Serological Testing

The microimmunofluorescence test (7) was used to detect *C. pneumoniae*-specific IgG, IgM, and IgA antibodies. Titters are expressed as reciprocals of serum dilution. All assays were performed by a single observer who was unaware of the case or control status of the specimens. Seropositivity to IgG, IgA, or IgM was defined *a priori* as a titer $\geq 16$. In the risk factor analyses, subjects with IgA titters $\geq 16$ were compared with those with undetectable titers (<8); subjects with titters of 8 were excluded.

### Statistical Analysis

Fisher’s exact test was used to compare differences in proportions of categorical variables; student’s t-test was used to compare differences in means of continuous variables. To examine the relationship between IgA seropositivity to *C. pneumoniae* and lung cancer, an unconditional logistic regression model was used to calculate the odds of an IgA titer $\geq 16$ after stratification by age (<60 or $\geq$60 years) with adjustment for smoking status (current or former), pack-years (<40 or $\geq$40), and educational level (less than high school, high school, or more than high school).
significant interaction by smoking status ($P = 0.26$ for interaction term of smoking and seropositivity). Among current smokers $< 60$ years, there was little difference by pack-years of smoking (data not shown). Among former smokers in that age group, the association with IgA seropositivity was limited to those who had quit within 15 years of diagnosis (OR, 2.1; 95% CI, 0.4–12.5).

Among the cases, IgA seropositivity rates did not vary significantly by stage at diagnosis (local, regional, or distant), histological type, or location of the malignancy (upper, middle, or lower lobe or main bronchus; data not shown).

### Discussion

In this analysis, we found that IgA seropositivity to *C. pneumoniae*, defined as a titer of $\geq 16$, was independently associated with lung cancer among subjects $< 60$ years of age but not among older subjects. Despite differences in the study design and serological definitions used, these results are very similar to those reported by Laurila *et al.* (4) in their prospective nested case-control study conducted among participants in the ATBC study, in which the association between serological evidence of infection (defined as IgA titer $\geq 16$ in both samples or IgA titer $\geq 16$ in the year 3 sample and immune complexes titer $\geq 4$ in both samples) and risk of lung cancer also was restricted to persons $< 60$ years of age. Both study populations included only male ever-smokers.

These findings are clearly not sufficient to conclude that there is a biological relationship between *C. pneumoniae* infection and lung cancer, but they suggest a hypothesis that persistent pulmonary infection with *C. pneumoniae* may be a risk factor for lung cancer among younger male smokers. If true, this risk could be a consequence of a chronic inflammatory stimulus. Chronic inflammation can play a role in the development of cancer, as demonstrated by the association of *Mycoplasma pneumoniae* infection to act as an inflammatory stimulus. *C. pneumoniae* infection among the cases preceded the disease.

Another potential limitation is the relatively low overall response rate for blood collection, particularly among the cases. This raises the possibility that the seropositivity rates found in these analyses are not representative of all cases from the underlying population. The low response rate among cases is primarily attributable to their short survival. To the extent that antibody status to *C. pneumoniae* is related to length of survival after lung cancer diagnosis, our results would over- or underestimate the association with lung cancer. However, we are not aware of any data that suggest such a relationship.

It has been suggested that smoking may be associated with IgG or IgA seropositivity to *C. pneumoniae*. Because all of the subjects in this study were current or former smokers and because the association persisted after adjustment for smoking variables, confounding by smoking status is an unlikely explanation for this apparent association, although it is possible that there could be residual confounding by another variable. The retrospective design of this study is also a limitation because it cannot be determined from these results whether the serological evidence of *C. pneumoniae* infection among the cases preceded the disease.

In summary, although these results are clearly not sufficient to conclude that *C. pneumoniae* infection is a cause of lung cancer, they do provide additional evidence in support of this hypothesis. They should be followed by larger prospective studies.
studies, that ideally would include women and nonsmokers, to further evaluate the potential for C. pneumoniae infection to act as a predisposing factor for lung cancer.

References
Association of *Chlamydia pneumoniae* Immunoglobulin A Seropositivity and Risk of Lung Cancer


Updated version  Access the most recent version of this article at:  
http://cebp.aacrjournals.org/content/9/11/1263

Cited articles  This article cites 19 articles, 3 of which you can access for free at:  
http://cebp.aacrjournals.org/content/9/11/1263.full#ref-list-1

Citing articles  This article has been cited by 9 HighWire-hosted articles. Access the articles at:  
http://cebp.aacrjournals.org/content/9/11/1263.full#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.