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., 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 bronchosopic diagnosis of lung cancer for cases with that of control groups of 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 IgA 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

1 The abbreviations used are: ATBC study, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; OR, odds ratio; CI, confidence interval; CHD, coronary heart disease.

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

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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.

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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, 338 were alive at the time of diagnosis for cases and 338 were alive at the time of interview; we obtained a blood specimen from 502 (85.8%) of those with non-proxy interviews, representing 84.8% of all completed interviews in these counties, 585 were alive at the time of interview, representing 81.4% of those with non-proxy interviews, taking into account the proportions of participants who completed the interview and provided a blood specimen, was 69.2% (96.8% of controls, taking into account the proportions of participants who completed the interview and provided a blood specimen, was 41.6% (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 was 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 control interviews. An additional 3.7% of case and 1.7% of control interviews were conducted with the assistance of a proxy. 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 study. Of the 584 cases with completed interviews in these counties, 585 were alive at the time of interview (i.e., were not proxy interviews) and could be approached for a blood specimen. We successfully obtained a blood specimen from 275 (81.4%) of those with non-proxy interviews, representing 47.1% of all cases who were interviewed. The overall response rate for cases, taking into account the proportions of participants who completed the interview and provided a blood specimen, was 41.6% (88.4% × 47.1%). Of the 592 controls with completed interviews in these counties, 585 were alive at interview; we obtained a blood specimen from 502 (85.8%) of those with non-proxy interviews, representing 84.8% of all controls who were interviewed. The overall response rate for controls, taking into account the proportions of participants who completed the screening and interview and provided a blood specimen, was 69.2% (96.8% × 84.3% × 84.8%).

Because 95.3% of cases in the study were Caucasian (reflecting the underlying population of the 11-county area) and 97.1% of the cases had ever smoked cigarettes, we restricted our analyses for this report to white ever-smokers. Among that group, all cases and controls <60 years of age and an ~50% random sample of those 60 years and older were selected to yield 148 cases and 148 controls. Of those, six subjects were included 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. Titers 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 ≥16. In the risk factor analyses, subjects with IgA titers ≥16 were compared with those with undetectable titers (<8); subjects with titers 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 ≥16 after stratification by age (<60 or ≥60 years) with adjustment for smoking status (current or former), pack-years (<40 or ≥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 ≥16 was detected in equal proportions, 80%, of cases and controls. IgM antibodies were not detected in any sample. IgA titer ≥16 was detected in 47% of cases and 38% of controls overall (P = 0.15; Table 2). Among cases, the prevalence of IgA titer ≥16 tended to decrease with age whereas among controls this proportion tended to increase with age.

In multivariate analysis, there was a significant interaction between age (<60 or ≥60 years) and IgA titer ≥16 (P < 0.01 for the interaction term of age group and seropositivity); therefore, the analysis was conducted after stratification by age group. Among persons <60 years of age, IgA titer ≥16 was independently associated with case status (Table 3). This association was not found among persons ≥60 years of age. Further stratification of persons <60 years of age by smoking status revealed a strong and statistically significant association between IgA seropositivity and lung cancer among current smokers but only a modest increase in risk among former smokers (Table 3). Among this age-group there was not, however, a

<table>
<thead>
<tr>
<th>Table 1 Characteristics of cases and controls</th>
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<tbody>
<tr>
<td>Cases</td>
</tr>
<tr>
<td>Mean age, yr (95% CI)</td>
</tr>
<tr>
<td>Current smoker (n, %)</td>
</tr>
<tr>
<td>Mean pack-years (95% CI)</td>
</tr>
<tr>
<td>Education (n, %)</td>
</tr>
<tr>
<td>Less than high school</td>
</tr>
<tr>
<td>High school</td>
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<td>More than high school</td>
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<table>
<thead>
<tr>
<th>Table 2 Prevalence of IgA seropositivity (titer ≥16) by age group and case status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
</tr>
<tr>
<td>N</td>
</tr>
<tr>
<td>All subjects</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>35–49</td>
</tr>
<tr>
<td>50–59</td>
</tr>
<tr>
<td>60–74</td>
</tr>
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</table>

* Comparing IgA titers ≥16 with negative titers.
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 gastroesophageal reflux disease and esophageal adenocarcinoma (8, 9). Inflammation resulting from persistent bacterial infection may also be associated with certain cancers. For example, IgG seropositivity to *Helicobacter pylori* is associated with an increased risk of gastric adenocarcinoma (10–12) and primary gastric non-Hodgkin’s lymphoma (13). The common pathway in the development of these cancers is believed to be infection resulting in chronic gastritis, which then leads to epithelial or lymphoid hyperplasia, increasing the risk of malignant transformation of those tissues.

Although *C. pneumoniae* infection has not been previously associated with malignancy, the organism has been implicated as a cause of immunopathology in other settings. In rabbit and murine models, experimental infection with *C. pneumoniae* has been documented to accelerate the progression of aortic atherosclerotic-like lesions (1, 2). Because atherosclerosis is largely an inflammatory process (14), this effect supports the potential for chronic *C. pneumoniae* infection to act as an inflammatory stimulus. *C. pneumoniae in vitro* infection of human alveolar macrophages and peripheral blood mononuclear cells has also been demonstrated to induce secretion of cytokines, including tumor necrosis factor-$\alpha$ and interleukin 1$\beta$ (15, 16). Furthermore, chronic or repeated infection with *C. trachomatis* has been implicated as an inflammatory stimulus in the pathogenesis of trachoma and pelvic inflammatory disease (17). Thus, it is plausible to consider induction of an inflammatory response as one potential mechanism by which *C. pneumoniae* infection could be causally associated with the development of lung cancer.

One factor limiting the interpretation of these results is the uncertain relationship between IgG or IgA antibodies and the presence of persistent *C. pneumoniae* infection. Although IgA is believed by some to be a more specific marker of chronic or persistent *C. pneumoniae* infection than IgG, because the half-life of IgA is only ~6 days compared with 23 days for IgG (18), and an association between elevated IgA titers and risk of cardiovascular disease has been reported in some studies (2, 19–21), the sensitivity and specificity of this marker have not been determined. The lack of a true gold standard for persistent infection hampers efforts to validate potential serological markers (22).

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 rates 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

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**Table 3**  Estimated odds ratio of lung cancer associated with *C. pneumoniae* IgA titers $\geq 16$ by age group and smoking status

<table>
<thead>
<tr>
<th>Age &lt;$\approx$ 60 yr</th>
<th>Cases</th>
<th>Controls</th>
<th>OR</th>
<th>Adjusted OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>n % IgA $\geq 16$</td>
<td>n % IgA $\geq 16$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>64</td>
<td>50</td>
<td>68</td>
<td>26</td>
</tr>
<tr>
<td>Current smokers</td>
<td>42</td>
<td>52</td>
<td>32</td>
<td>19</td>
</tr>
<tr>
<td>Former smokers</td>
<td>22</td>
<td>46</td>
<td>36</td>
<td>33</td>
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</table>

<table>
<thead>
<tr>
<th>Age $\geq$ 60 yr</th>
<th>Cases</th>
<th>Controls</th>
<th>OR</th>
<th>Adjusted OR</th>
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<td></td>
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<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>79</td>
<td>44</td>
<td>79</td>
<td>46</td>
</tr>
<tr>
<td>Current smokers</td>
<td>43</td>
<td>47</td>
<td>20</td>
<td>45</td>
</tr>
<tr>
<td>Former smokers</td>
<td>36</td>
<td>42</td>
<td>59</td>
<td>46</td>
</tr>
</tbody>
</table>

a Adjusted for smoking status (current or former), pack-years (<40 or $\geq$ 40), and education.

b Adjusted for pack-years (<40 or $\geq$ 40) and education.
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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

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