Chlamydia pneumoniae Infection and Risk of Lung Cancer

Alyson J. Littman,[1,2] Emily White,[1,2] Lisa A. Jackson,[1,3] Mark D. Thornquist,[2] Charlotte A. Gaydos,[4] Gary E. Goodman,[2,5] and Thomas L. Vaughan[1,2]

[1]Department of Epidemiology, University of Washington, Seattle, WA; [2]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; [3]Center for Health Studies, Group Health Cooperative, Seattle, WA; [4]Division of Infectious Diseases, Johns Hopkins University, Baltimore, MD; [5]Swedish Cancer Institute, Seattle, WA

Abstract

Infection with *Chlamydia pneumoniae* may be associated with an increased risk of lung cancer. We conducted a matched case-control study (508 pairs) nested within a large prospective study to investigate whether IgA antibody titers to *C. pneumoniae* measured by the microimmunofluorescence test are associated with lung cancer risk after controlling for confounders. Individuals with antibody titers ≥16 had 1.2 times the risk of lung cancer (95% confidence interval, 0.9-1.6) compared to those with lower titers. There was a significant trend (P = 0.007) of increasing odds ratios with increasing IgA titers primarily due to an odds ratio of 2.8 (95% confidence interval, 1.1-6.7) associated with titers ≥256. Lung cancer risk associated with IgA titers ≥16 was stronger among former smokers. To better understand predictors of IgA seropositivity, we also examined demographic, lifestyle, dietary, and medical correlates of IgA titers ≥16 among controls. Those with race not classified as White or Black were more likely to have IgA titers ≥16; there were no significant differences in seropositivity by smoking behaviors. In summary, the adjusted odds ratio for lung cancer associated with IgA titers ≥16 was compatible with a weakly positive association, although non-differential measurement error of antibody titers may have resulted in a conservative bias. Future studies using precise measures of chronic *C. pneumoniae* status are needed to better determine the role of this organism in the etiology of lung cancer. (Cancer Epidemiol Biomarkers Prev 2004;13(10):1624–30)

Introduction

There is accumulating evidence that infection with *Chlamydia pneumoniae* may be associated with an increased risk of lung cancer (1-6). Prospective and retrospective studies have reported 50% to 100% increased risks of lung cancer among those with elevated IgA antibody titers to this organism. Chronic inflammation and its sequelae may be part of the causal pathway.

We conducted a nested case-control study to further investigate this association while controlling for major lung cancer risk factors. Additional objectives were to identify possible modifying factors of the *C. pneumoniae*-lung cancer association and to describe histology-specific and site-specific associations. We used IgA antibody titers to *C. pneumoniae* as our exposure measure because IgA antibodies have a short half-life and thus have been proposed as a serologic marker of chronic or persistent *C. pneumoniae* infection (7, 8). To better understand the predictors of IgA antibody detection, we also examined the demographic, lifestyle, dietary, and medical correlates of IgA titers ≥16 among controls.

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Requests for reprints: Alyson J. Littman, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center and Department of Epidemiology, University of Washington, 1100 Fairview Avenue North, M4-B402, P.O. Box 19024, Seattle, WA 98109-1024. Phone: 206-667-7707; Fax: 206-667-4787. E-mail: alittman@fhcrc.org

Materials and Methods

Study Population. The present report is based on a case-control study nested within the β-Carotene and Retinol Efficacy Trial (CARET), a randomized, double-blind, placebo-controlled prevention trial that evaluated whether supplementation with β-carotene and vitamin A would reduce the risk of lung cancer among two high-risk groups: heavy smokers and persons with occupational exposure to asbestos. The design and findings of the study have been published elsewhere (9-11). Briefly, CARET was initiated with a pilot phase in 1985 and expanded 10-fold at six study centers throughout the United States (the ''efficacy'' study). CARET randomized 14,254 heavy smokers and 4,060 asbestos-exposed workers. Eligibility criteria for heavy smokers included being between ages 50 and 69 years and a current or former smoker (quit within the previous 6 years) with ≥20 pack-years of cigarette smoking history. Eligible asbestos-exposed participants were men ages 45 to 69 years, who were current or former cigarette smokers who quit within the previous 15 years. In addition, they had to have occupational exposure to asbestos beginning at least 15 years prior to recruitment into the trial and either 5 years of work in a high-risk trade or chest X-ray findings consistent with asbestos exposure. Subjects were randomized to receive a standard daily regimen of either 30 mg β-carotene plus 25,000 IU of retinyl palmitate or placebo. In January 1996, the active intervention was terminated prematurely because the group receiving retinyl

palmitate and β-carotene had a 28% excess lung cancer incidence and 17% excess overall mortality rate compared with the group receiving a placebo.

Data Collected at Baseline and Follow-up. At baseline, information on demographic factors, general health, health history, smoking history, and usual diet was collected. Participants identified the age when they started smoking, any period(s) of time when they did not smoke cigarettes and their duration(s), and average number of cigarettes they smoked. Pack-years of smoking were calculated as average number of cigarettes smoked per day divided by 20 multiplied by the number of years smoked. To assess usual dietary intake over the preceding year, we used a 110-item semiquantitative food frequency questionnaire that was developed at the Fred Hutchinson Cancer Research Center and is similar to the National Cancer Institute/Block Food Frequency Questionnaire (12).

Blood was collected from pilot participants every year and from efficacy participants at their first visit and every 2 years after randomization. Serum was separated by centrifugation and stored for a maximum of 2 weeks in brown glass freezer vials at −20°C prior to long-term storage at the −70°C at CARET Serum Bank (10).

Sources of initial reports of outcomes were received from participants or their next of kin at yearly or biannual contacts as well as from cancer registries, state boards of health, and the National Death Index. The CARET Internal End Points Committee reviewed all initial reports of lung cancer and obtained clinical records and pathology reports from the diagnosing physician/hospital. The committee used this information to determine the primary site, histology, date of histologic diagnosis, and relationship of the cancer to death if the patient had died (9).

Selection of Cases and Controls. In the present study, we selected 520 of the 836 confirmed lung cancer cases diagnosed through June 2000. To maximize the power to detect associations in important subgroups (i.e., women and those diagnosed at younger ages), we selected all women (n = 264) and all men ages <60 years at diagnosis (n = 84). About 30% of men over age 60 years (n = 172) were randomly selected after stratifying on 5-year age groups. One control subject without lung cancer was selected for each case from among those who were alive and being followed at the time of the case's diagnosis. The control subject was matched to the case for year of randomization, age at randomization (within 5-year age groups), gender, pilot or efficacy study, exposure cohort (heavy smoker or asbestos–exposed), and smoking status (current or former at baseline). Nine case-control pairs were omitted for the following reasons: insufficient blood sample available (n = 4), sample lost (n = 2), only a postdiagnosis blood sample available (n = 2), and missing information on smoking frequency or duration (n = 1). Furthermore, three pairs were concordant on all factors and were dropped from conditional logistic regression models, leaving 508 pairs. Participants with implausibly high or low energy intakes (<800 or >5,000 kcal/d for men and <600 or >4,000 kcal/d for women; n = 128; ref. 13) were excluded from the analyses on diet.

Serologic Testing. Serum collected at baseline or, for those without baseline serum available (n = 70), at the earliest time available prior to lung cancer diagnosis in the cases and a matched time in the controls was used to evaluate *C. pneumoniae* IgA and IgG antibody titers using the microimmunofluorescence test (14). Purified *C. pneumoniae* elementary bodies (strain AR39, Washington Research Foundation, Seattle, WA) were used as the antigen. Details of the test technique are described elsewhere (14). Only even fluorescence of the elementary bodies in the antigen dots was considered to be positive. After screening at a dilution of 1:16, the highest dilution demonstrating definite fluorescence was recorded as the titer (expressed as a reciprocal of the serum dilution). The antibody determinations of each case and matched control were tested simultaneously in the same titration series (batch) in a blinded fashion. By convention, *C. pneumoniae* titers (IgA or IgG) ≥16 were considered seropositive, which is consistent with the cutoff used in other studies (1, 2, 5, 6).

Statistical Analysis. We used conditional logistic regression, which takes into account the matched design, to estimate the odds ratios (OR) and 95% confidence intervals (95% CI) of lung cancer in relation to IgA and IgG antibody titers. The other characteristics listed in Table 1 were evaluated for confounding and were included in models if they changed the β coefficient for dichotomous IgA by >10%. Ultimately, all models were adjusted for education (college graduate or not), body mass index (continuous), years smoked (log transformed), and cigarettes smoked per day (continuous). Although we were concerned that storage time could potentially affect antibody titers, because participants were matched on randomization year, they were also effectively matched on the time between blood draw and microimmunofluorescence test.

The purpose of our main study was to determine whether IgA titers ≥16 were associated with an increased risk of lung cancer (identified as the ''case-control study''). In secondary analyses, we evaluated lung cancer risk by categorical IgA titer level and also conducted analyses with IgG antibody titers. The association between *C. pneumoniae* and lung cancer risk may differ depending on the presence of other factors (age, sex, intervention arm, etc.); consequently, we evaluated the presence and magnitude of multiplicative interactions by conducting analyses stratified on the characteristics of interest. In these analyses, former smokers were defined as individuals who had quit at least 1 year before baseline to reduce misclassification of those who only quit briefly at, or only shortly before, baseline (15). To examine whether *C. pneumoniae* may be associated with certain types (e.g., histologic type and lobe of origin) of lung cancer, we also constructed polychotomous regression models, which were adjusted for the confounding factors as well as the matching factors.

We used unconditional logistic regression to identify correlates of IgA seropositivity among controls (identified as the ''correlates of IgA seropositivity study''). In these analyses, seropositivity was our outcome variable. Models were adjusted for age, sex, race, pilot or efficacy study, and time between blood draw and microimmunofluorescence test.

Table 1. Characteristics of lung cancer cases and controls

Characteristics	Cases (n = 508)	Controls (n = 508)
Age at enrollment (y)*		
<55	23.1	23.1
55-59	27.8	27.8
60-64	25.1	25.1
>64	24.1	24.1
Sex and exposure cohort*,†		
Female—heavy smoker cohort	50.7	50.7
Male—heavy smoker cohort	33.7	33.7
Male—asbestos-exposed worker cohort	15.7	15.7
Race/ethnicity		
White	93.0	92.2
Black	3.5	2.7
Other/unknown	3.5	5.1
Education		
Less than high school graduate	16.5	12.3
High school graduate	26.8	24.2
Some college	40.9	36.7
College graduate or more	15.8	26.7
Study*		
Efficacy	84.2	84.2
Pilot	15.8	15.8
Study arm		
Placebo	42.5	46.6
Intervention	57.5	53.4
Body mass index (kg/m^2)		
<25	37.7	31.4
25-29	40.2	43.2
≥30	22.2	25.3
Current smoker at baseline*		
No	15.8	15.8
Yes	84.2	84.2
Pack-years of cigarette smoking		
<35	13.5	26.8
35-43	21.5	24.1
44-56	24.9	25.2
≥57	40.1	23.9
Cigarettes smoked per day		
<20	10.0	20.0
20	35.6	38.8
21-39	37.0	32.3
≥40	17.4	9.0
Years smoked		
<36	19.0	29.4
36-40	26.4	26.2
41-44	25.1	19.6
>44	29.6	24.9
Prior lung diseases		
Asbestosis	7.8	8.8
Asthma	10.4	8.6
Chronic bronchitis/ emphysema	20.0	14.9
Pneumonia	29.0	26.4
Tuberculosis	2.0	2.7
Histologic type‡		
Large cell carcinoma	12.5	—
Small cell carcinoma	18.4	—
Squamous cell carcinoma	19.6	—
Adenocarcinoma	25.6	—
Other	23.9	—
Site		
Main bronchus	2.0	—
Upper lobe	50.9	—
Middle lobe	6.3	—
Lower lobe	20.6	—
Lung, not otherwise specified	20.4	—

NOTE: 86 cases and 82 controls were missing data on education, 1 case and 2 controls were missing data on body mass index, and 21 cases and 23 controls were missing data on prior lung diseases. Percentages reported are of nonmissing values.

*Variables used to match controls to cases.

†Due to eligibility criteria, no women were in the asbestos-exposed worker cohort.

‡Histologic types were classified according to the *International Classification of Diseases for Oncology* (ICD-O; ref. 27) as follows: large cell carcinoma (ICD-O 8012), small cell carcinoma (ICD-O 8040-8045), squamous cell carcinoma (ICD-O 8050-8076), and adenocarcinoma (ICD-O 8140, 8211, 8230-8231, 8323, 8480-8490, 8550, 8570-8572).

Results

Case-Control Study. Table 1 gives distributions of various characteristics among cases and controls. Cases were matched to controls on 5-year age group, sex, exposure cohort, pilot or efficacy study, and smoking status at baseline and thus did not vary by these characteristics (Table 1). The median age of cases and controls was 59 years, and about half were women. Those who developed lung cancer were less likely to be college graduates and more likely to have been randomized to the intervention, to have a body mass index <25 kg/m^2, and to have a history of chronic bronchitis or emphysema. As expected, those who developed lung cancer had a heavier smoking history (cigarettes per day, years smoked, and pack-years). Cases tended to have a higher energy intake and percentage energy from fat but to consume fewer servings of fruits and vegetables compared with controls (data not shown). The most common histologic type was adenocarcinoma (26%) followed by squamous cell carcinoma (20%) and small cell carcinoma (18%); 51% of cancers arose in the upper lobe of the lung.

Table 2 provides the distributions of IgA and IgG antibody titers among cases and controls and their corresponding adjusted ORs and 95% CIs. Compared with controls, cases were more likely to have IgA titers ≥16 (55.4% versus 51.3%) and ≥256 (5.1% versus 2.5%). Those with antibody titers ≥16 had 1.2 times the risk of lung cancer (95% CI, 0.9-1.6) compared with those with lower titers. There was a significant trend (P = 0.007) of increasing ORs with increasing IgA titers primarily due to an OR of 2.8 (95% CI, 1.1-6.7) associated with titers ≥256. Because a history of chronic bronchitis or emphysema could be a confounder or in the causal pathway, we conducted analyses both with (OR, 1.3; 95% CI, 1.0-1.7), and without adjustment for it (Table 2). For IgG, the proportions of cases and controls with titers ≥16 were similar (63.8% and 64.2%, respectively), although cases were more likely to have IgG titers ≥256 than controls (21.7% versus 16.1%; Table 2). To determine the association between high IgG titers and lung cancer risk, independent of IgA titers, we also conducted analyses adjusting for IgA titers. In these analyses, results were

Table 2. Distribution of IgA and IgG antibody titers to *C. pneumoniae* among cases (*n* = 508) and controls (*n* = 508) and corresponding OR and 95% CI

Titer	IgA				IgG			
	Cases (%)	Controls (%)	OR	95% CI	Cases (%)	Controls (%)	OR	95% CI
Dichotomous analysis								
<16	44.6	48.7	1.0	—	36.2	35.8	1.0	—
≥16	55.4	51.3	1.2	0.9-1.6	63.8	64.2	0.8	0.6-1.2
Categorical analysis								
<16	44.6	48.7	1.0	—	36.2	35.8	1.0	—
16	12.7	15.5	0.9	0.6-1.4	6.1	6.9	1.0	0.5-1.8
32	19.4	17.4	1.3	0.9-1.8	11.7	12.7	0.9	0.6-1.4
64	10.6	10.2	1.3	0.8-2.1	11.9	14.7	0.7	0.4-1.1
128	7.6	5.7	1.3	0.7-2.4	12.3	13.9	1.0	0.6-1.5
≥256	5.1	2.5	2.8	1.2-6.4	21.7	16.1	1.5	1.0-2.2
P for trend			0.007				0.06	

NOTE: Models are based on conditional logistic regression with case-control pairs matched on year of randomization, age at randomization, sex and exposure cohort (female, male—heavy smoker cohort, and male—asbestos-exposed worker cohort), pilot or efficacy study, and smoking status at baseline (current or former) and adjusted for years smoked (log transformed), cigarettes smoked per day, education (college graduate or not), and body mass index (continuous).

attenuated and there was no longer evidence for a stronger association with high titers (OR, 1.1 for IgG ≥256 versus IgG <16).

Table 3 gives ORs for the association between IgA ≥16 and lung cancer risk in specific subsets of the cohort. Lung cancer risks associated with IgA ≥16 were greater for men in the asbestos-exposed worker cohort, individuals enrolled in the pilot study, and former smokers who had quit at least 1 year before baseline, although only the smoking status-IgA interaction approached statistical significance (*P* for interaction = 0.08). To be more certain that we had excluded those with subclinical lung cancer at the time blood was collected, we progressively eliminated individuals who were diagnosed within 5 years of blood sampling. There was little change in the ORs, suggesting that IgA titers measured at baseline represented pre–lung cancer levels. IgA seropositivity was more strongly associated with squamous cell carcinomas. There was also a suggestion, based on small numbers, of increased risk for cancers occurring in the main lobe (OR, 2.5; 95% CI, 0.6-10.1) and middle lobe (OR, 1.9; 95% CI, 0.9-4.1) of the lung.

Correlates of IgA Seropositivity Study. Table 4 gives the prevalence of IgA seropositivity among controls along with ORs and 95% CIs. Seropositivity increased somewhat with age (Table 4). Men in the smoker cohort were more likely to be seropositive than women or than men in the asbestos-exposed worker cohort. Those of ''other'' or unknown race were more likely to be seropositive than Caucasians. Seropositivity was lower among those who were obese and who had self-reported health that was not excellent. There was a suggestion of lower seropositivity with increasing time since quitting but little pattern of seropositivity with any other cigarette smoking variables or any dietary variables (data not shown).

Discussion

At least six other studies (1-6) have investigated the association between IgA antibodies to *C. pneumoniae* and lung cancer risk, two of which were prospective in design (2, 6). Despite differences in populations and study designs, ORs observed in the prospective studies were similar and ranged between 1.6 and 2.2 after adjustment for smoking. The four case-control studies (1, 3-5) also observed ORs of similar, or slightly greater, magnitude. In studies that used more than one cutoff for IgA titers, stronger ORs were observed for higher IgA titers (e.g., ≥64; refs. 2-4).

In comparison, our results for IgA seropositivity were generally weaker and compatible with there being no association. As expected, we observed no association with IgG seropositivity. ORs were statistically significantly elevated for IgA titers ≥256, suggesting that higher titers may be a better predictor of lung cancer risk than lower antibody titers. However, because we did not design the study to investigate this categorization, it needs replication in other studies. Furthermore, it is unclear biologically how higher titers may be related to severity or chronicity of infection.

In analyses limited to controls, we did not observe any differences in IgA seropositivity by smoking behaviors, except perhaps time since quitting, contrary to previous study findings (16, 17). This may be due to homogeneity of study participants in terms of their smoking, misclassification of smoking or *C. pneumoniae* seropositivity, or because there truly is no association.

Of the three studies that investigated histology-specific results (2, 5, 6), one study reported a stronger association with squamous cell and small cell carcinomas combined (6), whereas the others did not observe a statistically significant difference by histologic type (2, 5). We observed a stronger association for squamous cell and to a lesser extent, for small cell carcinomas and adenocarcinomas. Several studies have observed a stronger association between *C. pneumoniae* infection and lung cancer among those diagnosed at younger ages (<60 years; refs. 1, 5, 6); we found little support for that notion.

Prospective studies such as ours should minimize any influence of disease itself on titer levels, reduce selection bias, and generally include better adjustment of potential confounding factors. In case-control studies where blood

Table 3. OR and 95% CI for lung cancer associated with *C. pneumoniae* IgA antibody titers >16 compared with <16 in various subgroups

Subgroup	*n*	OR	95% CI	*P* for interaction
All	1,016	1.2	0.9-1.6	—
Age at diagnosis (y)				
<60	256	1.3	0.7-2.2	0.98
60-69	518	1.2	0.8-1.8	
≥70	242	1.3	0.7-2.3	
Sex and exposure cohort				
Female—heavy smoker cohort	514	1.2	0.8-1.7	0.43
Male—smoking cohort	342	1.1	0.7-1.8	
Male—asbestos-exposed worker cohort	160	1.9	0.9-3.7	
Study arm				
Placebo	452	1.2	0.8-1.9	0.99
Intervention	564	1.2	0.9-1.8	
Study				
Efficacy	854	1.2	0.9-1.6	0.46
Pilot	162	1.6	0.8-3.1	
Smoking status				
Former smoker (quit >1 y)	194	2.1	1.1-4.1	0.08
Current smoker	822	1.1	0.8-1.5	
Latency analysis (y)*				
>1	990	1.2	0.9-1.6	—
>2	880	1.3	1.0-1.8	
>3	762	1.2	0.9-1.7	
>4	648	1.2	0.8-1.6	
>5	546	1.3	0.9-1.8	
Histologic type†,‡				
Large cell carcinoma	128	0.7	0.4-1.3	—
Small cell carcinoma	188	1.4	0.9-2.2	
Squamous cell carcinoma	198	1.7	1.1-2.8	
Adenocarcinoma	260	1.3	0.9-1.9	
Other	242	1.0	0.6-1.5	

NOTE: Models are based on conditional logistic regression with case-control pairs matched on year of randomization, age at randomization, sex and exposure cohort (female, male-heavy smoker cohort, and male—asbestos-exposed worker cohort), pilot or efficacy study, and smoking status at baseline (current or former) and adjusted for years smoked (log transformed), cigarettes smoked per day, education (college graduate or not), and body mass index (continuous), expect where otherwise noted.

*Individuals that were diagnosed within 1, 2, 3, 4, or 5 years of blood draw were sequentially eliminated from analyses.

†Histologic types were classified according to the ICD-O (27) as follows: large cell carcinoma (ICD-O 8012), small cell carcinoma (ICD-O 8040-8045), squamous cell carcinoma (ICD-O 8050-8076), and adenocarcinoma (ICD-O 8140, 8211, 8230-8231, 8323, 8480-8490, 8550, 8570-8572).

‡Models are based on polytomous logistic regression and adjusted for matching factors and years smoked (log transformed), cigarettes smoked per day, education (college graduate or not), and body mass index (continuous).

Table 4. Proportion of controls with *C. pneumoniae* IgA titers ≥16 and corresponding OR and 95% CI

Characteristics	% with IgA ≥16	OR*	95% CI
Overall	51.3	—	—
Age at enrollment (y)			
<55	49.2	1.0	—
55-59	46.5	0.9	0.5-1.5
60-64	53.1	1.2	0.7-2.0
≥65	56.9	1.5	0.9-2.5
Sex and study cohort			
Female—heavy smoker cohort	47.1	1.0	—
Male—heavy smoker cohort	59.3	1.7	1.1-2.5
Male—asbestos-exposed worker cohort	47.5	1.2	0.7-2.0
Race			
White	50.3	1.0	—
Black	50.0	1.1	0.4-3.3
Other/unknown	69.2	2.5	1.0-5.9
Study			
Efficacy	53.5	1.0	—
Pilot	39.5	0.4	0.1-1.7
Study arm			
Placebo	51.7	1.0	—
Intervention	50.9	1.0	0.7-1.5
Body mass index (kg/m^2)			
<25	52.5	1.0	—
25-29	54.6	1.0	0.7-1.6
≥30	45.0	0.7	0.4-1.2
Prior lung diseases†,‡			
Asbestosis	41.9	0.6	0.2-1.5
Asthma	59.5	1.5	0.8-2.9
Chronic bronchitis/emphysema	54.8	1.2	0.7-1.9
Pneumonia	49.6	0.8	0.6-1.3
Tuberculosis	69.2	1.5	0.4-5.3
General health			
Excellent	65.2	1.0	—
Very good/good	50.8	0.6	0.3-1.0
Fair/poor	55.1	0.7	0.3-1.5
Smoking status			
Former (quit >1 y)	49.5	1.0	—
Current	51.7	1.0	0.6-1.5
Pack-years of cigarette smoking			
<35	53.3	1.0	—
35-43	43.9	0.6	0.4-1.1
44-56	51.2	0.8	0.4-1.3
>57	56.6	0.9	0.5-1.5
Cigarettes smoked per day			
<20	52.0	1.0	—
20	50.0	0.9	0.5-1.5
21-39	50.3	0.9	0.5-1.5
≥40	58.7	1.1	0.5-2.3
Years smoked			
<36	50.0	1.0	—
36-40	48.5	0.9	0.6-1.5
41-44	50.0	0.8	0.5-1.5
>44	56.7	0.9	0.5-1.7
Years since quit smoking§			
<4	58.8	1.0	—
4-6	52.6	0.9	0.4-2.3
≥6	37.9	0.7	0.2-2.7

*ORs adjusted for age at randomization, sex and exposure cohort (female, male—heavy smoker cohort, and male—asbestos-exposed worker), race, pilot or efficacy study, and years between blood draw and micro-immunofluorescence test.

†488 controls answered questions on prior lung diseases; 51.2% were seropositive.

‡Comparison group consists of controls without the lung disease.

§Among former smokers.

was sampled after diagnosis (1, 3-5), it is possible that lung cancer caused an elevation in *C. pneumoniae* titers. It is therefore unclear whether lung cancer cases were more susceptible to chronic infection with *C. pneumoniae* or whether chronic infection contributed to an increased risk of lung cancer. Serum was collected before diagnosis in our study. When we successively excluded cases that had blood drawn 1 to 5 years before diagnosis, results remained the same, suggesting that *C. pneumoniae* infection preceded disease onset.

Another issue in case-control studies is the short survival of persons diagnosed with lung cancer. To the extent that *C. pneumoniae* infection is related to length of survival after lung cancer diagnosis, results of studies that exclude those who died shortly after diagnosis, or were too sick to participate, may be biased. However, we are aware of no data that suggest such a relationship.

Because both age and smoking are strongly associated with lung cancer and possibly positively associated with *C. pneumoniae* IgA and IgG titers (16, 17), they are potentially important confounders. Inadequate control for these factors could result in a bias away from the null. In several case-control studies that found particularly strong associations (3, 4), controls were more likely to be younger and former or never smokers than cases, and investigators did not control for any measures of smoking. In our study, confounding due to cigarette smoking was addressed both in the design phase by restricting the study to heavy smokers (see eligibility criteria in Materials and Methods) and by matching on smoking status at baseline, and in the analysis phase by adjusting for daily number of cigarettes smoked and years of smoking. However, because there was little association between cigarette smoking and IgA seropositivity in our study, there was likely little confounding due to cigarette smoking.

Definition of ''chronic'' infection varied in each of the studies. For example, one study used a combined measure of IgA $\geq$16 and immune complexes (6), whereas others used IgA $\geq$64 (4) and/or IgG $\geq$512 (1, 3). Furthermore, it was unclear if the criteria were chosen a priori or based on the data. Data-driven determination of cut points could result in a bias away from the null. Conversely, nondifferential misclassification of IgA antibody titers could result in a bias toward the null. We can use information on the reliability of *C. pneumoniae* antibodies to determine how misclassification of *C. pneumoniae* titers might attenuate relative risks. For example, if the true relative risk for the association between lung cancer and IgA titers $\geq$16 were 2.0, reliability of the magnitude observed in our reliability study (κ = 0.39; ref. 18) would result in an attenuated relative risk of at most $2.0^{\sqrt{0.39}}$ or 1.5 (19). Thus, because of nondifferential misclassification, our results may underestimate the true association. It is unlikely, however, that the present study would have more misclassification and consequently a greater degree of attenuation due to misclassification than other studies.

A relationship between *C. pneumoniae* infection and lung cancer is biologically plausible. Although it is not known how infection with *C. pneumoniae* might induce or lead to the progression of lung cancer, chronic inflammation may be involved. Agents that cause inflammation, such as infectious agents, may cause prolonged irritation, resulting in cell death and increased mitotic activity. The subsequent cell division that occurs during repair of the damaged tissue may increase the risk of cancer at the affected site (20). For example, multiple studies have linked chronic infection with *Helicobacter pylori* to an increased risk of gastric adenocarcinoma (21-23). *C. pneumoniae* may play a similar role in lung cancer development. *C. pneumoniae* stimulates the release of inflammatory mediators such as tumor necrosis factor-α, interleukin-1β, and interleukin-8 (24). Chronic infection generally, and interleukin-8 specifically, may cause genetic damage. Interleukin-8 also acts as a promoter of tumor growth for human non-small cell lung carcinoma through its angiogenic properties. *C. pneumoniae* can impair or even block apoptosis of infected cells (25) by induction of interleukin-10 (26), resulting in chronic infection and an increased risk of malignant transformation of infected cells.

In summary, we found a more moderate overall association between lung cancer risk and elevated IgA titers than most previous studies. However, a key limitation of all such studies is the relatively modest reliability of the microimmunofluorescence test on which they are based (18) and the resulting conservative bias. Thus, to better understand the role of *C. pneumoniae* in lung cancer carcinogenesis, it is important that we identify measures of chronic *C. pneumoniae* infection that are valid and reliable as well as being inexpensive and minimally invasive to obtain. Due to the intracellular nature of *C. pneumoniae* (which makes it difficult to culture), the inaccessibility of lung tissue for sampling, and the lack of a gold standard for identification of chronic infection, development and evaluation of new measures and their role in lung cancer is challenging. Nevertheless, considering the public health importance of lung cancer, such efforts could be richly rewarded.

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