

Letter to the Editor

Lack of association between serum antibodies of *Chlamydia pneumoniae* infection and the risk of lung cancer

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Sir,

Chronic inflammation of the lung might contribute to carcinogenesis and there is epidemiological evidence that a history of pulmonary diseases such as pneumonia entails an increased risk of lung cancer.¹ Among respiratory infectious agents suspected to be responsible for such an effect is *Chlamydia pneumoniae*. An association between *C. pneumoniae* serum antibody titers and lung cancer risk has been reported in studies from Finland,^{2,3} Turkey,⁴ Sweden,^{5,6} the United States^{7,8} and Singapore.⁹ Only the study from Singapore addressed the role of *C. pneumoniae* on lung cancer specifically among nonsmokers.⁹ This study is the first to be conducted in a nonsmoking Caucasian population with a large sample size.

Within the framework of an international case-control study of lung cancer¹⁰ designed to screen and sample preferably pure nonsmokers (<400 cigarette-equivalents ever smoked), we selected cases and controls from 8 participating centers in 6 countries [Sweden, Germany (2 centers), France, Italy (2 centers), Russia, and Romania]. This included a total of 163 histologically confirmed cases of lung cancer and 190 controls (of whom 90 and 68 were never smokers, respectively). In Sweden, the 2 German centers and one of the Italian centers, controls were selected among residents in the same area of recruitment as the cases, while in the remaining centers controls were selected among hospital patients admitted for conditions not related to tobacco smoking in the same hospital of the cases or from general hospitals serving the same population. Information on smoking habits was collected *via* questionnaire; blood samples were collected and serum was separated shortly thereafter. Among cases, 90 had not smoked more than 400 cigarettes over their lifetime [including 57 adenocarcinoma (63.3%), 21 squamous cell carcinoma (23.3%), 7 small cell carcinoma (7.8%) and 5 large cell carcinoma (5.5%)]. Among the 73 lung cancer cases with a history of smoking >400 cigarettes (mean 17.8, range 1–90.9), 34 had squamous cell carcinoma (46.6%), 29 adenocarcinoma (39.7%), 8 small cell carcinoma (11.0%) and 2 large cell carcinoma (2.7%). The mean age was similar in cases and controls (65.4 and 62.7 years, respectively).

C. pneumoniae IgG and IgA antibodies were measured, blinded of case-control status, using a standardized microimmunofluorescence (MIF) assay optimized for the detection of *C. pneumoniae* (Focus Diagnostics©). All sera were screened for *C. pneumoniae* at 1:16 dilution and titered to end-point (1:64 and 1:256). An IgG titer of ≥ 16 was considered evidence for past or current *C. pneumoniae* infection. An IgA titer of ≥ 16 or greater is more likely to be a marker of chronic infection when compared with an IgG titer.^{2,7,8,11,12} A blinded reproducibility study was conducted by retesting a random sample of 40 specimens (11.3%) twice. Percent agreements for *C. pneumoniae* IgG and IgA antibodies were 77.5 and 92.5%, respectively.

Odds ratios (OR) of lung cancer and corresponding 95% confidence intervals (CI) were calculated based on multivariate logistic regression, after adjustment for age, sex, center and smoking status. Analyses were repeated on smokers and never-smokers separately, and after stratification on age (<65 and ≥ 65 years) and sex in separate regression models. Heterogeneity of results between study centers was evaluated using a likelihood ratio test.¹³ Tests for trend were performed using ordinal variables (0, 1, 2, 3) for increasing titer levels (0, 1/16, 1/64, 1/256, respectively).

The prevalence of IgG positivity was 78% among cases and 74% among controls (OR = 0.90, 95% CI: 0.52–1.57) (Table I). Corresponding OR estimates were 0.65 (95% CI: 0.20–2.13) among smokers and 0.86 (95% CI: 0.43–1.73) among never smokers. No significant heterogeneity between centers was noted for analyses among all cases and controls ($p = 0.9$), smokers ($p = 0.8$) or nonsmokers ($p = 0.7$). No dose-response

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TABLE I – ODDS RATIOS OF LUNG CANCER FOR *C. pneumoniae* POSITIVITY BY ANTIBODY TITER: OVERALL ANALYSIS AND NEVER-SMOKERS

IG titer	All subjects			Never smokers		
	ca/co ¹	OR ²	95% CI	ca/co	OR	95% CI
IgG						
Seronegative	35/49	1.00 ³	–	21/43	1.00 ³	–
Seropositive	128/141	0.90	(0.52–1.57)	69/115	0.86	(0.43–1.73)
1/16	31/35	0.85	(0.41–1.73)	20/26	0.95	(0.40–2.26)
1/64	66/77	0.90	(0.49–1.66)	37/66	0.85	(0.40–1.81)
1/256	31/29	0.96	(0.45–2.04)	12/23	0.75	(0.27–2.06)
		<i>p</i> for trend = 0.91			<i>p</i> for trend = 0.67	
IgA						
Seronegative	83/108	1.00 ³	–	52/89	1.00 ³	–
Seropositive	80/82	0.95	(0.59–1.53)	38/69	0.72	(0.39–1.32)
1/16	57/62	0.93	(0.56–1.56)	29/55	0.68	(0.40–1.32)
1/64	20/19	1.00	(0.48–2.19)	9/14	0.85	(0.30–2.43)
1/256	3/1	1.15	(0.10–12.8)	0/0	–	–
		<i>p</i> for trend = 0.94			<i>p</i> for trend = 0.42	

CI, confidence interval.

¹ca/co, number of cases and controls. ²OR, odds ratio of lung cancer, adjusted by sex, age, center and, in the overall analysis, smoking status. ³OR = reference category.

was suggested with increasing *C. pneumoniae* antibody titers (*p* for trend = 0.9) among all subjects. OR for IgA positivity (prevalence: 49% among cases and 43% among controls) were 0.95 (95% CI: 0.59–1.53) for the whole study population, and 1.73 (95% CI: 0.65–4.58) for smokers and 0.72 (95% CI: 0.39–1.32) for never smokers separately. No dose-response relationship for IgA was suggested in all subjects (*p* for trend = 0.9), although 3 cases and only one control had IgA antibodies detectable at the dilution level of 1/256. Again, no heterogeneity between centers was noted in stratified analyses (*p* = 0.2). When we stratified all cases and controls by age, *C. pneumoniae* IgG results were similar in subjects younger than 65 (OR = 0.57; 95% CI: 0.25–1.31) and those 65 years or older (OR = 1.25; 95% CI: 0.56–2.75). OR for IgA *C. pneumoniae* positivity were also similar for those <65 years of age (OR = 0.88; 95% CI: 0.42–1.83) and those ≥65 years of age (OR = 0.92; 95% CI: 0.48–1.77). There was no significant effect of IgG or IgA *C. pneumoniae* seropositivity in either women (OR IgG = 0.88; 95% CI: 0.48–1.60; IgA = 0.82; 95% CI: 0.49–1.38) or men (OR IgG = 1.51; 95% CI: 0.29–7.80; IgA = 1.86; 95% CI: 0.47–7.37).

This is the first study to investigate *C. pneumoniae* as an etiological factor for nonsmoking lung cancer with a reasonable number of nonsmoking cases in a European population. Our results do not support a role of *C. pneumoniae* infection in lung carcinogenesis. No effect of *C. pneumoniae* was found in combined analyses of all study participants and no clear effect was seen in subgroup analyses stratified by tobacco smoking, age or gender.

The reliability of our results are supported by the use of a multicenter study with a large sample size, a common protocol and standardized laboratory procedures for the ascertainment of type-specific *C. pneumoniae* serum antibodies using a MIF test (Focus Diagnostics©). This assay has been extensively validated and has shown a high sensitivity and specificity (~95–100%) that is comparable to other MIF assays.¹⁴

Overall, *C. pneumoniae* IgG seropositivity among controls in this study is consistent with the high prevalence among similarly aged controls in the United States study,⁸ but higher than

in studies from Finland,^{2,3} Turkey⁴ and Singapore.⁹ Some studies^{4,8} (including ours) used IgG > 16 as the cut-off to indicate presence of *C. pneumoniae* infection, while others used IgG > 32^{2,3} and IgG > 512.⁹

Most previous epidemiological studies that have examined the association between *C. pneumoniae* seropositivity and lung cancer have been limited to male smokers. There had been at least 8 studies that have described the serological association between *C. pneumoniae* IgA or IgG to lung cancer, 7 of which were conducted among smokers or adjusted for smoking status.^{2–8} Only one study among Chinese women in Singapore was limited to nonsmoking lung cancer cases,⁹ with results consistent with those presented here. The latter study⁹ found no association between chronic infection with *C. pneumoniae* and lung cancer (OR = 1.01; 95% CI: 0.55–1.83) among nonsmoking cases and nonsmoking controls. However, only relatively high antibody titers of IgA ≥ 64 and IgG ≥ 512 were taken as indication of chronic *C. pneumoniae* infection in that study, potentially underestimating past *C. pneumoniae* exposure.

A review article on the association between *C. pneumoniae* and lung cancer¹⁵ found that ORs adjusted for smoking were similar across studies using IgA ≥ 16 cut-off (range: 1.2–1.6),^{2,3,7,8} and higher using increasing IgA titers (range: 2.1–9.9).^{2,4,6} All previous studies cited in the review used MIF to detect *C. pneumoniae* specific IgG and IgA antibodies.

Our results suggest no association between lung cancer and *C. pneumoniae* seropositivity in both young and old subjects. The prevalence of *C. pneumoniae* IgG antibodies have been shown to attenuate with age due to a low production of IgG antibodies many years after initial infection.¹⁶ Given that study participants have a median age of 65 years in the present study, this may have attenuated the association between *C. pneumoniae* and lung cancer if *C. pneumoniae* positivity is misclassified by measurements after middle age.

Although serological testing to define chronic *C. pneumoniae* infection is not yet validated,¹⁷ elevated antibody titers ought to be consistent with a higher probability of more intense and/or more frequent exposure to *C. pneumoniae*.¹⁸ The lack

of an increased risk of lung cancer with higher *C. pneumoniae* MIF serum IgG or IgA antibody titers argues against a potential role for *C. pneumoniae* in lung carcinogenesis.

In conclusion, our study offers no support to the hypothesis that *C. pneumoniae* infection is an important cause of lung cancer in Europe, in particular among nonsmokers.

Yours sincerely,

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