Combined and interaction effect of Chlamydia pneumoniae infection and smoking on lung cancer: a case-control study in southeast China

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Abstract

Objective: This case-control study investigated the role of *Chlamydia pneumoniae* (Cpn) infection in the pathogenesis of lung cancer and the combined and interaction effect of *Chlamydia pneumoniae* infection and smoking or other environmental factors.

Methods: The study was comprised of 449 lung cancer patients and 512 age- and gender-matched healthy controls. All participants provided a 5-ml fasting peripheral venous blood sample for testing Cpn-specific IgG and IgA by using micro-immunofluorescence. Besides analyzing the associations between Cpn and lung cancer, combined effect analysis, logistic regression, and the excel table made by Andersson were used to analyze the combined and interaction effects of Cpn and environmental factors on lung cancer.

Results: Compared to those with no evidence of serum Cpn IgA or Cpn IgG, those with both Cpn IgG+ and IgA+ had 2.00 times the risk (95% CI: 1.34 - 3.00) of developing lung cancer. Smokers with Cpn IgG+ or IgA+ were associated with a significantly increased risk of lung cancer, the adjusted OR was 1.79 (95% CI: 1.10-2.91) or 2.27(95% CI:1.38-3.72), respectively. Those exposed to passive smoking with Cpn IgG+ or IgA+ also increased the risk of lung cancer, the adjusted OR was 1.82 (95% CI: 1.20-2.77) or 1.87(95% CI:1.22-2.87), respectively. The similar results were also observed among alcohol drinking people. Multiplicative and additive interactions were not observed between Cpn infection and environmental factors. The combined effects of Cpn IgG+ or IgA+ and smoking, passive smoking, family history of cancer were found on lung cancer.

Conclusion: The Cpn infection was potentially associated with primary lung cancer in the Chinese Han population and had combined effects with smoking, passive smoking, and the family history of cancer.

Background

Worldwide, lung cancer is the most commonly diagnosed cancer (11.6% of the total cases) and the leading cause of cancer death (18.4% of the total cancer deaths), with greater than 80% of lung cancers in western populations attributed to smoking (1). Recently, the researches on other causes of lung cancer are increasing, such as infection, respiratory diseases (2).

Infection is the third leading cause of cancer worldwide (3). Chronic inflammation resulting from various persistent infections is known to place a person at risk for malignancy. The etiologic role of chronic lung inflammation in lung cancer development has been established (4, 5) . Moreover, chronic lung infections can increase lung cancer risk independently and in conjunction with tobacco smoke exposure (4). Chlamydia species, including *Chlamydia pneumoniae* (Cpn), *Chlamydia trachomatis* (Ctr), and *Chlamydia psittaci* (Cps), can also cause persistent infections and chronic inflammation which may play an essential role in lung cancer pathogenesis. Cpn can cause pneumonia and other respiratory infections, and repeated or prolonged exposure to Chlamydia antigens may cause chronic obstructive pulmonary disease, asthma, and lung cancer. Several studies have examined the association between Cpn infection

and lung cancer (6-16). but failed to identify the combined and interaction effect of Cpn infection and environmental factors, which is of great value.

This study aimed to evaluate the role of Cpn infection in the pathogenesis of lung cancer and to investigate the combined and interaction effect of Cpn infection and environmental factors on lung cancer.

Methods

Cases and controls

Lung cancer cases were identified from the Department of Thoracic Surgery and Respiratory Medicine of The First Affiliated Hospital of Fujian Medical University, Fujian Medical University Union Hospital, and Fuzhou General Hospital of Nanjing Military Command between December 2006 and December 2016. Inclusion criteria: (1) newly diagnosed primary lung cancer by fiberoptic bronchoscopy or histopathologic evaluation, (2) lived in the Fujian province of China for more than ten years. Exclusion criteria: (1) pathologic diagnosis were lung inflammation, benign lesion, or secondary lung cancer, (2) could not answer the study questions.

Controls were frequency matched based on the age and gender of cases. During the same study period, healthy community dwellers were selected for the control group. Inclusion criteria: (1) lived in Fujian province for more than ten years, (2) no history of tumor, (3) no family member participate as a case of this study. In total, 449 lung cancer cases and 512 healthy controls were included in this study. The Institutional Review Board of Fujian Medical University (Fuzhou, China) approved this study, and all participants signed informed consent forms.

Survey content and variables

All epidemiological data were obtained by face to face interview by using standardized questionnaire, which collected information on baseline demographic characteristics, body mass index (BMI), smoking, passive smoking, alcohol consumption, tea use, history of lung diseases, family history of cancer, occupational physical activity, physical exercise, cooking oil fumes and pollution near the residence.

Smoking was defined as having smoked more than 100 cigarettes. Passive smoking was defined as non-smokers who were exposed to inhaled cigarette smoke or exhaled smoke more than once per day for more than 15 minutes per day. Alcohol consumption was defined as drinking at least one alcoholic beverage per week for more than six months, regardless of alcoholic drink type. Drinking tea was defined as consuming at least one cup per week for more than six months. A family history of cancer was defined as the occurrence of a malignant tumor in first-degree or second-degree relatives. Occupational physical activity was rated as low, moderate, or high intensity, following the Reference Standard of Labor Intensity recommended by the Chinese Nutrition Society in 2000 (17). Participants were asked about fumes in their kitchens during cooking for evaluating cooking oil fume exposure.

Experimental methods

All cases and controls provided a 5 ml fasting peripheral venous blood sample, using non-anticoagulation vacuum blood collection tubes. Samples were immediately processed by centrifugation at 2000 rpm for 10 minutes, followed by serum separation and storage at -80℃.

Cpn-specific IgG and IgA were tested by using a micro-immunofluorescence (MIF) kit (Chlamydia IgG SeroFIA kit and Chlamydia IgA SeroFIA kit, DADE Behring, Savyon Diagnostics, Israel). Positive chlamydia controls produced a moderate apple green fluorescent color, whereas negative controls did not fluoresce. A positive result for the presence of chlamydia showed a moderately dispersed apple green fluorescent color; strongly positive results had an intense glare apple green fluorescent color. No fluorescence of any color or a dark background indicated no chlamydial morphology. Although serum Chlamydia IgG and IgA antibody detection are the accepted diagnostic tests for Cpn, MIF test results are subjectively read with the naked eye. To minimize bias, two persons experimented: one skilled technician conducted the preliminary experiment, and the second person conducted a blind interpretation of the results. For quality control, 10% of samples were randomly selected for retesting. (Figure 1)

Statistical analysis

The Chi-square test was used to compare general characteristics for cases and controls. Stratified analysis and unconditional logistic regression were performed to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for chlamydia infection and lung cancer risk. The combined effects and multiplication interaction were analyzed by crossover analysis and logistic regression model. The excel table made by Andersson (18). was used to evaluate the additive interaction, including the relative excess risk of interaction (RERI), attributed proportion of interaction (API), the synergy index (S), and their 95% CIs. If there is no additive interaction, the 95% CI of the RERI and API contain 0, and the 95% CI of S contains 1. All analyses were performed using the SPSS 24.0 software package (IBM Corporation, Armonk, New York, USA). All *P* values were based on a two-sided test with an α of 0.05.

Results

Participant characteristics

A total of 961 patients enrolled in this study, including 449 cases and 512 controls. There were no baseline differences between groups concerning gender, age, ethnicity, marital status, tea-drinking, decoration within ten years, and ventilation status ($P > 0.05$). However, cases and controls did differ concerning educational level, occupation, BMI, smoking, passive smoking, alcohol consumption, history of lung diseases, history of other diseases, family history of cancer, occupational physical activity, physical exercise, cooking oil fumes and pollution near the residence ($P < 0.05$). Of the 449 cases of lung cancer, there were 277 (61.7%) with lung adenocarcinomas, 96 (21.4%) with squamous cell carcinoma, 38 (8.5%) with small cell carcinoma, 7 (1.6%) with adenosquamous carcinoma, 2 (0.4%) with large cell carcinoma, and 29 (6.4%) with other types (Table 1).

Chlamydia infection and lung cancer

The association between Chlamydia infection and lung cancer was observed in table2. Patients with serum Cpn IgG significantly increased lung cancer risk (OR=1.42; 95% CI = 1.02 - 1.96) . Those with serum Cpn IgA significantly increased lung cancer risk (OR=1.73; 95% CI = 1.25 - 2.38). Those with both Cpn IgG+ and IgA+ were statistically associated with an increased lung cancer risk(OR=2.00; 95% CI=1.34 - 3.00). The relationship between other chlamydia (Ctr and Cps) infection and lung cancer not observed.

Stratified analyses were carried out by age, gender, smoking, passive smoking, drinking, and family history of cancer. Adjustment was made for demographic characteristics and relevant factors. The effect between Cpn IgG+ and lung cancer was modified by gender (P=0.049). Among men, those with serum Cpn IgG+ were 1.85 times as likely (95% CI = 1.21 - 2.82) to develop lung cancer. However, among women, the adjusted OR was 0.90 (95% CI = 0.53 – 1.53). Among men, those with serum Cpn IgA+ were 1.93 times as likely (95% CI = 1.28 - 2.93) to develop lung cancer. Among women, the adjusted OR was 1.54 (95% CI = 0.91 – 2.61). Those aged 60 years and older with Cpn IgA were 2.42 times more likely to develop lung cancer (95% CI = 1.49 - 3.92). Similarly, among smokers, the risk of developing lung cancer was 1.79 times higher if IgG was positive (95% CI = 1.10 - 2.91) and 2.27 times higher if IgA was positive (95% CI = 1.38 - 3.72). The lung cancer risk of passive smokers was 1.82 times higher if IgG was positive (95% CI = 1.20 - 2.77) and 1.87 times higher if IgA was positive (95% CI = 1.22 – 2.87). Among alcohol drinkers, those with Cpn IgG+ were 2.45 times as likely to develop lung cancer (95% CI = 1.27 - 4.75), and those with Cpn IgA+ were 2.68 times as likely to develop lung cancer (95% CI = 1.40 - 5.13). (Figure 2).

Combined and interaction effects of Cpn IgG or IgA and environmental factors

After adjustment for possible confounding factors, the results showed that multiplicative and additive interactions were not observed between Cpn infection and environmental factors. However, the combined effects of Cpn IgG+ or IgA+ and smoking, passive smoking, family history of cancer were found on lung cancer. The ORs of Cpn IgG+ or IgA+ combined with smoking were 4.332 (95% CI = 2.430-7.723) and 6.264 (95% CI = 3.425-11.453), respectively. The ORs of Cpn IgG+ or IgA+ combined with passive smoking were 2.059 (95% CI = 1.235-3.433) and 3.291 (95% CI = 2.085-5.192), respectively. Furthermore, the ORs of Cpn IgG+ or IgA+ combined with the family history of cancer were 2.493 (95% CI = 1.474-4.215) and 2.594 (95% CI = 1.409-4.776), respectively. (Table 3)

Discussion

The results of this study showed that Cpn infection was associated with the risk of lung cancer. Patients with serum both Cpn IgG+ and IgA+ had 2.00 times the risk of developing lung cancer. The stratified analysis showed that smokers or drinkers with Cpn IgG+ or IgA+ were more likely to develop lung cancer. Besides, Cpn IgG or IgA had a combined effect on smoking, passive smoking, and family history of cancer.

Our results were consistent with the results of other studies. Several studies (6, 19, 20) supported that Cpn infection is associated with a higher risk for lung cancer. Furthermore, other studies (6-8) showed ORs of 1.2 to 2.8 after adjustment for smoking status indicated that chronic Cpn infection was an independent risk factor for lung cancer. Several case-control studies showed that Cpn infection increased the risk of lung cancer development (10-13, 16), but failed to show a correlation between serum Cpn antibodies and the risk (14, 15, 21).

Although it is unclear how Cpn infection would induce or cause lung cancer, the process may involve chronic inflammation. Chronic Cpn infections may prolong inflammatory mediator stimulation to increase cell necrosis, apoptosis, and mitosis. Thus, the relationship between Cpn infection and lung cancer seems biologically plausible. Furthermore, Cpn proteins have been shown to trigger lung cancer growth potential by altering host cellular replication, transcription, and DNA damage repair (22). During tissue repair, active cellular splitting can result in the occurrence, accumulation, and fixation of mutations, deletions, ectoplasias, and amplifications; these changes increase the risk of malignant transformation at the site of infection (23). Besides, cellular experiments also showed that Cpn infection could transform mesothelial cells, which in turn could increase lung cancer risk (24). Researchers have also established a Cpn infection-induced lung cancer model in rats (25).

Cpn infections are common among specific patient subgroups (e.g. young person (6, 11) , men (12, 13), and smokers (6, 8, 11)). Furthermore, the relationship between Cpn infection and lung cancer risk may vary in environmental factors (e.g. age, gender, smoking history). Our results showed that the OR values of Cpn Ig A or IgG were 2.27 (95% CI = 1.38-3.72) or 1.79 (95% CI = 1.10-2.91) among smokers, which were consistent with those studies (8, 11). The current study also suggested that passive smokers with Cpn infection had a higher risk of lung cancer. The same carcinogen from cigarette smoke of smoking and passive smoking may all induce lung cancer (26-28). Reactive nitrogen and oxygen species (RNOS) produced by smoking can activate NF-κB to promote the expression of inflammatory genes, and directly or indirectly activate the production of inflammatory mediators through regulation of various protein modifications and degradation (29). Therefore, Cpn combines with smoking may promote lung cancer via elevated levels of inflammatory factors that, thereby. Though many studies indicated that smoking might induce lung cancer by aggravating lung inflammation (29, 30), a further research on the underlying mechanism and molecular mechanism of Cpn infection in the pathogenesis of lung cancer are still required.

Moreover, the current study also showed, for the first time, that Cpn IgG (OR=2.45; 95%CI=1.27-4.75) and IgA (OR=2.68; 95%CI=1.40-5.13) were more closely associated with lung cancer among alcohol drinkers. Alcohol exposure reduces airway mucociliary clearance with a progressive desensitization of ciliary response. As a result this important innate primary defense mechanism is weakened, chronic alcohol exposure alters the adaptative immune response to pathogens and leads to an inflammatory response (31). Therefore, Cpn combines with alcohol drinking may also promote lung cancer via elevated levels of inflammatory factors. Furthermore, the combined effects of Cpn IgG+ (OR=2.493, 95% CI = 1.474-4.215) or IgA+ (OR=2.594, 95% CI = 1.409-4.776) and family history of cancer were found on lung cancer. HE et

al.(32) proposed that non-small cell lung cancer (NSCLC) patients with family history of cancer, especially family history of lung cancer, might have a significantly higher incidence of epidermal growth factor receptor (EGFR) activating mutation. And EGFR is an important predictive biomarker of EGFR tyrosine kinase inhibitors (TKIs) in NSCLC. Moreover, Cpn proteins have been shown to trigger lung cancer growth potential by DNA damage repair (22). Therefore, Cpn infection might combined with family history of cancer to induce lung cancer by mutation. But further studies are warranted to confirm the results and further explore the role of family history of cancer.

In this study, serum Cpn IgG and IgA were detected by MIF, which is the standard for serologic detection of Chlamydia infection. However, the use of MIF is limited by its subjectivity and reproducibility (33). Therefore, our experiment was conducted by two different people. A skilled technician performed the preliminary experiment, and the other person conducted a blind interpretation of the results. Furthermore, 10% of the samples were randomly selected for retesting. Previously published studies have had variable definitions for "chronic" chlamydial infection. For example, one study (6) used a combination of specific IgA titers (1:16 or higher) and immune complex titers (1:4 or greater), whereas others have used IgA titers of 1:64 or higher (10) or IgG titers of 1:512 or higher (12-14). Still, in several studies (7, 8, 11, 34, 35) IgG antibody titers of 1:16 or more were considered as the evidence of past or present Cpn infection, whereas IgA antibody titers of 1:16 or more likely indicated chronic infection. Thus, IgG and IgA antibody detection was used to explore the relationship between chlamydia and lung cancer in the current study.

The present study was the most extensive retrospective case-control study to evaluate the role of Cpn in lung cancer pathogenesis. Meanwhile, stratification and multivariate analysis were used to identify possible effect modifiers associated with Cpn and lung cancer. However, still several potential limitations in this study should be considered. Firstly, there were some unavoidable selection and recall biases. Secondly, it is hard to explore the causal inference between Cpn infection and lung cancer when the blood was collected after the cancer diagnosis to determine Cpn infection status. Thirdly, our results may underestimate the effect of the association between Cpn infection and lung cancer due to non-disaggregated misclassification bias caused by the pre-selected criteria for determining chlamydial infection. Despite these limitations, our findings are biologically plausible. Studies have suggested that higher infection rates in patients with cancer are often caused by the immunosuppressive effects of cancers (6). However, studies in which serum was collected before lung cancer diagnosis showed that the association between serum Cpn and lung cancer still existed when blood samples obtained 1 to 5 years before diagnosis were excluded, suggesting that Cpn infection pre-dated the cancer diagnosis (8).

Conclusion

In conclusion, our results showed that Cpn infection might be an independent risk factor for lung cancer and it had combined effects with smoking, passive smoking, and family history of cancer. However, making causal inferences about Cpn infection and lung cancer, well-designed cohort studies and randomized controlled trials are needed to minimize the effect of disease on antibody titers, reduce

selection bias, and better adjust for potential confounders. Modifications of these researches would allow tests to clarify the pathogenic role of Cpn infection in lung cancer.

Abbreviations

API: attributed proportion of interaction; BMI: body mass index; Cpn: Chlamydia pneumonia; Ctr: Chlamydia trachomatis; Cps: Chlamydia psittaci; CIs: confidence intervals; EGFR: epidermal growth factor receptor; MIF: micro-immunofluorescence; ORs: odds ratios; RERI: relative excess risk of interaction; RNOS: Reactive nitrogen and oxygen species; S: the synergy index; TKIs: tyrosine kinase inhibitors

Declarations

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Authors' contributions

XX, LZQ and HF conceived of the study. XWM and XQP carried out the experiments, participated in the drafted the manuscript. QML, and KSL collected samples. HF and CL participated in the design of the study and helped to review the manuscript. XWM and LZQ performed the statistical analysis. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by the Institutional Review Board of Fujian Medical University (Fuzhou, China). All participants signed informed consent forms.

Consent for publication

All authors have given their consent for the publication of this study.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Subject characteristics by case and control groups

Variables	Cases N (%) (N=449)	Controls N (%) (N=512)	χ^2	P
Age (years)			0.194	0.979
<45	39(8.7)	43(8.4)		
45 ~	197(43.9)	229(44.7)		
60 ~	192(42.7)	214(41.8)		
≥75	21(4.7)	26(5.1)		
Gender			3.290	0.070
Male	293(65.3)	305(59.6)		
Female	156(34.7)	207(40.4)		
Ethnicity			0.274	0.601
Han	443(98.7)	507(99.0)		
Others	6(1.3)	5(1.0)		
Education			90.131	<0.001
Primary school or less	231(51.4)	145(28.3)		
High school	174(38.8)	199(38.9)		
College or higher	44(9.8)	168(32.8)		
Marital status			0.204	0.651
Married	429(95.5)	486(94.9)		
Unmarried or others	20(4.5)	26(5.1)		
Occupation			75.731	<0.001
Agriculture, forestry, animal husbandry and fishery personnel	138(30.7)	80(15.6)		
Production transport workers	109(24.3)	89(17.4)		
Enterprises and institutions personnel	123(27.4)	260(50.8)		
Business service personnel	33(7.4)	58(11.3)		
Unemployed or others	46(10.2)	25(4.9)		
BMI (kg/m^2)			11.815	0.003
<18.5	32(7.1)	20(3.9)		
18.5 ~	278(61.9)	286(55.9)		
≥24.0	139(31.0)	206(40.2)		
Smoking			68.114	<0.001
No	188(41.9)	350(68.4)		
Yes	261(58.1)	162(31.6)		
Passive smoking			48.699	<0.001
No	131(29.2)	263(51.4)		
Yes	318(70.8)	249(48.6)		

Alcohol consumption			13.787	<0.001
No	308(68.6)	405(79.1)		
Yes	141(31.4)	107(20.9)		
Tea drinking			1.255	0.263
No	239(53.2)	254(49.6)		
Yes	210(46.8)	258(50.4)		
History of lung diseases			18.235	<0.001
No	381(84.9)	478(93.4)		
Yes	68(15.1)	34(6.6)		
History of other diseases			5.326	0.021
No	264(58.8)	338(66.0)		
Yes	185(41.2)	174(34.0)		
Family history of cancer			5.070	0.024
No	336(74.8)	414(80.9)		
Yes	113(25.2)	98(19.1)		
Occupational physical activity			52.066	<0.001
Light	144(32.1)	278(54.3)		
Median	169(37.6)	150(29.3)		
Heavy	136(30.3)	84(16.4)		
Physical exercise			62.254	<0.001
Not often	316(70.4)	231(45.1)		
Usually	133(29.6)	281(54.9)		
Cooking oil fumes			31.925	<0.001
No	84(18.7)	167(32.6)		
Few	231(51.4)	241(47.1)		
Some	106(23.6)	93(18.2)		
Heavy	28(6.2)	11(2.1)		
Decoration within ten years			0.646	0.421
No	278(61.9)	304(59.4)		
Yes	171(38.1)	208(40.6)		
Pollution near the residence			27.114	<0.001
No	368(82.0)	476(93.0)		
Yes	81(18.0)	36(7.0)		
Ventilation status			4.272	0.118
Bad	11(2.4)	8(1.6)		
General	48(10.7)	38(7.4)		
Well	390(86.9)	466(91.0)		

Pathological types			-	-
Adenocarcinoma	277(61.7)	-		
Squamous cell carcinoma	96(21.4)	-		
Adenosquamous carcinoma	7(1.6)	-		
Large cell carcinoma	2(0.4)	-		
Small cell carcinoma	38(8.5)	-		
Others	29(6.4)	-		

Table 2 The association between Chlamydia infection and lung cancer

Variables	Cases N (%)	Controls N (%)	OR(95%CI)	OR(95%CI)[a]
Cpn				
Cpn IgG				
-(<1:64)	121(26.9)	185(36.1)	1.00	1.00
+(≥1:64)	328(73.1)	327(63.9)	1.53(1.16-2.02)	1.42(1.02-1.96)
Cpn IgA				
-(<1:32)	265(59.0)	376(73.4)	1.00	1.00
+(≥1:32)	184(41.0)	136(26.6)	1.92(1.46-2.52)	1.73(1.25-2.38)
Cpn IgG or IgA				
both-	105(23.4)	161(31.4)	1.00	1.00
single+	176(39.2)	239(46.7)	1.13(0.83-1.55)	1.07(0.74-1.54)
both+	168(37.4)	112(21.9)	2.30(1.63-3.24)	2.00(1.34-3.00)
Ctr				
Ctr IgG				
-(<1:64)	376(83.7)	447(87.3)	1.00	1.00
+(≥1:64)	73(16.3)	65(12.7)	1.34(0.93-1.92)	1.04(0.69-1.58)
Ctr IgA				
-(<1:32)	443(98.7)	509(99.4)	1.00	1.00
+(≥1:32)	6(1.3)	3(0.6)	2.30(0.57-9.24)	1.32(0.30-5.86)
Ctr IgG or IgA				
both-	373(83.1)	446(87.1)	1.00	1.00
single+	73(16.3)	64(12.5)	1.36(0.95-1.96)	1.06(0.69-1.61)
both+	3(0.6)	2(0.4)	1.79(0.30-10.79)	1.12(0.17-7.58)
Cps				
Cps IgG				
-(<1:64)	406(90.4)	481(93.9)	1.00	1.00
+(≥1:64)	43(9.6)	31(6.1)	1.64(1.02-2.66)	1.28(0.73-2.22)
Cps IgA				
-(<1:32)	444(98.9)	508(99.2)	1.00	1.00
+(≥1:32)	5(1.1)	4(0.8)	1.43(0.38-5.36)	1.82(0.41-8.10)
Cps IgG or IgA				
both-	404(90.0)	478(93.4)	1.00	1.00
single+	42(9.4)	33(6.4)	1.51(0.94-2.42)	1.18(0.68-2.05)
both+	3(0.6)	1(0.2)	3.55(0.37-34.26)	4.02(0.36-45.59)

[a]Adjusted by age, gender, education, occupation, BMI, smoking, passive smoking, alcohol consumption, history of lung diseases, history of other diseases, family history of cancer, occupational physical activity, physical exercise, cooking oil fumes and pollution near the residence.

Table 3 The combined and interaction effects of Cpn IgG or IgA and other factors

Variables		Cases N (%)	Controls N (%)	OR(95%CI)[a]
Cpn IgG	Smoking			
-	No	57(12.7)	123(24.0)	1.000
-	Yes	64(14.3)	62(12.1)	2.449(1.285-4.667)
+	No	131(29.2)	227(44.4)	1.078(0.697-1.667)
+	Yes	197(43.8)	100(19.5)	4.332(2.430-7.723)
Cpn IgG× Smoking				0.632(0.325-1.229)
RERI(95%*CI*)				1.807(0.083-3.530)
API(95%*CI*)				0.417(0.124-0.711)
S(95%*CI*)				2.185(0.945-5.051)
Cpn IgA	Smoking			
-	No	123(27.4)	250(48.8)	1.000
-	Yes	142(31.6)	126(24.6)	2.941(1.754-4.931)
+	No	65(14.5)	100(19.5)	1.308(0.849-2.016)
+	Yes	119(26.5)	36(7.0)	6.264(3.425-11.453)
Cpn IgA× Smoking				0.619(0.318-1.207)
RERI(95%*CI*)				3.015(-0.034-6.064)
API(95%CI)				0.481(0.217-0.746)
S(95%*CI*)				2.340(1.200-4.560)
Cpn IgG	Passive smoking			
-	No	36(8.0)	83(16.2)	1.000
-	Yes	85(18.9)	102(19.9)	1.103(0.627-1.941)
+	No	95(21.2)	180(35.2)	0.861(0.506-1.465)
+	Yes	233(51.9)	147(28.7)	2.059(1.235-3.433)
Cpn IgG× Passive smoking				0.540(0.271-1.074)
RERI(95%*CI*)				1.095(0.440-1.750)
API(95%*CI*)				0.532(0.206-0.857)
S(95%*CI*)				-29.138(-)
Cpn IgA	Passive smoking			
-	No	80(17.8)	188(36.7)	1.000
-	Yes	185(41.2)	188(36.7)	1.724(1.178-2.525)
+	No	51(11.4)	75(14.6)	1.483(0.893-2.464)
+	Yes	133(29.6)	61(11.9)	3.291(2.085-5.192)
Cpn IgA× Passive smoking				0.705(0.360-1.380)
RERI(95%*CI*)				1.083(-0.261-2.427)
API(95%*CI*)				0.329(-0.002-0.660)
S(95%*CI*)				1.897(0.805-4.468)
Cpn IgG	Drinking			
-	No	91(20.3)	143(27.9)	1.000

-	Yes	30(6.7)	42(8.2)	0.706(0.367-1.360)
+	No	217(48.3)	262(51.2)	1.183(0.815-1.717)
+	Yes	111(24.7)	65(12.7)	1.434(0.869-2.366)
Cpn IgG× Drinking				0.667(0.309-1.443)
RERI(95%*CI*)				0.546(-0.167-1.258)
API(95%*CI*)				0.380(-0.063-0.824)
S(95%*CI*)				-3.918(-)
Cpn IgA	Drinking			
-	No	190(42.3)	296(57.8)	1.000
-	Yes	75(16.7)	80(15.6)	0.939(0.592-1.489)
+	No	118(26.3)	109(21.3)	1.500(1.031-2.180)
+	Yes	66(14.7)	27(5.3)	1.897(1.051-3.422)
Cpn IgA× Drinking				0.806(0.382-1.700)
RERI(95%*CI*)				0.458(-0.697-1.614)
API(95%*CI*)				0.242(-0.268-0.751)
S(95%*CI*)				2.046(0.321-13.037)
Cpn IgG	Family history of cancer			
-	No	83(18.5)	145(28.3)	1.000
-	Yes	38(8.5)	40(7.8)	1.704(0.927-3.133)
+	No	253(56.3)	269(52.5)	1.314(0.902-1.913)
+	Yes	75(16.7)	58(11.3)	2.493(1.474-4.215)
Cpn IgG× Family history of cancer				0.953(0.438-2.075)
RERI(95%*CI*)				0.474(-0.909-1.857)
API(95%*CI*)				0.190(-0.321-0.701)
S(95%*CI*)				1.466(0.441-4.868)
Cpn IgA	Family history of cancer			
-	No	195(43.4)	305(59.6)	1.000
-	Yes	70(15.6)	71(13.9)	1.921(1.223-3.019)
+	No	141(31.4)	109(21.3)	1.703(1.183-2.452)
+	Yes	43(9.6)	27(5.3)	2.594(1.409-4.776)
Cpn IgA× Family history of cancer				1.317(0.597-2.906)
RERI(95%*CI*)				-0.032(-1.759-1.695)
API(95%*CI*)				-0.012(-0.684-0.659)
S(95%*CI*)				0.980(0.334-2.878)

[a]Adjusted by age, gender, education, occupation, BMI, smoking, passive smoking, alcohol consumption, history of lung diseases, history of other diseases, family history of cancer, occupational physical activity, physical exercise, cooking oil fumes and pollution near the residence.

Figures

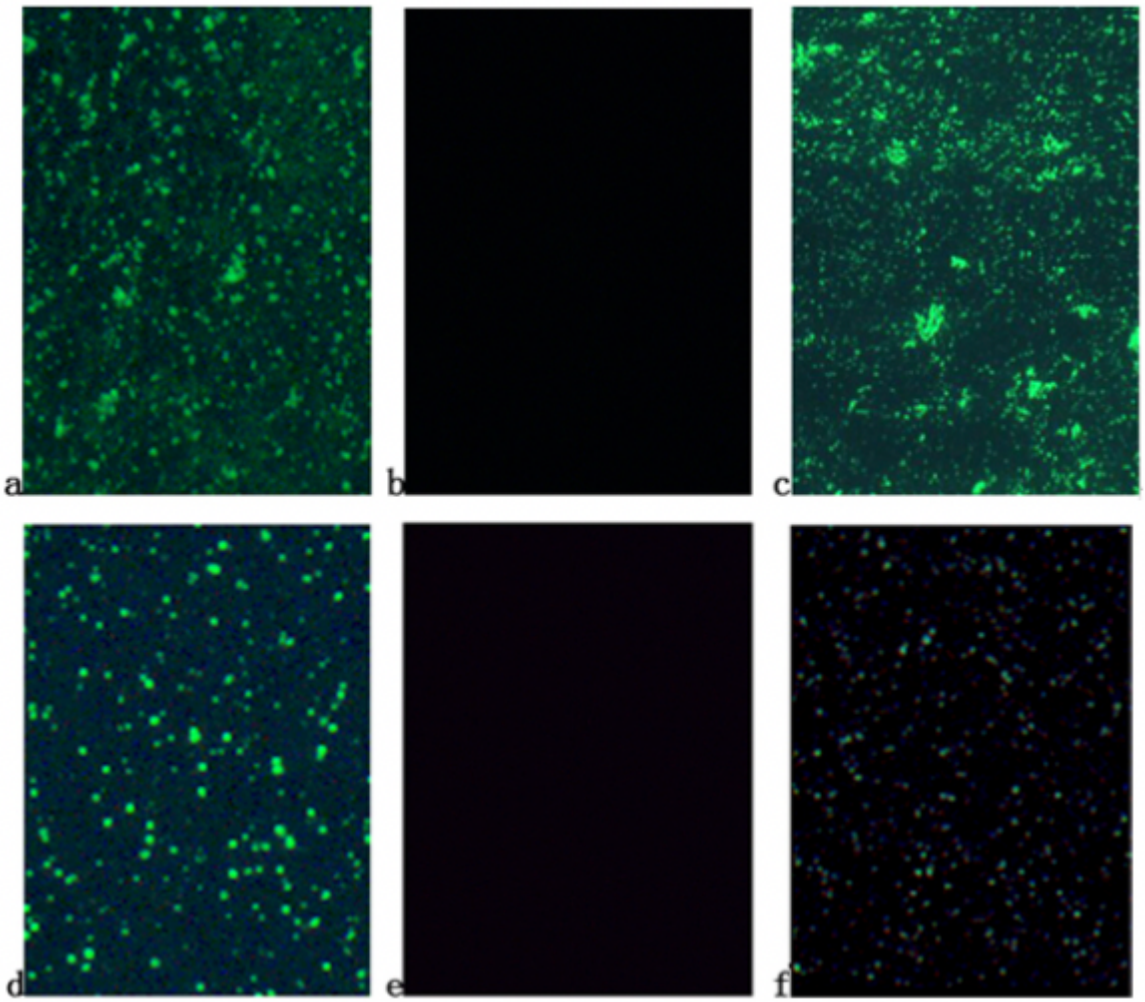

Figure 1

The detecting results of serum Cpn IgG and IgA by MIF method under the microscope (×400 times. a: IgG positive control; b: IgG negative control; c: IgG positive specimens; d: IgA positive control; e: IgA negative control; f: IgA positive).

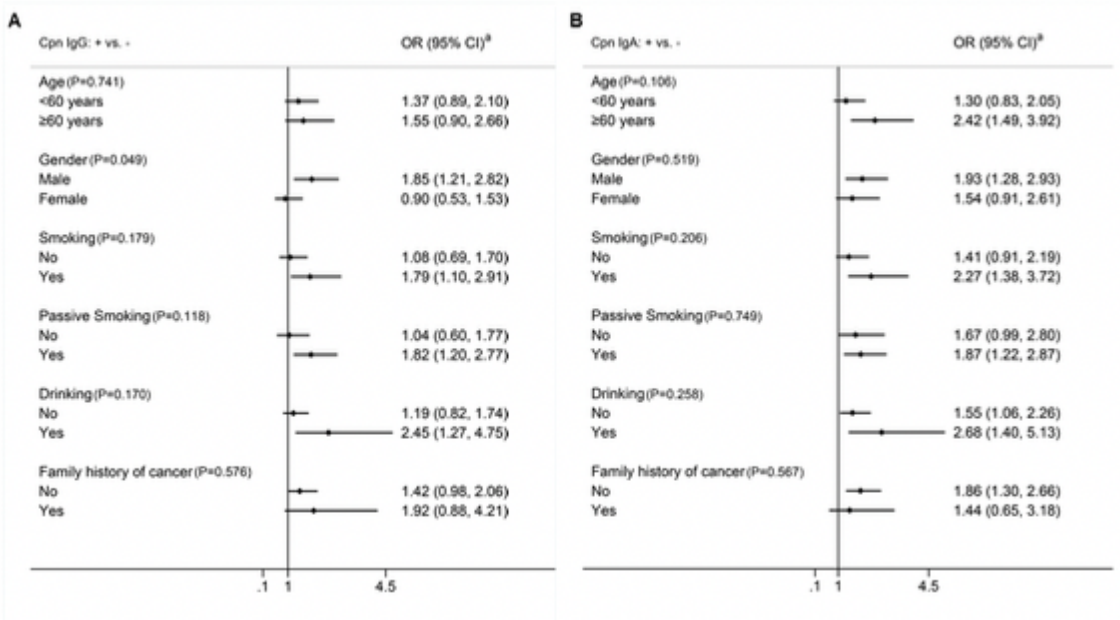

Figure 3

Stratified analysis of the association between Chlamydia pneumoniae infection and the risk of lung cancer. (A) and (B) are the results of IgG and IgA respectively. aAdjusted by age, gender, education, occupation, BMI, smoking, passive smoking, alcohol consumption, history of lung diseases, history of other diseases, family history of cancer, occupational physical activity, physical exercise, cooking oil fumes and pollution near the residence.