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Chlamydia pneumoniae infection and lung cancer risk: A meta-analysis

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ABSTRACT

Chlamydia pneumoniae (*C. pneumoniae*) is a common cause of acute respiratory infection and has been hypothesised to cause several chronic diseases, including lung cancer. Numbers studies were conducted to analyse the association between *C. pneumoniae* infection and risk of lung cancer, but no clear consensus had been found. To assess this relationship more precisely, a meta-analysis was performed. The electronic databases PubMed, Embase, Web of Science and CNKI were searched; Data were extracted and analysed independently by two investigators. Ultimately, 12 studies, involving 2595 lung cancer cases and 2585 controls from four prospective studies and eight retrospective studies were included. Overall, people exposed to *C. pneumoniae* infection had an odds ratio (OR) of 1.48 (95% confidence interval (CI), 1.32–1.67) for lung cancer risk, relative to those not exposed. *C. pneumoniae* infection was clearly identified as a risk factor for lung cancer in both prospective studies (OR, 1.16; 95% CI, 1.00–1.36) and retrospective studies (OR, 2.17; 95% CI, 1.79–2.63) and in both IgA $\geqslant$ 16 cutoff group (OR, 1.22; 95% CI, 1.06–1.41) and the IgA $\geqslant$ 64 cutoff group (OR, 2.35; 95% CI, 1.88–2.93). In conclusion, *C. pneumoniae* infection is associated with an increased risk for lung cancer, higher titre may be a better predictor of lung cancer risk.

1. Introduction

Lung cancer remains the deadliest cancer worldwide despite improvements in diagnostic and therapeutic techniques.[1] Its incidence has yet to peak in many parts of world, particularly in China, which has become a major public health challenge.[2] The mechanism of lung carcinogenesis is still not fully understood. Smoking status established as the most important single factor in causing lung cancer, other factors including occupational or environmental exposure to radon and asbestos, certain metals, air pollution, coal smoke, hormones, genetic susceptibility and chronic bacterial and parasitic infections (*Chlamydia pneumoniae*) have been implicated in lung carcinogenesis.[3,4]

Chlamydia pneumoniae (*C. pneumoniae*) is transmitted via respiratory secretions and is believed to cause 7–10% of community-acquired pneumonia (CAP) among adults.[5] The association of *C. pneumoniae* and atherosclerosis has been intensively studied since the time when serologic data were first presented suggesting a relationship.[6] The role of *C. pneumoniae*

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as a cocarcinogen in the pathogenesis of lung cancer has been investigated for more than 10 years through both clinical and laboratory researches. Laurila and colleagues[7] firstly hypothesised that *C. pneumoniae* might correlate with increased risk of lung cancer in 1997 based on the related observation case–control study. Several studies were then conducted to analyse the association between *C. pneumoniae* infection and risk of lung cancer, but the results have been inconsistent. A single study may be too underpowered to detect a possible small effect of *C. pneumoniae* infection on lung cancer risk, especially when the sample size is relatively small. Different types of study populations and study design may also contribute to the disparate findings. For the first time, we have performed a meta-analysis from all eligible studies published to date to clarify the association between *C. pneumoniae* infection and lung cancer risk.

2. Materials and methods

2.1. Publication search

The electronic databases PubMed, Embase, Web of Science and CNKI (China National Knowledge Infrastructure) were searched for studies to include in the present meta-analysis, using the terms 'chlamydia pneumoniae' or 'chlamydophila pneumoniae' (both genus names are used and refer to the same organism) and 'lung cancer'. An upper date limit of 1st August 2010 was applied; we used no lower date limit. We also reviewed the Cochrane Library for relevant articles. The reference lists of reviews and retrieved articles were hand searched simultaneously. When more than one of the same patient population was included in several publications, only the most recent or complete study was included in this meta-analysis.

2.2. Inclusion criteria

The included studies have to meet the following criteria: (1) evaluating the association between *C. pneumoniae* infection and lung cancer risk; (2) case–control studies; and (3) and supply the numbers (or percentage) of positivity for *C. pneumoniae* antibody in lung cancer cases and controls, respectively.

2.3. Data extraction

Information was carefully extracted from all eligible publications independently by two authors according to the inclusion criteria listed above. Disagreement was resolved by discussion between the two authors. The following data were collected from each study: first author's surname-year of publication, source of cases, source of controls, study design type, definition of chronic infection, the positivity or negativity for *C. pneumoniae* antibody and total numbers of cases and controls, respectively. The eligible studies were characterised in terms of timing of blood sampling in relation to cancer diagnosis: prospective study (blood sample was collected before diagnosis in cases and a comparable time in controls); retrospective study (blood was sampled at or after lung cancer diagnosis and at a comparable time in the controls). We did not define any minimum number of patients to include a study in our meta-analysis.

2.4. Statistical analysis

OR (odds ratio) with 95% CI (confidence interval) was used to assess the strength of an association between chronic *C. pneumoniae* infection and lung cancer risk. The pooled ORs for the risk were calculated. Subgroup analyses were done by study design type (prospective or retrospective study) and IgA titre level for defining *C. pneumoniae* chronic infection. Heterogeneity assumption was checked by the chi-square-based Q-test.[8] A *P* value greater than 0.10 for the Q-test indicates a lack of heterogeneity among studies, so the pooled OR estimate of the each study was calculated by the fixed-effects model (the Mantel–Haenszel method).[9] Otherwise, the random-effects model (the DerSimonian and Laird method) was used.[10] One-way sensitivity analyses were performed to assess the stability of the results, namely, a single study in the meta-analysis was deleted each time to reflect the influence of the individual data set to the pooled OR.[11] An estimate of potential publication bias was carried out by the funnel plot, in which the standard error of log (OR) of each study was plotted against its log (OR). An asymmetric plot suggests a possible publication bias. Funnel plot asymmetry was assessed by the method of Egger's linear regression test, a linear regression approach to measure the funnel plot asymmetry on the natural logarithm scale of the OR. The significance of the intercept was determined by the *t*-test suggested by Egger ($P < 0.05$ was considered representative of statistically significant publication bias).[12] All the calculations were performed using STATA version 10.0 (Stata Corporation, College Station, TX).

3. Results

3.1. Study characteristics

A total of 12 publications dealing with the association between *C. pneumoniae* infection and lung cancer risk met the inclusion criteria,[7,13–23] comprising four prospective studies and 8 retrospective studies, involving 2595 lung cancer cases and 2585 controls. One publication[24] had apparently overlapping cases with a second study[21]; thus, only the final report[21] was included. Table 1 presents the main characteristics of these studies. Among the 12 publications, 9 were published in English and 3 were in Chinese. The sample sizes ranged from 160 to 1264. Controls were mainly healthy populations and matched for age, sex and/or smoking status. In all included studies, the microimmunofluorescence (MIF) test was used to detect *C. pneumoniae*-specific IgA and IgG antibodies in serum, but the criteria for defining *C. pneumoniae* chronic infection were different. According to *C. pneumoniae* specific IgA antibody titre outoff, in 6 studies IgA $\geqslant$ 16(with or without immune complexes) was considered as the criterion for defining *C. pneumoniae* chronic infection, in the other 5 studies IgA $\geqslant$ 64 or more (without IgG titre) was used.

Table 1 – Main characteristics of the studies included in the meta-analysis.

First author (year)	Source of cases	Source of controls	Study design type	Definition of chronic infection[a]	Case positive/all	Control positive/all
Chaturvedi (2010)	USA	Cancer-free, age, sex and smoking status matched controls	Prospective	HSP-60 IgG ⩾ 50 IgA ⩾ 16 IgG ⩾ 16	227/671 201/671 356/671	245/593 174/593 293/593
Smith (2008)	International centres, European	No-smokers healthy populations	Retrospective	IgA ⩾ 64	80/163	82/190
Chen (2005)	China	Consecutive blood donors	Retrospective	IgA ⩾ 100	56/87	22/108
Koh (2005)	Female, Singapore	Free of cancer and pulmonary disease, age and sex matched control	Retrospective	IgG ⩾ 512 and IgA ⩾ 64	140/200	126/181
Littman (2004)	USA	Cancer-free, age, sex and Smoking status matched controls	Prospective	IgA ⩾ 16 or IgG ⩾ 16	281/508	260/508
Zhang (2004)	China	Healthy outpatients of the same period physical examinations	Retrospective	IgG and/or IgM positive	3/128	1/70
Anttila (2003)	Female, Finland	Cancer-free, age and sex matched control	Prospective	IgA ⩾ 16 or IgA ⩾ 64	15/58	46/287
Kocazeybek (2003)	Smokers, Turkey	Healthy hospital staff, relatives of the patients, blood donors or persons with similar age, sex and smoking habits	Retrospective	IgG ⩾ 512 and IgA ⩾ 40	62/123	25/123
Koyi (2001)	Sweden	Consecutive blood donors who were former or current smokers	Retrospective	IgG ⩾ 512 and IgA ⩾ 64	116/177	11/68
Chen (2001)	China	Cancer-free, age and sex matched pulmonary disease	Retrospective	IgA ⩾ 16	69/80	57/80
Jackson (2000)	Male smokers, USA	Men without cancer identified through random digit dialling	Retrospective	IgA ⩾ 16	68/143	56/147
Laurila (1997)	Male smokers, Finland	Cancer-free, age and locality matched controls	Prospective	IgA ⩾ 16 and IC[b] ⩾ 4	120/230	104/230

[a] Microimmunoflourescence (MIF) assay was measured the *C. pneumoniae* antibodies and antibody titre was used as the determination of end-point antibody.
[b] IC: immune complex.

3.2. *Meta-analysis results*

This meta-analysis was performed on 12 studies dealing with the association between *C. pneumoniae* infection and lung cancer risk. The results of the meta-analysis were reported in Table 2. Overall, the combined OR for all 12 eligible studies was 1.48 (95% CI = 1.32–1.67), and the test for heterogeneity was highly significant ($P = 0.001$), indicating a significant elevation in risk of lung cancer for those exposed to *C. pneumoniae* infection, compared with those without the exposure (Fig. 1). A random effect model was used for the analysis when heterogeneity existed among all studies. No significant heterogeneity was observed after stratification of study design type. Among 4 prospective studies, significantly increased risks were found (OR = 1.16, 95% CI = 1.00–1.36; $P = 0.359$ for heterogeneity), among 8 retrospective studies, significant association was also found (OR = 2.17; 95% CI = 1.79–2.63; $P = 0.000$ for heterogeneity) (Fig. 1). When stratified by the IgA titre level for defining *C. pneumoniae* chronic

Table 2 – Main results of pooled odds ratios (OR) with confidence interval (CI) in the meta-analysis.

	Number of the included studies	OR (95% CI)	*P*	*P* (*Q-test*)
Total	12	1.48 (1.32–1.67)	0.001	0.001
Prospective	4	1.16 (1.00–1.36)	0.002	0.359
Retrospective	8	2.17 (1.79–2.63)	0.011	0.000
IgA ⩾ 16	6	1.22 (1.06–1.41)	0.006	0.194
IgA ⩾ 64	5	2.35 (1.88–2.93)	0.000	0.001

P(Q-test): *P* value of Q-test for heterogeneity test; OR: odds ratio; CI: confidence interval.

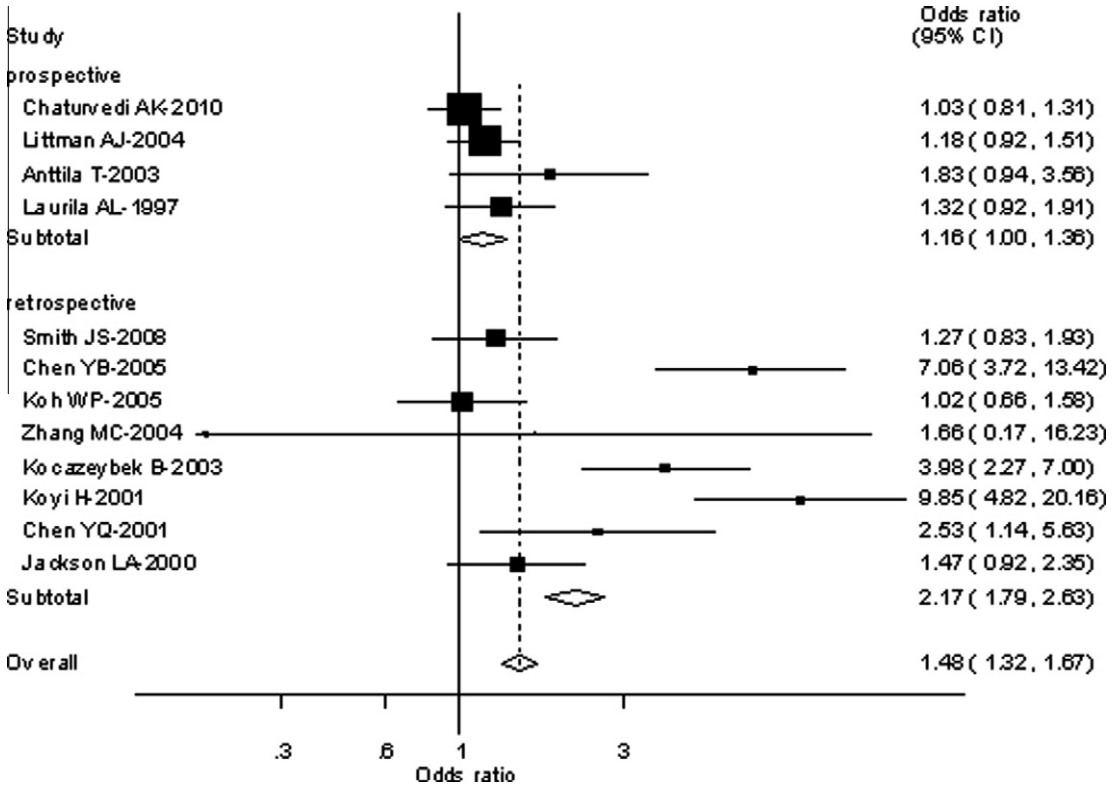

Fig. 1 – Forest plot (random-effects model) of lung cancer risk associated with *C. pneumoniae* infection stratified by the study design. Each box represents the OR point estimate and its area is proportional to the weight of the study. The diamond (and broken line) represents the overall summary estimate, with CI represented by its width. The unbroken vertical line is set at the null value [odds ratio (OR) = 1.0].

infection, significantly increased risks were found in 6 studies with IgA $\geqslant$ 16 cutoff (OR = 1.22, 95% CI = 1.06–1.41, P = 0.194 for heterogeneity); among other 5 studies with IgA $\geqslant$ 64 or more cutoff, the higher OR was found (OR = 2.35, 95% CI = 1.88–2.93, P = 0.001 for heterogeneity) (figure not shown).

3.3. *Sensitivity analyses*

A single study involved in the meta-analysis was deleted each time to reflect the influence of the individual data set to the pooled ORs, and the corresponding pooled ORs were not materially altered.

3.4. *Publication bias*

Begg's funnel plot and Egger's test were performed to access the publication bias of literatures. The shapes of the funnel plots did reveal some asymmetry (Fig. 2). Then, the Egger's test was used to provide statistical evidence of funnel plot symmetry (P = 0.02). These results indicated a potential for publication bias.

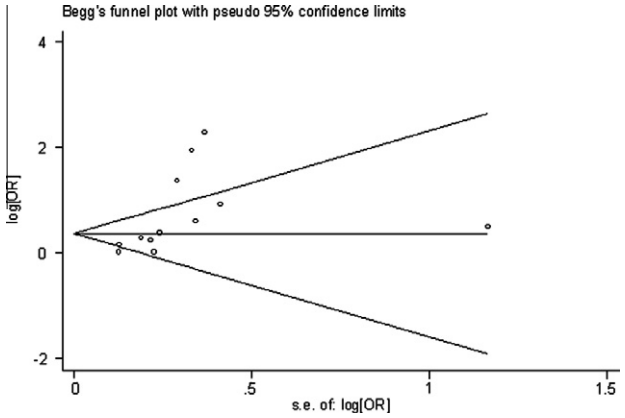

Fig. 2 – Begg's funnel plot of the association between *C. pneumoniae* infection and lung cancer risk.

4. Discussion

Meta-analytic methods are powerful tools for studying cumulative data from individual studies with small sample sizes and low statistical power. Pooling the effects from individual studies by a meta-analysis may increase the statistical power and can help detect modest risk differences among study groups. The large data set of this pooled analysis enabled us to investigate the association between *C. pneumoniae* infection and lung cancer risk that could not be addressed adequately in previous studies. Our meta-analysis summarised for the first time all the available data on the association between *C. pneumoniae* infection and lung cancer risk, including a total of 12 studies, involving 2595 lung cancer cases and 2585 controls. Our Results indicated a significant risk association between *C. pneumoniae* infection and lung cancer risk. In our

analysis of study design and the IgA titre level subgroups, we detected a significant association between *C. pneumoniae* infection and lung cancer risk in both retrospective and perspective studied, and we also showed that this association did not differ across different IgA titre level groups.

According to the timing of blood sampling in relation to cancer diagnosis, the included studies were divided to retrospective and prospective studies. A retrospective study looks backwards and examines exposures to suspected risk or protection factors in relation to an outcome that is established at the start of the study. Most sources of error due to confounding and bias are more common in retrospective studies than in prospective studies. To investigate whether there was the heterogeneity of different study design, we stratified the group by the study design, and found that there was significant heterogeneity among retrospective studies, but not among prospective studies, which showed that the heterogeneity among all the 12 studies may arise from the difference in study design. In 4 prospective studies, no significant heterogeneity ($P = 0.359$) was observed and the ORs (1.16, 95% CI = 1.00–1.36) were lower than the overall ORs (1.48) and the ORs (2.17) of the 8 retrospective studies. Although the perspective studies analysis showed a weaker risk association with lung caner exposed to *C. pneumoniae* infection, all the present studies showed a positive trend of lung cancer with increasing level of exposure to *C. pneumoniae* infection.

C. pneumoniae infection status was defined by serologic criteria based on levels of either antibodies or IC (immune complexes). The criteria used to define chronic *C. pneumoniae* infection ('exposed') varied widely. Some studies used a combination of measures (IgA antibodies and IC, or IgA and high IgG antibody titres). We stratified the group by the IgA titre level for defining *C. pneumoniae* chronic infection and found that *C. pneumoniae* infection was a risk factor in both IgA titre levels(IgA $\geqslant$ 16 and 64). However, we also observed a stronger risk association with *C. pneumoniae* infection pooled from IgA $\geqslant$ 64 titre level studies (OR = 2.35, 95% CI = 1.88–2.93) than pooled from IgA $\geqslant$ 16 titre level studies (OR = 1.22, 95% CI = 1.06–1.41). These results indicated a dose-response effect in which increasing lung cancer risk was associated with increasing IgA antibody titre, also suggested that higher titre may be a better predictor of lung cancer risk than lower antibody titres.

In all the included studies, *C. pneumoniae* infection has been associated with increased lung cancer risk among specific subgroups, such as young individuals,[7,23] men,[20,21] former smokers[17,23] and for squamous cell carcinomas or small cell carcinomas.[7,17] However, in the Chaturvedi study published in 2010, the elevated risk associated with the Chlamydia heat shock protein-60 (CHSP-60) seropositivity or antibody titres did not differ by age, sex, smoking status or lung cancer histology. Due to the limited studies of the specific subgroups, we could not conduct the subgroup analysis of meta-analysis. Therefore, stratified analyses of *C. pneumoniae* infection by smoking status, age, gender and histology, are certainly warranted in future studies.

Currently, the mechanism by which chronic *C. pneumoniae* infection may increase the risk of lung cancer was not still clear. According to Redecke and colleagues study, smoking may hasten *C. pneumoniae* to invade the lung, in this complex framework of interactions, superoxide oxygen radicals, TNF-a, IL1β and IL8 play an essential role, contributing to lung tissue and DNA damage that eventually results in carcinogenesis.[25] Another possible interpretation is that, *C. pneumoniae* infection may cause irregular apoptosis in tissues.[26]

Some limitations of this meta-analysis should be acknowledged. Firstly, heterogeneity is a potential problem when interpreting all the results of meta-analyses. Although we minimised the likelihood by performing a careful search for published studies, using the explicit criteria for study inclusion, performing data extraction and data analysis strictly, the significant between-study heterogeneity still existed in almost each comparison. The presence of heterogeneity can result from differences in the selection of controls, age distribution, prevalence lifestyle factors and so on. Although most of the controls were selected from healthy populations, some studies had selected controls among friends or family of lung cancer patients or patients with other diseases. Secondly, only published studies were included in this meta-analysis. The presence of publication bias indicates that non-significant or negative findings may be unpublished. Lastly, the included studies were case–control studies, only 4 were prospective studies, not cohort study, which provide stronger evidence of studying causal associations.

In conclusion, this meta-analysis involving 12 studies suggests that *C. pneumoniae* infection is associated with an increased risk of lung cancer, higher titre may be a better predictor of lung cancer risk. However, large sample studies including different specific subgroups with a careful matching between cases and controls should be considered in future association studies to confirm the results from our meta-analysis.

Conflict of interest statement

None declared.

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