Chronic *Chlamydomphila pneumoniae* infection in lung cancer, a risk factor: a case–control study

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The relationship between chronic *Chlamydomphila* (formerly *Chlamydia*) *pneumoniae* infection and lung carcinoma was investigated. A total of 123 patients who were smokers and diagnosed with lung carcinoma based on clinical and laboratory (radiological, cytological) findings were examined. Of these patients, 70 had small-cell, 28 squamous-cell and seven large-cell carcinomas, while 18 had adenocarcinoma. A total of 123 healthy persons matching patients in age, sex, duration of smoking and locality were chosen as controls. Blood samples (5 ml) were withdrawn at the time of diagnosis and 1 month later. The values between IgG $\geq$ 512 and IgA $\geq$ 40 were set as the criteria for chronic *Chlamydomphila pneumoniae* infections. In male patients with lung carcinoma, *Chlamydomphila pneumoniae* IgG antibody titres of $\geq$ 512 and IgA antibody titres of $\geq$ 40 were found at a higher rate than in the control group. This ratio was not significant for the female patients. In chronic *Chlamydomphila pneumoniae* infections, *Chlamydomphila pneumoniae* antibody titres with values IgG $\geq$ 512 and IgA $\geq$ 40 were found in a total of 62 (50.4 %) cases. Chronic *Chlamydomphila pneumoniae* infections were seen statistically more often in male patients with carcinoma who were aged 55 years or younger. This study supports the idea that chronic *Chlamydomphila pneumoniae* infection increases the risk of lung carcinoma.

Received 3 December 2001

Accepted 21 January 2003

INTRODUCTION

Chlamydomphila (formerly *Chlamydia*) *pneumoniae*, and previously also known as strain TWAR, is a Gram-negative bacillus and an obligatory intracellular parasite (Grayston, 1989; Everett *et al.*, 1999). It causes infection in more than 50 % of adults. It leads to a higher incidence of pneumonia in the elderly, while children younger than 5 years old have less clinical infection (Saikku, 1992). *Chlamydomphila pneumoniae* infections vary in different countries and populations, being endemic in the USA and epidemic in Scandinavian countries. The route of transmission is usually by aerosol and respiratory secretions (Grayston, 1989; Thom & Grayston, 1991). Since it is difficult to isolate the organism in culture, serological analysis is preferred in diagnosis. However, it could not be established as a routine method due to very few elementary substances in samples (Grayston *et al.*, 1990). PCR, known to be 25 % more sensitive than culture, is often used for diagnosis, but serum antibody determination, especially microimmunofluorescence (MIF), is the most frequently used method for the diagnosis of chlamydia infections (Gaydos *et al.*, 1993; Grayston, 1994). *Chlamydomphila pneumoniae* causes various infections, such as pneumonia, sinusitis, bronchitis, rhinitis, chronic obstructive pulmonary disease (COPD) or asymptomatic infection.

Abbreviations: COPD, chronic obstructive pulmonary disease; MIF, microimmunofluorescence.

The specific antibody titres of *Chlamydomphila pneumoniae* have been found to be high in myocardial infarction, stable angina pectoris and atherosclerosis cases. In addition, detection of *Chlamydomphila pneumoniae* in atherosclerotic plaques on coronary and carotid arteries, led to discussion about the cause–effect relationship between *Chlamydomphila pneumoniae* and atherosclerosis and coronary heart disease (Saikku *et al.*, 1988; Grayston *et al.*, 1993; Arat *et al.*, 1999). In the last 3 years, seroepidemiological studies have indicated a possible relation between *Chlamydomphila pneumoniae* infection and lung carcinoma and malignant lymphoma (Koyi *et al.*, 1999; Laurila *et al.*, 1997a). In this study, we aimed to evaluate the relationship between chronic *Chlamydomphila pneumoniae* infection and lung carcinoma by determining antibodies against *Chlamydomphila pneumoniae* in patients with lung carcinoma who are smokers.

METHODS

A total of 123 cases diagnosed as primary lung carcinoma with clinical symptoms and laboratory findings (radiological, cytological) consisting of 70 (56.91 %) small-cell carcinoma, 28 (22.76 %) squamous-cell carcinoma, 18 (14.63 %) adenocarcinoma and seven (5.69 %) large-cell carcinoma cases, were included in this study. The subjects were admitted to the Oncology and Lung Diseases Departments in the Florence Nightingale Group between 1 June 1998 and 1 July 2000. One hundred and one (82.11 %) of the cases were male and 22 (17.88 %) were female, with ages between 22 and 87 and a mean of 55 years (53

males were < 55, 48 were $\geq$ 55 years old; 10 females were < 55 and 12 were $\geq$ 55 years old). The duration of smoking before the diagnosis of primary lung carcinoma was between 3 and 58 years with a mean of 32 years. A control group of 123 people was chosen from among healthy hospital staff, relatives of the patients, blood donors or persons with similar age, sex and smoking habits. In the selection of the control group, the following criteria were taken into consideration: age, sex, living environment, smoking history, including the following: age of starting smoking, whether or not he or she is still a smoker, number of cigarettes per day, duration of smoking, and if smoking stopped, total time of smoking.

Of the control group, 101 (82.11 %) were males and 22 (17.88 %) were females. Their ages were 22–87 years with a mean of 55 years (53 males

were < 55, 48 were $\geq$ 55 years old; 10 females were < 55 and 12 were $\geq$ 55 years old) and smoking periods were between 5 and 58 years with a mean of 32 years. The main criteria accepted for the control group consisted of not having any medical treatment for a local or systemic disease and not being admitted to a hospital in the last 2 months (Table 1).

Blood samples (5 ml) were obtained from the patient group with primary lung carcinoma at the time of diagnosis and the second samples were obtained at the end of the following month. Similarly, the first blood samples were obtained from the control group at the time they were selected and the second samples were obtained at the end of the first month. Serological studies were performed with a *Chlamydomydia pneumoniae* MIF kit (Orgenium). In these kits, the elementary bodies of

Table 1. Baseline characteristics of lung carcinoma cases matched with the controls

Values given are number of cases or controls, with percentage in parentheses.

Characteristic	Male		Female	
	Cases (n = 101)	Controls (n = 101)	Cases (n = 22)	Controls (n = 22)
Age at diagnosis (years)				
< 50	31 (30)	31 (30)	10 (45)	10 (45)
50–59	24 (24)	24 (24)	4 (18)	4 (18)
60–69	24 (24)	24 (24)	6 (27)	6 (27)
70–79	15 (15)	15 (15)	2 (9)	2 (9)
80–89	6 (6)	6 (6)	—	—
$\geq$ 90	1 (1)	1 (1)	—	—
Total	101	101	22	22
Last recorded smoking status*				
Former smoker	14 (14)	14 (14)	13 (59)	13 (59)
Current smoker	87 (86)	87 (86)	9 (41)	9 (41)
Duration of smoking (years)				
< 10	18 (18)	18 (18)	7 (32)	7 (32)
10–19	30 (30)	30 (30)	9 (41)	9 (41)
20–29	22 (22)	22 (22)	3 (14)	3 (14)
30–39	17 (17)	17 (17)	2 (9)	2 (9)
40–49	11 (11)	11 (11)	1 (4)	1 (4)
$\geq$ 50	3 (3)	3 (3)	—	—
No. cigarettes (day⁻¹)				
< 5	1 (1)	1 (1)	1 (4)	1 (4)
5–9	4 (4)	4 (4)	3 (14)	3 (14)
10–19	31 (31)	31 (31)	14 (64)	14 (64)
$\geq$ 20	66 (66)	66 (66)	4 (18)	4 (18)
Living environment†				
Same province–district and street	97 (96)	97 (96)	19 (86)	19 (86)
Same province–district and neighbouring street	4 (4)	4 (4)	3 (14)	3 (14)

*While matching individually a lung carcinoma case with a control case by the history of patients with lung cancer at the last recording, the parameters of being a former or a current smoker and the number of cigarettes smoked per day between the beginning and the quitting of smoking in control groups were taken into consideration.

†While matching individually a lung carcinoma case with the control case for living environment, efforts were made to find a control case living in the same province–district and street as the lung carcinoma case. If this was not possible, efforts were made to find a control case living in the same province–district but on a street neighbouring the lung carcinoma case.

Chlamydomphila pneumoniae were used as an antigen on slides. In order to eliminate contradictory results, *Chlamydia trachomatis* and *Chlamydomphila psittaci* antigens were used as controls. Serum dilutions were started at 1/8 for IgG and at 1/10 for IgA and continued in twofold dilutions. As a conjugate, FITC-labelled goat anti-human IgA and IgG were used and all were evaluated by the same investigator (B. K.) using a fluorescence microscope with 40 × 0.65 oil-immersion objective. Seropositivity criteria for the diagnosis of chronic *Chlamydomphila pneumoniae* infection were IgG ≥ 512 and IgA ≥ 40 and for the community population titres of IgG ≥ 16 were accepted as the criterion for previous infection. In both the patient and the control groups, the highest result among the first or second blood samples was accepted as the final titre (Leinonen *et al.*, 1991; Saikku, 1993; Grayston *et al.*, 1989; Forsey *et al.*, 1986).

The Mc-Namar test was used in univariate analysis because patient and control groups were matched individually. Logistic regression method was used for the multivariate analysis. Forward conditional method was selected for this model.

RESULTS

Basic characteristics of the matched lung cancer group and patient group are shown in Table 1. The number of cases having infection with titres ≥ 16 was 98 (79.7%) for the patient group and 54 (43.2%) for the control group ($P < 0.001$), as shown in Table 2. There was a significant difference between male patients with lung cancer and their control group for specific *Chlamydomphila pneumoniae* antibody titres of IgG ≥ 16, IgG 512 and IgA 40 ($P < 0.001$). On the other hand, female patients and their control group showed no significant difference. However serological evidence of chronic *Chlamydomphila pneumoniae* infection was found more often in male patients with lung cancer than female patients (Table 3). The comparison of lung carcinoma cases with the control group, on the basis of percentage prevalence of the specific antibody seropositivity of *Chlamydomphila pneumoniae* at titres of IgG ≥ 512 and IgA ≥ 40 based on age and sex, is shown in Table 4. Titres indicative of chronic *Chlamydomphila pneumoniae* infection were significantly higher in male patients with lung carcinoma below age 55 than the control group [χ^2 28.66, $P < 0.001$, odds ratio

(OR) 18, CI (95%) 6.2–51.8]. Conversely, no difference was found between male patients with lung carcinoma over age 55 and the control group ($P > 0.05$). No significant difference was detected in the serological results between female patients and the female control group below age 55 and over age 55 years ($P > 0.05$). Variants included in the logistic regression model are shown in the legend to Table 5. According to this they were found to be significant. In the multiple logistic regression model, IgG ≥ 16 and IgA ≥ 40 were found significant. Results were determined for each variant in order for IgG ≥ 16 $\beta = 1.684$, $P < 0.0001$, OR 5.38, CI (95%) 2.939–9.866 and for IgA ≥ 40 $\beta = 1.684$, $P < 0.0001$, OR 4.583, CI (95%) 2.525–8.32. According to this, the risk of the raised antibody titres at IgG ≥ 16 and IgA ≥ 40 is greater in the patient group than in the control group. The number of the cases having infection with titres of IgG ≥ 512 and IgA ≥ 40 was 62 (50.4%) for the patient group; 41 lung carcinomas were of the small-cell type (Table 6).

DISCUSSION

Saikku *et al.* (1988, 1992) demonstrated the seroepidemiological correlation between coronary heart diseases and atherosclerosis and infection by *Chlamydomphila pneumoniae*, and proposed that chronic chlamydia infection of the upper and lower respiratory tract may initiate acute myocardial infarction and may be the cause of chronic arthritis in atherosclerosis. Grayston *et al.* (1993) and Kuo *et al.* (1993) have shown the presence of *Chlamydomphila pneumoniae* in atheromatous plaques. In the following years, the occurrence of *Chlamydomphila pneumoniae* in atherosclerotic plaques in arteries other than the coronaries, such as the carotids, was demonstrated by seroepidemiological, immunohistochemical and molecular biological methods, leading to implication of this bacterium in aetiology and pathogenesis (Yamashita *et al.*, 1998; Maass *et al.*, 1998). Studies concerning the role of *Chlamydomphila pneumoniae* in the pathogenesis of juvenile arthritis and reactive arthritis in distal

Table 2. Distribution of patient and control groups according to variables

Patients	Controls		
	IgG ≥ 16	IgG < 16	Total
IgG ≥ 16*	34	64	98
IgG < 16	18	7	25
Total	52	71	123
	IgG ≥ 512+IgA ≥ 40†	IgG < 512+IgA < 40	Total
IgG ≥ 512+IgA ≥ 40†	16	46	62
IgG < 512+IgA < 40	9	52	61
Total	25	98	123

* $P < 0.0001$.

† $P < 0.0001$.

Table 3. Distribution of patient and control groups according to sex

Patients	Controls					
	Male			Female		
	IgG $\geq$ 16	IgG < 16	Total	IgG $\geq$ 16	IgG < 16	Total
IgG $\geq$ 16	34*	54	88	6‡	4	10
IgG < 16	12	1	13	2	10	12
Total	46	55	101	8	14	22
	IgG $\geq$ 512+IgA $\geq$ 40	IgG < 512+IgA < 40	Total	IgG $\geq$ 512+IgA $\geq$ 40	IgG < 512+IgA < 40	Total
IgG $\geq$ 512+IgA $\geq$ 40	12†	42	54	4§	4	8
IgG < 512+IgA < 40	8	39	47	2	12	14
Total	20	81	101	6	16	22

* $P < 0.0001$.† $P < 0.0001$.‡ $P > 0.05$.§ $P > 0.05$.**Table 4.** Distribution of male patient and control groups according to the criterion of 55 years old

Patients	Controls					
	< 55 years*			$\geq$ 55 years†		
	IgG $\geq$ 512+IgA $\geq$ 40	IgG < 512+IgA < 40	Total	IgG $\geq$ 512+IgA $\geq$ 40	IgG < 512+IgA < 40	Total
IgG $\geq$ 512+IgA $\geq$ 40	6	36	42	5	7	12
IgG < 512+IgA < 40	2	9	11	7	29	36
Total	8	45	53	12	36	48

* $P < 0.0001$.† $P > 0.05$.**Table 5.** Results of logistic regression according to the forward conditional model

Abbreviations: B, beta regression coefficient; SE, standard error; Wald, test statistics used for the determination of the meaning of variables; d.f., degrees of freedom; Sig., significant; Exp (B), exponent; CI, confidence interval. Variables included in logistic regression model were age, sex, IgG16, IgG512, IgA40 and IgG 512+IgA 40.

	B	SE	Wald	d.f.	Sig.	Exp (B)	95.0 % CI for Exp (B)	
							Lower	Upper
IgG16(1)	1684	309	29.697	1	.000	5385	2939	9866
IgG40(1)	1522	304	25.042	1	.000	4583	2525	8320

Table 6. Distribution of chronic *Chlamydomphila pneumoniae* infection with IgG ≥ 512 and IgA ≥ 40 according to histological type of lung carcinoma

Lung carcinoma (n)	Infection	
	n	%
Small cell (70)	41	58.5
Squamous cell (28)	14	50
Adenocarcinoma (18)	6	33.3
Large cell (7)	1	14.28
Total (123)	62	50.4

extremities (Moazed *et al.*, 1996; Kol *et al.*, 1998) and the study of Blasi *et al.* (1997) on the relationship between sarcoidosis and *Chlamydomphila pneumoniae* showed correlations on the basis of seropositivity; however, *Chlamydomphila pneumoniae* was not detected by nested PCR in tissue samples. Recently, Anttila *et al.* (1998) have detected *Chlamydomphila pneumoniae*-specific antibodies at high titres in 72 patients with lymphoma. In all studies, the common question was whether *Chlamydomphila pneumoniae* had an effect in initiating the disease or merely shows affinity for pre-formed lesions. Most investigators (Kol *et al.*, 1998; Moazed *et al.*, 1996, 1997) accept the idea that the infection chain in the respiratory tract is initiated by the infection of monocytes. The agent, which proliferates in the monocytes and macrophages, provokes the pathogenesis and is responsible for the clinical symptoms. Clinical symptoms start with the antigenic response, followed by the infection of new monocytes, endothelial cells, and macrophages in the intima and smooth muscle cells in the media layer, so that cytokines and acute-phase proteins become active, producing chronic inflammation. In recent years, some investigators have suggested that *Chlamydomphila pneumoniae* has a different role in the aetiopathogenesis, for example the high *Chlamydomphila pneumoniae* antibody titres detected in lung carcinomas found in smokers (Laurila *et al.*, 1997a, b; Koyi *et al.*, 1999). In this study, chronic *Chlamydomphila pneumoniae* infection seropositivity was found in 50.81 % of the patient group, and small-cell and squamous-cell carcinomas were detected especially in the cases with chronic *Chlamydomphila pneumoniae* infection seropositivity. Laurila *et al.* (1997b) found evidence that *Chlamydomphila pneumoniae* infection was present especially in patients with small-cell and squamous-cell carcinomas selected among 230 smokers with lung carcinoma. Koyi *et al.* (1999) reported that in Sweden in 1993, seropositivity ratios were significantly different from the control group in 117 smokers with lung carcinoma. Korvenan *et al.* (1994) showed the significant correlation of seropositivity of *Chlamydomphila pneumoniae* with smoking. von Hertzen *et al.* (1998) suggested that smoking destroys lung and bronchial immunity, increases the secretion of IL4, and as a result, humoral immunity increases and cellular immunity is suppressed, so *Chlamydomphila pneumoniae* can localize easily in the lung. Some studies (Ohshima & Bartsch,

1994; Redecke *et al.*, 1998) explain the pathogenesis of *Chlamydomphila pneumoniae* infections as invasion of the lung with the help of smoking: superoxide oxygen radicals, TNF- α , IL1 β and IL8, which are produced and secreted by the monocytes activated by the antigen and the macrophages in the intima, then cause damage to lung tissue and DNA that results in carcinogenesis; in particular, IL8 is known to cause genetic damage. Another suggestion is that, by unknown mechanisms, *Chlamydomphila pneumoniae* infection causes irregular apoptosis in tissues (Fan *et al.*, 1998).

In this study, a significant difference was found between male patients who smoked and controls in IgG positivity at titres ≥ 16 ; male smokers had a greater level of contact with *Chlamydomphila pneumoniae* in the past ($P < 0.001$). In a recent study, it has been stated that in people below age 60 years, specific *Chlamydomphila pneumoniae* IgA positivity at titres of 216 is independently related to carcinoma (Jackson *et al.*, 2000). A group of investigators in Sweden has indicated that male patients particularly had a high level of contact with *Chlamydomphila pneumoniae* (IgG ≥ 32 positivity) in the past, and these patients had IgG ≥ 512 and IgA ≥ 64 values which were significantly higher than the control group: even 48 % of the young male patients were shown to have IgA titres of ≥ 512 (Koyi *et al.*, 1999). It has been reported that patients with lung carcinoma have very high titres of IgA. We have found the risk of the elevation of titration of *Chlamydomphila pneumoniae* antibody titres at IgA ≥ 40 is greater in the lung cancer group than the control group according to the multivariate regression analysis.

In this study, 54 % of the male patients and 36 % of the female patients were diagnosed as having chronic seropositivity for *Chlamydomphila pneumoniae*, and a significant difference was found in male patients when compared ($P < 0.001$) with the control group (Table 5). This difference was especially seen in male patients below the age of 55. In a Finnish study, the seropositivity ratios of chronic *Chlamydomphila pneumoniae* was found to be high in 231 male smoking patients below the age of 60. Similar results have been reported with studies dealing with male patients who were smokers below the age of 70 and above the age of 60 (Laurila *et al.*, 1997a; Koyi *et al.*, 1999; Jackson *et al.*, 2000).

However, age and sex are not thought to be factors in the multivariate analysis. As a result, the detection of chronic *Chlamydomphila pneumoniae* infection in young, smoking, male lung carcinoma patients, at high ratios in univariate analysis and the elevation of IgG ≥ 16 and IgA ≥ 40 titres compared with the control group, suggests that infection together with age, sex and smoking is a high risk factor in lung carcinoma development. It is not possible to conclude from this study whether chronic *Chlamydomphila pneumoniae* infection will increase the risk of lung cancer or whether patients with lung cancer will be at greater risk of picking up *Chlamydomphila pneumoniae*. In order to define the possible association between chronic *Chlamydomphila pneumoniae* infection and lung cancer, further studies with a larger number of cases are necessary.

ACKNOWLEDGEMENTS

I would like to thank Professor Dr Seyhan Çelikoğlu from the Kadir Has University, Florence Nightingale Hospital, Thoracic Diseases Dept, and Dr A. Suat Sarıbaş from the Cerrahpaşa Faculty of Medicine Microbiology and Clinical Microbiology Dept, and also my co-workers, Dr Mesut Gencay and Kadir Sağlam for their contributions to this study.

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