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Chlamydia pneumoniae infection in patients with chronic obstructive pulmonary disease

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SUMMARY

The prevalence of chronic *Chlamydia pneumoniae* infection was assessed in 54 patients with established chronic obstructive pulmonary disease (COPD), 41 of these with severe COPD (group I), 13 with mild to moderate COPD (group II), and in 23 patients with community-acquired pneumonia (controls, group III). Specific IgG and IgA antibody levels and circulating immune complexes (ICs) were measured in paired sera, and specific secretory IgA (sIgA) levels in sputum specimens. A polymerase chain reaction (PCR) test was used for the detection of *C. pneumoniae* in sputum. According to our definite diagnosis criterion, 65% of the COPD patients showed evidence of suspected chronic *C. pneumoniae* infection and the prevalence was still higher (71%) in patients with severe disease. The occurrence of specific markers of infection was invariably highest in patients with severe COPD, next-highest in patients with mild to moderate COPD and lowest in pneumonia patients. The association between COPD and *C. pneumoniae* infection persisted after controlling for the potential confounding factors.

INTRODUCTION

Several studies have shown that airway obstruction in smokers is associated with a chronic inflammatory reaction in small airways [1, 2]. However, airway obstruction develops only in a minority of smokers [3] although most of them develop an inflammatory reaction in the bronchioles [1]. It therefore seems obvious that other factors besides smoking must be involved in the pathogenesis of COPD.

Chlamydia pneumoniae, a ubiquitous intracellular pathogen, has the ability, unlike many other respiratory bacteria, to penetrate the epithelial layer and invade the interstitium [4]. Prolonged delay is part of the natural progression of chlamydial infection which suggests an innate ability of chlamydia to persist intracellularly [5]. Its persistence and the resulting immune response of the host have been recognized as the major factors in the pathogenesis of chlamydial disease [6]. The severe sequelae of chronic *C. trachomatis* infections are relatively well established [7, 8] whereas the complications of chronic *C. pneumoniae* infections in the human bronchi and alveoli are still poorly elucidated. Associations between *C. pneumoniae* and adult-onset asthma [9] and asthma in children [10] have been described, as has an association between *C. pneumoniae* and sarcoidosis [11]. We have previously shown that more than 50% of patients with established COPD have diagnostic levels of short-lived secretory IgA (sIgA) antibodies to *C. pneumoniae* in their sputum and that nearly all such

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patients also have elevated serum IgG and IgA antibody levels [12]. Since the cultures in chronic chlamydia infections frequently remain negative, the polymerase chain reaction (PCR) technique might be a useful method for detecting chlamydial nucleic acids directly. However, the lack of a generally accepted 'gold standard' criterion for chronic infections has complicated evaluation of this method.

The aim of the present study was to extend our earlier observations on the association between *C. pneumoniae* and COPD. We also wanted to evaluate the usefulness of the PCR technique with sputum specimens for diagnosis of *C. pneumoniae* infection in patients with lower respiratory tract disease.

SUBJECTS AND METHODS

Subjects and sample collection

The patients were enrolled in the study during September 1992 and April 1994 on admission to hospital. During this period no major *C. pneumoniae* epidemic occurred in Finland. The cases comprised 54 consecutive elderly patients with established COPD who either required hospitalization (40 patients) or visited an ambulatory chest clinic (14 patients) owing to various reasons associated with COPD, including acute exacerbation, hypoxaemia, increased dyspnoea and adjustment of medication. The patients were divided into two groups according to the severity of their disease by applying the criteria of the American Thoracic Society (1991) [13]:

Group I consisted of 41 patients whose forced expiratory volume in 1 s (FEV_1) was less than 50% of the predicted FEV_1 value, defined as having severe disease, or who had not been able to perform any function measurements for years due to advanced disease (10 of the 41 patients). The mean age in this group was 68 years, and the male–female ratio 27:14.

Group II comprised the remaining 13 COPD patients who had an FEV_1 value higher than or equal to 50% of the predicted value and who were defined as having mild to moderate disease. In one patient with dementia senilis it was not possible to measure the lung function due to poor co-operation but the patient was clinically evaluated as having moderate disease. The mean age of the patients in group II was 69 years; 8 patients were males, 5 females. Patients with current asthma or pneumonia were excluded from groups I and II.

Group III, the controls, consisted of 23 hospitalized patients with community-acquired pneumonia established by X-ray and defined clinical criteria (fever $\geqslant 38$ °C, sputum production and one of the following symptoms: cough, dyspnoea, chest pain, chills or an abnormal white blood cell count ($> 10 \times 10^9$/l). Patients with COPD or current asthma were excluded from this group as were patients who did not produce sputum. Also patients for whom paired sera were not available to allow assessment of seroconversions were excluded from the analysis. The mean age of the patients in group III was 45 years, and the male–female ratio 19:4. The clinical characteristics of the study population are shown in Table 1.

Informed consent was obtained from all the patients and the study protocol was accepted by the ethical committee of each participating hospital.

Serum and sputum samples were taken on admission; a second serum and, if possible, a second sputum sample 2 weeks later or, in the case of ambulatory COPD patients, 3 months later. Altogether 101 sera, 78 sputum specimens for sIgA measurements and 82 sputum specimens for PCR test were available from the COPD patients, and correspondingly 46 sera and 27 sputa for sIgA measurements and 22 sputa for the PCR test from the pneumonia patients. In addition, nasopharyngeal or pharyngeal swabs were obtained from 53 COPD and from 14 pneumonia patients for *C. pneumoniae* isolation.

Methods

Measurement of serum antibodies

C. pneumoniae-specific serum IgG and IgA levels were measured by the microimmunofluorescence (micro-IF) method of Wang and Grayston [14]. Elementary bodies of *C. pneumoniae* strain Kajaani 6 were used as antigen and fluorescein-conjugated anti-human IgG (Kallestad Diagnostic, Chaska, MN) and anti-human IgA (Sigma Chemical, St Louis) were used as conjugates. All the serum samples were treated with goat antibody to human IgG antibody (Gullsorb, Gull Laboratories, Salt Lake City, Utah) to remove IgG antibodies before IgA measurements [15]. Sera were tested in serial twofold dilutions from 1:8 (IgG) or 1:10 (IgA) to the end-point. The previously suggested criterion for chronic infection (IgG $\geqslant 128$ and concomitant IgA $\geqslant 40$) [16] was used, and both of the paired samples had to fulfil this criterion. For acute infection a fourfold titre change in any Ig class was considered diagnostic. All paired sera were analysed in parallel. The presence of *C. trachomatis* and *C.*

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Table 1. *Clinical characteristics of study population*

	Group I	Group II	Group II
Number (*N*)	41	13	23
Age			
Mean (S.D.)	68 (8·2)	69 (12·6)	45 (16·1)
Range	45–83	50–85	22–91
Sex ratio (M/F)	1·9 (27/14)	1·6 (8/5)	4·8 (19/4)
Lung function, mean (S.D.)			
FEV_1	0·86 (0·3)	1·78 (0·5)	na
FEV_1%pred	28·9 (8·7)	61·2 (11·2)	na
FEV_1/FVC%	37·8 (11·9)	63·4 (10·9)	na
Corticosteroids, *n*/*N* (%)			
Long-term*	32/41 (78·0)	9/13 (69·2)	na
Short-term†	2/41 (4·9)	1/13 (7·7)	na
Smoking *n*/*N* (%)			
Non-smokers	1/41 (2·4)	1/13 (7·7)	9/23 (39·1)
Ever-smokers‡	40/41 (97·6)	12/13 (92·3)	13/23 (56·5)
Data not obtained	0/41 (0)	0/13 (0)	1/23 (4·4)
Duration of smoking, years mean (S.D.)	41·5 (11·7)	48·2 (12·2)	14·6 (9·9)
Acute exacerbation, *n*/*N* (%)	26/41 (63·4)	7/13 (53·9)	
Concomitant heart disease§ *n*/*N* (%)	14/41 (34·2)	5/13 (38·5)	3/23 (13·0)

na, not assessed.
n, number of subjects meeting the criterion.
N, number of subjects examined.
FEV_1, forced expiratory volume in one second.
FEV_1%pred, FEV_1 percentage of the predicted.
FVC, forced vital capacity.
* $>$ 2 weeks, mostly months or years.
† $\leqslant$ 2 weeks.
‡ Current or ex-smokers.
§ Chronic coronary heart disease, acute myocardial infarction or both.

psittaci antibodies was tested as described previously [12].

Detection of C. pneumoniae*-specific immune complexes*

Circulating immune complexes (ICs) were measured as described elsewhere [17]. Briefly, 100 μl of the serum sample was added to an equal volume of 7% polyethylene glycol (PEG) (Fluka AG, Buchs, Germany) in sodium borate buffer (pH 8·4) and incubated overnight at +4 °C. The mixture was centrifuged at 3700 g for 15 min. The pellets were washed twice with 3·5% PEG in sodium borate buffer and the precipitates were finally dissolved to 100 μl volume of phosphate buffered saline (PBS), pH 7·4. The dissociated ICs were analysed for the presence of *C. pneumoniae* antibodies by the micro-IF test using Kajaani 6 strain as antigen and fluorescein-conjugated anti-IgG (Kallestad Diagnostic) as conjugate. Two-fold sample dilutions from 1:2 to 1:16 were used. A titre of 4 or higher was considered indicative of chronic infection.

Sputum antibody measurements

To measure sIgA antibodies in sputum we used the previously described and validated EIA method [12]. Briefly, homogenized sputum samples diluted 1:20 were added into the wells of antigen-coated (strain TWAR, Washington Research Foundation, Washington D.C.) microtitre plates (Maxisorp Immunoplates, Nunc, Roskilde, Denmark). After incubation, mouse monoclonal antibody to human secretory component (Sigma) was added and the plates were incubated overnight. Alkaline phosphatase anti-mouse conjugate (Jackson Immuno Research, West Grove, PA) was then added and finally, after incubation, dinitrophenyl phosphate substrate (Sigma). Absorbances were read at 405 nm using an

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ELISA reader (Labsystem Multiscan MCC 340, Labsystems, Helsinki, Finland).

Culture of C. pneumoniae

C. pneumoniae isolation was performed in HL cells applying the conventional technique of Kuo and Grayston (1990) [18] as described previously [12].

PCR

Sputum samples were treated with proteinase K (Sigma) and Nonidet P-40/Tween-20 (Sigma) at final concentrations of 100 μg/ml and 0·5% respectively. After incubation at 55 °C overnight DNA was purified using a commercially available QIAamp tissue kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. The oligonucleotide primers used, SC5 (5′TGCCTGT(AG)GGGAA(TC)-CC(AT)(GT)CTGA(AT)CCA3′ and SCH4 (5′GTC-GAAAAGA(AT)AGTC(TA)CC(GA)TAGTA3′), were purchased from the Institute of Biotechnology, Helsinki, Finland. The primers were originally published by Holland and colleagues [19] and were slightly modified by Dr S. Rasmussen (personal communication). The primers amplify at 145 bp sequence at the 5′-end of the *C. pneumoniae* omp1 gene. The PCR amplification mixture contained 1 mM deoxynucleoside triphosphates, 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8·3), 1·0 U *Taq* polymerase (Hytest, Turku, Finland), 50 pmol of each primer and 14 μl of the processed and diluted sample. The total reaction volume was 25 μl. AmpliWax beads (Perkin-Elmer Cetus, Norwalk, CN) were used to facilitate 'hot start' PCR [20]. After 5 min of denaturation at 94 °C, the samples were subjected to 40 cycles of denaturation (94 °C, 30 s), annealing and extension (60 °C, 45 s) using a Perkin-Elmer Cetus GeneAmp 9600 thermocycler. The final extension time was 6 min at 72 °C after cycling had been completed. The PCR products were separated by electrophoresis on 3% agarose gels containing ethidium bromide (0·2 μg/ml). DNA was transferred on to a nylon membrane (Immobilon N, Millipore, Bedford, MA). The blot was hybridized with a specific digoxigenin-labelled probe in 5 × standard saline citrate (SSC), 1·0% (w/v) Blocking Reagent for nucleic acid hybridization (Boehringer-Mannheim, Mannheim, Germany), 0·1% *N*-lauroylsarcosine and 0·02% sodium dodecyl sulphate (SDS) at 43 °C. The sequence of the probe was 5′CCATAT(TA)CT(GA)CCATCAATTAA3′. The hybridized probe was detected by alkaline phosphatase-labelled anti-digoxigenin Fab fragments using 5-bromo-4-chloro-3-indolyl-phosphate/nitro-blue tetrazolium (BCIP/NBT) (Bio-Rad, Hercules, CA) as substrate.

Control of PCR inhibition

Since sputum may contain inhibitors of the PCR reaction [21], 10 PCR-negative sputum specimens that were positive by at least two other diagnostic methods were tested for inhibition by adding *C. pneumoniae* DNA to the PCR reaction. The amount of DNA added corresponded approximately to 20 elementary bodies of *C. pneumoniae*. This DNA was amplified with the sample as described above. If no amplification product developed, the sample was considered to contain inhibitory components.

Diagnostic criterion for chronic *C. pneumoniae* infection

To evaluate the occurrence of *C. pneumoniae* infection in COPD patients, the diagnostic criterion for suspected chronic infection was defined. Diagnosis was considered definite if two of the three tests, PCR, serum micro-IF (elevated IgG and IgA levels) and sputum sIgA-EIA, gave a positive finding in either or both of the paired samples.

Statistical analysis

The chi-square test was used for comparing categorical data and Student's *t*-test was used for normally distributed data. To control the potential confounding factors associated with *C. pneumoniae* infection, multiple logistic regression analysis was used and the effect of each factor on chlamydia infection was expressed as an odds ratio. Confidence intervals were calculated at the 95% level. All analyses were carried out using SAS System Software Version 6·09 for OpenVMS (SAS Institute Inc., Cary, NC).

RESULTS

Marker positivity for *C. pneumoniae* infection

The proportions of sputum sIgA-, PCR- and circulating IC-positive subjects were highest in patients with severe COPD, next-highest in patients with mild to moderate disease and lowest in patients with pneumonia. Specific sputum sIgA antibodies were demonstrated in 28 (80%) out of 35 patients in group I and in 7 (58%) out of 12 patients in group II. PCR

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Table 2. *Proportions of* C. pneumoniae *marker-positive subjects among COPD and pneumonia patients, and the geometric mean titres (GMT) of IgG and IgA antibody levels in the first (I) and second serum samples (II)*

	Group I	Group II	Group III	*P*-value*
Marker, *n*/*N* (%)				
PCR†	23/39 (59·0)	4/10 (40·0)	3/14 (21·4)	0·047
Sputum sIgA†	28/35 (80·0)	7/12 (58·3)	3/22 (13·6)	0·0001
Immune complexes†	21/41 (51·2)	5/13 (38·5)	3/20 (15·0)	0·025
Serum antibodies‡	27/41 (65·9)	6/13 (46·2)		
GMT				ANOVA
IgGI/IgGII	119·6/109·7	79·2/93·4	47·4/66·0	0·01/0·19
IgAI/IgAII	59·0/52·4	22·3/22·7	7·2/7·2	0·0001/0·0001

n, number of patients fulfilling the positivity criterion.
N, number of patients with paired or single sample.
* For the difference between the COPD (group I and II) and pneumonia (group III) patients.
† Single sample or any of the paired samples positive.
‡ Marker for chronic infection (group I and II): elevated IgG ($\geqslant$ 128) and IgA ($\geqslant$ 40).

gave a positive finding in 23 (59%) out of 39 patients in group I and in 4 (40%) out of 10 patients in group II. For the presence of specific circulating ICs, the respective figures were 21 (51%) out of 41 and 5 (39%) out of 13 patients. Elevated serum IgG and IgA antibodies (IgG $\geqslant$ 128 and IgA $\geqslant$ 40) were found in 27 (66%) of the 41 patients with severe COPD (group I) and in 6 (46%) of the 13 patients with mild to moderate disease (group II). The differences in markers between groups I and II were not significant. In the pneumonia patients, by contrast, significantly lower proportions as compared to COPD patients were found for PCR, sputum sIgA and the ICs: 21% for PCR, 14% for sputum sIgA and 15% for the ICs. A similar pattern was observed in the GMTs of the serum IgA antibodies between the severity of the disease and the IgA level: the levels were highest in patients with severe COPD, intermediately elevated in patients with mild to moderate COPD and lowest in pneumonia patients ($P = 0{\cdot}0001$) (Table 2). Comparison of the GMTs of the IgG antibodies did not reveal as clear differences as in the IgA antibody levels, even though a significant difference was found between the groups in the first samples taken on admission ($P = 0{\cdot}01$).

One COPD patient showed a diagnostic titre change between the paired sera indicative of acute infection in the IgG immunoglobulin fraction (a titre rise from 32 to 128). In another COPD patient an IgG antibody to *C. trachomatis* was measured in paired samples at a titre of 128 concomitantly with high *C. pneumoniae* IgG and IgA antibody levels. No other diagnostic *C. trachomatis* or *C. psittaci* antibody levels were detected in any of the sera.

Among the pneumonia patients four seroconversions were observed in the IgG fraction, whereas none of the patients in this group met the serological criterion of chronic infection.

Stability of marker positivity

The frequency of changes in marker positivity during the study period was assessed in the COPD patients. The status of the serum marker based on both IgG and IgA antibody levels was unaltered in all 47 patients with paired sera during the entire study period. For circulating ICs, a stable result was similarly found in 94% of cases. In contrast to serology, changes in the positivity of the other two markers, sputum sIgA and PCR, were frequently observed between the paired specimens; in 30 and 23% of cases respectively, PCR and sIgA changed from negative to positive, whereas the opposite result, from positive to negative, was observed for PCR in 24% of the patients and for sIgA in 10% (Table 3).

Prevalence of *C. pneumoniae* infection

The overall prevalence of suspected chronic *C. pneumoniae* infection using the definite diagnostic criterion (see 'Methods') in the COPD patients was 65% (35 out of 54), and, according to the severity of COPD, 71% (29 of 41) for patients with severe COPD and 46% (6 of 13) for patients with mild to moderate COPD. The prevalence of acute *C. pneumoniae* pneumonia in group III was 17% (4 out of 23). In one of these four patients a positive PCR was observed and in another patient there was a positive sIgA finding

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Table 3. *Frequency of changes in marker positivity in COPD patients during the study period*

Marker	Pos → Neg *n/N* (%)	Neg → Pos *n/N* (%)	No change *n/N* (%)
Elevated IgG and IgA*	0/47 (0)	0/47 (0)	47/47 (100)
sIgA	3/31 (9·7)	7/31 (22·6)	21/31 (67·7)
PCR	8/33 (24·2)	10/33 (30·3)	15/33 (45·5)
ICs	1/47 (2·1)	2/47 (4·3)	44/47 (93·6)

n, number of patients fulfilling the marker positivity criterion.
N, number of patients with paired samples.
* IgG ⩾ 128 and IgA ⩾ 40.

in addition to a diagnostic titre change. No other cases with positive findings for at least two markers were found among the pneumonia patients.

Comparison of the PCR test to serum and sputum antibodies in COPD patients

In 18 of the 49 (37%) COPD patients for whom PCR data were available discrepant results were observed for serum micro-IF and the PCR test. In 11 cases the serum antibodies were elevated, but the PCR test was negative, and in seven cases the opposite result was obtained. In the former category, all the serum micro-IF-positive but PCR-negative patients also had diagnostic sputum sIgA antibody levels (data for sputum sIgA not available for three patients) and were considered definite positive cases. In the latter category, five patients had diagnostic levels of sputum sIgA antibodies in addition to a positive PCR result and were similarly considered definite positive cases. All those four patients who had IgG antibody levels equal to or higher than 1024 in any of the paired sera were PCR negative (Table 4). The PCR inhibition control test revealed no inhibitory elements in any of the sputum specimens tested.

In our laboratory PCR sensitivity and specificity were 75·8% (25/33) and 87·5% (14/16) respectively; serology sensitivity and specificity were 85·7 and 84·2% respectively defining a patient in whom at least two of three tests (serology, sputum, sIgA and PCR) were positive as a true positive case.

Potential confounding factors for *C. pneumoniae* infection

The potential confounding factors for *C. pneumoniae* infection in COPD patients were tested by multiple logistic regression analysis. None of the factors tested, namely age as a continuous variable, gender, smoking and the use of corticosteroid drugs as categorical variables, showed any significant confounding effect on the prevalence of *C. pneumoniae* infection, defined as serum micro-IF-positivity by the elevated IgG and IgA criterion and/or PCR-positivity (Table 5). Nor was any significant association found when serology alone was used to define chlamydia infection.

Culture

All the cultures of the COPD patients were negative, but in one pneumonia patient with a fourfold titre change and diagnostic levels of sputum sIgA antibodies but a negative PCR test result, the culture was positive.

DISCUSSION

Acute *C. pneumoniae* infections are rare among COPD patients; in 4–5% of acute exacerbations involvement of *C. pneumoniae* has been demonstrated by using diagnostic criteria for acute infection [22, 23]. In this study we have however provided further evidence for the previously suggested association [12] between COPD and *C. pneumoniae* infection that seems to be more chronic in nature. Seventy-one percent of the patients with severe COPD and 46% of those with mild to moderate COPD showed evidence of chlamydial involvement when our definite diagnosis criterion was used. This criterion was based on the presence of elevated serum IgG and IgA levels, sputum sIgA and specific DNA as detected by PCR.

Concomitantly elevated serum IgG and IgA level has proved to be a useful marker of suspected chronic *C. pneumoniae* infection in asthma patients [16], and it was reasonable to assume that this might be true for

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Table 4. *Discrepant results between the PCR and serum micro-IF tests in COPD patients*

Patient no.	Age/gender	Severity of COPD§	PCR*	Serum micro-IF† IgG	IgA	Result‡	sIgA-EIA*
606	61/M	Severe	+	128	10	−	−
				64	5		
502	55/F	Severe	+	16	5	−	+
				32	10		
506	70/F	Moderate	+	64	5	−	+
				128	10		
508	69/F	Severe	+	32	20	−	−
				32	20		
510	59/F	Severe	+	128	10	−	+
				128	10		
517	72/M	Severe	+	128	10	−	+
				64	10		
545	59/F	Severe	+	32	10	−	+
				32	10		
602	76/M	Severe	−	512	160	+	+
				1024	160		
528	76/F	Severe	−	1024	80	+	nd
				512	80		
533	70/M	Severe	−	128	160	+	+
				256	160		
534	83/F	Severe	−	256	80	+	nd
				128	160		
521	79/M	Severe	−	2048	640	+	+
				2048	640		
504	85/M	Moderate	−	128	40	+	+
				128	80		
526	79/M	Severe	−	1024	640	+	+
				1024	640		
525	80/F	Mild	−	540	40	+	+
507	65/M	Severe	−	128	40	+	+
				128	40		
523	65/M	Severe	−	128	160	+	+
				128	160		
608	55/M	Moderate	−	128	40	+	nd
				128	40		

sIgA-EIA enzyme immunoassay for secretory IgA.
nd, not done.
* Positivity is defined as a positive test result in either or both of the two samples.
† The titre of the first serum specimen is given above the second in each case.
‡ The 'elevated IgG ($\geqslant 128$) and IgA ($\geqslant 40$)' criterion for chronic infection is used to define serum micro-IF positivity.
§ Severity of COPD: defined as severe if $FEV_1 < 50\%$ of the predicted, moderate if $FEV_1 \geqslant 50\% < 70\%$ of the predicted, and mild if $FEV_1 \geqslant 70\%$ of the predicted.

COPD patients as well. It has been suggested that the presence of short-lived sIgA antibodies in secretion indicates on-going infection in several chlamydia infections even more reliably than serology [24, 25]. Furthermore, the PCR technique has proved to be more sensitive than culture in various infections caused by *C. trachomatis* [26–28]. As regards *C. pneumoniae*, the difficulty in finding a proper sample type for this technique, especially in suspected chronic infections, has meant that the evaluation of this method has proceeded slowly.

The different markers of chlamydial infection used in this study revealed significant differences between COPD and pneumonia patients. The occurrence of

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Table 5. *Odds ratios with 95% confidence intervals (CI) for potential confounding factors for* C. pneumoniae *infection* in COPD patients derived from the multiple logistic regression analysis*

Factor	Odds ratio	(95% CI)
Age	0·99	(0·92, 1·06)
Gender	1·28	(0·32, 5·15)
Smoking	0·56	(0·17, 1·82)
Corticosteroids	1·45	(0·68, 3·10)

* Defined as seropositivity (IgG $\geqslant$ 128 and IgA $\geqslant$ 40) and/or PCR-positivity.

the markers was highest in severely ill COPD patients and next-highest in patients with mild to moderate disease. This pattern was also found in GMTs of IgA antibodies. Our recent study of hospitalized and non-hospitalized COPD patients compared to age-adjusted disease-free controls has shown a similar relationship between the severity of COPD and the serum IgA level expressed as GMT. Overall, the difference in the GMT of IgA antibody levels between the elderly COPD patients and their age-matched controls without COPD, asthma or symptoms of chronic bronchitis was highly significant, and the association between the disease and the elevated IgA antibody levels persisted after controlling for smoking [29]. We are aware of the potential confounding effect of age when comparing COPD and pneumonia patients in the present study. However, the exclusion of data from pneumonia patients younger than 45 years did not abolish the differences in the GMTs of IgA antibody levels and in the proportions of PCR-, sputum IgA- and IC-positive subjects between COPD and pneumonia patients. In addition, we could not use healthy elderly persons as controls in the present study owing to sputum antibody measurements.

Assessment of the marker stability of patients with suspected chronic chlamydial infection revealed that serological markers including circulating ICs are stable for several months. In contrast, sputum sIgA and PCR showed more changes. The quality of successive sputum samples may vary considerably, emphasizing the importance of collecting at least two samples for analysis. We cannot rule out the possibility that medication, both corticosteroids and antibiotics, during hospitalization may affect chlamydial infection and sIgA production, as indicated by the higher proportion of sputum sIgA-positive patients at the end of the study compared to those at admission.

Discrepant results between serum micro-IF and PCR were obtained in 37% of the COPD patients. In the PCR-negative and serum micro-IF positive cases, high serum IgG and IgA antibody levels were present in addition to sputum sIgA antibodies. Circulating ICs were found in most of these cases, too. In the patients with the highest levels of serum IgG antibodies all the PCR tests were negative. This accords well with the results reported by Kuo and colleagues [30], who found that in patients with atherosclerosis the PCR test was negative in those subjects in whom the highest specific serum IgG antibody levels were detected. A high serum and sputum antibody level may indicate a more advanced infection and a more distinct Th2-type immune response observed in chronic disease [31]. Similarly, in cystic fibrosis, late in the inflammatory response, cytokines of the Th2-type immune response are induced, evidently to down-regulate the inflammation and limit further destruction of the tissues involved [32]. Antigen-shedding in the chronic stages may be minimal; the organism can persist quiescently for long periods inside the host cell, inflicting relatively little damage on it [33]. It is well known that isolation of chlamydia is difficult in chronic disease, and we were unable to isolate the organism in the nasopharyngeal or pharyngeal swabs of the COPD patients. Whether a specific 'cryptic', unculturable form of chlamydia exists [34] is still a matter of controversy. The serum micro-IF test has largely remained 'the method of choice', although it also has been criticized [35].

Comparison of the PCR and serum micro-IF test may not be warranted as it seems to us that they detect infection at different stages; PCR may detect cases with low or moderately elevated serum antibody levels at an earlier stage of the parasitic relationship, whereas in more advanced cases with a distinct Th2 response reflected in high levels of humoral antibodies, DNA detection by PCR is probably unsuccessful.

In acute community-acquired pneumonias *C. pneumoniae* accounts for 6–10% of cases during interepidemic periods [36] and in acute exacerbations of COPD, it is even rarer [22, 23]. Our results indicate that the involvement of *C. pneumoniae* in COPD is much more common than studies of acute exacerbation have suggested. Neither smoking nor any other potential confounding factor tested had any significant effect on *C. pneumoniae* prevalence. Recent studies on the association between *C. pneumoniae* and coronary heart disease have demonstrated, in accordance with our results, that smoking is not a

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significant confounding factor for *C. pneumoniae* seropositivity [37, 38].

Stable elevated serum IgA levels, the almost complete absence of seroconversions and the relatively frequent presence of circulating ICs in COPD patients support the hypothesis that the infection in these subjects might be chronic. Whether there is a causal relationship between *C. pneumoniae* infection and COPD or whether the severely ill patients are more susceptible to persistent *C. pneumoniae* infection cannot be determined on the basis of this study. Further intervention studies are required to clarify finally the question of cause and effect. Unlike the most frequently found bacteria in COPD patients, *C. pneumoniae* is not a common inhabitant of a normal nasopharynx. Neither does the intracellular nature of *C. pneumoniae* support a 'bystander' role for this agent. In conclusion, the findings reported here provide further evidence for an association between *C. pneumoniae* and COPD. The possible role of this micro-organism in the pathogenesis of COPD remains to be elucidated.

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