

Acute Purulent Exacerbation of Chronic Obstructive Pulmonary Disease and *Chlamydia pneumoniae* Infection

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In order to investigate the role of bacteria, including *Mycoplasma pneumoniae* and especially *Chlamydia pneumoniae* in acute purulent exacerbations of chronic obstructive pulmonary disease (COPD), we examined sputum specimens and acute and convalescent sera taken 26 d apart from 49 outpatients experiencing an acute purulent exacerbation of COPD. The sera were tested for antibodies to *C. pneumoniae* with the microimmunofluorescence test, and for antibodies to *M. pneumoniae* with the indirect fluorescence antibody test. Routine microbiologic culture of sputum yielded potentially pathogenic microorganisms in 12 of the 49 patients (24%). Three patients (6%) showed serologic evidence of recent *M. pneumoniae* infection. Seven patients showed high IgG titers of $\geq 1:1,024$ to *C. pneumoniae*, and an additional four had a fourfold increase in IgG titer, suggesting reinfection with *C. pneumoniae*. Sputum from two of these 11 patients also grew *Streptococcus pneumoniae*, and one grew *Moraxella catarrhalis*. Patients with and without serologic evidence of current *C. pneumoniae* infection showed no significant differences in clinical features or pulmonary function. The high incidence of infection with *C. pneumoniae* (the sole causal agent in 16% of cases, and the causal agent with other agents in 6%) provides insight into the importance of this organism among agents leading to exacerbations of COPD in Turkey. Mogulkoc N, Karakurt S, Isalska B, Bayindir Ü, Çelikel T, Korten V, Çolpan N. Acute purulent exacerbation of chronic obstructive pulmonary disease and *Chlamydia pneumoniae* infection.

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Chronic obstructive pulmonary disease (COPD) is a lifelong disease with significant cost and morbidity. In patients with COPD, acute bacterial infections of the respiratory tree are common and have a negative impact on quality of life and on the progression of the disease, particularly in more severely affected patients (1, 2). However, causal infective agents may not be isolated from up to 50% of purulent sputum samples from these patients (3). Although *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis* are traditionally recognized as major pathogens (4), recent studies have considered the possibility that *Chlamydia pneumoniae* is also involved in some exacerbations of COPD (4 to 16%) in both nonhospitalized and hospitalized patients (5-7). Because *C. pneumoniae* may contribute to disease progression through its toxic effect on bronchial epithelial cells, with ciliostasis, it seems increasingly important to detect infection by this organism (8). This prompted us to conduct a prospective study to investigate the incidence of bacteria, including *Mycoplasma pneumoniae*, and especially *C. pneumoniae*, in acute purulent

exacerbations of COPD in nonhospitalized patients attending the outpatient clinic at a university hospital in Turkey.

METHODS

Patient Selection

Inclusion criteria for the study were: (1) ambulatory status; (2) a history of COPD according to previously defined criteria (9); (3) an Anthonisen type-1 exacerbation (10); (4) purulent sputum, as defined by a Gram stain showing > 25 polymorphonuclear leukocytes and < 10 squamous epithelial cells per low-power field (lpf); and (5) written informed consent. Exclusion criteria were: (1) hospitalized status; (2) treatment with any antibiotic for 24 h or longer within 72 h before the baseline visit; (3) absence of an adequate sputum specimen as determined by Gram stain; (4) evidence of bronchiectasis and/or pneumonia; and (5) malignancy or severe immunosuppression. The first consecutive 49 patients who fully met these criteria, from among approximately 200 COPD patients attending the university hospital's clinics between November 1996 and September 1997, were included. After a thorough history and examination, including chest radiography, patients underwent baseline pulmonary function tests. Hematologic, microbiologic, and serologic investigations were done on the same day. Patients were treated with either oral ciprofloxacin, 500 mg twice daily, or oral trovafloxacin, 200 mg twice daily, for 10 d. All patients were reassessed clinically, hematologically, and bacteriologically at the end of treatment (Day 11) and at follow-up assessment (Day 26). According to clinical response on Day 11, eight patients additionally received oral amoxicillin, 500 mg thrice daily for 10 d. A further serum specimen for detection of *Chlamydia* species and antibodies to *M. pneumoniae* was collected on Day 26. Serologic and he-

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matologic analyses, together with bacteriologic confirmation, were performed in the same reference laboratory in Switzerland. All patients who were enrolled in the study completed it.

Laboratory Investigations

Bacteriology. Sputum samples were plated on blood and chocolate agar and incubated at 37° C for both 24 and 48 h. All microorganisms isolated were identified through standard laboratory methods (11).

Serology. All sera were investigated for the presence of IgG, IgM, and IgA antibodies to *Chlamydia* species (*C. pneumoniae*, *C. trachomatis*, and *C. psittaci*) with the microimmunofluorescence (MIF) test (MRL Diagnostics, Cypress, CA; the source of *C. pneumoniae* antigen was the English strain IOL-207). Serum antibodies against *C. pneumoniae* elementary bodies were detected with fluorescein-conjugated monoclonal goat antihuman Ig-subclass antibodies. Measurements of IgA were made after absorption with human IgG inactivation reagent. Seropositivity was defined as a titer of $\geq 1:64$ for IgG and IgA, and of $\geq 1:20$ for IgM for all *Chlamydia* species. An isolated IgM titer of $\geq 1:20$ was considered to be evidence of primary *C. pneumoniae* infection. An IgG titer of $\geq 1:1,024$ or a fourfold change in titer of IgG, IgM, or IgA was considered to be an indicator of reinfection. An IgG titer of 1:512 was regarded as suggestive of reinfection with *C. pneumoniae*. Past *C. pneumoniae* infection (chronic or preexisting antibody) was defined as an IgG titer of 1:64 to 1:256.

The presence of circulating IgG and IgM antibodies to *M. pneumoniae* was detected with an indirect fluorescence antibody (IFA) system (Zeus Scientific, Inc., Raritan, NJ). A fourfold change in titer of IgG or IgM, or an isolated IgG titer of $\geq 1:256$ or IgM titer of $\geq 1:16$, was considered evidence of recent infection.

Statistical Analyses

Statistical analyses were performed with the SPSS/PC + package, version 5.0.2 (SPSS Inc., Chicago, IL). For normally distributed data, analysis of variance (ANOVA) was used to compare group means; otherwise the Mann-Whitney U test was applied. Response to treatment was analyzed with the Pearson's chi-square test. Values are expressed as means \pm SD, and a statistical significance level of 0.05 was used.

RESULTS

The clinical characteristics of the patients are summarized in Table 1.

Bacteriologic Results

Potentially pathogenic microorganisms (PPMs), which are recognized as agents causing respiratory infections whether or not they belong to the oropharyngeal or gastrointestinal flora (12), were identified in 12 (24%) of the 49 patients. They consisted of *S. pneumoniae* (33.3% of PPMs), *H. influenzae* (33.3% of PPMs), *Haemophilus parainfluenzae* (8.4% of PPMs), and *M. catarrhalis* (25% of PPMs). Non-PPMs, which belong to the oropharyngeal or gastrointestinal flora not usually involved in respiratory infections in immunocompetent patients (12), were found in the remaining 37 (76%) patients (Table 2).

Serologic Results

Antibodies to *C. pneumoniae*. Forty-one of the 49 patients (83.7%) had specific IgG titers of $\geq 1:64$. None of the patients developed IgM antibodies.

Twenty-eight (57%) patients who had specific IgG titers to *C. pneumoniae* of 1:64 to 1:256 in paired sera were regarded as having had previous *C. pneumoniae* infection. Four patients showed a fourfold seroconversion to *C. pneumoniae* infection in the IgG antibody class, one of whom showed a simultaneous fourfold change in the IgA antibody class, indicating reinfection. IgG titers of $\geq 1:1,024$ were found in seven patients. Two patients had IgG titers of 1:512.

TABLE 1
CLINICAL CHARACTERISTICS OF PATIENTS

No. of subjects	49
Age, yr*	66.7 (7.8)
Male/female	39/10
Duration of acute symptoms, d*	8.3 (4.3)
Smoking, n/N (%)	
Nonsmokers	8/49 (16.3)
Ex-smokers	33/49 (67.4)
Current smokers	8/49 (16.3)
Respiratory rate/min*	19.5 (3.5)
Pulse rate/min*	89.5 (10.6)
Temperature > 37.5° C, n/N (%)	6/49 (12)
Leukocytes > 10.5 ($\times 10^9/L$), n/N (%)	5/49 (10)
Lung function*	
FEV ₁ , L	1.0 (0.4)
FEV ₁ , % pred	41.0 (16.9)
FVC, L	2.0 (0.6)
FVC, % pred	56.7 (15.4)
FEV ₁ /FVC	69.1 (14.6)
Dyspnea, n/N (%)†	
Mild	1/49 (2)
Moderate	23/49 (47)
Severe	25/49 (51)
Cough, n/N (%)†	
Mild	1/49 (2)
Moderate	27/49 (55.2)
Severe	21/49 (42.8)
Increased sputum, n/N (%)†	
Mild	3/49 (6.1)
Moderate	20/49 (40.8)
Severe	26/49 (53.1)
Wheeze, n/N (%)†	
Mild	2/49 (4)
Moderate	27/49 (55.2)
Severe	20/49 (40.8)
Chills, n/N (%)†	
Absent	34/49 (69.4)
Mild	9/49 (18.4)
Moderate	5/49 (10.2)
Severe	1/49 (2)

* Values are presented as mean (SD).

† Severity of symptoms was based on patients' perception.

Twenty of the patients with past *C. pneumoniae* infection had a low IgA titer, and eight patients showed an increased IgA titer at presentation, with fluctuating high titers in paired sera in only one patient. Nine of the 11 patients with serologic features of reinfection had a simultaneously increased IgA titer at presentation, with fluctuating high titers in paired sera in seven of these patients. Patients who had no seroconversion to IgG also had no increase in IgA titers (Table 3).

Antibodies to other *Chlamydia* species. Antibodies to *C. trachomatis* or *C. psittaci* were found in three patients at lower titers evidencing a cross-reaction.

Antibodies to *M. pneumoniae*. A fourfold increase in IgG titer to *M. pneumoniae* was found in two patients, with an IgG titer of $\geq 1:256$ in one indicating recent *M. pneumoniae* infection (6%). None of these patients had antibodies to *C. pneumoniae*. Five patients showed an IgG titer of 1:128 to *M. pneumoniae*, suggesting previous infection.

Serologic Results in Patients According to Bacteriologic Culture Status

In this study, of the 12 patients whose sputum yielded PPMs, three (6%) were seropositive and nine (18%) were seronegative for *C. pneumoniae*. Of the 37 patients whose sputum failed to yield a PPM on culture, eight (16%) showed evidence of reinfection with *C. pneumoniae*, three (6%) had serologic

TABLE 2
MICROORGANISMS ISOLATED FROM SPUTUM CULTURES
AND SEROLOGIC RESULTS FOR 49 PATIENTS

Organism(s)	n	Medication	Response to Treatment
PPM			
<i>Streptococcus pneumoniae</i>	4	3 Q/1 Q+A	Bacteriologic response (Day 11)* 4 Eradicated
<i>Haemophilus influenzae</i>			
Beta lactamase-positive	2	2 Q	2 Eradicated
Beta lactamase-negative	2	2 Q	2 Eradicated
<i>Haemophilus parainfluenzae</i>	1	1 Q	1 Eradicated
<i>Moraxella catarrhalis</i>			
Beta lactamase-positive	3	2 Q/1 Q+A	3 Eradicated
Total PPM	12		
Non-PPM[†]			
<i>Streptococcus viridans</i> group	25	20 Q/5 Q+A	Clinical response (Day 26) [‡] 23 of 37 patients cured, 9 improved (3 <i>C. pneumoniae</i> , 3 <i>M. pneumoniae</i> serology positive), 5 failed (all <i>C. pneumoniae</i> serology-positive patients).
<i>Neisseria</i> spp.	5	4 Q/1 Q+A	
<i>Corynebacterium</i> spp.	2	2 Q	
<i>Candida</i> spp.	1	1 Q	
<i>Micrococcus</i> spp.	2	2 Q	
<i>Streptococcus</i> group D	2	2 Q	
Total non-PPM	37		
Other			
<i>Chlamydia pneumoniae</i> [§]	11	6 Q/5 Q+A	Clinical response (Day 26) [‡] 3 Cured, 3 improved, 5 failed
<i>Mycoplasma pneumoniae</i>	3	2 Q/1 Q+A	3 Improved

* Bacteriologic response definition: Eradication = elimination of the original causative organism from expectorated sputum upon completion of therapy.

[†] Includes eight patients with *C. pneumoniae* and three patients with *M. pneumoniae* infection.

[‡] Clinical response definitions: Cure = resolution of signs and symptoms of acute exacerbation. Supporting information includes resolution of leukocytosis. Improvement = resolution of fever but incomplete resolution of other signs and symptoms of exacerbation. Failure = lack of resolution of any of the signs and symptoms of exacerbation.

[§] Includes three mixed infections; two with *S. pneumoniae* and one with *M. catarrhalis*. Q = quinolone alone (either ciprofloxacin or trovafloxacin); Q+A = quinolone + amoxicillin.

levels consistent with recent *M. pneumoniae* infection, and the remaining 26 were negative for both *C. pneumoniae* and *M. pneumoniae* (Table 4).

There was no statistically significant difference in lung function or leukocyte count of patients with and without evidence of *C. pneumoniae* infection (Table 5). There was also no statistically significant difference in duration of acute symptoms; fever; pulse rate; respiratory rate; severity of cough, dyspnea, wheezing; or increased sputum production according to etiologic agent ($p > 0.05$ in the two groups for all parameters applying the Mann-Whitney U test). All patients who were considered to represent failures of clinical treatment had serologic evidence of reinfection with *C. pneumoniae* ($p < 0.001$ applying Pearson's chi-square test).

DISCUSSION

This first prospective surveillance report of a serologic evaluation for *C. pneumoniae* infection in acute purulent exacerbations of ambulatory COPD in patients in Turkey has shown that 57% of patients had serologic evidence of past *C. pneumoniae* infection. This is comparable to the prevalence of

C. pneumoniae infection found in healthy blood donors of similar age and sex of the same institution, in Izmir, Turkey (61%) (13), and indicates a prevalence of the organism no higher than in certain other European countries such as The Netherlands (14), Finland (15), and Italy (16). Acute *C. pneumoniae* infection was demonstrated in 11 (22%) cases. The absence of an increase in IgM suggests reinfection rather than primary infection. This finding supports the concept that *C. pneumoniae* is an increasingly frequent cause of exacerbations of severe COPD even in nonhospitalized patients. However, in three patients, *C. pneumoniae* was associated with other PPMs: *S. pneumoniae* in two patients and *M. catarrhalis* in another. The frequent occurrence of coinfection could be explained by *C. pneumoniae* inducing ciliostasis, which then predisposes to infection with other respiratory pathogens (6, 8). All of the 11 patients were treated with either ciprofloxacin or trovafloxacin for 10 d. In addition, five of the 11 patients received amoxicillin for 10 d after completion of the first therapy (Table 2).

An interesting feature of our study was that high IgA titers at presentation were found in the vast majority of patients considered to have had reinfection with *C. pneumoniae*. None of the patients who were seronegative for *C. pneumoniae* had

TABLE 3
SEROLOGIC RESULTS FOR *Chlamydia pneumoniae*

IgA Status (at Presentation)	< 1:64	1:64–1:256	IgG Titers (4x)	≥ 1:1,024	1:512
< 1:64	8	20	1	1	1
≥ 1:64 (fluctuating*)	0	1	2 [†]	5	0
≥ 1:64 (stable [†])	0	7	1	1	1
Total patients	8	28	4	7	2

* In paired sera.

[†] One patient had a simultaneous fourfold change in IgA titer.

TABLE 4
SUMMARY OF THE ETIOLOGIC AGENTS
EXACERBATIONS OF COPD

	Bacterial Culture Positive (n)	Bacterial Culture Negative (n)	Total
<i>Chlamydia pneumoniae</i> +	3 (6%)	8 (16%)	11 (22%)
<i>Chlamydia pneumoniae</i> -	9 (18%)	29 (60%)*	38 (78%)
Total	12 (24%)	37 (76%)	49 (100%)

* Three patients showed serologic indication of recent *Mycoplasma pneumoniae* infection.

increased IgA titers. Surprisingly, 78% of patients with high IgA titers had fluctuating rather than stable IgA titers in paired sera, which may be considered evidence of reinfection, in addition to high IgG titers (Table 3). However, persistence of stable, elevated titers of IgA has been previously suggested to be an appropriate marker of an active, recurrent, or chronic carrier state (15, 17, 18).

Past studies evaluating the role of *C. pneumoniae* in COPD have shown an incidence of infection with the organism of 4% and 16% in nonhospitalized and hospitalized patients, respectively (5, 7).

Although there was no statistically significant difference in the clinical characteristics of patients with *C. pneumoniae* in-

fection and those without (Table 5), other authors have linked more severe COPD or pneumonia to higher geometric mean titers of IgG and IgA (6). We have found a similar severity of clinically apparent bronchospasm in approximately similar proportions of patients with *C. pneumoniae*, those with other infectious agents, and those remaining seronegative and culture negative, suggesting that the presence and severity of wheezing are not helpful in differentiating *C. pneumoniae* infections from others.

Information about the role of *M. pneumoniae* in patients with exacerbations of COPD is scarce. Our data showed that *M. pneumoniae* (6%) might have a role in this setting. This differs from reports in the recent literature, which failed to implicate this organism (5, 7).

The role of bacterial infection in COPD has been the subject of much debate. It is generally accepted that bacteria with or without copathogens are probably significant in producing exacerbations. The bacteria isolated in our study population were compatible with those isolated in other studies (4).

A diagnosis of acute *C. pneumoniae* infection is usually based on serologic criteria that include the presence of IgM antibodies and/or a fourfold rise in IgG antibody titers (19). The frequent occurrence of *C. pneumoniae*-specific immunoglobulin in adults limits the value of IgG alone as a diagnostic marker of acute infection. IgG titers of 1:512 may persist for prolonged (> 1 yr) periods, and it has therefore been pro-

TABLE 5
CHARACTERISTICS OF PATIENTS ACCORDING TO ETIOLOGIC AGENTS

Evaluated parameter	<i>Chlamydia pneumoniae</i> (n = 11)*	Other Organism (n = 12) [†]	No Organism (n = 26)
DAS, d [‡]	9.0 (4.6)	7.1 (3.7)	8.5 (4.5)
Respiratory rate/min [‡]	20.3 (3.6)	20.8 (3.3)	18.6 (3.3)
Pulse rate/min [‡]	88.8 (9.2)	92.3 (15.9)	88.9 (8.1)
Fever, °C [‡]	36.8 (0.4)	36.8 (0.5)	36.9 (0.4)
Leukocytes (× 10 ⁹ /L) [‡]	8.87 (2.3)	8.92 (2.1)	8.27 (1.6)
Lung function [‡]			
FEV ₁ , L	0.9 (0.4)	1.0 (0.5)	1.1 (0.4)
FEV ₁ , % pred	36.0 (14.4)	44.6 (21.5)	41.3 (15.8)
FVC, L	1.8 (0.5)	1.9 (0.6)	2.1 (0.7)
FVC, % pred	49.5 (10.9)	57.7 (16.9)	59.4 (15.9)
FEV ₁ /FVC	68.9 (13.4)	70.1 (14.9)	68.4 (15.4)
Dyspnea, n/N (%)			
Mild	—	—	1/26 (4)
Moderate	6/11 (55)	6/12 (50)	11/26 (42)
Severe	5/11 (45)	6/12 (50)	14/26 (54)
Cough, n/N (%)			
Mild	1/11 (9)	—	—
Moderate	7/11 (64)	5/12 (42)	15/26 (58)
Severe	3/11 (27)	7/12 (58)	11/26 (42)
Sputum, n/N (%)			
Mild	—	—	3/26 (12)
Moderate	9/11 (82)	5/12 (42)	6/26 (23)
Severe	2/11 (18)	7/12 (58)	17/26 (65)
Wheeze, n/N (%)			
Mild	—	1/12 (8)	1/26 (4)
Moderate	5/11 (45)	4/12 (33)	18/26 (69)
Severe	6/11 (55)	7/12 (59)	7/26 (27)
Chills, n/N (%)			
Absent	5/11 (46)	9/12 (76)	20/26 (77)
Mild	3/11 (27)	2/12 (16)	4/26 (15)
Moderate	3/11 (27)	1/12 (8)	1/26 (4)
Severe	—	—	1/26 (4)
Response to treatment [§]	3 C / 3 I / 5 F	8 C / 4 I	23 C / 3 I

Definition of abbreviations: C = cured; DAS = duration of acute symptoms; F = failed; I = improved.

* Three have other etiologic agents (two *Streptococcus pneumoniae*, one *Moraxella catarrhalis*).

[†] Includes nine patients with bacterial infection and three patients with recent *Mycoplasma pneumoniae* infection.

[‡] Values are presented as mean (SD).

[§] All treatment failures were observed in *C. pneumoniae* patients (p < 0.001 using Pearson's chi-square test).

posed that the arbitrary breakpoint of an IgG titer of $\geq 1:512$ be replaced by a titer of $\geq 1:1,024$ to indicate acute infection (19, 20). We applied this revised level of $\geq 1:1,024$ as indicative of reinfection to the paired sera in our study.

In summary, in a well-selected population with an acute purulent exacerbation of COPD, bacteria were cultured from 24% of patients and serologic studies showed *M. pneumoniae* infection in 6% and *C. pneumoniae* in 22%. Although we were unable to perform appropriate analyses to identify viral etiologic agents, it seems that *C. pneumoniae* is an important etiologic agent in exacerbations of COPD, being present in 16% of patients on its own and in 6% together with other infectious agents. We suggest that this finding be borne in mind when initiating antibiotic therapy. Studies addressing the isolation or demonstration of *C. pneumoniae* in the lung are likely to shed further light on the significance of acute infection and chronic carriage of this organism in COPD.

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