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Use of serology and polymerase chain reaction to detect atypical respiratory pathogens during acute exacerbation of chronic obstructive pulmonary disease

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Background/Aims: To use serological and multiplex polymerase chain reaction (PCR) assays to examine sputum samples from patients experiencing acute exacerbation of chronic obstructive pulmonary disease (AECOPD) for the presence of atypical pathogens, including *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella pneumophila*.

Methods: From September 2012 to February 2014, 341 patients with AECOPD attending outpatient clinics were enrolled as part of a randomized, double-blind, multicenter study. A commercial enzyme-linked immunosorbent assay was used to measure serum immunoglobulin M (IgM) and IgG antibody titers on the first day of the study and at 36 days post-enrollment. Multiplex PCR was used to test sputum samples for the presence of atypical pathogens. A urinary antigen test for *L. pneumophila* was performed on the first day.

Results: Nineteen patients (5.6%) showed serological evidence of acute infection with *M. pneumoniae*. Also, one and seven patients (2%) showed serological evidence of acute infection with *C. pneumoniae* and *L. pneumophila*, respectively. All DNA samples were negative for *M. pneumoniae*, *C. pneumoniae*, and *L. pneumophila* according to PCR. Only one urine sample was positive for *L. pneumophila* antigen, but serologic evidence was lacking.

Conclusions: Serological testing suggested that infection by atypical pathogens during AECOPD was relatively uncommon. In addition, PCR provided no direct evidence of infection by atypical pathogens. Thus, atypical pathogens may not be a major cause of AECOPD in South Korea.

Keywords: Pulmonary disease, chronic obstructive; Exacerbation; Atypical pathogen; Serology; Polymerase chain reaction

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is associated with considerable morbidity and mortality. The World Health Organization estimates that COPD will be the third leading cause of death worldwide by 2030 [1]. The overall prevalence of COPD in subjects aged ≥ 40 years in Korea is estimated to be 13.4% [2]. Acute exacerbation of chronic obstructive pulmonary disease (AECOPD) are the most important prognostic factor; such exacerbations are associated with short and long term reductions in quality of life and lung function, as well as an increased risk of death [3,4]. The etiology of AECOPD is multifactorial; the condition is caused by complex interactions between the host immune system, respiratory viruses, and airway bacteria, all of which lead to an increase in the inflammatory burden within the airway [5]. Previous data suggest that the etiology is unclear in nearly 30% of AECOPD cases; however, respiratory tract infection (50% to 60% of cases) and air pollution (10% of cases) are major causes [6].

Atypical respiratory pathogens usually include *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella pneumophila*, although the exact role of these pathogens in AECOPD is yet to be elucidated. Atypical pathogens are detected indirectly by serological assays, or directly by cell culture or polymerase chain reaction (PCR). Most studies used a single method [7-13], whereas a few combined methods [14-16]. Although serology may suggest that atypical pathogens play a significant role in AECOPD, interpreting serology results is tricky and yields variable results [7,8,17-25]. Also, culturing atypical pathogens is not easy. There are some data regarding the use of PCR for diagnosing atypical pathogens, and PCR appears superior to serology in this respect [26]. However, serology and PCR can yield discrepant results [14-16]. Indeed, a previous study revealed that, according to real-time PCR results, atypical pathogens do not play a significant role in stable COPD or AECOPD [9]. Thus, the role of atypical pathogens in AECOPD remains controversial. The present prospective study was designed to include patients with a moderate AECOPD (defined as an increase in symptoms that required treatment with antibiotics and/or corticosteroids, but not hospitalization); the aim was to use serology and multiplex PCR to determine the role of atypical pathogens in AECOPD.

METHODS

Study design and subjects

This was a *post hoc* analysis of a clinical trial examining the use of zabofloxacin versus moxifloxacin to treat patients with COPD exacerbation [27]. The study was a multicenter, randomized, double-blind, double-dummy, parallel-group, controlled, phase 3 clinical trial conducted at 31 university hospitals in South Korea. The first patient was enrolled in September 2012 and the last in February 2014.

Eligible patients were aged ≥ 40 years and had COPD as defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines (i.e., a post-bronchodilator forced expiratory volume in 1 second [FEV_1]/forced vital capacity < 0.7). Patients experiencing moderate exacerbation of COPD (defined as worsening of respiratory symptoms beyond normal day-to-day variations and leading to a change in medication but not requiring hospitalization), and who also had purulent sputum or an increased volume of sputum, were enrolled. Pregnant women; patients who received systemic antibiotics and/or antifungal agents within the last 72 hours; those with confirmed pneumonia (on chest X-ray) within 48 hours; and those with underlying septic shock, bronchiectasis, lung abscess, active tuberculosis, pulmonary malignancy, cystic fibrosis, empyema, or asthma were excluded.

Microbiological assays

Each patient provided a sputum sample on day 1 (visit 1), day 10 ± 3 (visit 3), and day 36 ± 7 (visit 4). A blood sample was obtained on day 1 and day 36 ± 7. The sputum specimens containing group 4 or 5 in Gram stain score were used for bacterial culture and group 3 to 5 were considered suitable for PCR. Paired serum samples were used for serological tests for *M. pneumoniae*, *C. pneumoniae*, and *L. pneumophila*. Urine samples for the urinary antigen test were also collected on day 1 and examined for the presence of *L. pneumophila* and Streptococcus pneumoniae. All samples were sent to the central Seegene medical foundation reference laboratory (Seoul, Korea). Serological assays for *M. pneumoniae* were performed using the *M. pneumoniae* immunoglobulin G (IgG)/IgM enzyme-linked immunosorbent assay (ELISA) (Vircell, Granada, Spain). The assay for *C. pneumoniae* was performed using the SeroCP™ IgG/IgM kit (Savyon Diag-

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nostics, Ashdod, Israel) and that for *L. pneumophila* was performed using the *L. pneumophila* serogroup 1 IgG/IgM ELISA (Vircell). All assays were performed according to the manufacturer's protocols. A definite acute infection was defined as a 4-fold or greater increase of the IgG titer between the acute (day 1) and convalescent (day 36 ± 7) serum specimens. A probable acute infection was defined as a positive IgM result on day 1. *Legionella* antigen was detected in urine samples using the Binax NOW *Legionella* Urinary Antigen Test (Binax, Portland, ME, USA). Sputum samples were tested for atypical respiratory pathogens (*M. pneumoniae*, *C. pneumoniae*, and *L. pneumophila*) using a multiplex PCR assay (Seeplex PneumoBacter ACE Detection kit; Seegene), according to the manufacturer's instructions. This multiplex PCR assay had a detection limit of 100 copies per reaction, and no cross-amplification of DNA from the 63 different pathogens was observed. The assay was also reproducible when PCR reactions were run on 5 different days. An acute atypical respiratory pathogenic infection was defined when serological tests, PCR, or the *Legionella* urinary antigen test was positive.

The study was approved by the Institutional Review Board of each hospital (2012-52), and all participants provided written informed consent. The study is registered with ClinicalTrials.gov (number NCT01658020) (Clinical Research Information Service http://ncrc.cdc.go.kr/cris; KCT0000532).

Statistical analysis

All statistical analyses were performed using SPSS for Windows version 21.0 (IBM Co., Armonk, NY, USA). Categorical variables were expressed as numbers and percentages, while numerical variables were expressed as the mean ± standard deviation. Differences between groups were compared using Student *t* test or the Mann-Whitney *U* test (continuous variables) or the chi-square test or Fisher exact test (categorical variables). Multiple logistic regression analysis was performed to identify independent factors that discriminate patients with atypical respiratory pathogens from those without. A $p < 0.05$ was considered statistically significant.

RESULTS

Demographic characteristics

In total, 428 COPD patients with moderate exacerbation of COPD were screened during the study period, and 345 met the inclusion criteria. Of these, 341 patients were included in this analysis; three patients were excluded because the drug was not administered and one patient was excluded because no serum samples were obtained for serology tests. The study group comprised 311 men and 30 women (mean age, 68.1 ± 7.9 years). Most patients (93.8%) had a smoking history and 88 (25.8%) were current smokers. The average post-bronchodilator FEV_1 % predicted value was 49.7%, and the mean COPD assessment test (CAT) score at screening was 22.9. The baseline characteristics of the study subjects are shown in Table 1.

Atypical respiratory pathogens

IgM and IgG antibodies specific for atypical respiratory pathogens were identified in 341 acute phase (day 1) and 327 convalescent phase (day 36 ± 7) serum samples. Of the 341 patients, 28 (8.2%) were positive for atypical respiratory pathogens; 10 of these patients also yielded at least one typical bacterial pathogen upon culture. By contrast, culture tests revealed that 119 patients (34.9%) were positive for typical respiratory pathogens.

Serological tests were positive for *M. pneumoniae* in 19 cases and one was positive for *C. pneumoniae*. Seven samples were positive for *L. pneumophila* in serological tests and one was positive in the urinary antigen test. However, PCR results for atypical respiratory pathogens were negative in all patients. The results of the serologic tests and PCR assays are shown in Table 2. Acute phase serum specimens from 16, 6, and 1 patient were positive for *M. pneumoniae*-, *L. pneumophila*-, and *C. pneumoniae*-specific IgM antibodies, respectively. Only four patients showed a 4-fold rise of the IgG titer between the acute and convalescent serum specimens. Of the 10 patients harboring typical bacterial pathogens, *Haemophilus influenzae* was isolated from five, *Klebsiella pneumoniae* from three, and *S. pneumoniae* from two. The serology and sputum culture results for the 28 patients are shown in Table 3.

There was no significant difference between patients with and without atypical respiratory pathogens in terms of age, gender, smoking status, lung function, COPD

Table 1. Baseline characteristics of the 341 patients with acute exacerbation of chronic obstructive pulmonary disease

Variable	Value
Age, yr	68.1 ± 7.9
Sex	
Male	311 (91.2)
Female	30 (8.8)
BMI, kg/m^2	22.1 ± 3.4
Smoking status	
Current	88 (25.8)
Former	232 (68.0)
Never	21 (6.2)
Pack-years	31.4 ± 25.7
Lung function	
FEV_1/FVC, %	46.6 ± 12.8
FEV_1, L	1.4 ± 0.5
FEV_1, % predicted	49.7 ± 17.7
FVC, L	3.0 ± 0.8
FVC, % predicted	75.6 ± 16.9
GOLD stage	
I	14 (4.5)
II	123 (39.6)
III	142 (45.7)
IV	32 (10.3)
CAT score (visit 1)	22.9 ± 7.3
Severity of dyspnea	
Mild	52 (15.3)
Moderate	213 (62.5)
Severe	76 (22.3)
Phenotype of COPD	
Chronic bronchitis	136 (39.9)
Non-chronic bronchitis	205 (60.1)
Comorbidity	
Hypertension	127 (37.2)
Diabetes mellitus	40 (11.7)
Heart failure	10 (2.9)
Atrial fibrillation	4 (1.2)
Cancer	50 (14.7)

Values are presented as mean ± SD or number (%).
BMI, body mass index; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; CAT, COPD assessment test; COPD, chronic obstructive pulmonary disease.

stage, CAT score, severity of dyspnea, respiratory secretions, COPD phenotype, and clinical outcome. Only body mass index was significantly different between the two groups. However, none of the variables tested was identified as a significant independent factor by multiple logistic regression analysis (data not shown). Similar results were observed for patients with and without *M. pneumoniae*. There was no difference between patients with *L. pneumophila* and those without. Statistical analysis of data from groups with and without *C. pneumoniae* was not performed because only one patient had a positive result. The characteristics and outcomes of the patients with and without atypical respiratory pathogens are shown in Table 4.

DISCUSSION

This study examined sputum samples from patients with AECOPD and used serological and multiplex PCR assays to assess the role of atypical pathogens. The serology results revealed that atypical pathogens were relatively uncommon in AECOPD. PCR revealed no direct evidence of atypical pathogens in AECOPD. Finally, we found no clinically significant differences between patients with and without atypical respiratory pathogens.

We enrolled patients with moderate AECOPD who attended outpatient clinics; however, most previous studies enrolled hospitalized patients with severe AECOPD [7,14,18,21,28,29]. Moderate AECOPD is defined as an increase in symptoms that requires treatment with antibiotics and/or corticosteroids, whereas a severe exacerbation is one that requires hospitalization [30]. Although our study was part of a multicenter trial, all laboratory tests were performed at a single central laboratory to avoid technical differences that may cause inconsistent results.

The role of atypical pathogens in AECOPD is controversial because *M. pneumoniae* and *C. pneumoniae* are common causes of respiratory tract infections, and both show high seroprevalence of IgG antibodies in the general healthy population (up to 60% and 70%, respectively) [31,32]. In the present study, the seroprevalence of IgG antibodies against *M. pneumoniae*, *C. pneumonia*, and *L. pneumophila* was 74.2%, 84.8%, and 2.6%, respectively. Also, there are different diagnostic criteria for detecting

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Table 2. Prevalence of atypical pathogens in the 341 patients with acute exacerbation of chronic obstructive pulmonary disease

	M. pneumoniae	*L. pneumophila*	*C. pneumoniae*	Total
Definitive diagnosis				
IgG titer, 4-fold rise	3 (0.9)	1 (0.3)	0	4 (1.2)
Urinary antigen	NA	1 (0.3)	NA	1 (0.3)
PCR	0	0	0	0
Probable diagnosis				
IgM (+)	16 (4.7)	6 (1.8)	1 (0.3)	23 (6.7)
Total	19 (5.6)	8 (2.3)	1 (0.3)	28 (8.2)

Values are presented as number (%).
M. pneumoniae, *Mycoplasma pneumoniae*; *L. pneumophila*, *Legionella pneumophila*; *C. pneumoniae*, *Chlamydia pneumoniae*; IgG, immunoglobulin G; NA, not applicable; PCR, polymerase chain reaction.

atypical pathogens by serology. A definitive diagnosis is based on a 4-fold increase in the IgG titer between the acute and convalescent phases; however, some studies report that the IgM response may be nonspecific or absent, particularly in adults [31,32]. One study defined elevated IgM levels on day 1 as a probable infection [15]. Another study defined an acute *M. pneumoniae* infection as a significant increase in the *M. pneumoniae* IgG titer, seroconversion in paired sera, or the presence of IgM antibodies against *M. pneumoniae* [26]. We defined a definite infection by atypical pathogens as a 4-fold or greater increase in the IgG titers and a probable infection as a positive IgM result on day 1.

Most previous studies used serologic techniques to detect atypical pathogens in AECOPD samples [7-13]. More recently, however, molecular techniques have been used to detect etiologic agents of AECOPD; indeed, several studies used PCR to detect atypical pathogens in AECOPD [9,14-16].

A previous study based on serologic assays reported a possible relationship between *M. pneumoniae* infection and AECOPD in 9% of hospitalized AECOPD cases [28]. Other reports estimate the prevalence of *M. pneumoniae* in AECOPD at between 0% to 16%, depending on the serologic method and diagnostic criteria used [7,11,15]. Recent microbiological studies of patients hospitalized for AECOPD identified *M. pneumoniae* in only 1.5% to 2.2% of cases using serologic methods [14,29]. Two previous studies of outpatient AECOPD did not detect *M. pneumoniae* infection by PCR [9,15]; however, serological evidence was obtained in 16% of cases [15]. Another prospective study attempted to detect *M. pneumoniae* in hospitalized AECOPD using both serologic and PCR-based methods. The serologic assay identified *M. pneumoniae* in two patients (2.2%) who were also PCR-positive; however, another 3/92 patients were PCR-positive alone [14]. A recent study prospectively examined 50 cases (43 cases of hospitalized AECOPD) using real-time PCR and conventional procedures, including sputum culture. PCR detected *M. pneumoniae* in four cases (8%) without any serologic evidence of infection [16]. Here, we found that none of the moderate AECOPD patients was PCR-positive, and that only 5.6% were positive for *Mycoplasma* infection according to serological tests.

According to serological tests, the incidence of *C. pneumoniae* among AECOPD is 4% to 16% [8,23-25]. Another recent study detected only one positive result for *C. pneumoniae* after serologic testing of 132 patients hospitalized for AECOPD [29]. Here, we identified only one patient with a *C. pneumoniae* infection by serology; this was the only patient that was IgM-positive at the initial visit. The PCR assay was negative. Indeed, another study found no PCR-based evidence of *C. pneumoniae* infection in outpatient AECOPD [9]. A recent prospective study diagnosed acute or presumed acute infection with *C. pneumoniae* in 4/92 hospitalized AECOPD patients (4.3%) by serologic testing; two of the four were also PCR-positive whereas one was positive by PCR only [14]. Another prospective study detected *C. pneumonia* in one patient by PCR, without any serologic evidence of infection [16].

Studies of the association between *Legionella* and AECOPD are relatively rare. A prospective study revealed

Table 3. Serology and sputum culture results for 28 acute exacerbation of chronic obstructive pulmonary disease patients with a definite or probable diagnosis of infection by acute atypical respiratory pathogen

Patient no.	Atypical pathogen	ELISA day 1		ELISA day 36		Sputum culture
		IgM	IgG	IgM	IgG	
	Mycoplasma pneumoniae					
1	IgM (+)	+	–	–	[a]	*Pseudomonas aeruginosa*
2		+	+	–	+	
3		+	–	+	–	
4		+	+	+	+	*Haemophilus influenzae*
5		+	+	+	+	
6		+	+	+	+	
7		+	+	+	+	*Streptococcus pneumoniae, H. influenzae*
8		+	+	–	+	*Klebsiella pneumoniae*
9		+	+	–	+	
10		+	+	+	+	
11		+	+	–	+	
12		+	+	+	+	
13		+	+	+	+	*H. influenzae*
14		+	+	+	+	
15		+	+	–	–	*H. influenzae*
16		+	+	–	+	*K. pneumoniae*
17	IgG 4-fold rise	–	–	–	+	
18		–	–	–	+	*H. influenzae*
19		–	–	–	+	
	Legionella pneumophila					
20	IgM (+)	+	–	+	–	
21		+	–	+	–	
22		+	–	+	–	
23		+	+	+	+	
24		+	–	[a]	–	
25		+	–	–	–	
26	IgG 4-fold rise	–	–	–	+	
27	Urinary antigen (+)	–	–	–	–	*S. pneumoniae, Staphylococcus aureus*
	Chlamydia pneumoniae					
28	IgM (+)	+	+	–	+	*K. pneumoniae*

ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G.
[a]Intermediate result.

serological evidence of *Legionella* spp. in 16.7% of hospitalized AECOPD patients [18]; however, another study detected *Legionella* non-pneumophila DNA in only one sputum sample out of 126 outpatient AECOPD samples by real-time PCR [9]. By contrast, another prospective AECOPD study did not detect *Legionella* spp. by PCR [14]. In the present study, we identified seven *L. pneumophila*-positive samples in serologic assays and one in the urinary antigen test; however, PCR was negative for all 341 patients.

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Table 4. Characteristics and outcomes of patients with and without atypical respiratory pathogens

Characteristic	Atypical pathogen-positive (n = 28)	Atypical pathogen-negative (n = 313)	*p* value	*M. pneumoniae*-positive (n = 19)	*M. pneumoniae*-negative (n = 322)	*p* value	*L. pneumophila*-positive (n = 8)	*L. pneumophila*-negative (n = 333)	*p* value
Age, yr	68.9 ± 5.3	68.0 ± 8.1	0.401	70.0 ± 4.6	68.0 ± 8.0	0.085	67.9 ± 5.6	68.1 ± 8.0	0.945
Sex			0.725			0.228			1.000
Male	25 (89.3)	286 (91.4)		16 (84.2)	295 (91.6)		8 (100)	303 (91.0)	
Female	3 (10.7)	27 (8.6)		3 (15.8)	27 (8.4)		0	30 (9.0)	
BMI, kg/m^2	20.5 ± 3.2	22.3 ± 3.3	0.009	20.0 ± 3.4	22.2 ± 3.3	0.005	21.6 ± 2.8	22.1 ± 3.4	0.656
Smoking status			0.603			0.560			0.673
Current	5 (17.9)	83 (26.5)		4 (21.1)	84 (26.1)		1 (12.5)	87 (26.1)	
Former	21 (75.0)	211 (67.4)		13 (68.4)	219 (68.0)		7 (87.5)	225 (67.6)	
Never	2 (7.1)	19 (6.1)		2 (10.5)	19 (5.9)		0	21 (6.3)	
Pack-years	28.8 ± 19.7	31.5 ± 26.1	0.817	31.1 ± 22.0	31.4 ± 26.0	0.981	19.5	31.5 ± 25.8	0.646
PFT (post-bronchodilator)									
FEV_1/FVC, %	44.0 ± 13.9	46.8 ± 12.6	0.300	46.6 ± 14.4	46.6 ± 12.7	1.000	46.0 ± 10.3	46.6 ± 12.8	0.890
FEV_1, L	1.5 ± 0.6	1.4 ± 0.5	0.326	1.3 ± 0.5	1.4 ± 0.5	0.422	1.5 ± 0.6	1.4 ± 0.5	0.541
FEV_1, % predicted	51.0 ± 15.6	49.6 ± 17.8	0.696	49.3 ± 15.8	49.7 ± 17.8	0.915	49.4 ± 13.6	49.7 ± 17.8	0.956
GOLD stage			0.785			0.895			0.778
I	1 (4.0)	13 (4.5)		0	14 (4.8)		0	32 (10.6)	
II	11 (44.0)	112 (39.2)		6 (35.3)	117 (39.8)		5 (62.5)	137 (45.2)	
III	12 (48.0)	130 (45.5)		9 (52.9)	133 (45.2)		3 (37.5)	120 (39.6)	
IV	1 (4.0)	31 (10.8)		2 (11.8)	30 (10.2)		0	14 (4.6)	
CAT score (visit 1)	24.3 ± 7.6	22.7 ± 7.3	0.286	25.4 ± 8.2	22.7 ± 7.2	0.117	22.3 ± 6.3	22.9 ± 7.3	0.805
Severity of dyspnea			0.173			0.318			0.382
Mild	1 (3.6)	51 (16.3)		1 (5.3)	51 (15.8)		0	52 (15.6)	
Moderate	21 (75.0)	192 (61.3)		15 (79.0)	198 (61.5)		5 (62.5)	208 (62.5)	
Severe	6 (21.4)	70 (22.4)		3 (15.8)	73 (22.7)		3 (37.5)	73 (21.9)	
Respiratory secretion			0.876			0.375			0.330
Mucoid	5 (17.9)	59 (18.8)		4 (21.1)	60 (18.6)		1 (12.5)	63 (18.9)	
Mucopurulent	14 (50.0)	141 (45.0)		11 (57.9)	144 (44.7)		2 (25.0)	153 (46.0)	
Purulent	9 (32.1)	113 (36.1)		4 (21.1)	118 (36.7)		5 (62.5)	117 (35.1)	
Phenotype of COPD			0.946			0.839			1.000

Table 4. Continued

Characteristic	Atypical pathogen-positive (n = 28)	Atypical pathogen-negative (n = 313)	*p* value	*M. pneumoniae*-positive (n = 19)	*M. pneumoniae*-negative (n = 322)	*p* value	*L. pneumophila*-positive (n = 8)	*L. pneumophila*-negative (n = 333)	*p* value
Chronic bronchitis	11 (39.3)	125 (39.9)		8 (42.1)	128 (39.8)		3 (37.5)	133 (39.9)	
Non-chronic bronchitis	17 (60.7)	188 (60.1)		11 (57.9)	194 (60.3)		5 (62.5)	200 (60.1)	
Other bacteria culture			0.925			0.498			0.270
Positive	10 (35.7)	109 (34.8)		8 (42.1)	111 (34.5)		1 (12.5)	118 (35.4)	
Negative	18 (64.3)	204 (65.2)		11 (57.9)	211 (65.5)		7 (87.5)	215 (64.6)	
Outcome			0.533			0.582			1.000
Cure	23 (82.1)	241 (77.0)		16 (84.2)	248 (77.0)		6 (75.0)	258 (77.5)	
Failure	5 (17.9)	72 (23.0)		3 (15.8)	74 (23.0)		2 (25.0)	75 (22.5)	

Values are presented as mean ± SD or as the number (%).
M. pneumoniae, *Mycoplasma pneumoniae*; *L. pneumophila*, *Legionella pneumophila*; BMI, body mass index; PFT, pulmonary function test; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; CAT, COPD assessment test; COPD, chronic obstructive pulmonary disease.

Although PCR is used to detect atypical pathogens in clinical laboratories, no clear guidelines regarding the true performance, drawbacks, and limitations of the technique have been published [14]. PCR has been used to test for *M. pneumoniae* infections for about 20 years, but the method has several limitations. First, PCR inhibitors in samples can lead to false-negative results. Second, contamination can cause false-positive results. Third, the time of sampling influences the results. Indeed, in contrast to serologic assays, the diagnostic accuracy of PCR may decrease at or beyond 7 days after disease onset [26,33]. The above could explain why PCR shows low sensitivity for detecting atypical pathogens in AECOPD. Finally, samples suitable for PCR may be difficult to acquire.

A previous study showed that PCR was superior to serology for the diagnosis of acute *M. pneumoniae* infection and identified a high rate of persistent infection [9]. The study examined *M. pneumoniae* infection in children aged 10 to 16 years who showed acute respiratory symptoms during a community outbreak of *Mycoplasma* infection. The results revealed that PCR testing of respiratory secretions may provide an early diagnosis and be more sensitive than serologic techniques.

Another prospective study was conducted to compare the diagnostic value of an indirect immunofluorescence assay with that of PCR for the diagnosis of *M. pneumoniae* in adults. PCR showed lower sensitivity than serology; therefore, the authors recommended the use of serology and PCR in parallel to confirm *M. pneumoniae* infections in adults with community acquired pneumonia [34].

A meta-analysis showed that commercial PCR tests generate consistent results with high specificity, but they show low/variable sensitivity for *M. pneumoniae*. These findings suggest that, although commercial PCR tests may be superior for diagnosing *M. pneumoniae* infection, they cannot completely replace serologic assays. Thus, PCR plus serology could be a good screening method for the reliable and accurate diagnosis of *M. pneumoniae* infection [35].

Although a previous study showed that the seroprevalence of *M. pneumoniae* infection in a study population was significantly higher than that in the control group, the role (if any) played by *M. pneumoniae* in AECOPD was not substantiated by culture isolation or PCR [15]. Thus, there is a need for more studies of well-defined patient

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populations with AECOPD to establish a correlation between serological evidence of *M. pneumoniae* infection and that provided by culture and PCR.

Few studies have examined correlations between different microbiologic techniques with respect to the detection of atypical pathogens [14-16]. Indeed, we found no correlation between serologic assays and PCR in this respect.

Another finding that we found interesting was that 10 of 28 moderate AECOPD patients (36%) who were positive for atypical pathogens also yielded at least one typical bacterial pathogen upon sputum culture. A previous study detected at least one additional respiratory pathogen (viral or bacterial) in 71% of hospitalized AECOPD cases with *M. pneumoniae* [7]. Another prospective study of patients with severe exacerbation and respiratory failure showed that 29% were mixed infections by bacterial pathogens [11]. The prevalence of mixed infections varied, depending on the detection method and pathogen examined. One pathogen may exacerbate infection by another pathogen, or two pathogens may act independently.

The aim of the current prospective study was to use a combination of techniques (serologic assays and PCR) to determine the role of atypical pathogens in 341 AECOPD patients. This is a large cohort when compared with those in previous studies. However, a limitation of the present study is that we did not perform direct culture of atypical pathogens.

In conclusion, the serological prevalence of atypical pathogens in AECOPD was relatively low. These results, when combined with the negative PCR results, suggest that atypical pathogens play no (or a very limited) role in AECOPD, and that detection of atypical pathogens is not necessary in South Korea.

KEY MESSAGE

1. The serology results revealed that atypical pathogens were relatively uncommon in acute exacerbation of chronic obstructive pulmonary disease (AECOPD).
2. Polymerase chain reaction revealed no direct evidence of atypical pathogens in AECOPD.
3. Atypical pathogens may not be a major cause of AECOPD in South Korea.

Conflict of interest

This study was supported by Dongwha Pharmaceuticals, Seoul, Korea. The sponsors of all the funding bodies had no role in the design of the study, the collection and analysis of the data, or the preparation of the manuscript.

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REFERENCES

1. World Health Organization. Burden of COPD [Internet]. Geneva: WHO, c2018 [cited 2018 Feb 27]. Available from: http://www.who.int/respiratory/copd/burden/en/.
2. Yoo KH, Kim YS, Sheen SS, et al. Prevalence of chronic obstructive pulmonary disease in Korea: the fourth Korean National Health and Nutrition Examination Survey, 2008. Respirology 2011;16:659-665.
3. Aaron SD, Vandemheen KL, Clinch JJ, et al. Measurement of short-term changes in dyspnea and disease-specific quality of life following an acute COPD exacerbation. Chest 2002;121:688-696.
4. Seemungal TA, Donaldson GC, Paul EA, Bestall JC, Jeffries DJ, Wedzicha JA. Effect of exacerbation on quality of life in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1998;157(5 Pt 1):1418-1422.
5. Rodriguez-Roisin R. Toward a consensus definition for COPD exacerbations. Chest 2000;117(5 Suppl 2):398S-401S.
6. White AJ, Gompertz S, Stockley RA. Chronic obstructive pulmonary disease. 6: The aetiology of exacerbations of chronic obstructive pulmonary disease. Thorax 2003;58:73-80.
7. Lieberman D, Lieberman D, Ben-Yaakov M, et al. Serological evidence of Mycoplasma pneumoniae infection in acute exacerbation of COPD. Diagn Microbiol Infect Dis 2002;44:1-6.
8. Mogulkoc N, Karakurt S, Isalska B, et al. Acute purulent exacerbation of chronic obstructive pulmonary disease and Chlamydia pneumoniae infection. Am J Respir Crit Care Med 1999;160:349-353.
9. Diederen BM, van der Valk PD, Kluytmans JA, Peeters MF, Hendrix R. The role of atypical respiratory pathogens in exacerbations of chronic obstructive pulmonary disease. Eur Respir J 2007;30:240-244.
10. Monso E, Ruiz J, Rosell A, et al. Bacterial infection in chronic obstructive pulmonary disease. A study of stable and exacerbated outpatients using the protected specimen brush. Am J Respir Crit Care Med 1995;152(4 Pt 1):1316-1320.
11. Soler N, Torres A, Ewig S, et al. Bronchial microbial patterns in severe exacerbations of chronic obstructive pulmonary disease (COPD) requiring mechanical ventilation. Am J Respir Crit Care Med 1998;157(5 Pt 1):1498-1505.
12. Donaldson GC, Seemungal TA, Bhowmik A, Wedzicha JA. Relationship between exacerbation frequency and lung function decline in chronic obstructive pulmonary disease. Thorax 2002;57:847-852.
13. Eller J, Ede A, Schaberg T, Niederman MS, Mauch H, Lode H. Infective exacerbations of chronic bronchitis: relation between bacteriologic etiology and lung function. Chest 1998;113:1542-1548.
14. Nakou A, Papaparaskevas J, Diamantea F, Skarmoutsou N, Polychronopoulos V, Tsakris A. A prospective study on bacterial and atypical etiology of acute exacerbation in chronic obstructive pulmonary disease. Future Microbiol 2014;9:1251-1260.
15. Varma-Basil M, Dwivedi SK, Kumar K, et al. Role of Mycoplasma pneumoniae infection in acute exacerbations of chronic obstructive pulmonary disease. J Med Microbiol 2009;58(Pt 3):322-326.
16. Shimizu K, Yoshii Y, Morozumi M, et al. Pathogens in COPD exacerbations identified by comprehensive real-time PCR plus older methods. Int J Chron Obstruct Pulmon Dis 2015;10:2009-2016.
17. Lieberman D, Dvoskin B, Lieberman DV, Kahane S, Friedman MG. Serological evidence of acute infection with the Chlamydia-like microorganism Simkania negevensis (Z) in acute exacerbation of chronic obstructive pulmonary disease. Eur J Clin Microbiol Infect Dis 2002;21:307-309.
18. Lieberman D, Lieberman D, Shmarkov O, et al. Serological evidence of Legionella species infection in acute exacerbation of COPD. Eur Respir J 2002;19:392-397.
19. Lieberman D, Lieberman D, Ben-Yaakov M, et al. Infectious etiologies in acute exacerbation of COPD. Diagn Microbiol Infect Dis 2001;40:95-102.
20. Ewig S. Legionella spp. in acute exacerbations of chronic

https://doi.org/10.3904/kjim.2017.279

obstructive pulmonary disease: what is the evidence? Eur Respir J 2002;19:387-389.
21. Lieberman D, Ben-Yaakov M, Lazarovich Z, Ohana B, Boldur I. Chlamydia pneumoniae infection in acute exacerbations of chronic obstructive pulmonary disease: analysis of 250 hospitalizations. Eur J Clin Microbiol Infect Dis 2001;20:698-704.
22. Hahn DL. Chlamydia pneumoniae, asthma, and COPD: what is the evidence? Ann Allergy Asthma Immunol 1999;83:271-288.
23. Blasi F, Legnani D, Lombardo VM, et al. Chlamydia pneumoniae infection in acute exacerbations of COPD. Eur Respir J 1993;6:19-22.
24. Beaty CD, Grayston JT, Wang SP, Kuo CC, Reto CS, Martin TR. Chlamydia pneumoniae, strain TWAR, infection in patients with chronic obstructive pulmonary disease. Am Rev Respir Dis 1991;144:1408-1410.
25. Miyashita N, Niki Y, Nakajima M, Kawane H, Matsushima T. Chlamydia pneumoniae infection in patients with diffuse panbronchiolitis and COPD. Chest 1998;114:969-971.
26. Nilsson AC, Bjorkman P, Persson K. Polymerase chain reaction is superior to serology for the diagnosis of acute Mycoplasma pneumoniae infection and reveals a high rate of persistent infection. BMC Microbiol 2008;8:93.
27. Rhee CK, Chang JH, Choi EG, et al. Zabofloxacin versus moxifloxacin in patients with COPD exacerbation: a multicenter, double-blind, double-dummy, randomized, controlled, phase III, non-inferiority trial. Int J Chron Obstruct Pulmon Dis 2015;10:2265-2275.
28. Papaetis GS, Anastasakou E, Tselou T, et al. Serological evidence of Mycoplasma pneumoniae infection in patients with acute exacerbation of COPD: analysis of 100 hospitalizations. Adv Med Sci 2010;55:235-241.
29. Boixeda R, Rabella N, Sauca G, et al. Microbiological study of patients hospitalized for acute exacerbation of chronic obstructive pulmonary disease (AE-COPD) and the usefulness of analytical and clinical parameters in its identification (VIRAE study). Int J Chron Obstruct Pulmon Dis 2012;7:327-335.
30. Jones P, Higenbottam T. Quantifying of severity of exacerbations in chronic obstructive pulmonary disease: adaptations to the definition to allow quantification. Proc Am Thorac Soc 2007;4:597-601.
31. Tuuminen T, Varjo S, Ingman H, Weber T, Oksi J, Viljanen M. Prevalence of Chlamydia pneumoniae and Mycoplasma pneumoniae immunoglobulin G and A antibodies in a healthy Finnish population as analyzed by quantitative enzyme immunoassays. Clin Diagn Lab Immunol 2000;7:734-738.
32. Kumar S, Hammerschlag MR. Acute respiratory infection due to Chlamydia pneumoniae: current status of diagnostic methods. Clin Infect Dis 2007;44:568-576.
33. Kashyap B, Kumar S, Sethi GR, Das BC, Saigal SR. Comparison of PCR, culture & serological tests for the diagnosis of Mycoplasma pneumoniae in community-acquired lower respiratory tract infections in children. Indian J Med Res 2008;128:134-139.
34. Martinez MA, Ruiz M, Zunino E, Luchsinger V, Avendano LF. Detection of Mycoplasma pneumoniae in adult community-acquired pneumonia by PCR and serology. J Med Microbiol 2008;57(Pt 12):1491-1495.
35. Zhang L, Zong ZY, Liu YB, Ye H, Lv XJ. PCR versus serology for diagnosing Mycoplasma pneumoniae infection: a systematic review & meta-analysis. Indian J Med Res 2011;134:270-280.