

Role of bacteria in acute exacerbations of chronic obstructive pulmonary disease

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Background and study objective: Infections are major causes of acute exacerbations of chronic obstructive pulmonary disease (COPD) which result in significant mortality and morbidity. The primary aim of the study was to determine the microbiological spectrum including atypical agents in acute exacerbations. The secondary aim was to evaluate resistance patterns in the microorganisms.

Methods: The sputum culture of 75 patients admitted to our clinic from January 1, 1999 to December 31, 2002 was evaluated prospectively, for aerobic Gram-positive and Gram-negative bacteria, and serologically for *Chlamydomphila pneumoniae* and *Mycoplasma pneumoniae*. Sensitivity patterns in potentially pathogenic microorganisms (PPMs) were also investigated.

Results: An infectious agent was identified in 46 patients, either serologically or with sputum culture. Pathogens most commonly demonstrated were: *Haemophilus influenzae* (30%), *Chlamydomphila pneumoniae* (17%), and *Mycoplasma pneumoniae* (9%). Mixed infections were diagnosed in 9 patients. PPMs showed a high resistance rate to commonly used antibiotics.

Conclusion: We have shown that microorganisms causing acute exacerbations of COPD are not only typical bacteria (46%) but also atypical pathogens (26%), with unpredictable high rates. Typical agents showed a high resistance to commonly used antibiotics.

Keywords: chronic obstructive pulmonary disease, acute exacerbation, infection, atypical pathogens, *Haemophilus influenzae*

Introduction

Chronic obstructive pulmonary disease (COPD) is one of the most prevalent disease in the world. Furthermore, the number of individuals affected has grown since the 1980s and this increase is expected to continue during the next 20 years (GOLD 2001). COPD is characterized by intermittent acute exacerbations associated with worsening symptoms and lung function. These acute exacerbations contribute considerably to mortality and diminished quality of life (Seemungal et al 1998).

Several etiologic factors alone or in combination may cause exacerbations of COPD. In 50%–70% of acute exacerbations of COPD, the pathophysiological basis is usually infectious (Ball 1995). *Haemophilus influenzae* is the most frequent bacterium isolated in all series followed by *Streptococcus pneumoniae*, and *Moraxella catarrhalis*, and *Pseudomonas aeruginosa* (Ball 1995; Monso et al 1995; Soler et al 1998; Miravitlles et al 1999). Some reports on the role of *Chlamydomphila pneumoniae* and *Mycoplasma pneumoniae* in acute exacerbations of COPD have appeared in recent years (Mogulkoc et al 1999; Lieberman et al 2001), showing that they are important causes of community-acquired pneumonia. However, these bacteria are usually almost totally absent from discussion on acute exacerbations of COPD. In the light of this knowledge, we prospectively designed a study to disclose the role of bacteria including *Ch. pneumoniae* and *M. pneumoniae* and sensitivity patterns of PPMs in acute exacerbations of COPD.

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Material and methods

Patient selection

All patients hospitalized with acute exacerbation of COPD in our clinic between January 1, 1999 and December 31, 2002 were prospectively evaluated. All patients were diagnosed as having COPD, according to the criteria of the American Thoracic Society (ATS 1995). Acute exacerbation was defined as the presence of an increase in at least two of the three following symptoms: dyspnea, cough and sputum production.

Admission to hospital was according to the clinical situation of the patient, or the presence of complicating factors such as respiratory failure by a senior chest physician, experienced in the management of COPD. Exclusion criteria were: (1) outpatient status; (2) treatment with any antibiotic within 48 h before admission; (3) absence of an adequate sputum specimen as determined by Gram stain; (4) evidence of bronchiectasis and/or pneumonia; (5) malignancy or severe immunosuppression; and (6) the need for mechanical ventilation. After a thorough history and physical examination, chest radiography and pulmonary function tests were performed. Hematologic, microbiologic, and serologic investigations were done on the same day. Serologic investigation for *Ch. pneumoniae* and *M. pneumoniae* was repeated 21 days later.

Laboratory investigations

Sputum cultures

At least one sample of spontaneously expectorated sputum for microbiological evaluation was obtained from all patients during admission. A Gram's stain of sputum in the area of maximal purulence was examined for polymorphonuclear leukocytes and epithelial cells. The criteria used to determine whether a sputum sample was acceptable for analysis were: a microbiological study using a low-magnification field ($\times 100$) revealed <10 epithelial cells and >25 leukocytes (Murray et al 1975). Sputum specimens not fulfilling these criteria were not cultured because they were not considered representative bronchial samples.

Sputum samples were plated on blood, eosin-methylene blue and chocolate agar and incubated at 37 °C for both 24 h and 48 h. All microorganisms isolated were identified through standard laboratory methods (Isenberg et al 1991).

Bacterial agents were classified into PPM or non-PPMs, as described by Cabello and colleagues (1997). PPMs were regarded as significant only if they achieved $>10^6$ cfu, except for *S. pneumoniae* where 10^5 cfu was deemed sufficient. A PPM had to grow in significant concentrations irrespective of presence of non-PPMs to be considered a potential causative agent of an exacerbation.

Serology

All sera were investigated for the presence of immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies to *Chlamydia* species, using indirect-microimmunofluorescence test (Vircell SL, Microgen Bioproducts Inc., Camberley, UK). Serum antibodies against *Ch. pneumoniae* elementary bodies were detected with fluorescein-conjugated monoclonal goat antihuman Ig-subclass antibodies. An isolated IgM titer of $\geq 1:16$, or IgG titer of $\geq 1:512$, or a fourfold increase in titer of IgG or IgM, was considered evidence of acute *Ch. pneumoniae* infection. Past *Ch. 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-microimmunofluorescence test (Zeus Scientific, Inc., NJ, USA). A 4-fold change in titer of IgG or IgM, or an isolated IgG titer of $\geq 1:128$ or IgM titer of $\geq 1:16$, was considered to evidence of acute infection.

Statistical analysis

Statistical analysis was performed with a statistical software system (SPSS, version 10.0; SPSS Inc., Chicago, IL, USA). Student's t-test and chi-square tests were used (p value of ≤ 0.05 was considered statistically significant).

Results

The study included 75 patients who had been diagnosed with acute exacerbation of COPD from a total of 156. The exclusion criteria were outpatient status (27 patients), prior antibiotic usage (21 patients), inappropriate sputum (15 patients), did not attend the control visit on day 21 (7 patients), inability to perform spirometric tests (7 patients) and pneumonia (4 patients). Table 1 summarizes the patients' clinical characteristics.

PPMs recognized as agents causing respiratory infections, whether or not they belong to the oropharyngeal or gastrointestinal flora, were identified in 34 (% 45) of 75 patients (Table 2).

Tables 3 and 4 show serological results for *Ch. pneumoniae* and *M. pneumoniae*.

There were mixed infections in 9 patients. In this mixed infection group, the most frequent micro-organisms were *Ch. pneumoniae* and *H. influenzae* (5 patients).

There was no statistical significance in terms of forced expiratory volume in one second (FEV_1) ($p = 1$), duration of hospital stay ($p = 0.258$), and requirement of noninvasive mechanical ventilation ($p = 0.0689$) between groups in which

Table 1 Characteristics of the patients*

No of subjects	75
Male/female	71/4
Age	61.1 (41–77)
Smoking	
Smokers	12 (16%)
Exsmokers	59 (78.6%)
Nonsmokers	4 (5.35%)
Pack-years	43.6 (6–100)
Number of exacerbations last year	2.3 (0–6)
Leukocytes ($\times 10^9/L$)	10.127
Lung function	
FEV ₁ , L	0.774 (0.4–1.63)
FEV ₁ , % pred	30.8 (13–59)
FVC, L	1.680 (0.51–2.4)
FVC, % pred	42.5 (20–86)
FEV ₁ /FVC	50.2 (35–71)
Arterial blood gases	
PaO ₂ (mmHg)	58.9 (37–75)
PaCO ₂ (mmHg)	43.2 (12–72)
SatO ₂ (%)	81.1 (70–97)
Patients using oxygen therapy	70
Patients using NIMV	40

Note: *Values are given mean (%) and mean (range).

Abbreviations: FEV₁, forced expiratory volume in one second; FVC, forced vital capacity; NIMV, noninvasive mechanical ventilation.

an etiological agent responsible for acute exacerbation was identified or not. Bacteriological and serological results did not differ among patients using noninvasive mechanical ventilation (NIMV). According to the sensitivity results of PPMs cultivated from the patients, *H. influenzae* was resistant to penicillin in 10 patients, and to ampicillin in 9 patients (Table 5). In 4 patients *S. pneumoniae* was sensitive to almost all of the commonly used antibiotics.

Discussion

Although there is no widely accepted definition of acute exacerbation of COPD, either in clinical practice or research, a recent consensus statement defined exacerbations as “a sustained worsening of the patient’s condition, from the stable state and beyond normal day to day variations that is acute in onset and necessitates a change in regular medication in a patient with underlying COPD” (Rodriguez-Roisen 2000). Patients who suffer the most exacerbations have significantly lower health status (Seemungal et al 1998) and there is also some evidence that exacerbation frequency predicts an accelerated decline in lung function (Kanner et al 2001).

Infections play a major role in the etiology of acute exacerbations (Ball 1995). Patients with COPD have significant impairment of their lung defence mechanisms and colonisation of bronchial system seems to be an important consequence of the disease, both in stable state and in exacerbations. For more than 50 years, researchers have been trying to explain the meaning of bacteria recovered from a sputum culture in a COPD patient really means. Does the isolation of these bacteria along with mucus hypersecretion contribute to the pathogenesis of the disease? Is it simply a bacterial colonisation, or should it be treated? Distinguishing between colonisation and infection is difficult in these patients. In most of the reported series, the predominant organisms responsible for infective exacerbations are *S. pneumoniae*, nontypical *H. influenzae*, and to some extent *Moraxella catarrhalis* (Ball 1995; Monso et al 1995; Soler et al 1998; Miravittles et al 1999). Our study results, in which the predominant bacteria isolated were *H. influenzae*, correlate with most of the previous ones in terms of bacterial etiology.

We have also investigated the role of *Ch. pneumoniae* and *M. pneumoniae* in acute exacerbations of COPD. We have seen an atypical etiology in 20 of 46 patients in whom an infectious etiology was determined from a total of 75 patients. This high percentage (26%) implicates the role of atypical agents in the acute exacerbations of COPD. In 13 patients (17%), the sole agent was *Ch. pneumoniae* or *M. pneumoniae* and there were mixed infections in 7 patients (9%) with other bacteria. Data in the literature is uncertain on the role of atypical pathogens in the etiology of acute

Table 2 Microorganisms isolated from sputum cultures and serologic results for 75 patients

PPMs	
<i>Haemophilus influenzae</i> ¹	23
<i>Streptococcus pneumoniae</i>	4
<i>Haemophilus parainfluenzae</i>	3
<i>Enterobacter aeruginosa</i>	1
<i>Serratia marcescens</i>	1
<i>Klebsiella oxytoca</i>	1
<i>Klebsiella pneumoniae</i>	1
<i>Acinetobacter</i>	1
Total PPM	35
Non-PPMs	40
Other	
<i>Chlamydomphila pneumoniae</i> ²	13
<i>Mycoplasma pneumoniae</i>	7

Notes: ¹Includes one mixed infection with *S. pneumoniae*; ²Includes eight mixed infections; five with *H. influenzae*, two with *M. pneumoniae* and one with *K. pneumoniae*.

Abbreviation: PPMs, potentially pathogenic microorganisms.

Table 3 Serologic results of *Chlamydomphila pneumoniae*

Serology	Patient (number)
IgM titer > 1:16	I
IgG titer > 1:512	I
A fourfold rise in IgG titer	II

exacerbations of COPD. Two studies of acute exacerbations (one in hospitalised patients and one in outpatients) found *Ch. pneumoniae* in approximately 5% (Beaty et al 1991; Verkooyen et al 1997). Two other recent studies have found *Ch. pneumoniae* to be associated with 24% and 34% of exacerbations (Mogulkoc et al 1999; Karnak et al 2001). Because *M. pneumoniae* infects mainly young adults, and immunity may have been gained by the time acute exacerbation of COPD occurs, this agent may have a less important role in the etiology of advanced-age COPD patients. It is not surprising to see in most studies, as in ours, less evidence of exacerbations with *M. pneumoniae* than *Ch. pneumoniae* (Mogulkoc et al 1999; Lieberman et al 2001). We found only one study related to the role of *Legionella* species in acute exacerbation of COPD (Lieberman et al 2002). This study demonstrated serological evidence of infection with *Legionella* species in 16.7% of patients. We would have preferred to include this species in our study in addition to viral etiologies. But, technical and economic problems prevented us from studying these agents.

Functional impairment is a predictor of the bacteria responsible for acute infective exacerbations in COPD patients. In one study, more than 56% of patients with a $FEV_1 \leq 50\%$ had Gram-negative-bacilli as etiology of acute exacerbation (Miravittles et al 1999). Our study population had a mean FEV_1 of 30%, an even worse functional impairment, well correlated with this study with a high gram-negative predominance (88%).

In this analysis of the sensitivity of micro-organisms to different antibiotics, we noted especially the poor efficacy of penicillin, ampicillin, amoxicillin-clavulanic acid, tetracyclin and erythromycin to most prevalent respiratory pathogens. Ciprofloxacin seems to be the most efficient drug for all microorganisms. The sensitivity results show severe functional impairment with Gram-negative predominance

Table 4 Serologic results of *Mycoplasma pneumoniae*

Serology	Patient (number)
IgM > 1:16	I
IgG > 1:128	3
A fourfold rise in IgG titer	3

Table 5 Antibiotic sensitivity patterns of potentially pathogenic microorganisms*

PPMs	Penicillin			Ampicillin			Amoxicillin-clavulanic acid			Erythromycin			Tetracycline			Ciprofloxacin			Cefuroxime			Cefepime			Piperacillin-tazobactam								
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R						
<i>H. influenzae</i>	13	-	10	14	-	9	23	-	-	23	-	-	16	2	5	23	-	-	21	2	-	23	-	-	23	-	-	23	-	-			
<i>S. pneumoniae</i>	4	-	-	4	-	-	4	-	-	4	-	-	4	-	-	4	-	-	4	-	-	4	-	-	3	1	-	4	-	-	4	-	-
<i>H. parainfluenzae</i>	1	-	2	1	-	2	3	-	-	3	-	-	2	1	-	3	-	-	3	-	-	3	-	-	3	-	-	3	-	-	3	-	-
<i>P. aeruginosa</i>	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-
<i>S. marcescens</i>	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-
<i>K. oxytoca</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-
<i>Acinetobacter</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>K. pneumoniae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	18	-	17	19	-	16	30	2	3	30	5	5	22	4	9	35	-	-	31	2	2	33	1	1	34	-	-	34	-	-	34	-	-

Abbreviations: S, sensitive; I, intermediate; R, resistant; H, influenzae; Haemophilus influenzae; S, pneumoniae; Streptococcus pneumoniae; H, parainfluenzae; H, parainfluenzae; S, marcescens; Serratia marcescens; K, oxytoca; Klebsiella oxytoca; K, pneumoniae; Klebsiella pneumoniae.

and also prior antibiotic usage during the lengthy course of COPD. These results were consistent with some studies in recent years (Miravittles et al 1999), which showed similar sensitivity patterns in COPD patients.

In conclusion, atypical pathogens as well as typical bacteria may have a role in acute exacerbations of COPD. High resistance to commonly used antibiotics for typical agents and mixed infections in these severe functionally impaired cases of COPD were remarkable. We recommend that these results be considered when choosing an antibiotic in severe cases of COPD, although further studies are needed for clarification.

Disclosure

The authors report no conflicts of interest in this work.

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