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Research Article

Role of Acute Chlamydophila Pneumoniae Infection in COPD Patients

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Abstract

Objective: To investigate the frequency of respiratory bacterial infections included the role of atypical bacteria such as *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* in hospitalized patients admitted to the emergency room for an acute episode of chronic obstructive pulmonary disease (COPD).

Methods: Prospective observational study conducted on 50 consecutive patients. Serum specimens were collected at days 0 and 15. *C. pneumoniae* and *M. pneumoniae* antibodies (IgM and IgG) were tested by commercial ELISA and immunofluorescence assay, respectively. At least one sample of spontaneously expectorated sputum for microbiologic evaluation was obtained in all patients during admission.

Results: No acute *M. pneumoniae* infection was recorded; by contrast 7 patients (14%) showed a profile compatible with a recent *C. pneumoniae* infection. Demographic and clinical parameters did not differ between patients with and without stigmata of recent *C. pneumoniae* infection. Eleven patients (22%) had positive sputum cultures, indicating the presence of bacterial infection. Pathogens most frequently isolated were: *Pseudomonas aeruginosa* (n = 4), *Haemophilus influenzae* (n = 2), and *Klebsiella pneumoniae* (n = 2).

Conclusion: *C. pneumoniae* is a pathogen that requires an adapted antimicrobial treatment. Its detection must always be performed considering its prevalence in patients presenting with acute COPD exacerbations.

Keywords: *Component; Chronic Obstructive Pulmonary Disease, Bacterial Infection, Chlamydophila Pneumoniae, Mycoplasma Pneumoniae*

Introduction

Chronic obstructive pulmonary disease (COPD) is an inflammatory process characterized by a progressive airflow limitation and the destruction of parenchyma. Patients affected by this disease exhibit frequent exacerbation episodes that favor airway inflammation and often lead to hospitalization. An episode of COPD exacerbation is characterized by increased sputum volume and purulence, worsening dyspnea and cough [1]. Bacterial and viral infections of the lower respiratory tract account for approximately 80% of all the exacerbation episodes [2,3].

Chlamydophila pneumoniae and *Mycoplasma pneumoniae* are common human pathogens causing asymptomatic, mild or severe upper and lower respiratory tract infections. These infections are usually not identified in general health care because the etiology of respiratory

infections is investigated in only a small proportion of patients, those who present a non-responsiveness to conventional antimicrobial therapy or severe pneumonia [4]. The role of these pathogens in exacerbation episodes of COPD is controversial [5,6]. The aim of this study was to provide recent prevalence data on the distribution of *C. pneumoniae* and *M. pneumoniae* IgG and IgM antibodies in a Tunisian cohort of COPD patients exhibiting an exacerbation of COPD, so that an adapted treatment could be established.

Patients and Methods

We conducted a prospective observational study in the Emergency Department of three University hospitals (Monastir, Mahdia and Sousse) located in the South-East part of Tunisia, from May 2013 to March 2015. The Ethics Committee in Research of Sousse hospital approved the study; a written informed consent was obtained from all the patients.

The study population consisted of patients older than 40 years of age diagnosed with COPD stages 1 - 4 as defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD), with an acute exacerbation (onset ≤ 14 days) [1]. Acute respiratory failure was defined as a worsening of dyspnea associated with at least two of the following characteristics: respiratory rate ≥ 24 breaths/min, arterial partial pressure of carbon dioxide ≥ 45 mmHg, and arterial pH ≤ 7.35. A chest radiograph confirming the absence of pneumonia was required. Exclusion criteria were as follows: outpatient status, evidence of bronchiectasis, pneumonia, malignancy or severe immunosuppression, and the need for mechanical ventilation. History, physical exam, blood gas and x-ray results were recorded for all the included patients. The patients received instrumental and medical therapy according to current guidelines [1].

Sputum specimens were collected systematically at entry. Semi-quantitative bacterial cultures were conducted at the Microbiology laboratory of the University hospital of Monastir as recommended [7]. A result was considered significant if a bacterium of interest was isolated with a count of at least 10^7 CFU/ml.

Four mL of blood specimen were collected systematically at entry and 15 days later. The serological tests were carried out in the Microbiology laboratory of the University hospital of Monastir. Serum IgG and IgM antibodies against *M. pneumoniae* were measured using a commercial indirect immunofluorescence assay (EUROIMMUN Medizinische Labordiagnostika AG, Lubeck, Germany) according to the manufacturer's instructions. The qualitative detection of IgG and IgM antibodies to *C. pneumoniae* was performed with a commercial ELISA technique (NovaTec, Immunodiagnostica GmbH, Dietzenbach, Germany), as recommended. According to standard serological rules [8], a patient was considered to exhibit a recent infection with one of those bacteria if IgM and IgG antibodies were present in the specimen taken at entry or if a seroconversion occurred between days 0 and 15. Serum specimens exhibiting IgM at day 0 without IgG at day 15 were considered false positive results.

Comparisons of qualitative variables were performed with the chi-square test and comparisons of means with the Mann-Whitney non-parametrical test for independent samples (SPSS version 18.1). A P value < 0.05 was considered significant.

Results

Episodes of COPD exacerbation from 50 patients were included consecutively from May 2013 to March 2015. IgM antibodies to *M. pneumoniae* were negative in all the patients. Specific IgG level was positive in 32 patients and negative in 18 of them. No seroconversion was observed (Table 1). IgM antibodies to *C. pneumoniae* were detected in 7 patients; 5 of them were also found positive for IgG in early and late serum specimens whereas an IgG rise was observed in one patient. One more patient exhibited a seroconversion for IgG antibodies to *C. pneumoniae* without IgM, which could correspond to a profile of acute reactivation. In summary, a serological profile of possible or probable acute infection with *C. pneumoniae* was noticed in 14% of the patients, whereas 56% of them exhibited a profile of past infection and 30% of them had no antibody at all against this bacterium (Table 1).

1st serum (day 0)		2nd serum (day 15)		Tentative interpretation	M. pneumoniae (number of patients)	C. pneumoniae (number of patients)
IgM	IgG	IgM	IgG			
-	-	-	-	No infection	18	15
-	+	-	+	Past infection	32	28
+	+	+	+	Acute infection	0	5
+	-	+	+	Acute infection	0	1
-	-	-	+	Reactivation	0	1

Table 1: *Prevalence of IGM and IGG antibodies to mycoplasma pneumoniae and Chlamydophila pneumoniae in two consecutive serum specimens from the 50 patients with acute episode of COPD included in the study.*

Regarding conventional cultures of sputum specimens from the 50 patients, 12 infections (as defined by a bacterial count of at least 10^7 CFU/mL) were recorded as follows: *Branhamella catarrhalis* (n = 1), *Escherichia coli* (n = 1), *Haemophilus influenzae* (n = 2), *Klebsiella pneumoniae* (n = 2), *Pseudomonas aeruginosa* (n = 4), *Staphylococcus aureus* (n = 1), *Streptococcus pneumoniae* (n = 1) and a co-infection by *H. influenzae* and *K. pneumoniae* (n = 1). With respect to the distribution of conventional bacterial infections according to the presence or absence of serological stigmata of *C. pneumoniae* acute infection, an infection with *S. pneumoniae* was recorded in the group of 7 patients characterized as recent *C. pneumoniae* infection. Table 2 lists the large number and variety of pathogens that were recovered from the sputum samples of these 50 patients.

Pathogen	Number
Conventional bacteriological cultures*	**12**
Escherichia coli	1
Klebsiella pneumoniae	2
Haemophilus influenzae	2
Pseudomonas aeruginosa	4
Staphylococcus aureus	1
Streptococcus pneumoniae	1
Branhamella catarrhalis	1
Serological test	
Bacteria	**7**
Chlamydophila pneumoniae	7

Table 2: *List of pathogens recovered from the sputum samples of the 50 patients included into the study.*

**: The result was considered positive for a threshold of 10^7 UFC/ml.*

Table 3 depicts the demographic, clinical and laboratory data of the 50 patients with or without serological stigmata of recent *C. pneumoniae* infection. No difference was recorded between the two populations for any of the tested items.

List of tested variables	Serological stigmata of acute *C. pneumoniae* infection		P value
	No	Yes	
	n = 43	n = 7	
Demographics			
Mean age (years)	67.3 ± 10.1	65.1 ± 11	NS
Ratio male/female	42/1	7/0	NS
Smoking habits (pack-year)	70.9 ± 116.1	64.2 ± 32.5	NS
Clinical findings at entry			
Body Mass Index (kg/m^2)	27.2 ± 5.1	26.7 ± 3.2	NS
Respiratory rate (beats per minute)	29.5 ± 14	27.6 ± 3.8	NS
Heart rate (beats per minute)	108.8 ± 21.1	119 ± 13.9	NS
Laboratory findings at entry			
PaO_2 (kPa)	17.6 ± 22.3	31.5 ± 25	NS
$PaCO_2$ (kPa)	6.8 ± 5.3	5.6 ± 1.2	NS
pH	7.3 ± 0.07	7.3 ± 0.04	NS
Percent oxygen saturation (SaO_2)	88.1 ± 11.7	87.9 ± 6.5	NS
White blood cell count ($c/mm^3 \times 10^3$)	11.9 ± 5.4	14.4 ± 8.4	NS
Hemoglobin (g/dL)	14.3 ± 4.6	14.2 ± 1.9	NS
Platelet count ($x10^3/\mu L$)	257.8 ± 108.2	287.5 ± 72.3	NS
C-Reactive Protein (mg/dL)	67.8 ± 66.1	45.8 ± 43.6	NS
Outcome			
Percent survived	97.0	100	NS

Table 3: *Comparison of different variables in 50 patients hospitalized for exacerbation of COPD with and without serological stigmata of acute infection of Chlamydophila pneumoniae. Most data are presented as mean ± standard deviation.*

PaO_2: partial pressure of oxygen in arterial blood.

$PaCO_2$: partial pressure of carbon dioxide in arterial blood.

NS: not significant by Mann-Whitney test for quantitative variables and by chi-square test for qualitative variables between the two groups at a P level of 0.05.

Discussion

From a large cohort of Tunisian patients exhibiting an acute episode of COPD, this study, mainly based on commercial serological tests, recorded an 14% rate of possible recent infection with *C. pneumoniae*. By contrast, no acute infection was documented for *M. pneumoniae*. The main limitation of our study is the absence of detection of those intracellular pathogens by nucleic acid testing as this kind of assay was not available in our laboratory at the time of the work. Interestingly, respiratory secretions from one patient with IgM antibodies could be tested retrospectively by PCR for *M. pneumoniae* and *C. pneumoniae* and were confirmed to be positive for the genome of the latter agent (data not shown). Indeed, molecular tests represent a good complement to serological testing [5]; they are considered faster, more sensitive and more specific than culture and serology [9], even if the presence of *C. pneumoniae* DNA without stigmata of serological infection can correspond to a persistent asymptomatic carriage of this bacterium [5,10]. The lack of standardization of commercial ELISA

kits for *C. pneumoniae* is another limitation of our study, with the possible occurrence of nonspecific IgM antibodies, especially when they are detected in the absence of specific IgG together with stigmata of another bacterial infection in the respiratory tract [11,12]. With regard to *C. pneumoniae* IgG, the sensitivity of different immunoassays compared to the microimmunofluorescence assay taken as gold standard ranged from 63 to 95% in the context of respiratory tract infection [5].

C. pneumoniae has been shown to be significantly involved in the occurrence of acute episodes of COPD, as illustrated by different studies from the literature using serological testing. As shown in table 4 summarizing a total of 453 patients with an acute episode of COPD (including the present study), the rate of positive cases for *C. pneumoniae* ranged from 4 to 34% with an overall mean of 13.1%, which is very close to the rate of 14% found in this study.

Reference	Period of study	Location	Serological assay	Total number of COPD patients	Number (%) of patients with acute *C. pneumoniae* infection
[13]	1999	Turkey	MIF	49	11 (22.4)
[14]	1999-2002	Turkey	MIF	75	13 (17.3)
[15]	1996	Finland	ELISA	29	2 (6.9)
[16]	2001	Turkey	MIF	38	13 (34.2)
[17]	2004	Italy	MIF	45	5 (11.1)
[10]	2005-2008	Greece	MIF	92	4 (4.3)*
[18]	2007	Greece	MIF and ELISA	75	7 (9.3)
This study	2013-2015	Tunisia	ELISA	50	7 (14)
Total				453	62 (14.9)

Table 4: *Prevalence of serological stigmata of acute infection with Chlamydophila pneumoniae during acute episodes of COPD in selected studies from the literature.*

Of interest, we show that no difference was observed between the groups of patients with and without evidence of serological acute *C. pneumoniae* infection in terms of demographics, clinical presentation, clinical outcome or detection of other bacterial agents at a significant level in the respiratory tract. However, these results pleads for the systematic investigation of stigmata of *C. pneumoniae* recent infection in patients with acute episodes of COPD since this pathogen would require an antimicrobial treatment based on compounds active on intracellular agents that are not recommended in primary intention in this clinical context [1].

By contrast to *C. pneumoniae*, our study identified no case of recent infection with *M. pneumoniae*. This pathogen is more rarely associated to acute episodes of COPD, although it was shown to be involved in a small proportion of them [6,10,13,14,17].

Conclusion

Our study conducted in a consecutive series of 50 Tunisian patients exhibiting a COPD exacerbation shows an overall prevalence of *C. pneumoniae* acute infection of 14%. Given this result, corroborated by those of studies from other parts of the world, it seems reasonable to recommend the diagnosis of this infection in this particular context with the aim of implementing an adequate antimicrobial treatment. However, the tools used for this diagnosis, based either on serology or on molecular testing, needs standardization in order to precise more accurately the real place of this bacterium in the pathophysiology of acute episodes of COPD.

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