Chlamydia pneumoniae infection in acute exacerbations of COPD


ABSTRACT: Chlamydia pneumoniae, strain TWAR, is a frequent causative agent of acute respiratory disease. We assessed the incidence and prevalence of Chlamydia pneumoniae infections in COPD.

We studied, from January 1990 to May 1991, 142 out-patients with acute purulent exacerbations of chronic obstructive pulmonary disease (COPD) and 114 healthy control subjects. Oropharyngeal swab specimens were collected at each exacerbation and analysed using a high definition monoclonal indirect fluorescent antibody test for Chlamydia pneumoniae identification. Immunoglobulins G and M (IgG and IgM) fractions of antibodies to Chlamydia pneumoniae were studied by microimmunofluor-scence test.

Prevalence of specific IgG was 63% in COPD, and 46% in controls (Chi-squared test p=0.007). Moreover, mean titre of IgG was significantly higher in COPD than in controls. Five patients were positive for specific IgM (≥1:16), and one had a fourfold increase of IgG titre; four of these patients had been treated with ciprofloxacin 1 g day⁻¹ for 10 days, and two with erythromycin, 3 g day⁻¹ for 14 days, with remission of signs and symptoms of exacerbation. Chlamydia pneumoniae identification was always negative.

Our data suggest that Chlamydia pneumoniae infection is a rather frequent event in COPD, since at least 4% of exacerbations may be associated with it.

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Over the past 40 yrs, an extensive literature has analysed the role of infectious agents in the pathogenesis of chronic obstructive pulmonary disease (COPD) and chronic bronchitis [1-5]. The causal role of infections in these conditions remains controversial and infectious exacerbations often result in incapacitation, hospitalization, respiratory failure and even death. Physicians continue to search for microbiological aetiologies to explain acute exacerbations, as well as for correct treatment [6]. Recently, attention has been directed toward new organisms, such as Chlamydia pneumoniae, as potential causative agents of exacerbations [7, 8]. Chlamydia pneumoniae is a newly described bacterium, known to be an important cause of acute respiratory illness in humans [9-11].

In order to assess the role of Chlamydia pneumoniae as a causative agent of acute exacerbations, we evaluated its presence in oropharyngeal swabs, as well as the titres of specific antibodies in patients with exacerbations of COPD and in healthy subjects from our area.

Patients and materials

The sample involved in the present study comprised 142 out-patients (83 males, 59 females, age range 30-88 yrs, mean 63±12 yrs), affected by acute purulent exacerbations of COPD, and 114 control subjects (64 males, 50 females, age range 30-85 yrs, mean 59±12 yrs) without history, signs or symptoms of pulmonary disease, recruited from our internal medicine out-patients’ department between January 1990 and May 1991.

Informed consent to participate to the study was obtained from all the subjects. Clinical information was found in patients’ case report forms, or recorded directly by the investigators. The patients had a mean follow-up of 5.3 months (range 2-12 months).

An acute exacerbation was considered as occurring when the patient experienced a worsening cough, accompanied by purulent or mucopurulent sputum, with or without fever and increasing dyspnoea.

Serum samples were taken at each acute exacerbation and 4–6 weeks later. Figure 1 shows the
percentage distribution, by month, of samples obtained in both COPD and control subjects. Oropharyngeal swab specimens were obtained from patients before and after treatment. Mean pulmonary function data of COPD patients are reported in table 1.

Methods

Throat swab specimens were applied to a slide, acetone-fixed at room temperature, and analysed using a high definition monoclonal indirect fluorescent antibody test (Cellabs Diagnostic PTY, Brookvale, NSW, Australia), for Chlamydia pneumoniae detection. A microimmunofluorescence test [12] was employed to detect immunoglobulin M (IgM) and immunoglobulin G (IgG), using TW AR antigen prepared by the Washington Research Foundation, Seattle, USA. Serological tests for Mycoplasma pneumoniae, Legionella pneumophila, and cytomegalovirus were also performed. Microimmunofluorescence results were classified, as reported previously [13]: a titre of \( \geq 1:16 \) in the IgM serum fraction indicated first Chlamydia pneumoniae infection; a titre of \( \geq 1:512 \) or a fourfold increase in the IgG fraction indicated reinfection; chronic or pre-existing antibody was suggested by a titre between 1:64 and 1:256 in the IgG fraction.

Sputum specimens were collected, avoiding oral contamination; suitability for culture was assessed according to Bartlett et al. [14]. Antibiotic susceptibility test was performed by the Bauer-Kirby procedure [15].

Patients with acute exacerbation were randomly assigned to receive ciprofloxacin, 500 mg b.i.d., or erythromycin, 1 g t.i.d., for at least 10 days.

![Percentage distribution of samples obtained in patients with acute exacerbations of COPD and in control subjects.](image)

Table 1. Mean pulmonary function data of patients with exacerbations of COPD

<table>
<thead>
<tr>
<th></th>
<th>Mean±sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV(_1)</td>
<td>1.38±0.52</td>
</tr>
<tr>
<td>FEV(_1) % pred</td>
<td>51±2.1</td>
</tr>
<tr>
<td>FVC (_l)</td>
<td>2.43±0.76</td>
</tr>
<tr>
<td>FVC % pred</td>
<td>76±2.2</td>
</tr>
<tr>
<td>FEV(_1)/FVC %</td>
<td>57±2</td>
</tr>
<tr>
<td>Pao(_2) mmHg</td>
<td>77±3</td>
</tr>
<tr>
<td>kPa</td>
<td>10.3±0.4</td>
</tr>
<tr>
<td>Paco(_2) mmHg kPa</td>
<td>40±2</td>
</tr>
</tbody>
</table>

COPD: chronic obstructive pulmonary disease; FEV\(_1\): forced expiratory volume in one second; % pred: percentage of predicted; FVC: forced vital capacity; Pao\(_2\): arterial oxygen tension; Paco\(_2\): arterial carbon dioxide tension.

Results

Chlamydia pneumoniae was not detected by indirect immunofluorescence in any oropharyngeal swab specimen.

Table 2 shows the results of serological tests for antibody to Chlamydia pneumoniae in patients with exacerbations of COPD and in control subjects. A significantly greater prevalence of positive results for IgG was recorded in the former group (Chi-squared test \( p=0.007 \)). Moreover, the mean titre of IgG was significantly higher in COPD than in control subjects (Student's t-test for unpaired data, \( p<0.005 \), with a geometric mean titre of 196 and 140, respectively. The geometric mean titre increased strikingly with age in patients with COPD, but not in controls (fig. 2).
CHLAMYDIA PNEUMONIAE INFECTION IN COPD

Table 2. Summary of microimmunofluorescence serological tests for Chlamydia pneumoniae infection in patients with exacerbations of COPD and in control subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>IgG +ve</th>
<th>IgG ≥1:512</th>
<th>IgG -ve</th>
<th>IgM ≥1:16</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPD (n=142)</td>
<td>90 (63%)</td>
<td>19 (14%)</td>
<td>52 (37%)</td>
<td>5 (4%)</td>
</tr>
<tr>
<td>Controls (n=114)</td>
<td>53 (46%)</td>
<td>6 (5%)</td>
<td>61 (53%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are number of subjects (%). p value=0.007 (Chi-squared test COPD versus controls (IgG positive versus IgG negative)). COPD: chronic obstructive pulmonary disease; IgG: immunoglobulin G; IgM: immunoglobulin M; +ve: positive; -ve: negative.

Table 3. Laboratory results in six patients with exacerbations of COPD attributed to Chlamydia pneumoniae

<table>
<thead>
<tr>
<th>Pt no.</th>
<th>Sex</th>
<th>Age yrs</th>
<th>Pharyngeal swab</th>
<th>Serum follow-up samples (days after onset)</th>
<th>IgM titre</th>
<th>IgG titre</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 F</td>
<td>80</td>
<td>-ve</td>
<td>12</td>
<td>16</td>
<td>256</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>2 F</td>
<td>69</td>
<td>-ve</td>
<td>18</td>
<td>16</td>
<td>256</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>3 M</td>
<td>63</td>
<td>-ve</td>
<td>13</td>
<td>64</td>
<td>1024</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>4 M</td>
<td>76</td>
<td>-ve</td>
<td>22</td>
<td>64</td>
<td>512</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>5 F</td>
<td>88</td>
<td>-ve</td>
<td>18</td>
<td>16</td>
<td>1024</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>6 M</td>
<td>54</td>
<td>-ve</td>
<td>15</td>
<td>0</td>
<td>128</td>
<td>C</td>
<td></td>
</tr>
</tbody>
</table>

E: erythromycin, 3 g·day⁻¹ for 14 days; C: ciprofloxacin, 1 g·day⁻¹ for 10 days. For further abbreviations see legend to table 2.

Figure 3 shows the percentage of positive IgG tests (≥1:64), recorded in COPD patients and in control subjects, according to age group. We found IgM titres ≥1:16, suggesting new infection in five patients (4%); high IgG titres ≥1:512 in another 19 patients (14%); and in one patient a fourfold increase in IgG titres suggesting reinfection.

All of these patients were seronegative for acute Cytomegalovirus, Legionella pneumophila, and Mycoplasma pneumoniae infections. None had clinical or radiological evidence of pneumonia.

Serological evolution and treatment of the six patients with microimmunofluorescence test positive for IgM, or with a fourfold increase in IgG titres, are shown in table 3.

No clinically relevant difference was recorded between exacerbations sustained by Chlamydia pneumoniae and those sustained by other agents.
Discussion

We found a high frequency of IgG anti-Chlamydia pneumoniae antibody, exceeding 63%, in patients with exacerbations of COPD, with a high titre (≥1:512) in 14% of all the patients tested (21% of IgG positive).

The high IgG titres observed suggest reinfecetion by Chlamydia pneumoniae. However, we cannot exclude a different aetiology of the exacerbation due to the presence of other microorganisms, usually involved in acute exacerbations.

An IgM titre ≥1:16 was observed in five patients. In another patient a fourfold increase of IgG titres was recorded. Despite the negative results of Chlamydia pneumoniae identification in pharyngeal swabs, the aetiological role of Chlamydia pneumoniae in the exacerbations of these patients is strongly suspected. The negative results of Chlamydia pneumoniae identification could be due to the prolonged storage of the samples in the deep freezer (4–6 months), or to the use of oropharyngeal instead of nasopharyngeal swabs as recommended by the manufacturer.

Four of the above-mentioned patients were treated with ciprofloxacin, 500 mg b.i.d., for 10 days and two with erythromycin, 1 g t.i.d. for 14 days. In all cases we obtained a remission of signs and symptoms of acute exacerbations.

Our data suggest that Chlamydia pneumoniae infection is a rather frequent event in COPD and that at least 4% of exacerbations could be associated with Chlamydia pneumoniae infection by this organism.

This would confirm the results reported by Beaty et al. [8], even if the incidence of infection seems to be lower in our population (4 versus 5%), probably due to the larger sample of patients with acute exacerbations studied (142 versus 44).

The prevalence of positive microimmunofluorescence tests for Chlamydia pneumoniae in COPD was remarkably higher than in control subjects, particularly in those aged over 50 yrs. These results are not consistent with those reported by Beaty et al. [8], due to the surprisingly high antibody prevalence (73%) in their control group. This could be explained, either by the low number of subjects (24) enrolled in their study, or by the periodicity of Chlamydia pneumoniae infection with time-related differences in antibody prevalence. The stronger prevalence recorded in our patient group could be due to either chronic infection by Chlamydia pneumoniae, as suggested by the increase of specific IgG prevalence and geometric mean titre with age, or to a higher rate of acute infection in such patients.

References