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Incidence of community-acquired pneumonia and *Chlamydia pneumoniae* infection: a prospective multicentre study

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Incidence of community-acquired pneumonia and Chlamydia pneumoniae *infection: a prospective multicentre study. J. Almirall, I. Morató, F. Riera, A. Verdaguer, R. Priu, P. Coll, J. Vidal, L. Murgui, F. Valls, F. Catalan, X. Balanzó.*

ABSTRACT: This one year prospective multicentre study was designed to determine the incidence of community-acquired pneumonia in adults. It was carried out in primary health care centres and three reference hospitals, located in the 'Maresme' region (Barcelona, Spain) serving a population of 39,733 subjects over 13 years of age. Patients suspected of having contracted community-acquired pneumonia were visited by their family doctors and referred to the three reference hospitals for confirmation of the diagnosis. Patients attending the emergency services of these hospitals were also included. Urine and blood samples were obtained for culture, antigen detection, blood count, serological tests, blood gases and biochemical profile.

The diagnosis of community-acquired pneumonia was made in 105 patients. Forty-six patients had an identifiable microbial etiology. *Chlamydia pneumoniae* was the most common pathogen (16 cases) followed by *Streptococcus pneumoniae* (13 cases) and *Mycoplasma pneumoniae* (8 cases).

In conclusion; the annual incidence rate of community-acquired pneumonia in adults in this area was 2.6 cases per 1,000 inhabitants and *Chlamydia pneumoniae* was the most frequent causative pathogen.

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Most of the epidemiological studies of community-acquired pneumonia have been carried out in patients admitted to hospital. Little information is available on virtually all outpatients treated by practising physicians, although WOODHEAD *et al.* [1] have estimated an annual incidence of 4.7 cases per 1,000 adults in Nottingham. New causative agents such as *Chlamydia pneumoniae* have emerged as significant pulmonary pathogens in adult patients with pneumonia requiring hospitalization [2], but a potential chlamidyal cause of the total cases of community-acquired pneumonia has not been investigated. We, therefore, have carried out a 1-year prospective study of all patients with community-acquired pneumonia in the adult population of the 'Maresme' region (Barcelona, Spain), independent of whether or not admission to hospital was required.

Patients and Material

Patients. From April 1990 to March 1991, 39,733 subjects over 13 years of age (49.5% men, 50.5% women, aged between 13–101 yrs), served by primary health care centres in four restricted geographical areas (Mataró, Arenys de Mar, Arenys de Munt and Argentona) of the Maresme region in Barcelona, were enrolled in a prospective study. Patients suspected of having contracted community-acquired pneumonia with fever and respiratory symptoms (cough with/without expectoration and/or chest pain) were seen by their own family physicians. When a presumptive clinical diagnosis of pneumonia was established by the family physician, the patient was referred to one of the three reference hospitals for confirmation of diagnosis. A definite diagnosis of pneumonia was a prerequisite for antibiotic therapy. Patients, from the area under study, with symptoms of pneumonia attending the emergency services of these hospitals were also included.

A diagnosis of pneumonia was made if there were suggestive clinical manifestations and if a pulmonary opacity was seen on the chest roentgenograms. Patients in whom pneumonia was secondary to concurrent disease (aspiration pneumonia, pulmonary tuberculosis) and patients with pneumonia acquired in a nursing home [3] or who had been discharged from hospital less than 7 days before the onset of symptoms, were excluded from the study. The initial respiratory rate and the Pa_{O_2}/F_{IO_2} index (abnormal value <300) [4] were used for the assessment of the seriousness of the clinical condition. Blood samples were obtained for culture (in patients with fever ≥38°C),

blood count, serological tests, blood gases and biochemical profile. Urine samples were taken for antigen detection. In addition, lower respiratory tract secretions were obtained by fibreoptic bronchoscopy (bronchoalveolar lavage, plugged double catheter) in immunocompromised patients with bilateral pulmonary involvement or in patients not responding to empirical antibiotic therapy. Patients were evaluated on the fifth day of illness and at monthly intervals until complete recovery. Chest roentgenograms were taken and a second blood sample was drawn 4 weeks after the initial visit.

Microbiological investigations. Standard laboratory methods were used for culture and identification of isolates from blood, lower respiratory tract secretions and pleural fluid. Urine specimens were frozen at -30°C and tested for *Streptococcus pneumoniae* and *Haemophilus influenzae* type b antigen in one batch towards the end of the study. In order to minimize possible nonspecific reactions, all samples were heated at 100°C for 3 min. Urine samples were centrifuged at 2,000 × g for 10 min and tests for antigen performed in both unconcentrated urine and urine concentrated approximately twenty-fold by means of a disposable ultrafilter (Minicon-B15 concentrator, Amicon, USA). *Haemophilus influenzae* type b capsular antigen was detected in urine and respiratory specimens with a commercially available latex kit (Bactigen, Wampole Laboratories, USA) according to the manufacturer's instructions. Pneumococcal capsular antigen was detected in urine and respiratory specimens by counterimmunoelectrophoresis (CIE) with pneumococcal Omniserum (Statens Serum Institut, Denmark). Continuous CIE was performed using a modification of the procedures of INGRAM *et al.* [5] and RYTEL [6] on 15×10 cm glass plates with 1 ml of 1% agarose solution (Agarose High-m_r, Bio Rad, USA) in sodium barbital buffer (pH 8.6). Electrophoresis was performed at 20 mA per plate (constant current) for 60 min. An additional examination of gel was performed after protein staining of the CIE plates with Coomassie brilliant blue R [7].

Serological tests. Paired sera were tested for evidence of complement fixing antibody to influenza A and B, parainfluenza, adenovirus, respiratory syncytial virus, *Chlamydia psittaci*, *Coxiella burnetti* and *Mycoplasma pneumoniae* [8]. The indirect fluorescent antibody technique was used to test for *Legionella pneumophila* serogroups 1–6 [9]. When varicella pneumonia was suspected, testing for antibodies was done by standard complement-fixation technique. *Chlamydia pneumoniae* and *Chlamydia trachomatis* specific antibodies were detected in paired sera at the Institut Fournier (Paris, France) by microimmunofluorescence according to the method described by TREHARNE *et al.* [10]. The chlamydial strains studied included IOL-207 (Institute of Ophthalmology, London) and L2 which were adapted to grow in HeLa 229 cells. The presence of chlamydial antibody in the IgM serum fraction was detected by microimmunofluorescence and immunoblot analysis (Western blot). Chlamydial antigens were separated by discontinuous sodium dodecylsulfate-polyacrylamide gel electrophoresis according to the method of LAEMMLI [11]. To rule out serologic-cross reactions, immunoblot analysis for *C. trachomatis* was also performed [12].

Diagnostic criteria. An etiological diagnosis was based on positive blood or pleural fluid cultures or urine antigen detection. A four-fold antibody titre rise for a virus, *Mycoplasma pneumoniae*, *C. psittaci*, *Coxiella burnetti*, or *Legionella pneumophila* was accepted as evidence of infection. A diagnosis of *C. pneumoniae* infection was established when there was a four-fold antibody titre rise or if there was antibody in the IgM fraction. In cases of paired IgM titres ≤ 1:64 without significant variation, the presence of IgM antibody was studied by Western blot.

Results

The diagnosis of community-acquired pneumonia was established in 105 patients with an annual incidence rate of 2.6 cases per 1,000 inhabitants. There were 69 men and 39 women (mean age 48 years; range 14–90 yrs), 18 (17%) patients were over 70 years of age. There was a seasonal distribution in the number of cases, 66.6% of episodes of pneumonia occurring between October and March. Regular cigarette smokers represented 43% of the patients, 9% had a history of excessive alcohol intake and 4% were parenteral drug abusers. Forty-one (39%) patients had underlying diseases (table 1).

Table 1. – Underlying disease in 105 patients with community-acquired pneumonia

Disease	No. patients
Chronic obstructive pulmonary disease	18
Diabetes mellitus	10
Cardiopathy	8
Neoplasm	2
Immunosuppression*	2
Chronic liver disease	1

*: Defined as presence of haematologic malignancy, malignant solid tumour, neutropaenia or chronic administration of corticosteroids.

The initial mean (±SD) respiratory rate was 24±8 breaths·min^{-1} (>30 breaths·min^{-1} in 19 patients). The Pao_2/Fio_2 index was 336±72.5 (<300 in 21 patients). Chest X-rays showed that 96 patients had unilateral involvement (alveolar pattern in 77% of cases), 9 had bilateral involvement and 11 had pleural effusions.

Of the 105 patients, 46 had an identifiable microbial etiology. A total of 54 pathogens were identified, a single pathogen in 38 (81%) patients (table 2) and two pathogens in 8 (17%) (Table 3). Serologic

tests were carried out in 83 patients and identified the causative organism in 39 cases (*C. Pneumoniae,* 16; *Mycoplasma pneumoniae,* 8; parainfluenza viruses, 5; adenovirus, 4; *Legionella pneumoniae,* 3; varicella virus, 2; respiratory syncytial virus, 1). Blood culture and urine CIE were performed in 84 patients and identified the causative organism in 13 (*Streptococcus pneumoniae*). Finally, bronchoalveolar lavage (BAL) and plugged double catheter were performed in two patients and identified the causative organism in both (*Pneumocystis carinii*). The relative frequencies of the pathogens identified are listed in tables 2 and 3. No correlation was found between the underlying diseases and specific microbiological aetiologies.

Table 2. – Distribution of 54 pathogens causing pneumonia in 46 patients and results of diagnostic tests

Pathogen	n	Serologic tests	Blood culture	Urine CIE	BAL PDC
Chlamydia pneumoniae	16	16/64			
Streptococcus pneumoniae	13		7/84	10/84	
Mycoplasma pneumoniae	8	8/83			
Parainfluenza	5	5/83			
Adenovirus	4	4/83			
Legionella pneumophila	3	3/83			
Pneumocystis carinii	2				2/2
Varicella virus	2	2/2			
Respiratory syncytial virus	1	1/83			

Results are expressed as number of patients with positive results/total number of patients submitted to diagnostic tests. CIE: counterimmunoelectrophoresis; BAL: bronchoalveolar lavage; PDC: plugged double catheter. Culture of the pleural fluid was performed in six cases with negative results.

Table 3. Pathogens causing dual infections in eight patients with community-acquired pneumoniae

Pathogens	No. Cases
Chlamydia pneumoniae +	
Adenovirus	3
Parainfluenza	2
Legionella pneumophila	1
Respiratory syncytial virus	1
Streptococcus pneumoniae + adenovirus	1

Chlamydia pneumoniae was the most common pathogen and accounted for 16 cases. *Streptococcus pneumoniae* was the second most common pathogen, accounting for 13 cases. *Mycoplasma pneumoniae* infection was diagnosed in eight cases. Chlamydial specific antibodies were detected by microimmunofluorescence in 30 (47%) of the 64 samples tested. The antibody in the IgM fraction was detected by microimmunofluorescence or Western blot in 16 cases which were classified as definitive. The remaining 14 cases in which elevated antibody titres in the IgM fraction were not detected, had serological evidence of previous infection with *C. pneumoniae*.

A total of 53 patients were admitted to hospital and in three cases admission to the intensive care unit was required. Of the 105 patients, one patient died due to pneumococcal sepsis. After 5 days of treatment, radiographic signs of improvement were seen in 82% of patients. The chest roentgenogram taken 4 weeks after the initial visit revealed persistent abnormalities in 3% of the patients.

In one of the three hospitals (the one which served the majority of patients) information was available regarding false positive and false negative diagnoses. On the basis of clinical manifestations, the rate of false positive diagnoses made by family physicians was 58% (45/78 evaluable patients). The diagnostic accuracy was 42% (33/78). On the basis of clinical, biological and roentgenographic findings, the rate of false positives was 9% (8/94 evaluable patients) among diagnoses made by physicians in the emergency service. The diagnostic accuracy at the hospital was 92% (86/94). The final rates of false positive and false negative diagnoses in 86 evaluable patients were 12% (10 cases) and 5% (4 cases), respectively.

Discussion

This study, carried out to determine the occurrence of community-acquired pneumonia in the adult population of the 'Maresme' region of Barcelona, reveals an annual incidence of 2.6 cases per 1,000 inhabitants, a figure lower than that reported by WOODHEAD *et al.* [1] in Nottingham. However after stratification according to age and area, differences between 40% and 173% were detected between the number of expected cases and those that actually occurred. This finding shows that variable incidences may be expected depending upon the population under study, even though the same follow-up procedures are used.

A total of 43% of patients were smokers and the daily alcohol consumption in 9% of subjects was 80 g or more. These figures are not substantially different from those found in the population at large [13]. Coinciding with the findings of other studies [1, 2, 14, 15], underlying diseases frequently occurred in our patients (39% of cases).

In this study, the initial respiratory rate and the Pao_2/Fio_2 index were taken as indicators of the severity of disease. Initial respiratory rate has already been proven to be a prognostic factor in a multicentre study carried out by the British Thoracic Society [16]; Pao_2/Fio_2 has been proposed as a useful indicator of the overall state of oxygenation and the degree of severity of pulmonary lesions [4]. Therefore, a respiratory rate of >30 breaths·min^{-1} in 19 patients and a Pao_2/Fio_2 index of <300 in 21 cases in our study indicates that the pulmonary infections investigated were relatively mild.

Fifty-three patients required hospitalization and only one died. The mortality rate of 1% is lower than that reported in other studies (8% and 21% depending on whether patients attended emergency services or whether they required hospitalization) [2, 15, 17]. WOODHEAD *et al.* [1] reported a mortality rate of 3%. In our study, however, pneumonias acquired in nursing homes (potentially more serious) were not included.

The most common cause of community-acquired pneumonia in this study was *C. pneumoniae*. Bearing in mind that testing for chlamydial antibodies was performed in only 64 patients, the real incidence of this pathogen may well be underestimated. The incidence of this organism in commmunity-acquired pneumonia has hitherto been unknown since only the prevalence of *C. pneumoniae* infection has been analyzed in studies carried out until now. FANG *et al.* [2] have shown that if diagnostic criteria are strictly applied, this pathogen is the primary cause of pneumonia.

In eight of the patients under study, two pathogens were identified. In seven of these cases one of the pathogens was *C. pneumoniae*. This represents 44% of the total number of 16 patients with serological evidence of *C. pneumoniae* infection. This finding is also reported in 33% of the cases studied by MARRIE *et al.* [18] if diagnoses established by sputum culture are excluded.

Population studies in adults have shown serologic evidence of previous *C. pneumoniae* infection in 20–60% of cases [18, 21] which indicates the high occurrence rate of this pulmonary pathogen. Of 301 patients with community-acquired pneumonia, in the study of MARRIE *et al.* [18] 32% had evidence of past infection. In Spain, GUERRERO *et al.* [22] reported a figure of 57% of specific antibodies in 103 paired sera from patients with community-acquired pneumonia admitted to hospital.

The cause of pneumonia was established in 44% of patients in our study. It should be noted that our diagnostic criteria were restrictive since bacteriological examination of sputum was not assessed, nor were invasive diagnostic procedures carried out systematically, nor were all diagnostic tests carried out in all patients.

One of the main problems encountered in epidemiological studies is that of erroneous diagnosis, and this often occurs in the diagnosis of pneumonias [23]. Of the initial diagnoses made by family physicians in our study 58% were false positives. This figure is difficult to compare since similar studies are lacking; the rate of this study could be high as the family physicians were particularly sensitive to this illness; hence, with a minium suspicion of community-acquired pneumonia the patient was referred to hospital for screening. A total of 8.5% of false positives were recorded among the initial diagnoses made by physicians in the emergency service. This figure is also difficult to evaluate for the same reasons. Studies usually either omit these data or analyze the relationship between the initial and final diagnoses. Only MARRIE and coworkers [15, 24] provided similar data to ours in two studies (7% and 23%). These, however, only refer to hospitalized patients so that the rate of erroneous diagnosis detected in our study may be considered to be acceptable.

The rate of error detected in final diagnoses varies between 5% and 36% in different studies [2, 16, 25]. This is mainly determined by the availability of complementary diagnostic techniques in hospital emergency services, which influences subsequent modifications in diagnoses as well as the clinical evolution of the patient. Even so, the rate of false positives in postmortem studies may reach 69% [15]. We found a rate 5% of false negative results but, at autopsy, undetected pneumonias have been reported in 53% of cases [26].

This study has provided information on microbial aetiologies of community-acquired pneumonia, allowing an updated approach to diagnosis and management. Despite the fact that only 46 patients had an identifiable microbial aetiology and that microbiological and serological tests were not performed in all patients, the relative frequency of different pathogens is considered to be close to the actual frequency in our setting. These data are also pertinent for the selection of more appropriate empirical antibiotic therapy.

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