EJ072200000034

J Microbiol Immunol Infect
2000;33:34-38

Chlamydia pneumoniae infection in community-acquired pneumonia in Taiwan

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Received: June 7, 1999 Revised: July 20, 1999 Accepted: September 23, 1999

Chlamydia pneumoniae is a common cause of pneumonia worldwide. This study examined the role of *C. pneumoniae* in 229 patients with community-acquired pneumonia. The ages of the patients ranged from 2 to 95 years. Sera were assayed for IgM and IgG antibodies with microimmunofluorescence (MIF). An IgM titer equal to or greater than 1:20 and IgG titer equal to or greater than 1:64 were considered positive. The prevalence of positive IgG among all of the patients was 75.1% (172/229). The seroprevalence was 81.8% (9/11) for patients in the 11- to 20-year-old group, 63.6% (14/22) in the 31- to 40-year-old group and 88.1% (52/59) in the 71- to 80-year-old group. All patients had a negative result for IgM antibody. Twenty-five of the patients had an IgG titer equal to or greater than 1:512, indicating the presence of current infection or reinfection. Age older than 60 years (vs. $\leq$ 60 years) was a risk factor for *C. pneumoniae* seropositivity in patients with community-acquired pneumonia ($p < 0.001$). Males had a significantly higher seroprevalence of *C. pneumoniae* infection ($p = 0.1010$). Patients older than 60 years were more likely to have *C. pneumoniae* infection ($p = 0.1107$). In this series, *C. pneumoniae* infection accounted for 10.9% (25/229) of community-acquired pneumonia. The most common clinical manifestations included fever (92%), productive cough (52%), white blood cell more than 10,000/mm^3 (56%), and bilateral pulmonary infiltrate (60%).

Key words: *Chlamydia pneumoniae*, community-acquired pneumonia, microimmunofluorescence (MIF)

Chlamydia pneumoniae formerly known as Taiwan acute respiratory agent (TWAR) is the third species of the genus *Chlamydia* and is a common cause of respiratory infections including pneumonia, bronchitis and pharyngitis worldwide [1,2]. The organism was first isolated from the conjunctiva of a Taiwanese child in 1965 and was recognized as a respiratory pathogen in 1983 [3]. The epidemiology of *C. pneumoniae* infection has been studied based on evidence of serology [4-8]. Previous studies have reported prevalence of *C. pneumoniae* infection of more than 50% among adults in many countries [4,5,7]. Studies in adults from different areas of the world have shown a higher prevalence in less developed tropical countries than in developed northern countries [9]. The geographic area of Taiwan covers both tropical and subtropical regions. A high prevalence of *C. pneumoniae* has been reported in the general population in Taiwan [10]. However, the prevalence of *C. pneumoniae* in community-acquired pneumonia has not been investigated previously in Taiwan.

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Materials and Methods

The study population included 229 patients with community-acquired pneumonia who were admitted to Tri-Service General Hospital from July 1997 through August 1998. During the study period, community-acquired pneumonia was diagnosed in a total of 544 hospitalized patients. These patients were enrolled in this study if a serum sample had been collected on admission.

The age of the patients ranged from 2 to 95 years old (mean, 59.5 years). Diagnosis of pneumonia was based on clinical and radiographic findings. Serum specimens were tested for the presence of antibodies to *C. pneumoniae*. A 10 mL blood sample was drawn and clotted at room temperature prior to centrifugation. Serum was collected in aliquots and stored at -70 °C.

Microimmunofluorescence (MIF) studies were performed using a two-stage sandwich procedure (Savyon, Diagnostics LTD, Israel) according to the manufacturer's instructions. Specific IgM, IgG and IgA

antibodies to *C. pneumoniae*, *C. trachomatis* and *C. psittaci* were measured. The sources of antigen were *C. pneumoniae* TW-183, *C. trachomatis* L_2 and *C. psittaci* (SZ-1), respectively. Each of the 21-well slides contained individual spots of antigen of these *Chlamydia* species. Serum was diluted to 1:64, 1:32 and 1:20 for initial screening of IgG, IgA and IgM antibody, respectively.

Serum was treated with IgG inactivation reagent before dilution to detect IgM antibody to avoid interference that can cause a false positive reaction [11].

An IgM titer equal to or greater than 1:20 was considered as indicating a primary *C. pneumoniae* infection. Serum positive for IgG (≥1:64) was tested again with a 1:512 dilution. A secondary *C. pneumoniae* infection was characterized by the absence of IgM response and an IgG titer equal to or greater than 1:512.

Results

IgM to *C. pneumoniae* was not found in any of the 229 patients. The prevalence of isolation of the three strains of *Chlamydia* is shown in table 1.

The age-specific prevalence of *C. pneumoniae* IgG is shown in table 2. The prevalence was 81.8% (9/11) in patients from 11 to 20 years old. A prevalence of 48% (12/25) was found in patients from 21 to 30 years old. Patients aged from 31 to 60 years old had a steady prevalence which ranged from 62% to 65%. IgG titer equal to or higher than 1:512 was noted in 10.9% (25/229). The prevalence of *C. pneumoniae* was significantly higher in the elderly group (> 60 years) than that in the younger group (≤ 60 years) ($p < 0.001$). *C. pneumoniae* IgG equal to or greater than 1:64 was found in a higher percentage of male patients compared with female patients (69.2%, 119/172 vs. 30.8%, 53/172; $p = 0.1010$). Patients older than 60 years (vs. ≤ 60 years) were more likely to have higher *C. pneumoniae* infection (IgG ≥ 1:512) ($p = 0.1107$).

C. pneumoniae infection was associated with underlying vascular occlusive diseases, for example, coronary artery disease (CAD), cerebrovascular accident (CVA), chronic obstructive pulmonary disease (COPD) and asthma (Table 3). Cigarette smoking has been considered a risk factor for *C. pneumoniae* infection. However, in this study none of these was risk factor for *C. pneumoniae* infection (IgG ≥ 1:512).

The clinical features of patients with *C. pneumoniae* pneumonia in this series are shown in table 4. Most of the patients had fever (92%) with productive cough (52%). Chest radiograph showed infiltrate over bilateral lung fields in 60% of patients, unilateral in 24% and pleural effusion in 16%. Six patients had respiratory failure and five of them were over 60 years old and had other underlying diseases. The reason for no documentation of cough (40% of patients) may have been due to unconsciousness or respiratory failure. Fifty-six percent of patients with *C. pneumoniae* pneumonia had a white blood cell (WBC) count over 10,000/mm^3.

Table 1. Seroprevalence of *Chlamydia* in patients with community-acquired pneumonia

Antibodies	No. of patients with positive serology [a] (%)		
	C. pneumoniae	*C. trachomatis*	*C. psittaci*
IgM (n = 229)	0	3 (1.3)	1 (0.4)
IgG (n = 229)	172 (75.1)	24 (10.5)	4 (1.7)
IgA (n = 185)	90 (48.6)	3 (1.6)	0

[a] IgM antibody titer ≥ 1:20, IgG antibody titer ≥ 1:64 and IgA antibody ≥ 1:32 were considered as positive results.

Discussion

Pneumonia and bronchitis are the most frequently

Table 2. Distribution of *C. pneumoniae* IgG titers among different age groups in patients with community-acquired pneumonia

Age (yr)	No. of patients	Distribution of IgG titers (%)			
		< 1:64	≥ 1:64 - < 1:512	≥ 1:512	≥1:64 in total
1-10	1	1 (100)	0	0	0
11-20	11	2 (18.2)	8 (72.7)	1 (9.1)	9 (81.8)
21-30	25	13 (52.0)	11 (44.0)	1 (4.0)	12 (48.0)
31-40	22	8 (36.4)	12 (54.5)	2 (9.1)	14 (63.6)
41-50	21	8 (38.1)	12 (57.1)	1 (4.8)	13 (61.9)
51-60	23	8 (34.8)	13 (56.5)	2 (8.7)	15 (65.2)
61-70	33	7 (21.2)	22 (66.7)	4 (12.1)	26 (78.8)
71-80	59	7 (11.9)	44 (74.6)	8 (13.6)	52 (88.1)
81-90	32	3 (9.4)	23 (71.9)	6 (18.8)	29 (90.6)
91-100	2	0	2 (100)	0	2 (100)
Total	229	57 (24.9)	147 (64.2)	25 (10.9)	172 (75.1)

Table 3. Risk factors for *C. pneumoniae* infection (IgG ≥ 1:512) in patients with community-acquired pneumonia

Underlying disorder[a]	*C. pneumoniae* IgG ≥1:512		*p*
	Yes (n = 25)	No (n = 204)	
CAD	16.0% (4/25)	8.3% (17/204)	0.3753
CVA [b]	20.0% (5/25)	10.3% (21/204)	0.2678
COPD	16.0% (4/25)	7.8% (16/204)	0.3235
Bronchial asthma	12.0% (3/25)	9.3% (19/204)	0.9433
Cigarette smoker	44.0% (11/25)	31.4% (64/204)	0.2964
Age (years)			
> 60	72.0% (18/25)	52.9% (108/204)	0.1107
≤ 60	28.0% (7/25)	47.1% (96/204)	
Sex			
Male	76.0% (19/25)	64.7% (132/204)	0.3675
Female	24.0% (6/25)	35.3% (72/204)	

[a]Some patients had more than one disorder
[b]Including recent or previous CVA
Abbreviations: CAD = coronary artery disease; CVA = cerebral vascular accident; COPD = chronic obstructive pulmonary disease

Table 4. Clinical features of 25 patients with *C. pneumoniae* pneumonia

Clinical feature	No. of patients (%)
Fever ≥ 38.0 °C	23 (92)
Cough	
Productive	13 (52)
Nonproductive	2 (8)
Not documented	10 (40)
WBC > 10,000/mm^3	14 (56)
Radiographic finding	
Unilateral	6 (24)
Bilateral	15 (60)
Pleural effusion	4 (16)
Respiratory failure	6 (24)

Abbreviation: WBC = white blood cell count

recognized illnesses associated with *C. pneumoniae* infection. Because *C. pneumoniae* is difficult to isolate from biologic specimens, the evidence supporting its etiologic role in pneumonia is mainly serologic. *C. pneumoniae* has been implicated as a cause of community-acquired pneumonia in 6% to 12% of cases [12-14]. It is associated with bronchial asthma, COPD [2,11], pharyngitis, CAD [17] and cerebrovascular occlusive disease [18]. The results of this study may have been affected by sample selection bias because 315 patients in the target population were not enrolled. However, the decision to collect a serum sample from each patient was not dependent on the clinical setting.

The results of this study demonstrate that *C. pneumoniae* caused 10.9% of cases of hospitalized community-acquired pneumonia in our hospital during the study period. The seroprevalence of *C. pneumoniae* infection was 75.1% among patients with community-acquired pneumonia in this series. A high seroprevalence of *C. pneumoniae* in aged patients was reported [10]. The high seroprevalence of *C. pneumoniae* infection in Taiwan may be attributable to its location in a subtropical region and its high population density. Karvonen *et al* [19] found that the antibody prevalence to *C. pneumoniae* was unevenly distributed in Finland and was strongly dependent on the size of the unit area and population density. Chlamydial IgG antibody titers usually decrease slowly; whereas IgA antibodies tend to disappear rapidly. Secondary chlamydial infection is characterized by an absence of IgM response and a prompt IgG and IgA response. IgA antibodies have been shown to be a reliable immunological marker of primary, chronic and recurrent infections. These antibodies usually decline rapidly to baseline levels following treatment. In the present study, 48.6% (90/185) of patients had positive test results for *C. pneumoniae* IgA antibody which is indicative of recent infection.

None of the 229 patients in this study had IgM antibody to *C. pneumoniae*. This result suggests that most patients had developed *C. pneumoniae* infection prior to hospitalization. *C. pneumoniae* infection is often subacute, with symptoms last for many days or weeks, and *C. pneumoniae* pneumonia develops slowly, and serum IgM antibody to *C. pneumoniae* may thus be at a very low titer at the time of onset of pneumonia. Hospitalized patients who develop *C. pneumoniae* pneumonia are often older and have chronic illness [20]. In the present study, most of the hospitalized patients were older than 60 years of age. *C. pneumoniae*

pneumonia was more common in patients aged 60 years (14.3%, 18/126 vs. 6.8%, 7/103; $p = 0.111$). A higher seroprevalence and a higher rate of *C. pneumoniae* infection in adult men than in adult women were also found .

An association between CAD/atherosclerotic syndromes and *C. pneumoniae* infection has been previously suggested by both seroepidemiologic studies and the demonstration of the presence of the organism in atheroma. An association between cerebral vascular disease and previous *C. pneumoniae* infection, and of stroke/transient cerebral ischemia and recrudescence of *C. pneumoniae* infection have also been reported [18]. The role of *C. pneumoniae* in the exacerbation of COPD was recently examined, and some cases were found to have been associated with recent chlamydial infection [15]. Hertzen *et al* [16] showed that high levels of *C. pneumoniae*-specific IgA antibodies were more frequently found in elderly male patients with COPD than in controls without the disease, suggesting an association between COPD and chronic *C. pneumoniae* infection. Blasi *et al* [15] also found that the prevalence of positive MIF test for *C. pneumoniae* in COPD was remarkably higher than in control subjects, particularly in those aged over 50 years. The results of the present study showed that COPD patients had a higher seroprevalence of *C. pneumoniae* antibody.

Infection with *C. pneumoniae* is much more common than that with other *Chlamydia* species and reinfection is also common [8]. The clinical significance of these frequent reinfections remains unclear and their clinical manifestations are not unique to pulmonary infection with *C. pneumoniae* [16]. Pacheco *et al* [19] reported four cases of community-acquired pneumonia due to *C. pneumoniae*, and all four presented with chronic cardiopulmonary disease. The symptoms and signs in their patients included fever, cough, pleuritic chest pain and leukocytosis. In the present study, fever (92%) was the most common manifestation. Other symptoms and signs included cough (productive 52%, nonproductive 8%. Leukocytosis with a WBC count over 10,000 was found in 56% of patients, and chest x-ray showed that bilateral infiltrate (60% of patients) was more common than unilateral infiltrate (24% of patients) and pleural effusion (16% of patients).

In conclusion, *C. pneumoniae* is an important cause of respiratory tract infection in Taiwan. Patients with community-acquired pneumonia had positive *C. pneumoniae* IgG in 75.1% of cases. *C. pneumoniae* infection accounted for 10.9% of cases of community-acquired pneumonia in this study. Most primary *C. pneumoniae* infections are probably acquired before 30 years of age. A high prevalence for *C. pneumoniae* infection was noted in aged patients (> 60 years) and *C. pneumoniae* reinfection accounted for 14.3% of community-acquired pneumonia in aged patients (> 60 years).

The most common clinical manifestations in the present study included fever, productive cough, leukocytosis (WBC > 10,000/mm^3) and bilateral lung infiltrate.

Acknowledgment

This study was supported by a grant from Tri-Service General Hospital (TSGH-C87-09).

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