PREVALENCE AND CLINICAL FEATURES OF *CHLAMYDIA PNEUMONIAE* PNEUMONIA AT SRINAGARIND HOSPITAL, KHON KAEN, THAILAND

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Abstract. Between October 2000 and December 2002, a prospective study was conducted among hospitalized community acquired pneumonia (CAP) patients admitted to Srinagarind Hospital, Khon Kaen, Thailand. The diagnosis of *Chlamydia pneumoniae* infection was based on serologic testing. The prevalence of *C. pneumoniae* among patients hospitalized with CAP was 8.7%; 24 cases of 276 hospitalized CAP patients. The mean age was 42.7 (range, 17-79) years and the male to female ratio was 1:2.4. More than half (54.2%) of them were without underlying disease. The mean duration of symptoms prior to admission was 5.5 (SD 3.7) days. Leukocytosis was found in 62.5% of patients. Localized patchy alveolar infiltration was the most common radiographic finding, followed by bilateral interstitial infiltration. Over half (52.4%) of the patients had a non-productive cough. Gram-positive diplococci or no organisms predominated in cases where adequate sputum was obtained. Dual infection was found in 45.8% of cases, mostly with *Streptococcus* spp or *Klebsiella pneumoniae.* Four patients (16.7%) had an initial clinical presentation of severe CAP; 3 of 4 had a dual infection. Ten patients (41.7%) received macrolides or a macrolide plus a third generation β-lactam at the beginning of management. Two patients (8.3%) did not improve clinically and were transferred home. The average hospital stay was 11.5 (range, 1-45) days. Parapneumonic effusions complicated 20.8% of the cases. Other complications included acute respiratory failure (16.7%), shock (8.3%), hospital-acquired pneumonia (8.3%), and acute renal failure (4.2%). We concluded that *C. pneumoniae* caused a wide variation of clinical presentations ranging from mild disease to severe CAP. Co-infection with other bacterial pathogens was a common finding. Use of macrolides or new fluoroquinolones as part of an initial therapeutic regimen should be considered to cover this organism.

INTRODUCTION

Community-acquired pneumonia (CAP) continues to be a major medical problem. In Thailand, pneumonia is the second most common infectious disease, but it causes the highest number of fatalities (Ministry of Public Health, 1998). Since CAP is potentially fatal, early appropriate antibiotic treatment is vital. *Chlamydia pneumoniae* is an atypical pathogen increasingly reported worldwide (Socan *et al*, 1999; Almirall *et al*, 2000; File, 2000). In Western countries, it has been found to account for up to 10% of CAP cases requiring hospitalization (Kauppinen and Saikku, 1995). The appropriate antibiotics to cover this organism include macrolides or the new-fluoroquinolone groups. Furthermore, *C. pneumoniae* has been reported to cause pneumonia in association with other microorganisms frequently (Liebermen *et al*, 1996). The diagnosis of *C. pneumoniae* infection usually requires a serology test, which is not performed on routine laboratory investigation.

The objectives of this study were: 1) to evaluate the prevalence of *C. pneumoniae* pneumonia in hospitalized CAP patients; and 2) to clarify the clinical presentation and treatment outcome of these patients.

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PATIENTS AND METHODS

Patients

This prospective study was carried out between October 2000 and December 2002 at Srinagarind Hospital, Khon Kaen University,

Khon Kaen, Thailand. Patients age 15 years or older admitted with CAP were included. The diagnosis of CAP was based on clinical signs and symptoms (presenting with three of five: fever, cough with or without productive sputum, dyspnea, pleuritic chest pain, and consolidation or crackles on physical examination), and radiographic pulmonary abnormalities (Thai Thoracic Society, 2001). The onset of symptoms and signs was less than or equal to 2 weeks. HIV positive patients, who were transferred from another hospital, or hospitalized within 3 weeks before admission were excluded.

Serum samples

A serum sample was obtained at the time of enrollment for serological testing. Convalescent sera were obtained 2-3 weeks later. All serum samples were immediately separated and stored at -20ºC until tested.

Serology for *C. pneumoniae* antibody detection

The Sero CP™-IgM (or IgG) Test is an enzyme immunoassay used for detecting IgM (or IgG) antibodies against *C. pneumoniae* in serum samples. The procedure was performed as described in the manufacturer's instructions. In brief, all the components of the reagent kit and specimens were brought to room temperature, and mixed before use. Fifty microliters of the two negative controls, one positive control and diluted sample serum (dilute serum 1:105 with diluent for IgM or IgG) each were dispensed into separated wells of the test strip and incubated for 1 hour at 37ºC in a moist chamber.

After incubation, the strips were washed with washing buffer 3 times then allowed to dry. Fifty microliters of horseradish peroxidase (HRP) conjugated anti-human IgM (or IgG) at a dilution of 1:300 was added into each well. The strips were further incubated for 1 hour at 37ºC in a moist chamber. After washing with buffer 3 times, 100 μl of tetramethylbenzidine (TMB) substrate was dispended into each well and incubated at room temperature for 15 minutes. The reaction was stopped by adding 100 μl of 1 M H_2SO_4. The absorbance at 450 nm wavelength was determined at 30 minutes.

For the test to be valid, it had to meet the following criteria: 1) The absorbance of the positive control should be ≥0.8; and 2) The average absorbance of the negative control should be >0.1 and ≤0.4

Calculation of cut-off value (COV) and cut-off index (COI)

The COV and COI were calculated according to the following formula:

COV = NC x 2; where NC = the average absorbance of negative control.

COI = Absorbance of the serum sample/COV

Interpretation of results

If the OD was <COV and the COI was <1.0, the result was negative (*ie* no IgM or IgG antibody was detected). If the COV≤OD≤1.1 x COV, and the COI was between 1 and 1.1, the result was borderline (*ie* a low level of IgM or IgG antibody). If the OD>1.1 x COV and the COI was >1.1, the result was positive (*ie* a relevant level of IgM or IgG antibody). A current *C. pneumoniae* infection was diagnosed when IgM antibody levels were significant or a current or previous infection was diagnosed when IgG antibody levels were significant.

If the initial and convalescent titers were both borderline the specimen was considered negative.

To differentiate between a past and current infection when testing for serum IgG, a rise in COI in the second sample by at least 40% was considered a current infection.

Ethics

This research was approved by the Ethics Committee of the Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

Statistical analysis

Descriptive statistics were used. The means and standard deviations were calculated for continuous data, and the numbers and percentages were calculated for the categorical data.

RESULTS

During the study period, 276 patients hospitalized with CAP were enrolled. A current infection with *C. pneumoniae*, as specified previously, was confirmed in 24 patients (8.7%). Seven were male and seventeen were female, with a male to female ratio of 1:2.4. The average patient age was 42.7 (SD 18.0) years, with

a minimum of 17 and a maximum of 79 years. Seven patients (29.2%) were farmers, and 13 (54.2%) of them had no underlying disease (Table 1).

The mean duration of symptoms prior to admission was 5.5 (SD 3.7) days. The mean white blood cell count on the first day was 11,925 cells/mm^3, with a range of 3,200 to 22,800 cells/mm^3. The most common pattern seen on initial chest radiograph was localized patchy alveolar infiltration (50%), commonly found in the lower lobes. Bilateral interstitial infiltration was detected in 37.5%, and lobar infiltration was seen in 12.5%. Four of twenty-four patients (16.7%) had pleural effusions detected on chest radiographs (Table 2). Thirteen patients (54.2%) had no sputum production. The microscopic findings on Gram's staining in the 11 sputums tested revealed 6 with gram-positive diplococci, 3 with no organism, and 2 with inadequate sputums (Table 2).

Four patients (16.7%) presented with severe CAP on initial presention, as defined by the American Thoracic Society (ATS) criteria (Niederman *et al*, 2001), which require one of two major criteria or two of three minor criteria. The major criteria are the need for mechanical ventilation or the presence of septic shock. The minor criteria are systolic blood pressure <90 mmHg, multilobar involvement, or Pao_2/Fio_2 <250.

Dual infections were found in 11 patients (45.8%); 3 with *C. pneumoniae* and *Streptococcus* spp, 3 with *C. pneumoniae* and *K. pneumoniae*, 2 with *C. pneumoniae* and *M. pneumoniae*, 1 with *C. pneumoniae* and *H. influenzae*, 1 with *C. pneumoniae* and *E. coli*, and 1 with *C. pneumoniae* and *B. pseudomallei* (Table 3). Three of the four severe CAP patients had dual infections: *C. pneumoniae* and *Streptococcus* spp, *C. pneumoniae* and *H. influenzae*, and *C. pneumoniae* and *B. pseudomallei*. Only one patient had *C. pneumoniae* as the sole pathogen.

Ten patients (41.7%) received macrolide or third generation β-lactam plus macrolide antibiotics at the beginning of management. The others were treated with a single agent: penicillin, third generation β-lactam, or a β-lactam-lactamase inhibitor antibiotic. Hospitalization averaged 11.5 (SD 10.0) days. Two patients did not improve clinically and were transferred to home. Twenty-two patients were discharged with clinical improvement. The complications with *C. pneumoniae* pneumonia included parapneumonic effusion (5), acute respiratory failure necessitat-

Table 1
Characteristics of 24 patients hospitalized with *C. pneumoniae* CAP.

Characteristic	N=24
Age, years mean (SD)	42.7 (18.0)
Male:female ratio	1 : 2.4
Incubation, days mean (SD)	5.5 (3.7)
Occupation N (%)	
Farmer	7 (29.2)
Employee	5 (20.8)
Business	3 (12.5)
Student	3 (12.5)
Government service	3 (12.5)
Retired	2 (8.3)
Monk	1 (4.2)
Healthy N (%)	13 (54.2)
Underlying disease N (%)	11 (45.8)
Diabetes mellitus	2 (8.3)
Systemic lupus erythrematosus	2 (8.3)
Chronic renal failure	1 (4.2)
Rheumatic heart disease	1 (4.2)
Myasthenia gravis	1 (4.2)
Chronic lymphoblastic leukemia	1 (4.2)
Grave's disease	1 (4.2)
Panuveitis	1 (4.2)
CA nasopharynx	1 (4.2)

Table 2
Initial laboratory findings.

Laboratory results	N=24
White blood cell count, cells/mm^3	
Mean (SD)	11,925 (5,590.8)
Range	3,200 - 22,800
Patients with WBC >10,000 cells/mm^3	
N (%)	15 (62.5)
Patients with no sputum production N (%)	13 (54.2)
Results of sputum Gram's staining N (%)	
Inadequate	2 (8.3)
No organism	3 (12.5)
Gram-positive diplococci	6 (25)
Results of chest radiograph N (%)	
Localized patchy alveolar infiltration	12 (50)
Bilateral interstitial infiltration	9 (37.5)
Lobar infiltration	3 (12.5)
Pleural effusion	4 (16.7)

Table 3
Dual infection in 11 patients.

Dual infection	N
C. pneumoniae and *Streptococcus* spp	3
C. pneumoniae and *K. pneumoniae*	3
C. pneumoniae and *M. pneumoniae*	2
C. pneumoniae and *H. influenzae*	1
C. pneumoniae and *E. coli*	1
C. pneumoniae and *B. pseudomallei*	1
Total	11

Table 4
Treatment outcomes.

Outcome	N= 24
Hospitalization (days)	
Mean (SD)	11.5 (10.0)
Range	1-45
Outcome of treatment N (%)	
Improved	22 (91.7)
Not improved	2 (8.3)
Complication N (%)	
Parapneumonic effusion	5 (20.8)
Acute respiratory failure	4 (16.7)
Shock	2 (8.3)
Hospital-acquired pneumonia	2 (8.3)
Acute renal failure	1 (4.2)

ing mechanical ventilation (4), shock (2), hospital-acquired pneumonia (2), and acute renal failure (1) (Table 4).

DISCUSSION

The prevalence of *C. pneumoniae* in our hospitalized CAP patients was 8.7%, which is lower than previously repored in Thailand (16.3%) (Wattanathum *et al*, 2003), higher than Japan (3.4%) (Ishida *et al*, 1998), and the same as in the western countries (about 10%) (Kauppinen and Saikku, 1995). More than half the patients had no underlying disease. The ages of patients ranged between young adults and the elderly. *C. pneumoniae* was more likely to infect healthy patients in no specific age group, though, the elderly had more severe clinical manifestations.

In general, *C. pneumoniae* causes mild, atypical pneumonia (Cook and Honeybourne, 1995; Miyashita *et al*, 2002). In our study, 16.7% of *C. pneumoniae* pneumonia presented with severe CAP. Most of them had a dual infection with other bacterial pathogens, such as *Streptococcus* spp, *H. influenzae*, or *B. pseudomallei.* Severe pneumonia due to *C. pneumoniae* has been previously described (Consentini *et al*, 1996; Panagou *et al*, 1996). The clinical presentation varied between a mild disease and life-threatening adult respiratory distress syndrome. *C. pneumoniae* has been reported to cause pneumonia in association with other respiratory pathogens, mainly *S. pneumoniae* (Lieberman *et al*, 1996; Miyashita *et al*, 2002). In our study, 45.8% of the *C. pneumoniae* pneumonia patients had a concomitant infection with other microorganisms. Therefore, inappropriate antibiotics might have been given if the decision depended on only bacterial culture results and serological testing for *C. pneumoniae* was not done. This is a possible reason for increased morbidity and prolonged hospitalization (Stahl *et al*, 1999; Brown *et al*, 2003).

There were no signs or symptoms that are unique to *C. pneumoniae* pneumonia. More than half the patients had a non-productive cough. This is a clinical feature often found in atypical pathogen infections (Lieberman, 1999). Leukocytosis and localized patchy alveolar infiltration were common findings, which cannot be used to differentiate pneumonia due to *C. pneumoniae* from other etiologies (Kauppinen *et al*, 1996; File *et al*, 1999). In patients who were able to expectorate appropriate specimens, if the Gram's staining showed polymorphonuclear leukocytes without an organism, this could indicate *C. pneumoniae* infection. However, the results of Gram's staining of sputum from our patients may mislead the clinician in inappropriate antibiotic use, because most of them showed gram-positive diplococci.

The mortality rate for *C. pneumoniae* is relatively low. A meta-analysis by Fine *et al* (1996) determined that patient mortality for *C. pneumoniae* was 9.8% (range, 4.5% to 15.8%). In our study, 8.3% of the patients' clinical symptoms did not improve, so they were transferred home. Parapneumonic pleural effusion was a complication in about 20% of the patients, the same as in a previous report (Kauppinen *et al*,

1996). Hospitalization averaged 10 days, but varied widely from 1 to 45 days. Since *C. pneumoniae* is an intracellular organism, β-lactams are ineffective. Macrolides, tetracyclines, and the new fluoroquinolones have shown excellent activity against this organism. They are effective in reducing the length of symptoms and hospitalization (Kauppinen and Saikku, 1995; Stahl *et al*, 1999; Brown *et al*, 2003). Only 40% of our patients received a macrolide or a β-lactam plus a macrolide for initial treatment.

In conclusion, *C. pneumoniae* is a common atypical pathogen in CAP requiring hospitalization. It is often a co-pathogen in CAP. The clinical spectrum varies from mild disease to adult respiratory distress syndrome necessitating mechanical ventilation. The radiographic and laboratory manifestations of the disease are similar to those of CAP caused by other pathogens, therefore, reliable etiological differentiation cannot be based on these factors alone. There is a possibility of shortening treatment time by using appropriate initial empiric antimicrobial therapy until laboratory results can be obtained to guide more specific therapy.

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