
Acute primary *Chlamydophila pneumoniae* bronchitis and bronchial hyperresponsiveness in young nonasthmatic Thai military recruits

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Background: A correlation between chronic *Chlamydophila pneumoniae* infection and chronic airway diseases has been suggested by several studies, but direct evidence to support the link between acute *C pneumoniae* infection and new-onset asthma is insufficient.

Objectives: To determine the association between *C pneumoniae* infection and subsequent bronchial hyperresponsiveness (BHR) and hence asthma.

Methods: We studied 110 Thai military conscripts during an epidemic of *C pneumoniae* infection in Thailand, from November 1, 1998, through February 28, 1999. The diagnosis was based on a standardized microimmunofluorescence technique. Spirometry and methacholine challenge tests (MCTs) were conducted. This cohort study excluded all conscripts with preexisting positive MCTs.

Results: Ninety-three percent of the conscripts presented with an acute cough of a mean duration of 15.2 days. The pattern of serologic response revealed that 83% had acute primary infections and 10% had acute reinfections. Mean forced expiratory volume in 1 second was 99.5%, with an improvement of 1.8% after bronchodilator administration. Only 3.6% revealed small airways disease (forced expiratory flow between 25% and 75% of <65%) on spirometry. Methacholine challenge tests failed to demonstrate BHR in all conscripts, and none had developed a new-onset wheeze (physician-diagnosed asthma) at up to 2 years of follow-up.

Conclusions: This study demonstrates that cough in patients with acute primary *C pneumoniae* infection is not associated with BHR among previously healthy adults. The pathogenetic mechanism by which organisms cause coughing and wheezing in acute bronchitis seems to be different among causative respiratory pathogens.

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INTRODUCTION

Acute bronchitis is considered to be an inflammatory disease of the lower airways that is characterized by an acute cough concurrent with constitutional symptoms initially and followed by airway hyperreactivity of 1 to 3 weeks' duration.^{1,2} It is frequently caused by respiratory viruses, *Bordetella pertussis*, or *Mycoplasma pneumoniae*.^{1–3} Only 15% to 30% of patients with acute bronchitis in primary care studies exhibit evidence for reversible airway obstruction that implies bronchial hyperresponsiveness (BHR).⁴ On a few occasions, this entity is clinically indistinguishable from occult

asthma and may trigger asthmatic exacerbation among susceptible patients.^{5,6}

Chlamydophila pneumoniae, an obligatory intracellular bacteria, can cause both upper respiratory tract infections (URTIs) and lower respiratory tract infections (LRTIs). In patients with *C pneumoniae* mild and asymptomatic disease is commonly encountered, whereas among patients without pneumonia acute bronchitis is a frequent diagnosis.^{7–9} Hahn et al¹⁰ first reported an association between *C pneumoniae* and asthma. Most of their study population had serologic evidence of possible *C pneumoniae* chronic infection or reinfection but not acute primary infection. They postulated that repeated or persistent infection contributes to chronic airway diseases. However, most previous studies of this association did not exclude other copathogens.¹¹

Most studies that describe the inflammatory characteristics of postinfectious airways include pulmonary function test (PFT) results; however, these studies generally make conclusions on the pathophysiology of acute bronchitis based on the original studies of influenza A virus and *M pneumoniae*, assuming that *C pneumoniae* would produce the same results.^{12–15} To our knowledge, only 1 case report has described the inflammatory characteristics of the airways and provided PFT results of a previously healthy individual in detail.¹⁶ Our

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study is the first, to our knowledge, to conduct a detail investigation of clinical manifestations and PFT results in conscripts with sole *C pneumoniae* infection.

MATERIALS AND METHODS

This study was performed on a group of military conscripts from 1 garrison in Bangkok, Thailand, from November 1, 1998, through February 28, 1999. All candidates with a positive methacholine challenge test (MCT) result (provocation concentration that caused a 20% reduction in forced expiratory volume in 1 second [FEV₁] $\leq$ 8 mg/mL and at least 15% reversibility in FEV₁ after bronchodilator administration) were eliminated from the pool of recruits, whether or not they had clinical asthma. A total of 110 conscripts, undergoing a 24-month compulsory military training, were enrolled in the study. They were all 21-year-old men who underwent interviews with the standardized questionnaire, modified and validated from the International Union Against Tuberculosis and Lung Diseases questionnaire,¹⁷ in addition to periodic examination and monitoring for clinical asthma by the same physician (W.S.) every 3 months for up to 2 years. All laboratory tests were conducted at Phramongkutklao Hospital and King Chulalongkorn Memorial Hospital in Thailand.

Physiologic and Radiographic Evaluation

The study protocol was approved by the ethic review board, and written informed consent was obtained. The patients underwent PFTs, including bronchodilator reversibility, with an automated body plethysmograph (SensorMedics, Yorba Linda, California) according to the American Thoracic Society guidelines¹⁸ 3 weeks after the onset of respiratory symptoms. The diagnosis of small airways disease was made whenever the forced expiratory flow between 25% and 75% (FEF_{25%-75%}) was less than 65% predicted.^{19,20} Chest radiography was performed in all conscripts to exclude pneumonia and other relevant conditions in the same period.

Microbiological Studies

Paired serum samples from centrifuged venous blood samples, collected at enrollment and 8 weeks later, were immediately frozen at -70°C . Microimmunofluorescence, using formalinized elementary bodies of *C pneumoniae* (a gift from J. Thomas Grayston, Washington Research Foundation, Seattle), *Chlamydia trachomatis*, and *Chlamydophila psittaci* as antigens for IgG, IgM, and IgA titer measurement, was performed to exclude serologic cross-reactivity. The clinical conditions and serologic study results were interpreted as an acute primary infection whenever the IgM titer was 1:16 or greater in either sample or when 4-fold increases were present; as an acute reinfection whenever the IgG titer was 1:512 or greater in either sample or when 4-fold increases without increases of IgM titer were present; and as a possible chronic persistent infection whenever the IgG titer was 1:8 to 1:256 accompanied by an IgA titer of 1:16 or greater.²¹ Rheumatoid factor was absorbed before IgM and IgA titrations to exclude a nonspecific reactivity. All serum samples

were screened for the presence of IgM and IgG antibodies to *M pneumoniae* by a microparticle agglutination assay (Serdia Myco II kit, Fujirebio Inc, Tokyo, Japan). Positive results were confirmed by an enzyme-linked immunosorbent assay (ELISA).

Streptococcus pneumoniae, *B pertussis*, and *Bordetella parapertussis*

To exclude infections caused by these organisms, oropharyngeal swabs were inoculated onto Regan and Rowe media (charcoal agar plate supplement with 10% sheep red blood cells and 40 $\mu\text{g/mL}$ of cephalexin), incubated at 37°C and subcultured after 72 hours, and slide agglutination with specific antisera to *B pertussis* and *B parapertussis* were used for identification. In addition, the antibody titer against *B pertussis* was assayed by the ELISA technique using pertussis toxin and filamentous hemagglutinin as antigens in all patients. Acute *B pertussis* infection was defined as a 4-fold increase in titer on paired serum samples.

Viral Study

Serum samples obtained at presentation and 8 weeks later were also analyzed by ELISA and indirect hemagglutination assay for antibody to the following agents: influenza virus type A and B; parainfluenza virus types 1, 2, and 3; adenovirus; respiratory syncytial virus; and herpes simplex virus. Positive test results were defined by a 4-fold increase in the titer. An indirect immunofluorescence test was used to detect antibodies to *Legionella* species.

Allergy Skin Tests

Allergy skin tests were performed by the skin prick method with 12 common standardized allergen extracts (Greer Laboratories, Lenoir, North Carolina) in all conscripts. Histamine and buffered saline were used as positive and negative controls, respectively. A 15-minute wheal greater than 3 mm was interpreted as a positive result. Patients with at least 1 positive skin test result were considered to be atopic or allergic.

Methacholine Challenge Tests

Standardized MCTs were performed with sequential methacholine concentrations of 0 (isotonic sodium chloride solution), 0.03, 0.06, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, and 25.0 mg/mL, using the 5-breath dosimeter method recommended by the American Thoracic Society.²² A volume of 1 mL was administered by dosimeter during 5 inhalations. Spirometry was evaluated 1 and 3 minutes later, and the interval between doses was 5 minutes. The procedure was discontinued if the FEV₁ decreased by at least 20% from the baseline level or the FEV₁ was 1.5 L or less. The provocation concentration that caused a 20% reduction in FEV₁ was calculated and labeled as a positive result whenever the concentration was 8 mg/mL or less and at least 15% reversibility was found in FEV₁ 10 minutes after bronchodilator administration (2 puffs of 100 μg of salbutamol via metered-dose inhaler). This procedure was performed in all patients at 3 weeks after the onset of respiratory symptoms.

Statistical Analyses

Statistical analyses were performed with SPSS (version 10; SPSS Inc, Chicago, Illinois) and Epi Info (version 6; Epi Info, Centers for Disease Control and Prevention, Atlanta, Georgia) statistical software. The χ^2 test with Yates correction was applied for comparisons, and $P < .05$ was considered statistically significant.

RESULTS

Two military conscripts developed community-acquired pneumonia and were hospitalized for 5 days when they were recruited and arrived at this garrison. After discharge from the hospital, they returned to the garrison and used a common bedroom and dining room with others during recovery.

Serologic studies of both conscripts were performed, and the results confirmed that both of them had an acute primary *C pneumoniae* infection without other concomitant respiratory pathogens. Subsequently, almost all conscripts in this building had acute respiratory illnesses with a mean incubation period of 22.8 days (range, 21–30 days) without administration of antibiotics. The summary of the important clinical data are given in Table 1.

Of the 110 patients, 82 (74.5%) were current smokers with a smoking history of 5 pack-years. Forty-two patients (38.2%) had a skin prick test result positive for allergy, with results positive for clinical allergic rhinitis in 7.3%. All chest radiographs revealed no abnormalities.

Seventy-three percent of the conscripts with acute respiratory illness were classified as having URTIs, whereas 27% developed LRTIs. None had developed asthmatic symptoms or new-onset wheeze at up to 2 years of follow-up.

Cough was the most common presenting symptom in both groups, and the duration of cough was significantly longer ($P < .01$) in cases of LRTI when compared with cases of URTI, particularly in patients who demonstrated small airways disease on PFT results. Persistent dry cough of 2 to 4 weeks' duration is the most prominent symptom. The mean duration of cough was longer in smokers (15.2 days; range, 3–42 days) vs nonsmokers (14.6 days; range, 3–33 days), those with LRTIs (20.3 days; range, 7–42 days) vs URTIs (14.7 days; range, 3–28 days), and those with small airways disease apparent on PFT results (19.1 days; range, 7–42 days) vs those without small airways disease (14.7 days; range, 3–33 days). Cough in acute bronchitis of those with LRTIs tended to be productive (70%), whereas a dry cough occurred in those with URTIs. Other reported symptoms were rhinitis, hoarseness, headache, and malaise.

The results of microbiological and serologic tests for other bacteria, *M pneumoniae*, *Legionella* species, and respiratory viruses were negative, but those for *C pneumoniae* were positive. Almost all conscripts (83.1%) were diagnosed as having acute primary infection and 10% had an acute reinfection. The prevalence of possible persistent antibody (IgA) to *C pneumoniae* was 84.7%, with a geometric mean titer and median IgA level of 80.9 and 64, respectively. This high prevalence rate lasted throughout the 3-month study period.

Table 1. Demographics and Baseline Characteristics of the 110 Male Study Participants

Characteristics	Finding
Mean age, y	21
Smoking history of 5 pack-years, No. (%)	82 (74.5)
URTI/LRTI, No. ^a	80/30
Mean $\pm$ SD duration of cough, days	15.2 $\pm$ 7.1
Mean $\pm$ SD FVC, % predicted	98.53 $\pm$ 9.86
Mean $\pm$ SD FEV ₁ , % predicted	99.45 $\pm$ 10.10
Mean $\pm$ SD FEV ₁ reversibility, % baseline ^b	1.78 $\pm$ 2.88
Mean $\pm$ SD FEV ₁ /FVC ratio, %	86.7 $\pm$ 4.2
Mean $\pm$ SD FEF _{25%-75%} , % predicted	83.06 $\pm$ 19.14
FEF _{25%-75%} <65%, No. (%)	4 (3.6)
Mean $\pm$ SD PEFR, % predicted	102.41 $\pm$ 14.84
Acute primary <i>Chlamydia pneumoniae</i> infection/acute reinfection, % ^c	83/10
PC ₂₀ >8 mg/mL on MCT, No. (%)	110 (100)

Abbreviations: FEF_{25%-75%}, forced expiratory flow between 25% and 75%; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; LRTI, lower respiratory tract infection; MCT, methacholine challenge test; PC₂₀, provocative concentration that caused a 20% reduction in FEV₁; PEFR, peak expiratory flow rate; URTI, upper respiratory tract infection.

^a URTI is defined as rhinorrhea, sore throat, cough with or without sputum, and/or fever accompanied by normal breath sounds and normal chest radiography results; LRTI is defined as bronchitis, a productive cough with or without fever, and adventitious lung sounds with normal chest radiography results; and pneumonia is defined as acute respiratory symptoms accompanied by the presence of a new-onset infiltrate apparent on a chest radiograph.

^b Measured at 20 minutes after 2 puffs of 100 μ g of salbutamol via metered-dose inhaler.

^c The prevalence of *C pneumoniae* seropositivity (IgG $\geq$ 1:16) among healthy Thai adults with a mean age of 48.8 years (range, 16–90 years) was 88.2%, whereas the prevalence among 20-year-old medical students was 81.6%.

No statistical correlations ($P > .05$) were found between geometric mean titer IgA and both URTI and LRTI or the presence of small airways disease.

The mean and median values of FEV₁ were 99.5%, with a reversibility of 1.8%. Only 3.6% revealed small airways disease (FEF_{25%-75%} <65%) on spirometry. Results of MCTs failed to demonstrate BHR (provocation concentration that caused a 20% reduction in FEV₁ of >8 mg/mL) in all conscripts (Table 1). All conscripts were examined and monitored for symptoms and signs of asthma every 3 months for up to 2 years, and none developed a new-onset wheeze (physician-diagnosed asthma).

DISCUSSION

BHR is defined as an exaggerated bronchoconstrictor response to a wide variety of stimuli that generally elicit little or no response in healthy individuals. Clinically, methacholine or histamine has been administered as an aerosolized provocative agent to elicit this airway hyperreactivity.²² Currently, airway inflammation is accepted as the cause of

BHR.²³ Not only aeroallergens but also several respiratory viruses are believed to induce BHR.^{24,25} Even though high prevalence rates (up to 27%) of BHR can be found in healthy white adults,^{26,27} the prevalence of BHR (3.31%) and asthma (2.91%) among Thai adults is substantially lower than that of reported western countries.²⁸

Originally, several respiratory viruses were reported to be the causative organisms of wheezing and infection-mediated BHR.^{24,25,29} Subsequently, other atypical pathogens, such as *M pneumoniae* and *C pneumoniae*, have been reported to be associated with wheezing as well.^{10,11} These organisms may induce airway inflammation and hence the risks of developing allergy and asthma.³⁰ Current data demonstrate that these respiratory pathogens may coordinate with epithelial cells, endothelial cells, leukocytes, and dendritic cells in activating and perpetuating the airway inflammation through secretion of relevant cytokines, inactivation of inhibitory mediators, and alteration in neural control of the airways, leading to BHR.^{24,29–32}

Acute viral illnesses can induce inflammatory infiltrate and bronchiolar edema, contributing to bronchiolitis.^{25,33} Influenza A and respiratory syncytial virus can temporarily produce airway epithelial damage and lead to a new-onset wheeze.^{29,30} Little et al¹² demonstrated that healthy college students can develop transient BHR for at least 7 weeks after a nonpneumonic influenza A infection. A retrospective medical record review by Williamson et al⁶ demonstrated that one third of patients with acute bronchitis made return visits for treatment of a persistent cough and a 10-fold increase in the rate of subsequent visits for asthma. At least 2 mechanisms were postulated to be the causes: cholinergic hyperresponsiveness caused by sensitization of rapidly adapting airway receptors to inhaled irritants through a vagally mediated mechanism that could be prevented by atropine and an exaggerated immune response caused by developing specific IgE antibodies to the aforementioned respiratory viruses.^{13,14,24,34,35}

M pneumoniae can cause URTI, bronchitis, and obliterative bronchiolitis. It can present as prolonged cough of 4 to 6 weeks' duration.^{2,3,36} Mok et al³⁷ performed PFTs serially in 50 children with preceding *M pneumoniae* respiratory infections with a median follow-up time of 2.5 years (range, 1.5–9.5 years), and almost half of them had recurrent wheezing or asthma. They demonstrated an impairment of small airways function with normal large airway parameters. However, exercise challenge test results were negative among these children except in those known to have asthma. This organism has also been proposed to be involved in the asthma pathogenesis. However, the true mechanism of mycoplasma-mediated BHR and the link between this infection and asthma pathogenesis has not been established to date.^{38,39}

Controversies exist about whether acute *C pneumoniae* infection is associated with new-onset wheeze and late-onset asthma.¹¹ Acute persistent cough without wheezing is the predominant symptom among epidemiologic studies^{8,9,40} in contrast to the development of recurrent wheezing after re-

spiratory virus infections.^{29,41–43} Hahn et al⁴⁴ described 10 adult patients with de novo wheezing, all of whom had acute *C pneumoniae* infections based on serologic criteria. Four had spontaneous resolution of wheezing, 5 developed persistent asthma, and 1 developed chronic bronchitis. In addition, they demonstrated that the development of chronic asthma may be related to persistent *C pneumoniae* infection.⁴⁵ However, their data did not exclude the possibility of preexisting bronchial hyperreactivity or other markers for occult asthma in patients who developed chronic respiratory illness after infection. Most recently, 1 study⁴⁶ performed in a longitudinal fashion concluded that patients with serologically diagnosed chronic *C pneumoniae* infection did not have a higher risk for new-onset asthma, but this infection was found to accelerate the loss of lung function significantly among new nonatopic asthmatic patients. However, virtually all previous studies did not perform laboratory tests to exclude copathogens as the cause of infection-induced BHR.

Pizzichini et al¹⁶ conducted MCTs in addition to the serial PFTs in 1 patient and concluded that BHR was not associated with an acute *C pneumoniae* infection. This patient had a nonproductive cough accompanied by normal FEV₁ that resolved spontaneously by day 11. Analyses of induced sputum samples revealed increased lymphocyte counts followed by an increase in neutrophils and a decrease in the CD4/CD8 ratio. Another report demonstrated that acute *C pneumoniae* infection in patients with acute asthma produced increased neutrophils and sputum eosinophilic cationic protein levels⁴⁷ similar to the animal and in vitro studies demonstrated by Yang et al.^{48,49}

To our knowledge, this study is the largest to describe BHR in apparently healthy individuals who are acutely infected with sole *C pneumoniae*. Our study definitely shows that acute primary infection caused by *C pneumoniae* is not associated with BHR. This finding is important, because it raises doubt about the theory that BHR is caused by airway inflammation alone. However, this study could not exclude the possibility that BHR developed later or that clinical asthma could occur after 2 years or later in patients infected with chronic *C pneumoniae*. We postulate that although respiratory infections can induce airway inflammation, only certain specific pathogens, timing of infection, and the genetic predisposition of the patients are also involved in the induction of asthma.³⁸ This study also emphasizes the complexity of the pathogenesis of BHR, which deserves additional investigation.

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