



Seroprevalence of *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* in children with chronic cough

Kronik öksürüklü çocuklarda *Chlamydia pneumoniae* ve *Mycoplasma pneumoniae* serolojisi

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ABSTRACT

Objective: Chronic cough is one of the most common symptoms in children. Post infectious causes following *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Bordetella pertussis* infections play important role in development of chronic cough. Aim of this study was to evaluate *M. pneumoniae* and *C. pneumoniae* serology and diagnosis and treatment in children with chronic cough according to American College of Chest Physicians (ACCP) guideline.

Materials and Methods: Pulmonary function tests (PFTs) and chest x-rays were obtained in all patients after detailed medical history and physical examination. Blood specimens for *M. pneumoniae*, *C. pneumoniae* serologies were analyzed by enzyme-linked immunosorbent assay (ELISA). Patients were re-evaluated in 2-4 weeks intervals until cough disappeared.

Results: The study included 41 children, between 6 and 14 years of age. The mean age was 8.00 ± 1.96 year. PFTs and chest x-rays were within normal limits in all children. The children with wet cough, comprising 68% of the patients, received 10-day course of Clarithromycin, while those with dry cough were treated with inhaled steroid. *M. pneumoniae* IgM positivity was found in 17.07% (7/41), *C. pneumoniae* IgM positivity in 2.85% (1/35), *M. pneumoniae* IgM and/or IgG positivity in 41.46% (17/41), and *C. pneumoniae* IgM and/or IgG positivity in 25.7% (9/35) of patients. Seropositive patients with *M. pneumoniae* and/or *C. pneumoniae* were diagnosed as protracted bronchitis, upper airway cough syndrome and asthma-like disease according to the guideline.

ÖZ

Giriş: Kronik öksürük çocuklarda sık görülen bir yakındır. Postinfeksiyöz nedenler, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* ve *Bordetella pertussis* infeksiyonlarını takiben daha sık görülen bir yakındır. Bu çalışmada kronik öksürüklü çocuklar American College of Chest Physicians (ACCP) tarafından önerilen algoritmaya göre tanı ve tedavi edilerek, *M. pneumoniae* ve *C. pneumoniae* serolojisi değerlendirilmiştir.

Gereç ve Yöntem: Dört haftadan uzun süren öksürük yakınması ile başvuran hastalardan ayrıntılı öykü ve fizik muayene yapıldıktan sonra solunum fonksiyon testi (SFT) yapıldı ve akciğer grafisi çekildi. Hastalar ACCP algoritmasına göre değerlendirilip tanı ve tedavi uygulanırken *M. pneumoniae* ve *C. pneumoniae* serolojisi kan örneklerinden ELISA ile çalışıldı. Hastalar öksürükleri düzeleneye kadar 4-6 hafta ara ile değerlendirildi.

Bulgular: Çalışmaya 6-14 yaş arasında 41 hasta alınmıştır. Hastaların yaş ortalaması 8.00 ± 1.96 yıldır. SFT ve akciğer grafisi hepsinde normal sınırlardaydı. Hastaların %68'inde yaş öksürük vardı. ACCP algoritmasına göre bunlara 10 gün klaritromisin (15 mg/kg/gün), kuru öksürüğü olan hastalara ise inhale steroid (flutikazon 125 µg 2 x 1) verildi. Serolojik değerlendirmede; IgM ve/veya IgG pozitifliği *M. pneumoniae* için %41.46 (17/41), *C. pneumoniae* için %25.7 (9/35), IgM pozitifliği *M. pneumoniae* için %17.07 (7/41)'sinde, *C. pneumoniae* için %2.85 (1/35) olarak saptandı. Hastalar sıklık sırasına uzamış bronşit, üst hava yolu kaynaklı öksürük sendromu ve astım benzeri hastalık olarak tanı aldı.

Conclusion: In school-age children with chronic cough, *M. pneumoniae* and *C. pneumoniae* seropositivity is in high rates. These microorganisms may cause chronic inflammation in airway and this condition clinically appear as protracted bronchitis, upper airway cough syndrome and asthma-like disease.

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Key words: Postinfectious causes, chronic cough, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*

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Sonuç: Okul çağı kronik öksürüklü çocuklarda *M. pneumoniae* seropozitifliği yüksek orandadır. Bu mikroorganizmalar hava yollarında kronik inflamasyona neden olabilir. Bu durum klinikte uzamış bronşit, üst hava yolu kaynaklı öksürük ve astım benzeri hastalık olarak görülmektedir.

(*Asthma Allergy Immunol* 2015;13:124-129)

Anahtar kelimeler: Postinfeksiyöz nedenler, kronik öksürük, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*

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INTRODUCTION

Chronic cough is a problem frequently encountered in children resulting in parental distress^[1,2]. The diagnosis becomes easier in the presence of specific clinical findings. In childhood, postinfectious causes play a major role in chronic cough. The pathogenesis of the postinfectious cough is not completely understood. It is thought to be the result of inflammation and the epithelial damage in the upper and lower airways, with or without transient airway hyperresponsiveness^[3].

In children, a specific infectious agent causing postinfectious cough remains unidentified in most cases. Respiratory viruses, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Bordetella pertussis* and *Moraxella catarrhalis* have all been implicated^[4-5].

This study aims to evaluate the role of *M. pneumoniae* and *C. pneumoniae* seropositivity in children with chronic cough and to ascertain the clinical diagnosis and response to treatment according to American College of Chest Physicians (ACCP) 2006 guideline^[6].

MATERIALS and METHODS

This study was conducted in Pediatric Allergy Unit of Izmir Dr. Behcet Uz Children's Hospital. School age children between 6 to 14 years, inclusive, who had been coughing longer than 4 weeks and having unremarkable chest x-ray and pulmonary function test (PFTs) were recruited. A comprehensive clinical history, including the length of cough, its association with physical effort, presence of dry or productive cough, and family history of atopic disease was obtained. Pathological findings were evaluated and clinical examination was performed. Patients with findings indicating pulmonary or cardiac diseases, known immune deficiency, neuromotor developmental deficit, or a previous diagnosis and treatment of asthma were all excluded. Permission was taken from the parents and local ethic committee for the study.

Patients were evaluated on the basis of 2006 ACCP guideline. Pulmonary function test and chest x-ray were

performed in all patients after the medical history and physical examination. They were reevaluated in 2 to 4 weeks intervals until cough disappeared. At study entry, a serum sample was analyzed for *M. pneumoniae* and *C. pneumoniae* antibody status using the enzyme-linked immunosorbent assay (Euroimmun-Germany, ELISA kits). Only one sample per patient was analyzed.

Definition of the Diagnostic Categories

Diagnostic Classification

1. Asthma-asthma-like conditions: Cough with variable airflow limitation demonstrated by bronchodilator responsiveness and/or response to inhaled steroid (Budesonide 400 µg/day) within 2-4 weeks.
2. Protracted bronchitis: History of chronic moist cough and response to antibiotic therapy (Clarithromycin 15 mg/kg/day, 10 days) with resolution of cough within 2-4 weeks.
3. Gastroesophageal reflux disease (GERD): Detection of reflux via gastroesophageal scintigraphy in children suffering from cough and responding to treatment (Lansoprazole 15 mg/day) within 2-4 weeks.
4. Upper airway cough syndrome (UACS): A medical history matching with the diagnosis, detection of postnasal discharge in physical examination and/or nasal mucosal oedema, hyperaemia, faintness, response to antihistamine, nasal saline and/or to nasal steroid in 2-4 weeks.
5. Bronchiectasis: Demonstrating bronchiectasis by thorax HRCT in patients with chronic wet cough and abnormal chest radiogram who did not respond to antibiotic therapy (Clarithromycin 15 mg/kg/day, 10 days) within 2-4 weeks.
6. *M. pneumoniae* infection: Chronic cough, presence of *M. pneumoniae* IgM antibodies via ELISA and improvement of symptoms with single clarithromycin treatment.

Statistical Analysis

Normality of distribution of variables were tested with Kolmogorov-Smirnoff test. Results were presented as mean ± standard deviation or percentages (%). We

used Mann-Whitney U test to compare the differences between groups in count variables. Qualitative variables were assessed with chi-squared test. A p value < 0.05 (2-tailed) was accepted as significant. Data were analysed using SPSS for Windows version 15.0.

RESULTS

The study included 41 children, 27 of whom were female (65.9%). The mean age was 8.00 ± 1.96 years. 68% of the patients had wet cough. The demographic characteristics of the patients are shown in Table 1.

In all patients, PFTs and chest x-rays were found normal. Clarithromycin (15 mg/kg/day for 10 days) was given to patients who had productive cough or were

diagnosed as sinusitis. Inhaled steroid (Budesonide 400 mcg/day) was given to patients with dry cough.

M. pneumoniae IgM and IgG serology was assessed in all patients. IgM and/or IgG positivity was found in 17 (41.46%) of them. Seven cases had IgM positivity, three cases had both IgM and IgG positivity and seven cases had IgG positivity.

The seven cases with *M. pneumoniae* IgM positivity were distributed according to the algorithm as follows: three UACS, two asthma-like, one prolonged bronchitis + asthma-like and one case of *M. pneumoniae* respiratory infection. Cases with UACS received antihistamines and nasal steroids. At follow-up, two weeks later, clarithromycin was added to the treatment because the patients complaints changed suggesting sinusitis with productive cough and purulent postnasal discharge. In the control visit, cough had improved. In two cases who were evaluated as asthma-like, inhaled steroids were initiated. One case with prolonged bronchitis did not respond to clarithromycin, and developed dry cough, so inhaled steroid was initiated and the symptoms resolved (Figure 1. Evaluation of diagnosis and treatment of patients with *M. pneumoniae* IgM and/or IgG positivity).

C. pneumoniae serology was assessed in 35 patients. Among those, one case (2.8%) had IgM and 8 cases

Table 1. Demographic characteristics of the patients

Total number	41
Male (%)	14 (34.1)
Age ± SD (years)*	8.00 ± 1.96
Length of cough ± SD (month)*	2.51 ± 2.18
Wet cough (%)	28 (68)
Presence of atopic disease (%)	13 (31.7)
Passive smoking (%)	28 (56.4)

* Average ± standard deviation.

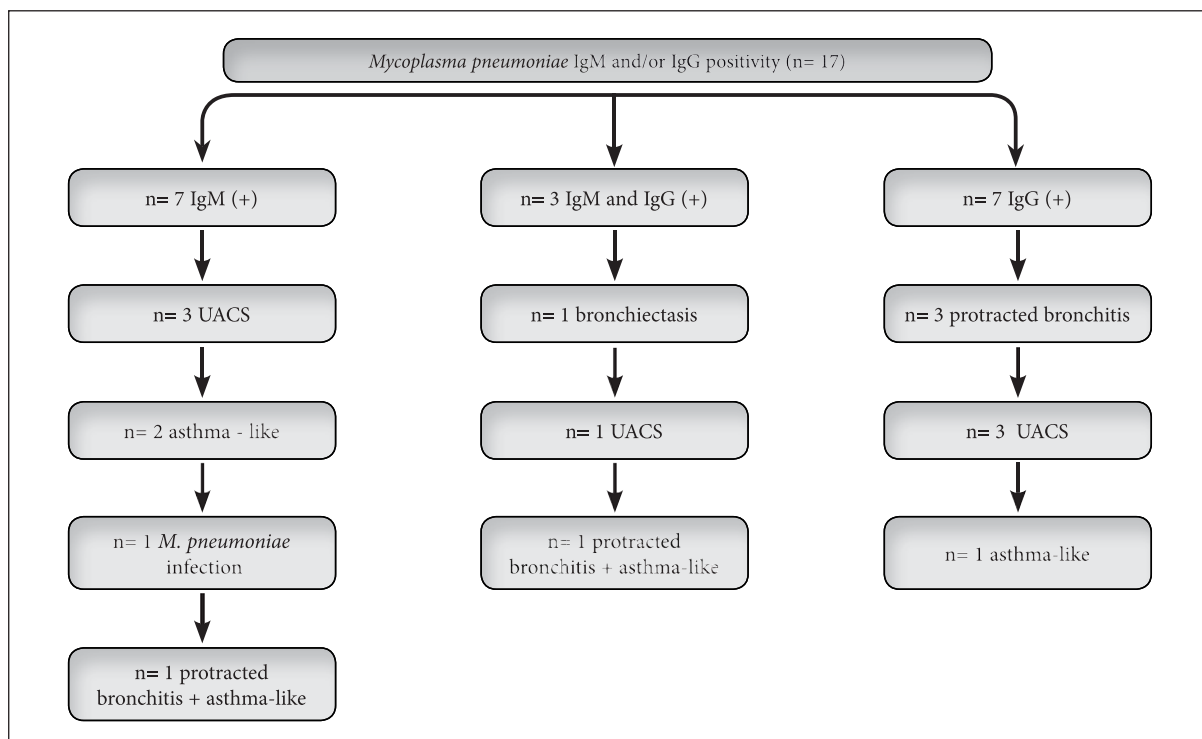


Figure 1. The evaluation of the diagnosis with *M. pneumoniae* IgM and/or IgG positivity *M. pneumoniae* IgM and/or IgG positivity (n= 17).

(22.8%) had IgG positivity. None of the cases had IgG and IgM positivity together.

The *C. pneumoniae* IgM positive patient was diagnosed with UACS and had specific symptoms suggesting GERD, as well. Following a diagnosis of GERD by scintigraphy, omeprazole was initiated. In the control visit at two weeks, his symptoms had improved. *C. pneumoniae* IgG positivity was present in 8 cases. (Figure 2. Evaluation of diagnosis and treatment of patients with *C. pneumoniae* IgM and/or IgG positivity.)

The duration of cough and its characteristics and the presence of familial atopic disease didn't significantly differ between seropositive and seronegative patient groups ($p > 0.05$).

In five of the 35 patients evaluated for both *C. pneumoniae* and *M. pneumoniae*, both serologies were positive. One had *M. pneumoniae* IgM positivity and *C. pneumoniae* IgG positivity, two had *M. pneumoniae* and *C. pneumoniae* IgG positivity, two had *M. pneumoniae* IgM and IgG and *C. pneumoniae* IgG positivity.

DISCUSSION

In this study, in children with chronic cough, IgM positivity for *M. pneumoniae* and *C. pneumoniae* were found, 17.07% and 2.8%, respectively. IgG positivity which may also show the presence of persistent infection was 24.39% for *M. pneumoniae* and 22.8% for *C. pneumoniae*. IgM and/or IgG positivity for any one of the agents was 51.21%. This suggests that in school children

with chronic cough, atypical pathogens have a major role. When these patients are evaluated according to the 2006 ACCP guideline, these patients can be classified as having asthma-like disease, UACS or protracted bronchitis.

C. pneumoniae and *M. pneumoniae* are common causes of acute lower and upper respiratory infection in children. However, the patients may also remain totally asymptomatic. In some cases of persistent cough lasting more than two weeks, unresponsive to conventional treatment, the clinicians consider these two microorganisms in their diagnosis. *M. pneumoniae* infections are reported to account for 70% of all cases aged 9-15 years. The 20 % of children infected with *C. pneumoniae* are also found co-infected with *M. pneumoniae*^[7,8].

Although many methods (e.g ELISA, MIF, PCR) are used for diagnosis, the most reliable diagnostic test is the enzyme immunoassay^[9,10,11]. The diagnosis of these organisms is clinically based on serologic response because culturing of these agents is difficult and time-consuming. There is no universal agreement upon gold standard serological assay for detection of antibody. The sensitivity of the serological response to *M. pneumoniae* infection depends on whether the sera are collected early or late after the onset of disease. Usually, a second serum sample is needed to demonstrate seroconversion in 2-4 weeks interval^[11]. In our study, only one serum sample was analyzed because the patients' complaints were chronic.

While the pathogenesis of the postinfectious cough is not known, it is thought to be the result of extensive disruption of epithelial integrity and widespread airway inflammation of the upper and /or lower airways with or without transient airway hyperresponsiveness^[12]. Therefore, it is likely that transient inflammation of the lower airways is important for the pathogenesis in some patients with postinfectious cough. This speculation is based on the fact that cough may be induced by the heightened responsiveness of the cough receptors, by bronchial hyperresponsiveness as seen in cough-variant asthma, or by impaired mucociliary clearance from the disruption of the epithelial lining of the airways. Since airway inflammation causes mucus hypersecretion, retained secretions resulting from excessive mucus production and decreased clearance may be another important mechanism of cough. In addition, persistent inflammation of the upper airway, particularly the nose and paranasal sinuses, can contribute to postinfectious cough^[13].

Another mechanism to consider in postinfectious cough is gastroesophageal reflux. The vigorous coughing

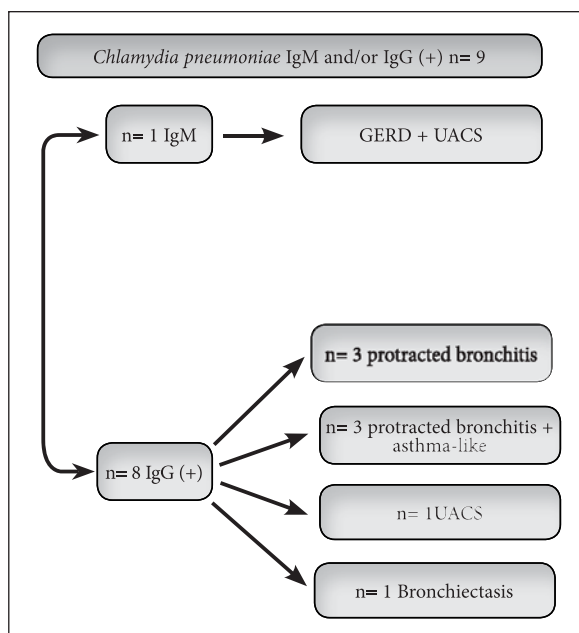


Figure 2. The evaluation of the diagnosis with *C. pneumoniae* IgM and/or IgG positivity.

that follows may induce or aggravate preexisting reflux disease because of the high abdominal pressures that are generated^[14,15]. In our study, the *C. pneumoniae* IgM positive patient who was diagnosed with UACS also had symptoms suggesting GERD. This patient's complaint was resolved with antireflux treatment.

C. pneumoniae is an obligate intracellular organism, whereas, *M. pneumoniae* is an extracellular pathogen. Both may cause prolonged symptoms. Chronic infections related to *C. pneumoniae* were shown to cause ciliary dysfunction of bronchial cells and epithelial damage^[16,17]. On the other hand, *M. pneumoniae* was suggested to cause disruption in ciliated epithelial cells by adhering airway mucosa. Both pathogens may cause pneumonia, interstitial pneumonia, bronchitis, bronchiolitis and pharyngitis in children as well as adults^[18-20].

In a study evaluating *M. pneumoniae* and *C. pneumoniae* seroprevalence in stable asthma and chronic obstructive pulmonary disease, *M. pneumoniae* was seropositive in 11.1% and *C. pneumoniae* in 8.3% of cases in the asthma group^[21]. In children with chronic persistent asthma, *C. pneumoniae* IgG was detected in 40.62%^[22].

In another study examining *C. pneumoniae* infection in adults with persistent cough, serological findings for acute Chlamydial infections were reported in 6.5% of cases, while *M. pneumoniae* was positive in 18.5% of cases^[4].

In a study evaluating *M. pneumoniae* and *C. pneumoniae* serologies and treatment in children with chronic and recurrent cough, 67 patients aged between 2,5 and 16 years old, *M. pneumoniae* was positive in 29%, *C. pneumoniae* in 6%, and *M. pneumoniae* IgG in 47.8% of cases. In the same trial, more than one cause was also determined. While some of the cases improved without clarithromycin, others improved with only anti-asthmatics and nasal steroids^[18]. In our study, IgM positivity for *M. pneumoniae* and *C. pneumoniae* were found to be 17.07% and 2.8%, respectively. IgG positivity which may also show the presence of persistent infection was 24.39% for *M. pneumoniae* and 22.8% for *C. pneumoniae*. IgM and/or IgG positivity for any one of the agents was 51.21%.

In a study from Turkey that investigated serologic markers of *C. pneumoniae* in healthy children, IgM and IgG seropositivity in *C. pneumoniae* was 1.2% and 18.7% respectively^[24]. In another study that investigated the role of *M. pneumoniae* and *C. pneumoniae* in children with community-acquired pneumonia in İstanbul, *M. pneumoniae* infection was diagnosed in 27%, *C. pneumoniae* infection in 2%^[25]. The results of our study revealed similar seropositivity in children with chronic cough.

This study evaluates the serology of *M. pneumoniae* and *C. pneumoniae* in children with chronic cough. Usually, in diagnosis of an acute infection, it is recommended that two serum samples should be obtained in 2-4 weeks interval. However, we analyzed one serum sample, because these patients' complaints were chronic. High seropositivity was found for these agents that could be considered to match with the results in community acquired pneumonia. Actually, the serological results should be compared with those in healthy school children. This study did not include a control group. When the children's respiratory complaints are chronic, they should be evaluated in a diagnostic algorithm to start antibiotics effective against atypical agents, if necessary.

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