ORIGINAL PAPERS

Beata M. Sobieszczańska[1, D, C], Urszula Kasprzykowska[1, A, B], Anna Duda-Madej[1, A, B],
Anna Secewicz[1, B], Joanna Marciniak[2, B], Grażyna Gościniak[1, E, F]

Relevance of Serology for *Mycoplasma Pneumoniae* Infection Among Children with Persistent Cough

[1] University of Medicine, Department of Microbiology, Wroclaw Medical University, Poland
[2] University of Medicine, 1st Department and Clinic of Paediatrics, Allergology and Cardiology, Wroclaw Medical University, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of article; G – other

Abstract

Background. *Mycoplasma pneumoniae* is an important cause of upper and lower respiratory tract infections. Cough and tracheobronchitis are the commonest features of *M. pneumoniae* infection but diagnosis based on clinical symptoms that may be due to other respiratory pathogens is impossible. Thus laboratory testing for *M. pneumoniae* is particularly important. Correct and rapid diagnosis of *M. pneumoniae* infections is of prime importance to introduce appropriate antibiotic treatment.
Objectives. Evaluation of the incidence of IgM and IgG antibodies specific to *M. pneumoniae* among children with pneumonia and/or chronic cough.
Material and Methods. Serum samples from 148 children with a history of chronic cough (lasting at least one month), recurrent respiratory tract infections, allergic rhinitis, and/or inflammatory changes on X-chest ray. First, all sera were screened for specific anti-*M. pneumoniae* antibodies using agglutination test following the detection of specific IgM and IgG anti-*M. pneumoniae* antibodies using immunoenzymatic assays.
Results. Out of the 148 serum samples, 57 (38.5%) gave positive screening results. However, the presence of *M. pneumoniae*-specific IgM and/or IgG antibodies was confirmed by immunoenzymatic assays in only 30 (52.6%) of these 57 positive samples. These results indicated that in as many as 27 (47.4%) out of the 57 serum samples screened, false-positive results occurred.
Conclusions. Evaluation of acute- and convalescent-phase sera is necessary to make possible accurate interpretation of the serological testing results (**Adv Clin Exp Med 2014, 23, 2, 185–190**).

Key words: *Mycoplasma pneumoniae*, serology, persistent cough.

Mycoplasma pneumoniae (*M. pneumoniae*) belongs to the family *Mycoplasmataceae*, order *Mycoplasmatales* and class *Mollicutes*. All members of the class *Mollicutes*, including *M. pneumoniae*, characterize the lack of a cell wall, making these microorganisms resistant to β-lactam antibiotics that inhibit bacterial cell wall synthesis. Moreover, the lack of a rigid cell wall confers pleomorphic nature of these organisms, inability to stain with Gram dyes and their substantial susceptibility to desiccation. Mycoplasmas belong to the smallest microorganisms capable of a cell-free mode of living. Their small size allows mycoplasmas to pass through 0.45 μm pore size filters and make them undetectable by light microscopy [1, 2].

There are several *Mycoplasma* species inhabiting a human's respiratory and urogenital tract (Fig. 1). Although all these species can colonize mucosal surfaces only some of them are involved in respiratory and urogenital tract infections in humans. Mycoplasmas belong to extracellular pathogens but require close association with host cell to survive. A complex, specialized attachment organelle with most prominent P1 cytoadhesin enable mycoplasmas to reside on epithelial surfaces and protects them from mucocilliary clearance. According to Rottem [3] close contact with the host
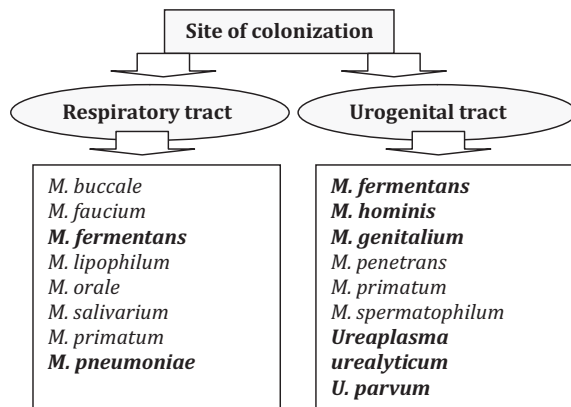
Fig. 1. Species of *Mycoplasmataceae* family inhabiting mucosal surfaces of the respiratory and urogenital tract in humans [1]. Species involved in human infections are marked in bold

cell facilitates *M. pneumoniae* to fuse with and enter host cells establishing later or chronic infections, although the extent to which the organism invades the host cells *in vivo* is not known.

M. pneumoniae is an important cause of upper and lower respiratory tract infections as well as a wide spectrum of extrapulmonary manifestations, involving almost all organs of the human body and including neurological (e.g. *M. pneumoniae* is an important antecedent to Guillain-Barre syndrome, cranial nerve palsy, cerebellar ataxia, polyradiculopathy), hepatic, renal (e.g. IgA nephropathy, acute glomerulonephritis, renal failure) cardiac diseases, hemolytic anemia, polyarthritis, erythrema multiforme or Stevens-Johnson syndrome. Although most commonly *M. pneumoniae* infections occur among children 5–15 years of age, they can be involved in diseases in older individuals and in children below 5 years of age [4–6]. In Poland, the epidemiological data from the period 1994–1996 showed that *M. pneumoniae* caused from 2 to 16% of all respiratory tract infections; however, the percentage of infections increases every 4–5 years during epidemics and may reach even 60% [7]. According to surveillance data from European countries during the autumn of 2011 there was an increase in reporting rates of M. pneumoniae infections in northern but not in southern Europe [8].

M. pneumoniae may account for as many as 25% of the clinical manifestations of the upper respiratory tract illness. The most common manifestations include sore throat, hoarseness, fever, nonproductive cough, headache, chills, coryza, myalgia and general malaise [1, 2, 9]. Children under 5 years of age are most likely to manifest coryza and wheezing but progression to bronchopneumonia is relatively uncommon in this age group. However, *M. pneumoniae* may cause up to 5% of cases of bronchiolitis in young children. In contrast, older children are more likely to develop bronchopneumonia. Mild and asymptomatic infections are particularly common in adults though bronchopneumonia develops in 3 to 10% of infected individuals [1]. Community acquired pneumonia due to *M. pneumoniae* is usually mild and self-limiting (hence term walking pneumonia) but severe pulmonary involvement requiring hospitalization can occur in otherwise healthy children and adults. Pleural effusion, pneumatocele, lung abscesses, pneumothorax, bronchiectasis, chronic interstitial fibrosis and acute respiratory-distress syndrome have been reported [2, 9]. *M. pneumoniae*-associated respiratory tract infections may promote the exacerbation of asthmatic symptoms, which is related to the immune response against these microorganisms [10, 11]. *M. pneumoniae* infection was found to increase the production of interleukin-4 (IL-4) and interferon gamma (IFNγ), indicating a predominant Th2-like cytokine response and creating favorable condition for IgE production [12]. Stelmach et al. [13] have shown serum IgE increase in children after acute infection with *M. pneumoniae*. Furthermore, lipoproteins and glycoproteins present in the cell membrane and cytoplasm of *M. pneumoniae* are potent inducers of cytokines that through their molecular mimicry can elicit autoimmunity.

Nonproductive cough is one of the most prominent clinical symptom of *M. pneumoniae* infection. According to Kiciński et al. [14] *M. pneumoniae* infection was confirmed in 9.4% of 1710 children up to 6 years of age with coughing as a predominant clinical symptom of pneumonia. Wang et al. [15] have detected *M. pneumoniae* infection among 12.9% of children with a persistent cough. In the study we analyzed the incidence of IgM and IgG antibodies specific to *M. pneumoniae* among children with pneumonia and/or chronic cough hospitalized in 1st Department and Clinic of Paediatrics, Allergology and Cardiology of the Wroclaw Medical University, Poland.

Material and Methods

Serum samples from 148 children were obtained from 1st Department and Clinic of Paediatrics, Allergology and Cardiology, Wroclaw Medical University. The ages of the patients ranged from 18 months to 17 years (median age, 7 years). All patients had a history of chronic cough (lasting at least one month), recurrent respiratory tract infections, allergic rhinitis, and/or inflammatory changes on X-chest ray.

Mycoplasma Screening Assay

First, all sera were screened for specific anti--*M. pneumoniae* antibodies. The Serodia Myco II gelatin particle agglutination test (Fujirebio, purchased from Mast Diagnostica, Poland) was performed according to the manufacturer's instructions. The test mainly measure IgM (and in a lesser extent IgG) antibody for *M. pneumoniae* and is based on the principle that gelatin particles sensitized with *M. pneumoniae* cell-membrane components are agglutinated by specific antibodies.

Mycoplasma IgM and IgG Antibody by Enzyme-Linked Immunosorbent Assay (EIA)

For detection of specific IgM and IgG anti-*M. pneumoniae* antibodies the EIA-Platelia (Bio-Rad Laboratories, Poland) was performed. The antigen included in the test contains a high proportion of membrane proteins and is enriched in P1 cytoadhesin and some other cytoadherence-associated proteins. The test was performed manually according to the manufacturer's recommendations. The test result was validated as instructed in the kit manual. A specimen was considered positive for IgM antibodies to *M. pneumoniae* when the absorbance value of the specimen was equal to or greater than that of the cutoff serum specimen included in the kit. The values of IgG antibodies to *M. pneumoniae* were validated and transformed to arbitrary units (AU) from 1 to 100 determined with the kit standards, and the results were interpreted as recommended by the manufacturer. The specimen AU values were determined from the calibration curve and were interpreted as follows. A value of < 10 U for a single serum specimen was considered insignificant, a value of > 10 and < 40 U was considered low, and a value of ≥ 40 U was considered high.

Results

Out of the 148 serum samples, 57 (38.5%) gave positive screening results. However, the presence of *M. pneumoniae*-specific IgM and/or IgG antibodies was confirmed by EIA assays in only 30 (52.6%) of these 57 positive samples (Fig. 2). These results indicated that in as many as 27 (47.4%) out of the 57 serum samples screened, false-positive results occurred.

Among these 30 serum samples positive in EIA-Platelia assay, *M. pneumoniae*-specific IgM antibodies were present in 6 (10.5%) serum
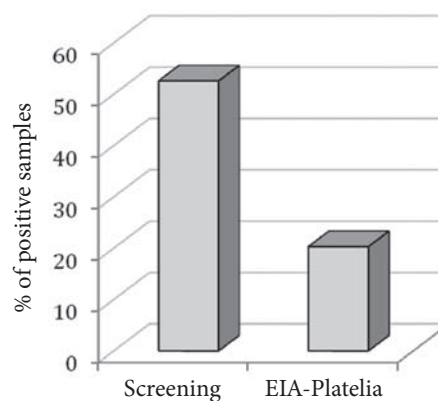

Fig. 2. The incidence of specific anti-*M. pneumoniae* antibodies determined by screening test and EIA--Platelia assay. Out of the 148 serum samples examined 57 (38.5%) was positive, but in only 30 (20.3%) samples the presence of specific antibodies was confirmed by EIA-Platelia test

samples. Both, IgM and IgG antibodies were present in 3 (5.3%) of serum samples examined (Table 1). Taking into consideration that all these IgM-positive samples were obtained from children presenting symptoms of the lower respiratory tract infection, i.e. persistent cough, an interstitial inflammatory changes on X-chest ray, these serologic assay results indicated an acute infection. As many as 19 (33.3%) serum samples showed high levels of IgG without the accompanying IgM. In all these cases the interpretation of these results is difficult as may indicate recent remote infection as well as an acute infection. Hence, to discriminate between acute and remote infection the second serum sample should be examined. In 8 (14%) of samples there were only low levels of IgG antibodies, indicating a remote infection. Thus, although all these children showed clinical signs of the lower respiratory tract infection, most likely *M. pneumoniae* was not the cause of these symptoms. On the other hand, taking into consideration that even among children re-infections may occur, the low levels of IgG antibody may indicate anamnestic response which can be confirmed by re-examination of the IgG level after two weeks. These cases perfectly illustrate the importance of serological tests in all patients with suspected mycoplasmal pneumonia as well as the necessity to re-examine doubtful results of serologic tests e.g. low levels of IgG without accompanying IgM antibodies.

Discussion

Coughing and tracheobronchitis are the commonest features of *M. pneumoniae* infection, but diagnosis based on clinical symptoms that may be

Table 1. The incidence of positive and negative results of screening and immunoenzymatic tests

Screening assay Titre	№ (%) of positive results out of 148 sera	№ (%) of samples out of 57 sera positive in screening assay						
		IgM+	IgM-	IgG+ < 40AU	IgG+ > 40AU	IgG-	IgM+ IgG+	IgM- IgG-
80	24 (16.2)	1 (4.2)	23 (95.8)	2 (8.3)	5 (20.8)	17 (70.8)	0	15 (62.5)
160	25 (16.9)	4 (16)	21 (84)	5 (20)	9 (36)	11 (44)	2 (8)	10 (40)
> 160	8 (5.4)	1 (12,5)	7 (87.5)	1 (12.5)	5 (62.5)	2 (25)	1 (12,5)	2 (25)
Total	57 (38.5)	6 (10,5)	51 (89.5)	8 (14)	19 (33.3)	30 (52.6)	3 (5,3)	27 (47.4)

AU – the specimen arbitrary unit values.

due to other respiratory pathogens is impossible, thus laboratory testing for *M. pneumoniae* is particularly important. Correct and rapid diagnosis of *M. pneumoniae* infections is of prime importance in order to introduce appropriate antibiotic treatment [16].

Culture of *M. pneumoniae* from clinical specimens is highly specific but time-consuming, difficult and relatively insensitive. Moreover, colonization of the upper respiratory tract by *M. pneumoniae* in asymptomatic individuals has been reported, making the clinical value of a positive culture results uncertain [17]. Therefore, laboratory diagnosis of *M. pneumoniae* infection is usually established through serological or molecular testing. Polymerase chain reaction (PCR), similarly to culture characterize high specificity, but low or variable sensitivity, thus PCR cannot replace serology although, may be performed in conjunction with serology [1, 16]. Several commercial tests utilizing passive agglutination, indirect immunofluorescence and immunoenzymatic assays are available and widely used for the detection of antibodies specific to *M. pneumoniae* in human sera. The complement fixation test, being as it is unspecific and insensitive, is no longer accepted [18]. However, several factors i.e. time of serum sample collection or patient's age, influence on the results of serologic tests as well as on the results interpretation. Moreover, the interpretation of serological tests results is complicated by the fact that low levels of antibody specific to *M. pneumoniae* are found in sera of healthy individuals, probably because of past *M. pneumoniae* infections or repeated exposures on *M. pneumoniae*. Thus, in *M. pneumoniae* infection it is difficult to set up criteria to determine acute or remote infection [19, 20].

Following an initial infection, specific IgM antibodies are rapidly produced, indicating acute or recent primary *M. pneumoniae* infection. IgM antibodies appear 7 to 10 days after infection, peak after 3 to 6 weeks, followed by a gradual decline over months to years [1]. However, specific IgM antibodies do not always indicate an acute infection, as they can persist for up to a year after the *M. pneumoniae* infection. In addition, IgM antibodies may not be present if the serum sample is obtained too early in the infection. Moreover, an IgM response may be either minimal or undetectable when adults are infected and during re-infection [16, 18]. Thus, a negative result of IgM evaluation does not exclude current infection, especially in patients over the age of 45 years. Since children have fewer than adults opportunities for repeated exposures to *M. pneumoniae*, the presence of specific IgM antibodies has a high reliability in pediatric patients with a recent infection of at least a week's duration, even on a single serum sample [1, 20]. The overall data showed that the frequency of the carriage *M. pneumoniae* in symptomless children is low, ranging from 4.6% to 13.5% [2].

Specific IgG antibodies appear about 2 to 3 weeks after IgM occurrence and maximal response for IgG occur during the fifth week after onset of disease. An elevated IgG is frequently interpreted as evidence of acute infection, whereas low levels of IgG can indicate either an early stage of the current infection or a past illness. However, specific IgG antibodies may remain elevated for extended periods and thus do not discriminate between a current or remote *M. pneumoniae* infection. On the other hand, IgG is produced more quickly as an anamnestic response to re-infection; therefore, a second serum sample collected with an interval of 2 to 3 weeks should be examined, when a fourfold or greater increase of specific IgG titer evidences current infection [16, 21, 22]. Unfortunately, correct interpretation of tests results in paired sera with a rise in IgG antibody titer, delays the diagnosis.

Importantly, the serologic response may not reach detectable levels, thus producing false-

negative results, if a patient is successfully treated with antibiotics early in the course of the disease. Moreover, Uldum et al. [23] have shown that children younger than 10 years may have negative IgG whereas sera of children infected with *M. pneumoniae* with high IgG value may have reduced by 20 to 40% IgM reaction, causing a risk of a false-negative IgM reactions.

Moreover, considering that many commensal *Mycoplasma* species, commonly colonizing the human oropharynx, can produce a cross-reaction with *M. pneumoniae* antibodies, serologic test results should be interpreted with caution and in association with clinical symptoms [1]. Mycoplasmal adhesion molecules exhibit homology with human CD4 and class II major histocompatibility complex lymphocyte proteins, which could generate autoreactive antibodies [1, 24]. In the study as many as 47.4% of serum samples showed false-positive results in a screening test, indicating that cross-reacting antibodies are common in children and may affect the actual number of infections detected. Although the detection of antibodies specific to *M. pneumoniae* allows us to eliminate most false-positive results, this still does not help to interpret the serologic tests results correctly.

The analysis presented in this study of serologic tests results perfectly illustrates the difficulties in the interpretation of the results, arising from a single serum sample survey. In pediatric patients the presence of IgM antibody is a good indicator of an acute *M. pneumoniae* infection, even in a single serum sample. However, the presence of IgG without accompanying IgM makes the interpretation of the result difficult. Even high levels of IgG antibody may result from a remote infection, but may also indicate acute infection without IgM. The presence of specific IgG without IgM, combined with clinical signs of the lower respiratory tract infection, may suggest *M. pneumoniae* infection and incorrect treatment. After all, many other infectious agents e.g. bacteria, viruses and fungi, can cause similar to *M. pneumoniae* infection clinical signs and symptoms. Although treatment with macrolides (e.g. erythromycin, clarithromycin, roxithromycin, josamycin and most effective azithromycin) and ketolides (telithromycin), tetracycylines (e.g. minocycline) and chinolones (e.g. levofloxacin, gatifloxacin, moxifloxacin, gemifloxacin) that are active against mycoplasmas may be effective against other pathogens of the respiratory tract, in many cases they can also lead to antibiotics abuse [4]. Taking into account the impact of mycoplasmal infections on the host immune system e.g. and their association with allergies, asthma and immune response disturbances, correct diagnosis and treatment is of particular importance. Thus, evaluation of acute- and convalescent-phase sera could make accurate interpretation of the serological testing results possible.

References

[1] **Waites KB, Talkington DF:** *Mycoplasma pneumoniae* and its role as a human pathogen. Clin Microbiol Rev 2004, 17, 697–728.

[2] **Principi N, Esposito S:** Emerging role of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in paediatric respiratory-tract infections. Lancet 2001, 1, 334–344.

[3] **Rottem S:** Invasion of mycoplasmas into and fusion with host cells. In Molecular biology and pathogenicity of mycoplasmas. Eds.: Razin S, Herrmann R, Kluwer Academic/Plenum Publishers, New York 2002, 391–402.

[4] **Kashyap S, Sarkar M:** *Mycoplasma pneumonia*: clinical management. Lung India 2010, 27, 75–85.

[5] **Narita M:** Pathogenesis of neurologic manifestations of *Mycoplasma pneumoniae* infection. Pediatr Neurol 2009, 41, 159–166.

[6] **Sharma MB, Chaudhry R, Tabassum I, Ahmed NH, Sahu JK, Dhawan B, Kalra V:** The presence of *Mycoplasma pneumoniae* infection and GM1 ganglioside antibodies in Guillain-Barre syndrome. J Infect Dev Ctries 2011, 5, 459–464.

[7] **Załęska-Ponganis J, Jackowska T:** Atypowe bakteryjne zapalenie płuc u dzieci. Post Nauk Med 2008, 9, 582–588.

[8] European Centre for Disease Prevention and Control, 2012/http://ecdc.europa.eu/en/press/news/Lists/News/ECDC_DispForm.aspx?List=32e43ee8-e230-4424-a783-85742124029a&ID=554

[9] **Esposito S, Principi N:** Asthma in children: are chlamydia or mycoplasma involved? Paediatr Drugs 2001, 3, 159–168.

[10] **Biscardi S, Lorrot M, Marc E, Moulin F, Boutonmat-Faucher L, Heilbronner C, Iniguez JL, Chaussain M, Nicand E, Raymond J, Gendrel D:** *Mycoplasma pneumoniae* and asthma in children. Clin Infect Dis 2004, 38, 1341–1346.

[11] **Specjalski K:** Rola zakażeń *Chlamydia pneumoniae* i *Mycoplasma pneumoniae* w przebiegu astmy. Pneumol Alergol Pol 2010, 78, 284–295.

[12] **Zaki MS, Raafat D, Metaal AA:** Relevance of serology for *Mycoplasma pneumoniae* diagnosis compared with PCR and culture in acute exacerbation of bronchial asthma. Am J Clin Pathol 2009, 131, 74–80.

[13] **Stelmach I, Podsiadlowicz-Borzecka M, Grzelewski T, Majak P, Stelmach W, Jerzynska J, Poplawska M, Dziadek J:** Humoral and cellular immunity in children with *Mycoplasma pneumoniae* infection: a 1-year prospective study. Clin Diag Lab Immunol 2005, 12, 1246–1250.

[14] Kiciński P, Wiśniewska-Ligier M, Woźniakowska-Gęsicka T: Zapalenie płuc o atypowej etiologii u dzieci do 6 roku życia. Przegl Pediatr 2011, 41, 15–21.

[15] Wang K, Chalker V, Berningham A, Harrison T, Mant D, Harnden A: *Mycoplasma pneumoniae* and respiratory virus infections in children with persistent cough in England: a retrospective analysis. Pediatr Infect Dis J 2011, 30, 1047–1051.

[16] Zhang L, Zong ZY, Liu YB, Ye H, Lv XJ: PCR versus serology for diagnosing *Mycoplasma pneumoniae* infection: a systematic review and meta-analysis. Indian J Med Res 2011, 134, 270–280.

[17] Karppelin M, Hakkarainen K, Kleemola M, Miettinen A: Comparison of three serological methods for diagnosing *Mycoplasma pneumoniae* infection. J Clin Pathol 1993, 46, 1120–1123.

[18] Talkington DF, Shott S, Fallon MT, Schwartz SB, Thacker WL: Analysis of eight commercial enzyme immunoassay tests for detection of antibodies to *Mycoplasma pneumoniae* in human serum. Clin Diag Lab Immunol 2004, 11, 862–867.

[19] Jacobs E, Bennewitz A, Bredt W: Reaction pattern of human anti-*Mycoplasma pneumoniae* antibodies in enzyme-linked immunosorbent assays and immunoblotting. J Clin Microbiol 1986, 23, 517–522.

[20] Petitjean J, Vabret A, Gouarin S, Freymuth F: Evaluation of four commercial immunoglobulin G (IgG)- and IgM-specific enzyme immunoassays for diagnosis of *Mycoplasma pneumoniae* infections. J Clin Microbiol 2001, 40, 165–171.

[21] Lui FC, Chen PY, Huang FL, Tsai CR, Lee CY, Lin CF: Do serological testes provide adequate rapid diagnosis of *Mycoplasma pneumoniae* infection? Jpn J Infect Dis 2008, 61, 397–399.

[22] Sillis M: The limitations of IgM assays in the serological diagnosis of *Mycoplasma pneumoniae* infections. J Med Microbiol 1990, 33, 253–258.

[23] Uldum SA, Jensen JS, Sondergard-Andersen J, Lind K: Enzyme immunoassay for detection of immunoglobulin M (IgM) and IgG antibodies to *Mycoplasma pneumoniae*. J Clin Microbiol 1992, 30, 1198–1204.

[24] Root-Bernstein RS, Hoobs SH: Homologies between mycoplasma adhesion peptide, CD4 and class II MHC proteins: a possible mechanism for HIV-mycoplasma synergism in AIDS. Res Immunol 1991, 142, 519–523.

Address for correspondence:

Beata M. Sobieszczańska
Department of Microbiology
Wroclaw Medical University
Chałubińskiego 4
50-346 Wrocław
Poland
Tel.: 071 784 13 08
E-mail: bmsobie@gmail.com