The Art and Science of Diagnosing Mycoplasma pneumoniae Infection

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Mycoplasma pneumoniae causes a significant burden of disease in children as both upper and lower respiratory tract infections (URTI and LRTI). A positive pharyngeal polymerase chain reaction (PCR) or serology for M. pneumoniae can be found in 4–39% of children hospitalized with community-acquired pneumonia (CAP). Since the introduction of the pneumococcal conjugate vaccine, M. pneumoniae has been reported to be the most common bacterial cause of CAP among hospitalized U.S. children.5

M. pneumoniae is transmitted by respiratory droplets through close contact. The incubation period can be as long as 1–3 weeks. M. pneumoniae infection is generally mild and self-limiting. However, patients of every age can develop severe CAP or extrapulmonary manifestations. The lack of a cell wall makes M. pneumoniae resistant to cell wall synthesis inhibitors such as β-lactam antibiotics. Antibiotics effective against M. pneumoniae include macrolides, tetracyclines and fluoroquinolones. However, a Cochrane review concluded that there is insufficient evidence to draw any definitive conclusions about the efficacy of antibiotics for M. pneumoniae LRTI in children. Macrolides are extensively used worldwide, and this has led to alarming resistance rates among Streptococcus pneumoniae and M. pneumoniae.5 Reported macrolide-resistant M. pneumoniae (MRMP) prevalence is particularly high in Asia with over 90% in some regions, resulting in therapy refractory M. pneumoniae CAP. Efficacy data and tailored prescription of antibiotic treatment are needed to minimize further selection of MRMP. Unfortunately, currently, there is no single diagnostic method that confirms active M. pneumoniae infection in CAP.

This review focuses on the diagnosis of M. pneumoniae infections in children and discusses clinical and microbiologic features that may help identifying M. pneumoniae as the cause of CAP.

**CLINICAL ASSESSMENT**

Clinical assessment – the art in diagnosing M. pneumoniae infection

The term “walking pneumonia” had been introduced to denote the mild form of CAP in most patients with M. pneumoniae infection. These patients can generally be managed in primary care. Physicians often rely solely on clinical suspicion in such cases.

**EPIDEMIOLOGY**

M. pneumoniae occurs endemically worldwide. Infections can be observed throughout the year, but tend to be more common in summer and early fall. Epidemic peaks can be observed every 3–7 years, whereas climate and geography may not be relevant.5,6 Outbreaks of M. pneumoniae infections have been reported within families, schools, institutions and military bases. Clinicians should be particularly aware of M. pneumoniae as potential cause of CAP during M. pneumoniae epidemics. M. pneumoniae infections can occur in all ages. However, M. pneumoniae CAP is reported to be most frequent among school-age children 5–15 years of age.12,5

**SIGNS AND SYMPTOMS**

In addition to the presentation at school-age, children with CAP due to M. pneumoniae have been found to present with a significantly longer duration of fever...
EXTRAPULMONARY MANIFESTATIONS

Additional clinical features of M. pneumoniae infection include extrapulmonary manifestations, which can affect almost every organ, including the skin and the nervous, hematologic, cardiovascular and musculoskeletal systems. These manifestations are caused either by direct local effects of M. pneumoniae or after dissemination or indirect immune-mediated effects.

Skin manifestations occur in up to 25% of all M. pneumoniae infections, including mainly nonspecific exanthems, urticaria, and (less commonly) erythema nodosum. There are also rare but distinct pediatric M. pneumoniae-associated skin disorders such as erythema multiforme, Stevens-Johnson syndrome, and M. pneumoniae-associated mucositis.

Encephalitis and Guillain-Barré syndrome constitute the most severe neurologic manifestations, where M. pneumoniae infection is thought to be causative in up to 10% and 21% of patients, respectively. We recently demonstrated that M. pneumoniae triggers antibodies against the major myelin antigen galactocerebroside (GalC), and showed that anti-GalC IgG is critical for the development of Guillain-Barré syndrome following M. pneumoniae infection. Because the detection rate of M. pneumoniae by PCR in cerebrospinal fluid (CSF) of M. pneumoniae encephalitis patients is low (0–14%), a significant proportion of the cases may be immune-mediated as well. In fact, we also demonstrated anti-GalC IgG antibodies in serum and CSF of patients with encephalitis and severe Guillain-Barré syndrome with additional CNS symptoms, which suggests that these antibodies are also involved in the development of M. pneumoniae-associated CNS disease.

The presence of extrapulmonary manifestations in children with CAP significantly increases the probability of M. pneumoniae infection.

LABORATORY PARAMETERS

CAP patients with uncomplicated M. pneumoniae infection often have normal or only slightly raised absolute leucocyte and neutrophil counts, as well as lower C-reactive protein levels than children with CAP caused by other bacterial organisms.

NONRESPONSE TO EMPIRICAL β-LACTAM ANTIBIOTICS

The British Thoracic Society guidelines recommend amoxicillin as first choice for oral antibiotic therapy in children with suspected bacterial CAP. They also advise that macrolide antibiotics may be added at any age in case of very severe disease or if there is no response to first-line empirical treatment. In children with CAP who do not recover within a few days as would be expected in viral infection, and who do not respond to β-lactam antibiotics, clinicians should consider M. pneumoniae CAP.

CHEST RADIOGRAPH

The radiographic presentation of “atypical” pneumonia due to M. pneumoniae is extremely variable. Bilateral, diffuse infiltrates are common, pleural effusions can occur, but none of the radiographic findings associated with M. pneumoniae CAP are specific.

NONRESPONSE TO EMPIRICAL β-LACTAM ANTIBIOTICS

Diagnostic tests – the science in diagnosing M. pneumoniae infection

Children with moderate-to-severe CAP and/or presence of risk factors (underlying disease or immunodeficiency) should be referred to secondary care for further assessment. Microbiologic diagnosis should be attempted in those children. Current guidelines recommend PCR and serologic tests to diagnose M. pneumoniae infections. An overview of diagnostic tests is shown in Table 1.

PCR

PCR is considered as the new “gold standard” with a superior sensitivity and shorter turnaround time than culture. Nucleic acid amplification techniques for the detection of M. pneumoniae DNA or RNA differ in the choice of target genes used (e.g., P1 gene, 16S rDNA, 16S rRNA etc.), (PCR vs. isothermal amplification techniques), and detection formats (conventional vs. real-time, monoplex vs. multiplex). In the recent past, research focused on the evaluation of commercially available tests, multiplex assays, and strain typing methods.

Importantly, like many other respiratory pathogens, M. pneumoniae can be carried in the upper respiratory tract of asymptomatic children. Detection rates in children without symptoms of a respiratory tract infection vary from 3% or less to 56%. It appears that the mere presence of M. pneumoniae in the upper respiratory tract may not necessarily indicate respiratory disease.

CULTURE

Culture is not used for routine diagnosis because it is labor-intensive, needs special enriched broth or agar media, and the incubation period can take up to 3 weeks.

RAPID ANTIGEN TEST

Rapid antigen tests have a limited sensitivity because of a detection limit of approximately 1 × 10⁵ colony-forming units (CFU)/ml. Although they have a lower sensitivity than PCR, the detection time is faster and only less-trained staff is required compared with culture.

SEROLOGY

The sensitivity of specific serologic tests depends on the time point of the first serum sample and on the availability of paired sera collected 2 weeks apart to evaluate seroconversion and/or ≥ 4-fold antibody titer increase (“gold standard”). Specific serum immunoglobulin (Ig) M can be detected within 1 week after initial infection and about 1–2 weeks before IgG. Reinfection in adults can lead directly to an IgG response and may lack production of IgM. Specific serum IgA rises, peaks and decreases earlier than IgM, but is less frequently detected.

The previously used serologic tests are complement fixation tests, particle agglutination assays, and immunofluorescent assays, which were based on crude M. pneumoniae antigen extracts. Since M. pneumoniae contains large amounts of glycopolipids that elicit cross-reactive antibody responses (manuscript submitted), enrichment for adhesion protein P1 or protein extracts without glycopolipids has been used to improve the test performance of enzyme immunoassays (EIAs).

Intriguingly, one study reported that IgM as well as IgG and IgA could be detected by EIA in single serum samples of asymptomatic M. pneumoniae PCR-positive children. The antibody response in these children...
may simply reflect a previous encounter with M. pneumoniae and is not necessarily related to the concurrent presence of M. pneumoniae in the upper respiratory tract.

Overall, no single diagnostic test or combination of tests is capable of differentiating carriage from infection in a clinically relevant time frame.

**ANTIBODY-SECRETING CELL RESPONSE**

The humoral immune response is highly specific for the infecting pathogen. However, the use of convalescent sera is not helpful in clinical settings, because of the time delay that is inevitable when waiting for a titer increase. The specific B cell response is more rapid and short-lived, and thus an optimal target for determining infectious etiology in CAP patients. It can be detected by measuring antibody-screening cells (ASCs) with an enzyme-linked immunospot assay. A recent study found that M. pneumoniae-specific ASCs indeed circulate in peripheral blood only during CAP, while serum antibodies remain at high levels over months (unpublished data). The detection of ASCs could therefore potentially serve as a future diagnostic tool discriminating M. pneumoniae infection from carriage.

In conclusion, clinicians need to be aware of the implications and clinical significance of a positive PCR and/or serology test result for M. pneumoniae. Rather than relying on diagnostic test results alone, clinicians need to interpret these results in combination with clinical features and a lack of response to β-lactam antibiotics.

### REFERENCES


### Table 1. Overview of Diagnostic Tests for M. pneumoniae

<table>
<thead>
<tr>
<th>Method</th>
<th>Test</th>
<th>Target/Antigen Description</th>
<th>Antibodies/Cells</th>
<th>Specimen(s)</th>
<th>Performance</th>
<th>Diagnostic Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct detection of M. pneumoniae</td>
<td>PCR</td>
<td>Different target genes</td>
<td>—</td>
<td>Respiratory specimen, other bodily fluids or tissues</td>
<td>High sensitivity, high specificity</td>
<td>Routine</td>
</tr>
<tr>
<td>Rapid antigen test</td>
<td></td>
<td>Different antigens (e.g., adhesion protein P1)</td>
<td>—</td>
<td>Respiratory specimen</td>
<td>Moderate-high sensitivity, moderate-high specificity</td>
<td>(Routine)‡</td>
</tr>
<tr>
<td>Culture</td>
<td></td>
<td>—</td>
<td>—</td>
<td>Respiratory specimen</td>
<td>Low sensitivity, high specificity</td>
<td>Advanced</td>
</tr>
<tr>
<td>Non-specific serologic tests for M. pneumoniae</td>
<td>Cold agglutinin test (&quot;bedside test&quot;)</td>
<td>—</td>
<td>Cold agglutinins (IgM)</td>
<td>Serum</td>
<td>Low sensitivity, low specificity</td>
<td>(Routine)†</td>
</tr>
<tr>
<td>Specific serologic tests for M. pneumoniae</td>
<td>CFT</td>
<td>Antigen extracts with glycolipids and/or proteins</td>
<td>Igs (no discrimination between isotypes)</td>
<td>Serum</td>
<td>Less sensitive and less specific than EIA</td>
<td>(Routine)‡</td>
</tr>
<tr>
<td></td>
<td>PA</td>
<td></td>
<td>IgM and/or IgG</td>
<td>Serum</td>
<td>Sensitivity and specificity comparable with EIA</td>
<td>(Routine)‡</td>
</tr>
<tr>
<td></td>
<td>IFA</td>
<td></td>
<td>IgM, IgG, IgA</td>
<td>Serum</td>
<td>Less sensitive and less specific than EIA (subjective interpretation)</td>
<td>(Routine)‡</td>
</tr>
<tr>
<td></td>
<td>Immunoblotting</td>
<td>Proteins (e.g., adhesion protein P1) and/or glycolipids</td>
<td>Serum</td>
<td>High sensitivity, high specificity, Moderate-high specificity</td>
<td>Routine</td>
<td></td>
</tr>
<tr>
<td>Specific ASC response for M. pneumoniae</td>
<td>ELISPOT</td>
<td>Proteins (e.g., adhesion protein P1) and/or glycolipids</td>
<td>IgM, IgG, IgA ASCs</td>
<td>Blood (PBMCs)</td>
<td>High sensitivity, high specificity (confirmatory assay) §</td>
<td>Advanced</td>
</tr>
</tbody>
</table>

*Not available worldwide.
†Historical test: cold agglutinins are IgM antibodies that target the I antigen of human erythrocytes during M. pneumoniae infection and precipitate when a blood sample is placed in an anticoagulated tube on ice for around 30 seconds; replaced by specific serologic tests.
‡Largely replaced by EIA.
CFT, complement fixation test; ELISPOT, enzyme-linked immunospot assay; IFA, immunofluorescent assay; Ig, immunoglobulin; PA, particle agglutination; PBMC, peripheral blood mononuclear cell.

Table adapted from Meyer Sauteur et al.Bold and italicized text is added to highlight key points in the natural text.


