**Mycoplasma pneumoniae and Its Role in Pediatric Lower Respiratory Tract Infections**

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**Overview**

- *M. pneumoniae* review
- *M. pneumoniae*’s role in pediatric LRTIs
- Current breakthroughs in identification and potential ramifications

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**Mycoplasma pneumoniae**

- *M. mycoides* discovered 1898
- *M. pneumoniae* emerged as a significant, human pathogen – 1940s
- 1960s - Singular cause of cold agglutinin – associated primary atypical pneumonia

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**M. pneumoniae characteristics**

- SMALL
- Class of Mollicutes
  - Latin: Mollis (soft) and cutis (skin)
- Lack typical cell walls
- Terminal attachment structure

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Spherical colonies of *M. pneumoniae*
Pulmonary Manifestations of *M. pneumoniae*

- Tracheobronchitis
- Pneumonia
- Wheezing
- Uncommonly: pleural effusion, lung abscess, pneumothorax, bronchiectasis and RDS

Pathophysiology of *M. pneumoniae* LRT Infection

- Attaches to host cell epithelium
- Attachment provides protection from clearance by mucociliary mechanisms
- Produces local cytotoxic effects
- Phagocytosis and progression to cytopathic effects
- Inflammation via cell infiltrates and inflammatory cytokines

Clinical Presentation

- Similar to viral or other atypical infections
- Scattered wheezes and coryza
- Scattered wheezes or rhonchi

HEISKANEN-KOSMA, TARJA; KORPPI, MATTI; JOKINEN, CAMILLA; KURKI, SEIJA; HEISKANEN, LEENA; JUVONEN, HELVI; KALLINEN, SAKARI; STEN, MARJA; TARKIAINEN, AIRI; RONNBERG, PIRJO-RIITTA; KLEEMOLA, MARJAANA; MAKELA, P; LEINONEN, MAIJA

Diagnosis

- General Laboratory Features
- Radiographic Findings
- Pathological Findings
- Microbiological Tests
- No rapid, cost-effective diagnostic test

General Laboratory Features

- Leukocytosis
- Elevated ESR
- Sputum may show mononuclear cells or neutrophils
- No hepatic or renal abnormalities
- Cold agglutinins

Radiographic Findings

- Extremely variable
- Diffuse, reticular infiltrates
- Unilateral disease vs. bilateral
- Lobar consolidation

Radiographic Findings of *M. pneumoniae*

<table>
<thead>
<tr>
<th>Finding</th>
<th>M. pneumoniae infection (n=68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperinflation</td>
<td>10</td>
</tr>
<tr>
<td>Peribronchial wall thickening</td>
<td>3</td>
</tr>
<tr>
<td>Perihilar linear opacities</td>
<td>41</td>
</tr>
<tr>
<td>Reticulo – nodular infiltrate</td>
<td>27</td>
</tr>
<tr>
<td>Segmental/Lobar consolidation</td>
<td>19</td>
</tr>
<tr>
<td>Bilateral consolidation</td>
<td>5</td>
</tr>
<tr>
<td>Effusion</td>
<td>4</td>
</tr>
</tbody>
</table>

Adapted from Principi and Esposito The Lancet 2001
Pathologic Findings

- Lesions of the epithelial lining of mucosal surfaces
- Ulceration and damage to ciliated epithelium of bronchi and bronchioles
- Edema of bronchial and bronchiolar walls
- Infiltrates of key, inflammatory cell types
- Type II pneumocyte hyperplasia

Microbiological Tests

- Lack of rapid and accurate diagnostic laboratory tests to detect *M. pneumoniae*
- Culture
- Antigen detection techniques
- PCR
- Serology

To Treat or Not to Treat

- Cochrane Database of Systematic Reviews 2005 and reviewed 2009:
- 1352 children enrolled from 6 studies
- Conclusions:
  - Interpretation was limited due to inability to extract relevant data
  - Antibiotic choice and dose varied
  - Variable diagnostic criteria (PCR vs. serology)
  - Clinical response did not differ between the macrolide treated groups vs. non-macrolide tx

Asthma Pathophysiology
Pathogenesis of Asthma

Genetic Factors
- Cytokine Response Profiles
  - Age
  - Environmental Factors
  - Allergens
  - Pollution
  - Infections
  - Microbes
  - Stress

Altered Innate and Adaptive Immune Responses

Lower Airway Targeting
- LRI
  - RSV/PIV
  - Adenovirus
  - Mycoplasma

Persistent wheezing and asthma

Inflammatory Response in Asthma

- **Key Cells**
  - Dendritic cells
  - T lymphocytes (CD4+)
  - Eosinophils
  - Mast cells (TNFalpha; IL-8)
  - Neutrophils

- **Key Cytokines**
  - IL4 – increase CD4+ lymphocytes, IgE
  - IL5 & 13 – increases goblet cells, airway reactivity, and eosinophils

Cytokine Balance

Factors favoring the Th1 phenotype
- Presence of other siblings
- Early exposure to dirt, moss, Weissella, or nutrition A infection
- Rural environment

Factors favoring the Th2 phenotype
- Widespread use of antibiotics
- Western lifestyle
- Urban environment
- Dust contamination to house dust mite and cockroach

Association of *M. pneumoniae* with Initiation, Promotion, and Exacerbation of Asthma
Cytokine Profile in Children with Acute *M. pneumoniae* Infection and Wheeze

- Esposito *et. al.* studied 25 children with an acute episode of wheezing.
- Population:
  - *M. pneumoniae* + wheezing (n = 15)
  - *M. pneumoniae* – wheezing/asymptomatic (n=10)
  - Healthy controls (n=16)
- Results:
  - Children with wheezing and acute *M. pneumoniae* infections had a statistically significant increase in IL-5 compared to children with *M. pneumoniae* who were asymptomatic and to controls without wheezing.

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*Cytokine Profile in Children with Acute *M. pneumoniae* Infection*

- Objective: investigate the pattern of cytokine response during an episode of acute LRTI caused by *M. pneumoniae*
- Results: Significantly increased IL-4 and IL-4/IFNγ in BAL of mycoplasma infected patients


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*M. pneumoniae* and Acute Asthma Exacerbation

- Children hospitalized with asthma exacerbation have significantly higher rates (20% vs. 5.2%) of *M. pneumoniae* infection
- High rate (50%) of *M. pneumoniae* infection in first time exacerbation

Biscard, S. *et al.* Mycoplasma pneumoniae and Asthma in children.
CID. May 2004

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*M. pneumoniae* and Promotion of Asthma

- Same study
- Higher rates of recurrent exacerbation in those + for *Mycoplasma pneumoniae*
Chronic Infection with *M. pneumoniae*

- Latent or chronic states of infection is facilitated by an intracellular existence
- Ability to evade therapeutics and immune mechanisms
- Clinical significance of this is unknown

Severe Asthma: Chronic Respiratory Infection

- National Jewish Medical and Research Center: 55 patients
  - Stable asthma
  - Moderate severity
- Mycoplasma pneumoniae
  - PCR (+) in 23/55 (42%)
  - Randomized to clarithromycin vs placebo (6 weeks)
- Only PCR (+) patients improved
- Follow-up study: clarithromycin x 3 months reduced ICS with improved asthma control

Kraft et al: Chest 2002; 121: 1782-88

The Role of *M. pneumoniae* in Inflammation and Bronchial Hyper-reactivity

- Animal data reveal the persistence of *M. pneumoniae* infection

Panels A/C = Placebo
Panels B/D = telithromycin

*mpn 372 (CARDS Toxin)*

- Kannan et al. (Infection and Immunity 2005;73(5):2828-34)
  - mpn 372 encodes a 68 KDa protein
  - possesses ADP ribosyltransferase (ART) activity
  - similarities to pertussis toxin
- Respiratory Diseases Branch, CDC 2008 – “…a newly described toxin gene represents a superior target for detecting *M. pneumoniae* DNA in clinical specimens”
**Similarity Between MPN372/CARDS TX and Pertussis Toxin S1 Subunit**

<table>
<thead>
<tr>
<th></th>
<th>Identity</th>
<th>Positive</th>
<th>Gap</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPN372/CARDS TX*</td>
<td></td>
<td>65/239 (27%)</td>
<td>42/239 (17%)</td>
</tr>
<tr>
<td>PTX-S1</td>
<td></td>
<td>99/239 (41%)</td>
<td></td>
</tr>
</tbody>
</table>

BORPE: 1   DPPATVYRYDS
RPPEDVFQNGFTAWGNNDNVLE
LITGRSCQVGSSNSAFV
STS
SSRRYTE 60

MYCPN: 3   NPVRFVYRVDL
RSPPEIEFHEGFSTLGDVRNFNE
I----LSTNFGRSYFI
STS
ETPTAAI 58

BORPE: 61  VYLEHRMQEAVEAERAGRGTGHFIGYIYEVRADNNFYGAASSYFEYVD------TYGD1 114
+E+V+V+V+V+V+V++A++   +   +   +

MYCPN: 112  RFFGSWLREYV-PEHPRR------AYLYEIRADQHYNARATGENLLDRMRQRQVVFDSG 111
+E+I+N+R           +  G  ETT

MYCPN: 157  ---TTEYSNARYVQAQQRRPNPYTSRRSVASIVGTLVRMAPVVGACMARQAESSEEAM 212
+E+Y+T+N+R          +   +   +

MYCPN: 172  RINEPEMHNPHYQELQTQANDQPWLPTPGIA----TPVLSIFQAMAYVYIESTRADL 226
+E+    +  A  T   YQ+E+I   N+R   V           +  G  ETT

**Effect of rCARDS TX on CHO Cell Morphology**

![Effect of rCARDS TX on CHO Cell Morphology](image)

Kannan and Baseman PNAS April 25, 2006 vol. 103 no. 17 6729

**Effect of rCARDS TX on Baboon Tracheal Epithelium**

![Effect of rCARDS TX on Baboon Tracheal Epithelium](image)

Kannan and Baseman PNAS April 25, 2006 vol. 103 no. 17 6729

**M. pneumoniae in Acute Exacerbation of Asthma**

<table>
<thead>
<tr>
<th>PCR Positive Subject</th>
<th>CARDS Ts</th>
<th>P-1 Adhesin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>CARDS Ts</td>
<td>P-1 Adhesin</td>
</tr>
<tr>
<td>AEA (18)</td>
<td>10 (55%)</td>
<td>21 (54%)</td>
</tr>
<tr>
<td>NALD (36)</td>
<td>7 (19%)</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

PCR* for Mycoplasma: 12% AEA (acute exacerbation of asthma) vs. 11% NALD (non-asthmatic lung disease).
Odds ratio 5.8 (95% CI 2.19-16.83; p < 0.001).
Only 4% were PCR positive by P-1 adhesin.
**M. pneumoniae in Refractory Asthma**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>CARDS Tx</th>
<th>P-1 adhesin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractory Asthma</td>
<td>14/44</td>
<td>1/44</td>
</tr>
<tr>
<td></td>
<td>(31.8%)</td>
<td>(2.3%)</td>
</tr>
<tr>
<td>Non-Asthmatic Lung Disease</td>
<td>7/63</td>
<td>1/63</td>
</tr>
<tr>
<td></td>
<td>(11%)</td>
<td>(1.6%)</td>
</tr>
</tbody>
</table>

Odds ratio 3.7 (95% CI 1.23 – 12.04); p = 0.008

CARDS Tx = MPN 372
P-1 Adhesin = MPN 141

**Mycoplasma pneumoniae** infection in Pediatric Patients with Acute Asthma Exacerbation or Difficult to Control Asthma (Refractory Asthma)

**Aim 1**

Determine the presence of CARDS TX and P1 proteins in serum, nasal, and throat swabs (and sputum if obtained) in patients with an acute exacerbation of asthma; assess the antibody response to CARDS TX and P1 in these patients; perform PCR analysis for unique DNA sequences of cards tx and p1 genes in these samples; and analyze immune parameters in this patient population. Evaluation will be obtained at baseline and 2-3 months after exacerbation if the patient is infected during an exacerbation. Patients who remain positive at 2-3 months, will be followed serially until PCR for CARDS TX and/or P1 is negative on two serial measurements.

**Aim 2**

Determine the prevalence of CARDS TX and P1 proteins in serum, nasal and throat swabs (and sputum if obtained) in patients with difficult to control or refractory asthma; assess the antibody response to CARDS TX and P1 in these patients; perform PCR analysis for unique DNA sequences of cards tx and p1 genes in these samples; and analyze immune parameters in this group of patients. All patients will be followed at 6 month intervals for 12 months to assess for *M. pneumoniae* associated markers.
Summary

• Identification of *M. pneumoniae* infection is challenging using today’s detection techniques.
• *M. pneumoniae* causes significant LRT disease in children and actual prevalence is likely underestimated.
• The role of *M. pneumoniae* infection in acute and chronic asthma is being further investigated using a novel CARDS toxin.

Thank You!

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