Lung Function and Bronchial Responsiveness After 
*Mycoplasma pneumoniae* Infection in Early Childhood

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**Summary.** *Mycoplasma (M.) pneumoniae* has been associated with exacerbation of symptoms in asthmatic school children and adults; and an etiological role in asthma has been suggested. The purpose of this study was to investigate whether infection with *M. pneumoniae* in early childhood has a long-term influence on lung function and bronchial responsiveness. In a retrospective, clinical cohort-study children younger than 5 years-of-age when PCR-tested for *M. pneumoniae* were enrolled. Sixty-five children with clinical symptoms suggesting infection with *M. pneumoniae* during an epidemic season completed a clinical follow-up examination including lung function testing (28 PCR-positive and 37 PCR-negative). In addition to the PCR-test for *M. pneumoniae* all respiratory tract specimens were additionally tested for other atypical bacteria and for viruses by PCR. Lung function was measured as specific airway resistance by whole-body plethysmography and bronchial hyperresponsiveness was assessed by cold, dry air hyperventilation. Neither baseline lung function nor bronchial response to cold dry air hyperventilation differed between *M. pneumoniae*-positive and -negative children: mean baseline lung function were 1.17 versus 1.21 (kPa sec), *P* = 0.45; and mean change in specific resistance was 13% versus 9%, *P* = 0.42. In conclusion, *M. pneumoniae* infection in early childhood was not associated with long-term effects on lung function and bronchial hyperresponsiveness 2 years after infection. *Pediatr Pulmonol.* 2008; 43:567–575. © 2008 Wiley-Liss, Inc.

**Key words:** asthma, pathogenesis; plethysmography, whole body; respiratory tract infections, bacteria and virus; polymerase chain reaction; cohort study; bronchial hyperreactivity.

**INTRODUCTION**

*Mycoplasma pneumoniae* infections occur endemically with epidemic outbreaks every 4–7 years. In school-age children it is a major cause of community-acquired pneumonia, but in preschool children the bacterium is usually considered less important due to a low frequency of severe disease.1 However, evidence has begun to accrue indicating that this view may be too simplified.2 Hospitalization due to community acquired pneumonia is relatively frequent also in children under 5 years of age;3,4 and late complications have been demonstrated by histopathological changes in high-resolution CT scans5 or reduced pulmonary diffusion capacity.6

*M. pneumoniae* may precipitate wheezing in asthmatic children and adults2,7-10 and a possible causal relation has been suggested.10,11

We aimed to investigate a possible long-term effect of *M. pneumoniae* infections on lung function and bronchial responsiveness in early childhood. In this study, we assessed specific airway resistance (sRaw) by whole body plethysmography, and bronchial responsiveness to hyperventilation with cold, dry air in 3- to 7-year-old children

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previously PCR-tested for this infection during an epidemic.

MATERIALS AND METHODS

The study was approved by the local ethics committee of Greater Copenhagen [(KF) 01-090/00].

Study Design

The study was a retrospective, clinical cohort study established by selecting patients having specimens submitted to a central laboratory in Copenhagen for diagnostic PCR-testing for *M. pneumoniae* during the epidemic winter season of 1998 and 1999. At the time of the epidemic in 1998, the Mycoplasma laboratory at Statens Serum Institut was the only place in Denmark performing diagnostic testing for *M. pneumoniae* by PCR on a routine basis. Thus, when the epidemic was publicly announced, the laboratory received a very large number of specimens for diagnosis of *M. pneumoniae* by PCR. The specimens were submitted from all types of health care facilities around the country, but the majority came from GP’s. The PCR-testing was requested by the doctor submitting the specimen. Practically all specimens from children sampled by GP’s were throat swabs. The smaller number of specimens submitted from hospitals were either throat-swabs or aspirates from the lower respiratory tract. The specimens were tested within a few days after arrival in the laboratory, and results were reported back to the doctors when the test was finalized.

After the acceptance of the study by the ethical committee, the families of the study participants were invited based on addresses obtained through the Central Office of Civil Registration.

Clearly, this design has the disadvantage of loss of information about the acute disease phase. As a rough measure of disease severity or disease burden, we obtained data from the Danish National Patients’ Registry to control for major differences between the two groups. This gave records of hospital admissions concerning infectious respiratory symptoms, asthmatic symptoms, or atopy-related ICD-10 codes registered during the period from infancy until November 2002 when data were collected, giving a mean observation period of 46 months after PCR testing for *M. pneumoniae*. Only finalized hospital contacts are registered; thus ambulatory contacts that were still on-going by the end of the observation period are not included.

Children under the age of 5 at the time of *M. pneumoniae* PCR with an address in the Greater Copenhagen area were eligible for a clinical follow-up examination. All eligible patients with a positive *M. pneumoniae* PCR (Mpn-Pos) were matched in a ratio of 1–2 according to age and sex with children tested PCR-negative (Mpn-Neg). The age difference between cases and controls was less than 6 months, and controls were matched to be included with less than 2 months separation in time whenever possible. It was not possible to match patients according to clinical setting (hospitals or general practitioners) to any reasonable degree. The matching in a 1–2 ratio was chosen in order to ensure a sufficient number of participants in the Mpn-Neg group, since a lower acceptance rate to invitation was expected in this group. The children were scheduled for clinical examination according to age starting with the oldest. A 4-week period free of any symptoms of respiratory tract infection prior to the examination was required. The study design has been illustrated in Figure 1.

Clinical Examination

(a) Medical history and physical examination focusing on atopic predisposition; past or present atopy-related symptoms and infectious upper or lower respiratory symptoms.

(b) Lung function was determined as specific airway resistance (sRaw). Methods and equipment for sRaw have previously been described in detail. All sRaw was measured as the relationship between simultaneous variations of respiratory flow and variations of pressure in a constant-volume whole body plethysmograph, and sRaw was calculated at the maximal changes in plethysmographic pressure during inspiration and expiration. Cold air challenge was performed as a single-step 4-min isocapnic hyperventilation test using −15°C dry air mixed with 5% CO₂ as described previously. The mean value of duplicate measurements (a) before challenge was used as baseline and (b) 3–5 min after challenge was used as measurement of response. An increase of 20% or more in sRaw was considered indicative of bronchial hyper-responsiveness.

(c) Skin prick testing was performed using the Soluprick test kit (ALK-Abello A/S, Hørsholm, Denmark) for allergens of the standard inhalation panel: birch, grass, mugwort, dog, cat, horse, two house dust mites, and two molds. Positive and negative controls were included. The test was carried out only in children cooperating with the lung function test.

Microbiological Testing

The respiratory tract specimens, predominantly throat swabs, were sent to Statens Serum Institut for routine *M. pneumoniae* PCR and were tested upon arrival using an inhibition controlled *M. pneumoniae* specific PCR detecting the P1 adhesion gene. All suspected positive test results required confirmation with an independent PCR test using primers detecting a different part of the P1 gene in order to out-rule PCR contamination. The specificity of the *M. pneumoniae* PCR is greater than...
99%, and the sensitivity is calculated to be 98.4% when using a combination of cultural and serological criteria.

In addition to this, the specimens were tested in a panel of PCRs for bacteria and viruses that were considered clinically relevant as differential diagnoses: Chlamydophila pneumoniae, Bordetella pertussis, and B. parapertussis, Ureaplasma urealyticum and U. parvum, RS virus, influenza A and B viruses, parainfluenza-1, -2 and -3 viruses, and adenovirus. For sample preparation for bacterial PCRs, DNA from clinical material is released in Chelex 100 slurry (Biorad, Hercules, CA) as described in detail previously. All primary bacterial PCRs included an inhibition control and all positive results were confirmed by secondary, confirmatory tests; either by an unrelated primer-pair (PCRs for C. pneumoniae, B. pertussis, and Ureaplasma) or by re-testing with the same primer-pair on material from a new sample preparation of the original material to rule-out contamination (B. parapertussis). All bacterial PCRs were carried out as hot-start PCRs. The Ureaplasma PCR is a TaqMan real-time PCR using primers detecting the urease gene and probes distinguishing between the two species Ureaplasma urealyticum and U. parvum (previously biovars). All bacterial PCRs except for the Ureaplasma PCR are accredited according to ISO 17025 as routine diagnostic tests.

The viral PCRs are used for routine clinical testing in the Danish WHO Influenza virus reference laboratory according to ISO17025. Adenovirus DNA was identified using a nested PCR with gel-electrophoresis detection. All PCRs for RNA-viruses were performed as real-time reverse transcriptase PCR (RT/PCR) specific for each of the viruses listed above. Single-step RT/PCR was performed for 1–2 virus per capillary tube using a Light-Cycler instrument (Roche, Hvidovre, Denmark). Sample RNA was extracted by QIAamp Viral RNA Mini Kit (Qiagen, Ballerup, Denmark) and the RT/PCR was performed using Master Hybridisation probes mix (Roche) according to the manufacturer’s instructions. Specificity and sensitivity was tested using the respective cultured virus, clinical samples and international blinded quality control panels.

Data From the Danish National Patients’ Registry

The Danish National Patients’ Registry includes data from all hospitalizations and completed outpatient registrations in Denmark based on ICD10-codes and unique personal registration numbers. Registrations concerning infectious respiratory or atopy-related ICD10-codes were obtained for all children of parents consenting to the study. These data were obtained as a marker for differences in morbidity in the Mpn-Pos versus the Mpn-Neg group, and thus to help validate data. The registry data were obtained in the fall of year 2002, well after completing the clinical examinations.
Statistical Methods

The SAS 8.2 for Windows25 (SAS Institute, Cary NC) and Proc-StatXact 6 for SAS users26 (Cytel Software Corporation, Cambridge MA) were used for the statistical analyses.

Numerical data were analyzed by exact Mann–Whitney tests supplemented with Hodges–Lehmann estimates27 (with exact 95% confidence limits),26 Tables 1 and 3. The Hodges–Lehmann estimate of the difference between the Mpn-Pos and the Mpn-Neg groups is the median of all possible differences between a value from the Mpn-Pos and a value from the Mpn-Neg groups for each variable.

Categorical/ordinal data were dichotomized and analyzed by Fisher’s exact tests, supplemented with estimated odds ratios with exact 95% confidence intervals,26 Table 2.

RESULTS

A total of 249 children were invited for the study (84 Mpn-Pos and 165 Mpn-Neg). Parents of 99 children (43 Mpn-Pos and 56 Mpn-Neg) gave written informed consent to the study protocol. Eighty-two showed up for the clinical examination. The reasons for the missing examination of 17 children were: 8 did not show up as booked; 4 had frequent respiratory tract infections; and 5 were cancelled for various reasons other than recurrent respiratory tract problems.

Sixty-five children (79%) completed the lung function test program; baseline values were achieved in further 5 children, leaving 12 children without lung function measurements, but with a full medical history.

Initially, a matched statistical analysis was intended; however, due to the relatively small number of children completing the study, a large proportion of the patients eventually seen had lost their match. Consequently, it was decided to use the statistical methods described in the Materials and Methods Section.

Clinical Examination

The two groups of children (Mpn-Pos and Mpn-Neg) were similar in terms of: sex; height; atopic predisposition (43 Mpn-Pos and 56 Mpn-Neg) gave written informed consent to the study protocol. Eighty-two showed up for the clinical examination. The reasons for the missing examination of 17 children were: 8 did not show up as booked; 4 had frequent respiratory tract infections; and 5 were cancelled for various reasons other than recurrent respiratory tract problems.

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### TABLE 1—Characteristics of Children With a Full Lung Function Test (LF), Numerical Data

<table>
<thead>
<tr>
<th></th>
<th>Mpn-Pos</th>
<th>Mpn-Neg</th>
<th>P-value</th>
<th>Theta</th>
<th>CIlow</th>
<th>Chigh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at PCR (months)</td>
<td>28 41</td>
<td>37 47</td>
<td>0.14</td>
<td>5.0</td>
<td>–1.6</td>
<td>13.2</td>
</tr>
<tr>
<td>Age at LF (months)</td>
<td>28 71</td>
<td>37 74</td>
<td>0.20</td>
<td>3.2</td>
<td>–1.9</td>
<td>10.0</td>
</tr>
<tr>
<td>PCR-LF interval (months)</td>
<td>28 28</td>
<td>37 27</td>
<td>0.11</td>
<td>–1.0</td>
<td>–1.9</td>
<td>0.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>28 21</td>
<td>37 23</td>
<td>0.04</td>
<td>2.0</td>
<td>0.0</td>
<td>4.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>28 117</td>
<td>37 119</td>
<td>0.11</td>
<td>4.0</td>
<td>–1.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Antibiotics, age-adjusted2</td>
<td>25 0.7</td>
<td>32 0.6</td>
<td>0.93</td>
<td>0.0</td>
<td>–0.3</td>
<td>0.4</td>
</tr>
</tbody>
</table>

P-value, for the Mann–Whitney statistic; Theta, Hodges–Lehmann estimate for the difference between Mpn-Pos and Mpn-Neg; CIlow, exact lower limit of the 95% CI for Theta; Chigh, exact upper limit for the 95% CI for Theta.

### TABLE 2—Characteristics of Children With a Lung Function Test, Categorical Data

<table>
<thead>
<tr>
<th></th>
<th>Mpn-Pos</th>
<th>Mpn-Neg</th>
<th>P-value</th>
<th>OR</th>
<th>CI95%</th>
<th>ORlow</th>
<th>CI95%</th>
<th>ORhigh</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHR</td>
<td>6 28 21</td>
<td>9 37 24</td>
<td>1.00</td>
<td>0.9</td>
<td>0.2</td>
<td>3.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (male)</td>
<td>11 28 39</td>
<td>19 37 51</td>
<td>0.45</td>
<td>0.6</td>
<td>0.2</td>
<td>1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atopic predisp1</td>
<td>19 27 70</td>
<td>22 36 61</td>
<td>0.59</td>
<td>1.5</td>
<td>0.5</td>
<td>5.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atopic history2</td>
<td>8 28 29</td>
<td>15 37 41</td>
<td>0.43</td>
<td>0.6</td>
<td>0.2</td>
<td>1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furry pets3</td>
<td>7 28 25</td>
<td>15 37 41</td>
<td>0.29</td>
<td>0.5</td>
<td>0.1</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tobacco4</td>
<td>10 28 36</td>
<td>8 37 22</td>
<td>0.27</td>
<td>2.0</td>
<td>0.6</td>
<td>7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td>7 28 25</td>
<td>14 35 40</td>
<td>0.28</td>
<td>0.5</td>
<td>0.1</td>
<td>1.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Registry data5</td>
<td>8 28 29</td>
<td>7 37 19</td>
<td>0.39</td>
<td>1.7</td>
<td>0.5</td>
<td>6.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P-value, for Fisher’s exact test; BHR, bronchial hyperresponsiveness; Cough, without concurrent signs of respiratory infections.

1 Atopic predisposition as past or present asthma, allergic rhinitis or eczema in first-degree relatives.
2 Atopic history as past or present eczema or recurrent asthmatic episodes or allergy. 7/84 (8.8%) children have asthma.
3 Present exposure to furry pets at home.
4 Present, daily exposure to tobacco smoke.
5 Registered hospital contacts at the Danish National Registry of Patients for atopy related illness or respiratory tract infections.

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from first-degree relatives (parents and siblings); history of atopy-related symptoms (eczema, allergy, recurrent asthmatic episodes); present, daily exposure to tobacco or furry pets; history of coughing in between respiratory infections; past or present treatment with antibiotics (Tables 1 and 2); and inhaled \( \beta_2 \)-agonists or steroids, or ointments for eczema; atopic findings (eczema, positive skin prick testing); history of lower or upper respiratory tract infections (such as pneumonia, otitis media, pharyngitis or recurrent colds) (data not shown).

The two groups were also similar regarding age at PCR-testing, age at lung function test and cold air challenge, and the interval between PCR-testing and lung function testing (Table 1). Overall, 77% of the children were tested within a time range of 24–29 months after infection (71% of the Mpn-Pos and 81% of the Mpn-Neg children). All statistical procedures were performed identically on the whole population (\( n = 65 \)) and on the 77% examined within the 5-month time range with similar findings in the sub-population and the whole population.

No differences were found between the two groups in baseline lung function, bronchial responsiveness to cold air challenge as measured by sRaw or the degree of responsiveness (Table 3 and Figs. 2 and 3). Also, the same proportion of children in the two groups exhibited bronchial hyperresponsiveness to cold air challenge (Table 2).

**Microbiological Examination**

A total of 90 positive PCR results were found among all children included. In 67 children at least one microbe was found and 20 children had more than one positive PCR. Details for the 65 children with full lung function tests are given in Table 4. The most prevalent microbes, apart from \( M. \ pneumoniae \), were adenoviruses and RS virus. Other microbes were detected only in very small numbers. These findings did not allow for any statistical calculations.

**Data From the Danish National Patients’ Registry**

A total of 27 (13 Mpn-Pos and 14 Mpn-Neg) of the 99 children included in the study had a record at the Danish National Patients’ Registry within the relevant ICD10 codes. Only six children (five Mpn-Pos and one Mpn-Neg) had relevant hospital admissions related in time to the Mpn-PCR test. The Mpn-Neg child, who was not seen, had RS virus pneumonia with asthmatic bronchitis. The five Mpn-Pos all had pneumonia; four of these were seen, and two obtained full lung function measurements, which showed no bronchial hyperresponsiveness (both had had

**TABLE 3—Lung Function Data**

<table>
<thead>
<tr>
<th></th>
<th>Mpn-Pos</th>
<th></th>
<th>Mpn-Neg</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>( P )-value</td>
<td>Theta</td>
<td>( CI_{low} )</td>
</tr>
<tr>
<td>sRaw (kPa sec)</td>
<td>28</td>
<td>1.17</td>
<td>0.28</td>
<td>37</td>
<td>1.21</td>
<td>0.26</td>
<td>0.45</td>
<td>0.1</td>
</tr>
<tr>
<td>delta sRaw (%)</td>
<td>28</td>
<td>13.4</td>
<td>24.5</td>
<td>37</td>
<td>9.0</td>
<td>18.4</td>
<td>0.49</td>
<td>( -3.0 )</td>
</tr>
</tbody>
</table>

\( P \)-value, for the Mann–Whitney statistic; Theta, Hodges–Lehmann estimate for the median difference between Mpn-Pos and Mpn-Neg; \( CI_{low} \), exact lower limit of the 95% CI for Theta; \( CI_{high} \), exact upper limit for the 95% CI for Theta; sRaw, specific airway resistance as measured by whole body plethysmography; delta sRaw, change in sRaw before and after cold air challenge.

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**Fig. 2.** Baseline lung function: measured as specific airway resistance (sRaw, kPa sec) with whole body plethysmography. The figure shows the distribution of sRaw in the \( M. \ pneumoniae \) positive and \( M. \ pneumoniae \) negative groups (each \( \times \) represents an individual).

**Fig. 3.** Bronchial responsiveness to hyperventilation with cold, dry air measured as change in specific airway resistance (\( \Delta sRaw \), %) before and after a 4-min isocapnic cold air challenge at a ventilation rate equivalent to body weight (\( l \times min^{-1} \)). The figure shows the distribution in the \( M. \ pneumoniae \) positive and \( M. \ pneumoniae \) negative groups (each \( \times \) represents an individual).
Adenovirus was found either alone or in combination with M. pneumoniae. RS virus and Adenovirus were common, with 4, 6, and 10% of children, respectively. Only one child had U. parvum, and no other bacteria were found.

At least two children had atelectases from their M. pneumoniae pneumonia and one also had asthmatic bronchitis. Of the 65 children with full lung function measurements, only one in each group had recurrent contacts and both included asthmatic symptoms. All together, the only apparent difference between the Mpn-Pos and Mpn-Neg groups was the frequency of pneumonias in the Mpn-Pos group (Table 2).

To estimate potential participation bias, that is, if results might be influenced by a higher proportion of more severe disease in the participating Mpn-Neg children, data from the Danish National Patients' Registry were used in combination with laboratory data: Four of the five children hospitalized with M. pneumoniae pneumonia had specimens submitted from hospitals and one sample was from a general practitioner. The Mpn-Neg child with pneumonia was tested by a general practitioner and later hospitalized. Hence, information about the health care setting (specimen from hospital or general practitioner) was used to estimate participation bias with regards to disease severity. No support was found for participation bias as a confounder of our conclusions on bronchial hyperresponsiveness as judged by the health care setting (Table 5).

### DISCUSSION

This study found no long-term influence on lung function or bronchial responsiveness from M. pneumoniae infection in preschool children (ages 0–5 years).

M. pneumoniae is a well-known trigger of symptoms in asthma patients, and has also been associated with onset of wheezing. Findings of elevated total serum IgE, IgE to common allergens, and IgE to M. pneumoniae and IgE to common allergens during the infection have associated the infection with the asthmatic reactions, although this might also indicate boosting in already sensitized individuals. Increased cytokine levels (IL-4 and IL-4/IFN-γ ratio) in BAL fluid have suggested a TH2-like cytokine response favoring IgE production. An etiological role in the pathophysiology of asthma has also been suggested based on findings of colonization or chronic infection in adults with chronic asthma.

Our findings do not support a role of early infection with M. pneumoniae in the development of recurrent wheeze or asthma. No differences between the M. pneumoniae infected and the control group were detected with regard to baseline lung function or bronchial responsiveness to cold air (Table 3, Figs. 2 and 3). This clearly suggests that a previous infection with M. pneumoniae in preschool children had not influenced lung function 2–3 years later.

We determined the specific airway resistance (sRaw) by whole-body plethysmography before and after cold air challenge to evaluate lung function and bronchial responsiveness. This method has been shown to be a valid measure of lung function in young children and it has a high discriminatory power between asthmatic and non-asthmatic responses. In this study full measurements were achieved in 79% of the participants seen for examination.

The lung function test was performed several months after the infection, and our timing can obviously be questioned. A short-term difference between groups would have been overlooked because the children were examined on average 2 years after the infection. On the other hand, it could be argued that any potential difference in lung function that has disappeared after 2–3 years cannot be considered a true long-term effect, which was the main focus of the present study.

The number of children studied is small, and thus, it could be argued that the inability to demonstrate a difference between groups could be due to a type II error.
Indeed, with a 24% prevalence of bronchial hyperresponsiveness in the MpnNeg group, the prevalence should have been doubled (48%) in the MpnPos group to reach statistical significance. However, the fact that the prevalence of bronchial hyperresponsiveness was 24% in the MpnNeg group as compared to 21% in the MpnPos group makes it highly unlikely that an enlargement of the groups would have enabled us to demonstrate a significant adverse effect of *M. pneumoniae* infection in early childhood in relation to asthma (Fig. 3).

A true adverse effect of a *M. pneumoniae* infection could be obscured if the *M. pneumoniae* negative children had a higher predisposition to hyperresponsiveness due to unbalanced participation bias between groups. No such differences were found, and the prevalence of atopic predisposition from first-degree relatives was comparable to recent data from a Danish birth-cohort of 562 children (51%). Only 99 consented of 249 invited. This probably reflects a general recruitment bias to the study but does not affect the comparison between the Mpn-Pos and -Neg groups. The retrospective design of the present study did not enable collection of clinical data on acute disease other than data of hospital registrations. This might be considered critical in controlling for differences between groups or as basis for sub-group analyses. The cohort design of selecting by infectious agent and not by disease presentation was, however, essential in addressing the issue of etiology. We considered the lack of acute disease data justified, since the design allowed us to address a relatively narrow age group, which was laboratory tested during an epidemic season with a broader epidemiological representation than during endemic seasons.

Our study design has the disadvantage of missing clinical data about the acute disease phase. As a rough measure of disease severity or disease burden, we obtained data from the Danish National Patients’ Registry to control for major differences between the two groups. Only six children (five Mpn. positive and one Mpn. negative) had admissions related in time to the *M. pneumoniae* PCR test.

The design benefits from the broad representation of children as opposed to a hospital setting, but only prospective, population based epidemiological cohort studies can address the issue of etiology in a conclusive manner.

Based on earlier studies, one would have expected to find an influence of an earlier *M. pneumoniae* infection. We found no difference in bronchial hyperresponsiveness between groups, but more hyperresponsive children than expected from a previous study. However, the population in that study was not representative, and the true prevalence of bronchial hyperresponsiveness in the background population is not known for this age group. On the other hand, the proportion of children with asthma (8–9%)...
and atopic predisposition corresponds well with prevalences for both in the Danish population.

Obviously, any effect of other microbiological agents (e.g., RS virus) causing infection in the *M. pneumoniae* negative children could theoretically have interfered with our results if these microbes had a significant influence on lung function. However, the baseline lung function in both study groups was comparable with that observed in other studies. Furthermore, the long follow-up period would tend to minimize such an effect, since most of the children would have experienced several viral infections during the follow-up period, whereas the risk of an *M. pneumoniae* infection during the intervening period was much less likely due to the low incidence of *M. pneumoniae* infections after epidemics, including this epidemic (Fig. 4). We are aware of the discussion of chronic infection or a possible carrier state with *M. pneumoniae* and found a very low prevalence of both pathogens, even when specimen collection was performed in an epidemic period. Indeed, we tested samples from two other cohorts for the presence of *M. pneumoniae* or *C. pneumoniae* and found no relation was seen between any microbe or combinations of microbes and lung function.

In conclusion, we found that *M. pneumoniae* lung infection in young children of 1–5 years, is not associated with long-term influence on lung function and bronchial hyperresponsiveness 2–3 years after the infection. Further investigations of *M. pneumoniae* in asthma etiology should be undertaken as population cohort studies rather than case–control studies to allow for a full description of various disease patterns and potential complications.

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