Association between Chlamydia pneumoniae antibodies and wheezing in young children and the influence of sex

E Normann, J Gnarpe, B Wettergren, C Janson, M Wickman, L Nordvall

Background: The proposed association between Chlamydia pneumoniae (Cpn) infection and wheezing needs further clarification.

Methods: Serum samples obtained from 1581 children aged 4 years in a population based cohort were tested for antibodies to Cpn and IgE antibodies to common allergens. Data on environmental factors and disease were collected prospectively from birth.

Results: The occurrence of IgG antibodies to Cpn at 4 years of age was associated with reported wheezing at different ages; however, these findings were most often not significant. In girls, the occurrence of anti-Cpn IgG was associated with wheezing at the ages of 1, 2, and 4 years (odds ratios [ORs] 3.41 (95% confidence interval [CI] 1.46 to 7.96), 2.13 (95% CI 1.02 to 4.44), and 2.01 (95% CI 1.14 to 3.54), respectively), and even higher ORs were observed for each age category when only high level antibody responses to Cpn were analysed. At the time of blood sampling the association between anti-Cpn IgG and wheezing was restricted to girls without atopic sensitisation (OR 2.39 (95% CI 1.25 to 4.57). No associations with wheezing were detected in boys, in whom IgE sensitisation was inversely associated with the presence of anti-Cpn IgG (OR 0.49 (95% CI 0.26 to 0.90).

Conclusions: This study suggests an association between evidence of earlier Cpn infection and a history of wheezing in young girls. Infection with Cpn may be an important risk factor for wheezing and possibly for non-atopic asthma, predominantly in girls.


**Asthma:** More than three episodes of wheezing over the last year or more than one episode over the last year if the child had been given inhaled steroids.

**Eczema:** Dry skin with itchy rashes in characteristic areas during the last year or a doctor’s diagnosis of atopic dermatitis after the age of 2 years.

**Suspected allergic rhinitis:** Non-infectious rhinitis during the last year or rhinitis combined with itchy red eyes.

**IgE sensitised:** Serum IgE antibody levels >0.35 kUA/l to any of the following allergens: house dust mite, cat, horse, dog, pollen from birch, timothy or mugwort, mould, hen’s eggs, wheat, fish, cow’s milk, soy, or peanuts. For sensitisation to inhalants positive Phadiatop tests were used and for food sensitisation positive fx5 tests were used (Phadia CAP).

**RESULTS**

With the exception of eczema, higher prevalences of study diagnoses were reported for boys. One hundred and fifty nine children (10.1%) had detectable anti-Cpn IgG levels, with no significant difference between the sexes. The results for all study variables are shown in table 1.

The occurrence of anti-Cpn IgG was associated with a history of wheezing, most evident when reported at 2 years of age (table 2).

Only 42 children had anti-Cpn IgA, which made possible associations between serology and clinical diagnoses difficult to study. Few children with anti-Cpn IgA had any of the study diagnoses reported at 4 years of age. Children with IgA antibodies to Cpn had a reduced risk of reactive airway disease and of being IgE sensitised at the time of blood sampling. None of the associations in table 2 remained statistically significant after adjusting for multiple statistical comparisons.

When the sexes were examined separately, an observed association between anti-Cpn IgG and wheezing was restricted to girls (table 3). The respective ORs calculated for boys and girls regarding IgE sensitisation were also different in that the occurrence of IgG antibodies to Cpn was associated with a lowered risk of IgE sensitisation in boys and an increased risk of IgE sensitisation in girls. When the statistics in table 3 were adjusted for multiple comparisons, including stratification by sex and the nine different outcomes, the lowest ordered p value had to be <0.0028 to be considered as significant; this requirement was true only for the association between anti-Cpn IgG and wheezing in girls at 1 year of age.

Post hoc analyses were performed to further scrutinise indicated associations. The associations found between anti-Cpn IgG and wheezing in girls were unchanged after adjusting for IgE sensitisation (data not shown). When stratifying for IgE sensitisation, anti-Cpn IgG was associated with an increased risk of wheezing in non-IgE sensitised girls at 4 years of age (OR 2.39 (95% CI 1.25 to 4.57), p = 0.0064) but not in IgE sensitised girls (OR 1.13 (95% CI 0.34 to 3.70)). No such association was found for boys, with or without sensitisation. IgE sensitisation was a risk factor for wheezing in 4 year old boys and for asthma in both sexes (data not shown).

To test whether high levels of anti-Cpn IgG would affect the results, another cut off level was chosen (IgG >1/512), yielding a group which comprised 10% of Ig positive children with raised titres. Sixteen children had anti-Cpn IgG above this level, 10 of which were girls. In girls a high level of anti-Cpn IgG was associated with an even higher risk for airway disease: wheezing at 1 year (OR 10.8 (95% CI 2.60 to 44.7)).

### Table 1 Prevalence of study variables in children at the age of 4 years according to sex

<table>
<thead>
<tr>
<th>Variables (no of children)</th>
<th>Boys (n = 827)</th>
<th>Girls (n = 754)</th>
<th>OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diagnoses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheeze at 1 year (n = 88)</td>
<td>57/820 (7.0%)</td>
<td>31/743 (4.2%)</td>
<td>1.72 (1.09 to 2.69)</td>
</tr>
<tr>
<td>Wheeze at 2 years (n = 139)</td>
<td>82/813 (10.1%)</td>
<td>57/740 (7.7%)</td>
<td>1.34 (0.94 to 1.92)</td>
</tr>
<tr>
<td>Wheeze at 4 years (n = 285)</td>
<td>167/824 (20.3%)</td>
<td>118/749 (15.8%)</td>
<td>1.36 (1.05 to 1.76)</td>
</tr>
<tr>
<td>Asthma (n = 148)</td>
<td>94/821 (11.4%)</td>
<td>54/749 (7.2%)</td>
<td>1.66 (1.17 to 2.37)</td>
</tr>
<tr>
<td>Suspected allergic rhinitis</td>
<td>49/818 (6.0%)</td>
<td>20/737 (2.7%)</td>
<td>2.28 (1.34 to 3.89)</td>
</tr>
<tr>
<td>Eczema (n = 313)</td>
<td>155/825 (18.8%)</td>
<td>158/753 (21.0%)</td>
<td>0.87 (0.68 to 1.12)</td>
</tr>
<tr>
<td>Sensitisation to inhalants</td>
<td>154/827 (18.6%)</td>
<td>85/754 (11.3%)</td>
<td>1.80 (1.35 to 2.40)</td>
</tr>
<tr>
<td>Sensitisation to foods (n = 234)†</td>
<td>128/827 (15.5%)</td>
<td>106/754 (14.1%)</td>
<td>1.12 (0.85 to 1.48)</td>
</tr>
<tr>
<td>Any IgE sensitisation (n = 371)</td>
<td>214/827 (25.9%)</td>
<td>157/754 (20.8%)</td>
<td>1.33 (1.05 to 1.68)</td>
</tr>
<tr>
<td><strong>Anti-Cpn Ig</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Cpn IgG (n = 159)</td>
<td>85/827 (10.3%)</td>
<td>74/754 (9.8%)</td>
<td>1.05 (0.76 to 1.46)</td>
</tr>
<tr>
<td>Anti-Cpn IgA (n = 42)</td>
<td>16/827 (1.9%)</td>
<td>26/754 (3.4%)</td>
<td>0.55 (0.29 to 1.04)</td>
</tr>
</tbody>
</table>

*Odds ratios are given using girls as the reference.
†Sensitisation to inhalants = Phadiatop >0.35 kUA/l; sensitisation to foods = fx5 >0.35 kUA/l.
trachomatis in children, nor were there any IgM antibodies to whether some wheezing children are prone to exhibit strong antibody response relationship. It is tempting to speculate whether one or several Cpn infections trigger both a strong antibody response pronounced, indicating a dose-sensitised. This association was even stronger when the evidence of earlier Cpn infection and wheezing in girls at 1, 2 and 4 years remained statistically significant after adjustment for multiple testing (p = 0.0026). The results of this study suggest an association between asthma (OR 5.78 (95% CI 1.44 to 23.2)). The association in girls between high levels of anti-Cpn IgG and wheezing at 1, 2 and 4 years (OR 5.54 (95% CI 1.56 to 19.6)), and wheezing at 2 years (OR 3.51 (95% CI 0.71 to 17.4)).

**DISCUSSION**

The results of this study suggest an association between evidence of earlier Cpn infection and wheezing in girls at 1, 2 and 4 years of age, especially in girls who were not IgE sensitised. This association was even stronger when the antibody response was pronounced, indicating a dose-response relationship. It is tempting to speculate whether one or several Cpn infections trigger both a strong antibody response and wheezing in some individuals or, alternatively, whether some wheezing children are prone to exhibit strong antibody responses to Cpn. No corresponding association was found for boys. Instead, boys exhibited an inverse relationship between anti-Cpn IgG and IgE sensitisation to common allergens. Pre-school children are frequently infected by Cpn, and there is no reason to believe that exposure to the agent would differ between the sexes. In the present study almost the same proportion of boys and girls had antibodies to Cpn, indicating equal exposure. Most exacerbations of wheezing in young children are probably triggered by respiratory tract infections, some of which may be Cpn infections.

Table 2: Association between study diagnoses and anti-Cpn IgG and IgA in children at 4 years of age

<table>
<thead>
<tr>
<th>Diagnoses</th>
<th>IgG+ and IgA– OR (95% CI)</th>
<th>IgG+ and IgA– OR (95% CI)</th>
<th>IgG– OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheeze at 1 year</td>
<td>10/117 [8.6]</td>
<td>1.68 (0.84 to 3.35)</td>
<td>1.89 (0.66 to 5.45)</td>
</tr>
<tr>
<td>Wheeze at 2 years</td>
<td>16/116 [13.8]</td>
<td>1.75 (1.00 to 3.07)</td>
<td>1.93 (0.79 to 4.70)</td>
</tr>
<tr>
<td>Wheeze at 4 years</td>
<td>30/117 [25.6]</td>
<td>1.61 (1.04 to 2.50)</td>
<td>0.78 (0.32 to 1.87)</td>
</tr>
<tr>
<td>Asthma</td>
<td>16/117 [13.7]</td>
<td>1.57 (0.90 to 2.75)</td>
<td>0.76 (0.23 to 2.51)</td>
</tr>
<tr>
<td>Suspected allergic rhinitis</td>
<td>6/116 [5.2]</td>
<td>1.16 (0.49 to 2.73)</td>
<td>0.04/0.06</td>
</tr>
<tr>
<td>Eczema</td>
<td>217/1422 (15.3)</td>
<td>1.9/117 (16.2)</td>
<td>3/42 [7.1]</td>
</tr>
<tr>
<td>Sensitisation to inhalants</td>
<td>210/1422 (14.8)</td>
<td>20/117 [17.1]</td>
<td>4/22 [9.5]</td>
</tr>
<tr>
<td>Sensitisation to foods</td>
<td>339/1422 (23.8)</td>
<td>28/117 [23.9]</td>
<td>4/22 [9.5]</td>
</tr>
</tbody>
</table>

*Odds ratios are given using seronegative values (Ig–) for each diagnosis as the reference.
†Number of children with diagnosis/total number of children within category (%).
‡Sensitisation to inhalants = Phadiatop >0.35 kUA/l; sensitisation to foods = fx5 >0.35 kUA/l.

Table 3: Association between study diagnoses and anti-Cpn IgG in children at 4 years of age according to sex

<table>
<thead>
<tr>
<th>Diagnoses</th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheeze at 1 year</td>
<td>6/85 [7.1]</td>
<td>8/74 [10.8]</td>
</tr>
<tr>
<td>Wheeze at 2 years</td>
<td>12/84 [14.3]</td>
<td>10/72 [13.9]</td>
</tr>
<tr>
<td>Wheeze at 4 years</td>
<td>17/85 [20.0]</td>
<td>19/74 [25.7]</td>
</tr>
<tr>
<td>Suspected allergic rhinitis</td>
<td>4/84 [4.8]</td>
<td>27/2 [23.8]</td>
</tr>
<tr>
<td>Sensitisation to inhalants</td>
<td>12/84 [14.3]</td>
<td>10/74 [13.5]</td>
</tr>
<tr>
<td>Any IgE sensitisation</td>
<td>13/85 [15.3]</td>
<td>19/74 [25.7]</td>
</tr>
</tbody>
</table>

*Number of children with diagnosis/total number of children within category [%].
†Test of homogeneity of ORs between sexes.
‡Statistically significant also when adjusting for multiple testing (p = 0.0026).
§Sensitisation to inhalants = Phadiatop >0.35 kUA/l; sensitisation to foods = fx5 >0.35 kUA/l.
anti-Cpn IgG and longstanding asthma in adults.14 As in the present study, the association between wheezing and an immune response to Cpn was significant only for women and was most apparent for those without IgE sensitisation. The scientific literature is limited with regard to data on differences between the sexes with respect to immune response to various microbes in relation to wheezing. von Hertzen et al detected a difference between sexes when studying Mycobacterium tuberculosis where previous infection was associated with a reduced risk for subsequent allergic disease and asthma in females.20 The present study cohort has previously been investigated with serology to Epstein-Barr virus and cytomegalovirus, but no differences between the sexes were found (M Wickman, personal communication).14 15

There may be several different explanations for the observed differences between the sexes. In spite of the large study population, the power may have been insufficient to detect an existing association in boys. A contributing factor could be that the association was masked in boys due to the Carter effect.22 This theory is based on the observation that some factors involved in diseases with multiple risk factors may be more easily detected in subjects lacking one dominating risk factor. Consequently, it may be easier to confirm a risk factor common for both sexes in the sex with the lowest prevalence of disease (in this case girls) because they are less influenced by another more important risk factor.23 Of the possible factors of importance in this context, atopic allergy appears particularly plausible since boys were more often IgE sensitised than girls. A possible association between Cpn infection and reactive airway disease in boys might therefore have needed a larger study population to be detected.

Given that there is a true sex difference, this might be explained by differences in respiratory tract reactivity upon Cpn infection as well as in differences in immune responses to Cpn. It has been suggested that girls are more susceptible to selected non-allergen effects in their airways, and are thus possibly more susceptible to reactive airway disease when infected with Cpn.24 25 Another explanation could be that some individuals, most often girls, might have an impaired ability to eliminate Cpn when infected.26 If persistently infected over an extended period of time, the immune system might respond with higher levels of antibodies to Cpn which might not be protective. Such persistent Cpn infection might induce a chronic inflammatory response and thus wheezing in some individuals.

The prevalence of non-atopic asthma is increasing, especially in girls.27 Chronic or repetitive infections with, for example, Cpn and increased susceptibility to airway irritants in females could possibly cause a higher prevalence of asthma in teenage girls than in boys.21 The results obtained from this and other studies suggest that the sexes develop their airway disease based on different prerequisites and/or on different time scales.26 29

In the present study there were some indications of an inverse association between anti-Cpn Ig and IgE sensitisation to common allergens. These findings are in agreement with two recent follow up studies reporting a lower prevalence of allergy in children previously infected with Cpn.20 30 The findings fit with the “hygiene hypothesis”, which suggests that infections in early life may protect against atopic disease.32 However, others have reported a positive association between atopy and Cpn infection.33 34 The latter studies differ from the present in that they correlated evidence of allergy with detection of bacteria, thus indicating an ongoing infection. Those studies did not include observations derived from earlier infections and this might possibly explain the disparate results compared with those of the present study.

This study cannot establish whether the observed associations between wheezing and positive serology to Cpn are due to acute or chronic infection with this bacterium. There is little information available on how to interpret serological reactions to Cpn infection in children. The detection of anti-Cpn IgG reflects earlier exposure to Cpn, but some children do not develop levels of antibodies detectable by MIF, especially when they are very young.27 35 In this study, children without anti-Cpn IgG were not tested for IgA antibodies because we have never found a child with anti-Cpn IgA who did not also have IgM or IgG antibodies to Cpn.36 In adults, anti-Cpn IgA is suggested to be associated with persistent infection but there is no information regarding children, even though the results of this study may indicate some interesting findings in children with anti-Cpn IgA. No method for bacterial detection was included in the present study since host reactivity to the agent was the major parameter of interest.

A cohort study such as this has important limitations. Children who continue to participate in a longitudinally designed investigation may be different from those who drop out—that is, in this study continuing children possibly had more respiratory tract diseases. This creates a problem for the ability to formulate generalisations but may improve the efficiency of analyses of associations between asthma and environmental risk factors including infections. However, it cannot be excluded that such a selection bias may introduced unknown confounding factors into this study. Importantly, those who donated blood did not differ with respect to parental educational level and important risk factors, but there was a tendency for children with wheeze to remain in the study.14 In addition, findings in subgroups may be misleading by selection, but it appears unlikely that such biases would be associated with the sex of the children. It is also important to acknowledge the increased risk of getting significant results obtained by chance when performing multiple statistical tests. For that reason, the Simes procedure was carried out on the different groupings of analyses and only a few associations between girls and wheezing remained significant after adjusting for multiple comparisons. Furthermore, even though the size of the study population was large, subgrouping was associated with an important loss of power. Some important differences may have been too small to be detected, particularly when the low sensitivity of the serological method is considered. MIF is, however, considered as the gold standard for serological testing to this organism and it is commonly applied in studies of seroprevalence.37 The study is also limited by the fact that only one blood sample was obtained from each child and that the serological response reflects a previous event, making this information retrospective. Since a low prevalence of self-produced antibodies to Cpn can be expected in even younger children when using MIF, an earlier blood sampling in this study cohort was not justified. Korppi et al studied younger individuals, which may be the reason why they could not detect an association between Cpn and asthma.

In summary, this study of young children suggests an association between evidence of earlier Cpn infection and wheezing, but only in girls. This result implies that, even when subjects are very young, the influence of sex should be included in studies on reactive airway diseases.38 This cohort of children will be followed longitudinally to further determine the role of Cpn infection on asthma and allergy.

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