Lung function and bronchodilator response in 4-year-old children with different wheezing phenotypes

Ellie Oostveen¹, Sandra Dom², Kristine Desager³, Margo Hagendorens³, Wilfried De Backer¹, Joost Weyler²

¹Dept. of Pulmonary Medicine, ²Dept. of Epidemiology and Social Medicine, ³Dept. of Pediatrics, Antwerp University Hospital and University of Antwerp, Belgium

Corresponding author:
Ellie Oostveen, PhD
Dept. of Pulmonary Medicine, Antwerp University Hospital
Wilrijkstraat 10, B-2650 Edegem-Antwerp, Belgium
Tel + 32 3 8213357, Fax + 32 3 8214447
Email: Ellie.Oostveen@uza.be

Running title: Lung function in wheezing preschool children
ABSTRACT

Persistent wheeze is a common chronic disease in early childhood and later may progress to asthma. However, the association between the pre- and the post-bronchodilator lung function and the wheezing phenotype in preschool children is not known.

Children 4 years of age involved in a prospective birth cohort study (in Antwerp, Belgium) on perinatal factors and the occurrence of asthma and allergies were invited to participate in lung function measurements with the forced oscillation technique. The wheezing phenotype was assessed via (bi)annual questionnaires. Wheezing phenotype and baseline respiratory impedance data were available for 325 children, 96% of whom underwent bronchodilation tests. The baseline resistance at 4 Hz was higher in children with early-transient (11.0 hPa.s.L⁻¹, n=127), or persistent wheeze (11.9 hPa.s.L⁻¹, n=54) than in children who never wheeze (10.3 hPa.s.L⁻¹, n=144). After bronchodilation, the resistance decreased on average by 22%. The decrease was greater among the persistent wheezers than among those who never wheezed (3.4 vs. 2.3 hPa.s.L⁻¹).

The baseline lung function was poorer and the bronchodilator response was greater in 4-year-old children with persistent wheeze than in those who never wheeze or who have early-transient wheeze, implying a higher bronchomotor tone in the former group.

Keywords: childhood asthma, cohort studies, forced oscillations, preschool children, wheezing.
INTRODUCTION

Persistent wheezing is a common chronic disease in young children, and a major cause of preschool morbidity. The prevalence of recurrent days with cough, wheeze and/or breathlessness in children aged between 1 and 5 yrs has been reported to be 32%, and as many as 24% of preschool children suffer from weekly symptoms despite current treatment (1).

Atopy and increased airway responsiveness in young children have been associated with persistent wheeze and asthma in later life (2-4). Furthermore, abnormalities in pulmonary function during infancy have been demonstrated to be an important determinant of subsequent respiratory symptoms and lung function, independently of atopy and airway responsiveness (5-7). It was recently reported that a persistent wheeze and a low airway function at school age are independently associated with chronic asthma in early adulthood (8). These findings have increased our awareness of the impact of respiratory events in early life on the development of respiratory disease in adulthood. At present, however, children at high risk of developing persistent asthma are still inadequately identified (9).

Recent advances in diagnostic technologies have led to the standardization of a number of techniques for the assessment of lung function in the preschool age group (10). The use of these techniques in preschool children with recurrent wheeze may have important implications for the correct identification of children at risk of developing severe asthma in later life. Brussee et al. (11) recently reported that 4-year-old children with persistent wheeze exhibited higher interrupter resistances
as compared with age-matched children who never wheezed or those with the early-
transient wheezing phenotype.

Asthma is at least partially defined by abnormalities in pulmonary function,
including variable airway obstruction. In addition to reporting on baseline lung
function measures (respiratory resistance and reactance as determined with the
forced oscillation technique) and the bronchodilator responsiveness in 4-year-old
children, we also determined the association between the baseline and post-
bronchodilator resistance and reactance and the phenotypes of childhood wheezing.

METHODS

Study design and study population

The Prospective Cohort on the Influence of Perinatal Factors on the Occurrence of
Asthma and Allergies (PIPO) study is a prospective birth cohort study involving
1128 children. Between June 1997 and December 2001, pregnant women in the
Antwerp region in Belgium were invited to participate by completing a screening
questionnaire. Data on demography, respiratory symptoms and risk factors for
asthma were collected through the use of postal questionnaires: the first
questionnaire covered the first year of life, with subsequent questionnaires at 6-
month intervals up to the age of 4 years. The questionnaires were based on validated
ISAAC questionnaires (12). At 4 years of age, the children included in the PIPO
study were invited for medical examination and lung function testing at the
University of Antwerp Hospital. The children who regularly used bronchodilators
were asked to abstain from their use on the examination day, whereas the use of
inhaled corticosteroids was not interrupted. The atopic status of the child and the parents was assessed from specific IgE levels against common food and inhalant allergens, determined from blood samples. All parts of the study were approved by the University Ethics Committee, and written informed parental consent was obtained before each assessment.

**Lung function measurements**

*Forced oscillation technique*

Respiratory impedance (Zrs) was measured in the frequency range 4-32 Hz, by using a home-made setup that met the ATS/ERS requirements (10, 13). Measurements were performed according to recent international guidelines (10). Recordings lasted for 16 s, and the average of 3-5 acceptable Zrs data was used for further analysis.

*Measurement protocol*

Children sitting upright on their parent’s lap were measured with their head in a neutral position. The parent supported the child’s chin and cheeks with both hands. The child was instructed to breathe normally while watching a video film.

*Reversibility testing*

After the baseline Zrs measurement, 200 µg of salbutamol was administered through a spacer (OptiChamber, Respironics without an additional face mask or a mouthpiece). The Zrs measurement was repeated 15 min thereafter. The pre- and post-bronchodilator measurements were performed by the same technician.

*Short-term reproducibility*

In 50 children, chosen at random, the short-term reproducibility of the Zrs data was assessed by repeating the baseline measurement after a 15-min time interval.
Wheezing phenotype

Symptoms of wheeze were assessed through core questions from the ISAAC questionnaire (12). According to the history of wheeze reported by the parents in the (bi)annual questionnaires, the children were divided into those who never wheeze, and those with early-transient, late-onset or persistent wheeze (2, 11).

Data analysis

Statistics

From the measured Xrs data, the "area under the reactance curve" (AX), i.e. the integrated area of all Xrs data below zero up to fres, was determined. Data are expressed as means (SD) unless otherwise specified. Statistical analyses were performed by using SPSS for Windows, Version 15.0 (SPSS Inc., Chicago, Ill, USA). After testing the data for normality, the difference between the two baseline measurements was analyzed with paired t-tests. The differences between the four groups of wheezing phenotype were assessed with one-way ANOVA, post-hoc LSD analysis or Mann-Whitney analysis (or chi square). Significance was accepted at the 0.05 level.

The Online Depository provides additional details on the measurement technique, the questionnaires, the definitions of the wheezing phenotypes and the analysis of adjustment for potential confounders.

RESULTS
Baseline Zrs data were obtained on 535 children and post-bronchodilator Zrs data on 501 children (the online supplement includes a flowchart of the children participating in the study). In 203 children (38%), the wheezing phenotype could not be determined because of incomplete or missing questionnaires. These children were excluded from the study group. The remaining children (n=332) were divided into those who never wheeze (n=144, 43%), and those with an early-transient (n=127, 38%), a late-onset (n=7, 2%) or a persistent wheezing phenotype (n=54, 16%). The group of children with late-onset wheeze was considered too small for further analysis. In 313 of the remaining 325 children (=96%), the post-bronchodilator response was also assessed. The general characteristics of the subgroups are listed in Table 1. The anthropometric characteristics and gender distribution were not different in the children with the different wheezing phenotypes. In comparison with the group who never wheeze, a significantly higher proportion of the persistently wheezing children were atopic, and had used antibiotics at 4 years of age, while the children with early transient or persistent wheeze suffered from lower respiratory tract infections at 4 years of age significantly more frequently.

The short-term reproducibility of Zrs was assessed in 27 boys and 23 girls. This group of children did not differ significantly from the study group (n= 325 children) in terms of the distribution of the wheezing phenotypes (in 12 children, the wheeze phenotype was undetermined; 18 children had never wheezed, 16 children had early-transient and 4 children had a persistent wheeze phenotype) or the value of R4 or AX (see below). The results of the two baseline measurements of respiratory resistance (Rrs) and reactance (Xrs) are depicted in Figure 1. The Rrs data obtained 15 min apart were not significantly different, except that R6 and R8 were slightly lower (by ~ 0.4 hPa.s.L⁻¹) in the second baseline measurement (p<0.05). A small
upward shift was found in the second baseline Xrs curve relative to the first at almost all data points (p<0.01); the Xrs data from the second baseline measurement were on average 0.4 hPa.s.L⁻¹ larger than those from the first measurement.

The baseline Rrs and Xrs in the groups of children with different wheezing phenotypes are reported in Figure 2. Rrs displayed a slight negative frequency dependence in the children who never wheeze. Rrs increased in magnitude and the negative frequency dependence of Rrs became progressively more marked in the groups of children with the early-transient or persistent wheezing phenotypes. In comparison with the children who never wheeze, Xrs became more negative and exhibited an ever higher resonance frequency (fres), in the sequence early-transient and persistent wheezing phenotype (see Table 1 of the online supplement). Since the largest differences in Zrs at baseline between the wheezing phenotype groups were observed in the low-frequency range, our subsequent analysis was focused on those frequencies. R4, R6, R8, X4, X6, X8 and AX were analyzed. Figure 3 presents the values of R4 and AX at baseline and after bronchodilation. The children with early transient, late-onset or persistent wheeze had baseline values of R4 and AX that were significantly larger than those of the children who never wheeze. Further, the children with persistent wheeze had significantly larger baseline R4 values than the children with early-transient wheeze.

Bronchodilation significantly decreased the values of R4 and AX in all the groups of children. On average, R4 was decreased by 22% by bronchodilation. However, for
the children with persistent wheeze, the decrease in R4 after bronchodilation was significantly larger as compared with the children who never wheeze and those with early-transient wheeze (p< 0.05). After bronchodilation, the values of R4 were still significantly larger in the children with late-onset or persistent wheeze than in those who never wheeze (p<0.02). Relative to the group who never wheeze, the decreases in AX were significantly larger in the children with early-transient and persistent wheeze, and accordingly the post-bronchodilator values of AX were comparable in the three groups of children with the different wheezing phenotypes (see Figure 3 and Table 2).

The characteristics of the children who never wheeze were used to determine the cutoff values for a significant bronchodilator response. From the 5th percentiles, the cutoff points were set to be absolute decreases of 5.5 hPa.s.L⁻¹ and 31.0 hPa.L⁻¹ for R4 and AX, respectively, and relative decreases of 43% and 81%, respectively. As compared with the relative change, expressing the bronchodilator response in absolute change proved to be a more sensitive means of differentiating between the groups (see Table 3). Significantly more children with a persistent wheeze (13%) than children who never wheeze (4%) responded positively to bronchodilation when the absolute change in R4 was considered (p<0.05). When the absolute change in AX was used to define a positive response, significantly more children with an early-transient (14%) or persistent wheeze (23%) responded significantly to bronchodilation than did the children who never wheeze (4%). The combined
changes in resistance and reactance at 4 Hz after bronchodilation in the different groups of wheeze phenotype followed the same path (Figure 4).

DISCUSSION

Although there was a considerable overlap in lung function data between the groups, our study did reveal significant differences both in the baseline respiratory function and in the bronchodilator response between preschool children with different wheezing phenotypes. At 4 years of age, the children with early transient wheeze yielded higher baseline resistance values as compared with those who never wheezed, although 43% of the children with early transient wheeze had experienced only one wheezing episode during the first 3 years of life. The children with persistent wheeze, of whom two-thirds had experienced several episodes of wheeze during their 4th year of life, displayed a poorer baseline lung function than that of the children who never wheeze or those with early-transient wheeze, and their bronchodilator response was larger.

There was a considerable dropout rate in our study: half of the children who entered the cohort at birth were not available for examination at 4 years of age and 38% of the remaining had incomplete or missing questionnaires. However, despite the loss of data, we feel that the dropout does not affect or limit the interpretation of our results. We did not intend to study lung function and wheeze phenotype in a representative sample of the population; the purpose of our study was rather to
investigate the association between lung function and wheezing phenotype. From the description of our study population (Table 1), it is clear that the mothers of the children were relatively highly educated (which may explain the low smoking incidence) and atopic mothers were more likely to enroll their child into our birth cohort. We do not consider this non-representative selection at entry as a drawback; on the contrary, it favored a more homogeneous distribution of the children among the wheeze phenotype groups.

Following the Tucson definition (2), the ERS Task Force on wheezing disorders in preschool children recently described cutoff ages to define the wheeze phenotype of the child at 3 and 6 yrs of age (14), whereas we defined cutoff points at 3 and 4 years of age. Many previous epidemiological studies, however, employed different definitions of the wheeze phenotype; cutoff ages of 3 and 5 years (15, 16), 3 and 7 years (17) and 4 and 10 years (18) have been described. Our definition of wheeze phenotype was similar to that employed by Brussee et al. (11).

The baseline reproducibility of $R_{rs}$ indicated isolated, small decreases (~3%) in $R_6$ and $R_8$ and a significant upward shift of the $X_{rs}$ curve throughout the whole frequency range at the second baseline measurement as compared with the first (see Figure 1). The most probable explanation appears to be a change in chest wall compliance due to a more relaxed state of the young children, who had adapted
somewhat to the new experimental situation. Although there was a systematic difference in Xrs between the two baseline measurements, the change was small relative to that induced by bronchodilation: the relative increases in X4 and X6 were 6% and 12%, respectively, whereas the changes induced by bronchodilation were 24% and 35%, respectively.

Besides the analysis of the raw Rrs and Xrs data points at low frequency, we also analyzed AX, all Xrs data points below f_{res} thus being taken into account. As such, AX can be regarded as an index reflecting the elastance of the respiratory system, and it actually has the dimension of elastance. Its recent introduction was due to the fact that AX is not greatly influenced by the measurement noise of the individual Xrs data points (19). Indeed, AX discriminated between the groups with different wheezing phenotypes better or with more power (see Table 2) than did X4, X6 or X8.

In contrast with most previous FOT measurements in children, we obtained highly reproducible Zrs data at 4 Hz (see the online supplement), and hence we chose to analyze R4. It should be noted, however, that similar results were obtained when R6 and R8 were considered (see Table 2). Our results are in close agreement with those of Brussee et al. (11), who prospectively investigated the interrupter airway resistance (Rint) in children 4 years of age; they employed similarly defined wheezing phenotypes, but included 2.5 times more children in their study than we did. They found that children with persistent wheeze had a higher baseline Rint than those who never wheezed. In contrast, our FOT resistance also differentiated the
group of children with early transient from the group of children who never wheeze, and the group of children with persistent wheeze from the group of children with early-transient wheeze. This suggests that resistance measurements with FOT are more sensitive than Rint measurements in children of this age. In accord with this, Delacourt et al. (20) concluded that FOT is more sensitive than Rint measurements for the detection of obstruction and its reversibility in children with asthma or a chronic cough.

Lowe et al. (21) studied the specific airway resistance (sRaw) in 3-year-old children taking part in a prospective birth cohort. sRaw was found to be significantly higher in the children who had had > 2 wheezing episodes than in the children who had never wheezed. In our study, the children who had stopped wheezing by 3 years of age (the children with early transient wheeze) still had a significantly higher respiratory resistance at 4 years of age as compared with the children who had never wheezed at all.

As far as we are aware, the bronchodilator response has not been assessed previously in preschool children with different wheezing phenotypes. We detected significant differences in the responses to a short-acting bronchodilator: the children with persistent wheeze displayed a larger decrease in Rrs than those children who never wheeze or who have early transient wheeze (Table 2). It is noteworthy that the baseline Xrs values and the change after bronchodilator administration also distinguished the different wheezing phenotype groups. In contrast with Rrs at low
frequency, the post-bronchodilator Xrs data were all very similar among the groups with different wheezing phenotypes. How should these data to be interpreted?

A higher baseline Rrs was always associated with a lower Xrs at low frequency, and thus with higher values of AX and fres (Table 2). Further, the effect of the short-acting bronchodilator was an upward shift in Xrs, which was largest in the groups with the lowest baseline Xrs values. There are a number of mechanisms that may explain the concomitant changes in Rrs and Xrs. Perhaps the most probable explanation for lower Xrs values in small children with larger values of airway resistance is the effect of the upper airway shunt (22). This shunt effect, exerted by the elastic properties of the soft tissues of the cheeks and floor of the mouth, increases as the airway resistance is increased. It results in a phase shift between pressure and flow, especially at low frequency, with the net effect that Xrs decreases as the airway resistance increases. A similar effect is obtained when the peripheral airway constriction in these young children is sufficiently severe and inhomogeneous to cause peripheral time constant differences and an altered frequency dependence of the pulmonary impedance (23). This mechanism was earlier offered as the most likely explanation for the increase in apparent tissue compliance observed after bronchodilation in adult asthmatics (24). Both of the above-mentioned mechanisms explain the parallel changes observed in Rrs and Xrs after bronchodilation. Indeed, all the wheezing phenotype groups exhibited a joint path of bronchodilator-induced changes in R4 and X4 (Figure 4). Children with more constricted airways display higher resistances associated with more negative reactances. The bronchodilator-induced changes in both resistance and reactance are
larger in these children, which results in a rather uniform post-bronchodilator impedance among the groups.

The bronchodilator responsiveness in our groups of 4-year-old children was remarkably large. In the children who never wheeze an average decrease of 22% in R4 was observed after bronchodilation, indicating a significant bronchomotor tone at baseline. When we used the coefficient of repeatability of R4 (10) as a cutoff value as many as 35% of the children who never wheezed demonstrated a significant positive bronchodilator response. To the best of our knowledge, the bronchodilator responsiveness of children who never wheeze has not been assessed previously in a prospective study. In cross-sectional studies, however, the bronchodilator response of healthy young children has been reported to be significant. Thamrin et al. (25) and Malmberg et al. (26) reported similar decreases (19-21%) when higher doses of salbutamol were used, whereas Hellinckx et al. (27) and Nielsen and Bisgaard (28) observed much smaller changes (10-13%).

The threshold for R4 to detect a significant bronchodilator response as determined in the children who never wheeze was -43% of the baseline value. Similar cutoff values were determined for R6 and R8 (-41% and -43%, respectively; see Table 2 of the online supplement). These cutoff values are close to previously published threshold values in healthy preschool children, which ranged from -28% to -42% (25-28). On use of this threshold value, the number of responders in the groups of different wheezing phenotypes were similar (Table 3). In accord with this finding, both
Thamrin et al. (25) and Hellinckx et al. (27) reported that use of this threshold did not allow the distinction of children with asthma or wheeze from healthy preschool children. We are aware of only one study in which preschool children at risk of persistent asthma were investigated because of repeated wheezing episodes at a young age. Marotta et al. (29) reported that children with suspected asthma exhibited a larger relative change in $R_5$ in comparison with children without asthma, but with a comparable baseline lung function.

When we used a cutoff value based on the absolute change to detect a positive bronchodilator response, significant differences were observed between the wheezing phenotype groups, suggesting that limits based on absolute change are more sensitive than limits based on relative change (Table 3). A higher baseline airway resistance, caused by an increased bronchomotor tone, will be associated with a larger absolute response to a bronchodilator (Figure 4). Consequently, the expression of the change relative to the initial level will tend to homogenize the different responses to bronchodilation in subjects with different baseline bronchomotor tones.

Turner et al. (5) demonstrated that a reduced pulmonary function at 1 month of age and airway responsiveness are independently related to persistent wheeze at 11 years of age. Moreover, it was recently concluded that the pattern of wheeze and the lung function level at 6 years of age determine the respiratory symptoms and respiratory function in adolescence (30). The two factors that may explain the relationship between a reduced lung function at an early age and an increased
wheeze during childhood are: 1) wheeze due to narrow, small airways, and 2) altered airway wall properties that may result in a more pronounced wheeze. Asymptomatic infants with a history of wheeze have been reported to have a lower airway wall compliance than that of healthy age-matched controls (31). A recent study reported that reticular basement membrane thickening is already detectable in the airways of preschool children who suffer from a severe, recurrent wheeze (32). A diminished lung function was detected in infants 1 month of age who subsequently exhibited episodes of wheezing or coughing, suggesting that alterations in the airways may precede the onset of wheeze (33). The findings of Brussee et al. (11) and the results of our own study uniformly indicate that 4-year-old children with persistent wheeze manifest an increased airway resistance as compared with children who never wheeze. Moreover, our results on the bronchodilator response point to narrower airways being associated (at least partially) with an increased bronchomotor tone.

In summary, our study has demonstrated that children at 4 years of age with various wheezing phenotypes possess different baseline lung functions. Children with persistent wheeze have an elevated baseline respiratory resistance, and their bronchodilator response is more marked than that of children who never wheeze or who have early-transient wheeze.
Acknowledgments

We are grateful to the children and parents participating in the study and to all PIPO study personnel, in particular Chris Daenen, Rita Vroom, Danielle Stappers, Frederik Willekens and Ria Heyndrickx, for their contributions to this study.

This study was supported by grants from the Research Foundation – Flanders (FWO), Belgium (number 7.0015.00) and the Flemish Government (PBO98/26/143).
References


Figure legends

Figure 1. Short-term reproducibility of Zrs data obtained from 50 4-year-old children. Average values (and SD) of baseline resistance (Rrs) and reactance (Xrs) are depicted. The two baseline measurements were made 15 min apart.

*, **, ***: significantly different at the p< 0.05, p< 0.01 and p<0.001 level, respectively.

Figure 2. Average resistance (Rrs, top panel) and reactance (Xrs, bottom panel) in the groups of children with different wheezing phenotypes: those who never wheeze (n= 144), those with early-transient wheeze (n= 127) and those with persistent wheeze (n= 54).

Figure 3. Baseline and post-bronchodilator values of resistance at 4 Hz (R4) and area under the Xrs curve (AX) in the groups who never wheeze, or who have early-transient or persistent wheeze. Average values and SEM are given. Significance of the difference between groups: *: p<0.05. **: p<0.01, ***: p<0.01. Full lines: comparisons between baseline values; broken line: comparison between post-bronchodilator values.

Figure 4. Relationship between baseline (closed symbols) and post-bronchodilator values (open symbols) of reactance at 4 Hz (X4) and resistance at 4 Hz (R4) in the groups of children who never wheeze, or who have early transient wheeze or persistent wheeze. Average values and SEM are given.
### Table 1. General characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Never</th>
<th>Early</th>
<th>Persistent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>325</td>
<td>144</td>
<td>127</td>
<td>54</td>
</tr>
<tr>
<td><strong>Sex, % boys</strong></td>
<td>53</td>
<td>49</td>
<td>55</td>
<td>59</td>
</tr>
<tr>
<td><strong>Age [yrs, mean (SD)]</strong></td>
<td>4.4 (0.2)</td>
<td>4.4 (0.2)</td>
<td>4.4 (0.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Height [cm, mean (SD)]</strong></td>
<td>106.4 (4.5)</td>
<td>106.1 (4.3)</td>
<td>106.0 (3.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Weight [kg, mean (SD)]</strong></td>
<td>17.6 (2.1)</td>
<td>17.5 (2.3)</td>
<td>17.6 (2.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Education level of the mother (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low</td>
<td>21</td>
<td>19</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>high</td>
<td>79</td>
<td>81</td>
<td>78</td>
<td>77</td>
</tr>
<tr>
<td><strong>Atopy of the mother (%)</strong></td>
<td>43</td>
<td>45</td>
<td>43</td>
<td>35</td>
</tr>
<tr>
<td><strong>Parental smoking at 4 yrs (%)</strong></td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td><strong>Siblings at 4 yrs (%)</strong></td>
<td>87</td>
<td>85</td>
<td>87</td>
<td>92</td>
</tr>
<tr>
<td><strong>Exposure to pets at 4 yrs (%)</strong></td>
<td>57</td>
<td>58</td>
<td>55</td>
<td>59</td>
</tr>
<tr>
<td><strong>Eczema of the child (%)</strong></td>
<td>53</td>
<td>50</td>
<td>55</td>
<td>58</td>
</tr>
<tr>
<td><strong>Rhinitis of the child (%)</strong></td>
<td>76</td>
<td>73</td>
<td>75</td>
<td>86</td>
</tr>
<tr>
<td><strong>Atopy of the child (%)</strong></td>
<td>28</td>
<td>23</td>
<td>29</td>
<td>41 *</td>
</tr>
<tr>
<td><strong>LRTI at 4 yrs (%)</strong></td>
<td>15</td>
<td>7</td>
<td>15 *</td>
<td>36 ***</td>
</tr>
<tr>
<td><strong>Antibiotics at 4 yrs (%)</strong></td>
<td>46</td>
<td>39</td>
<td>46</td>
<td>65 ***</td>
</tr>
</tbody>
</table>

Education level of the mother: high if the mother had completed a bachelor or master education; otherwise low. Atopy of the mother/child was defined as a specific IgE level $\geq 0.35$ kU/L at the medical examination at 1 or 4 years. Parental smoking: regular exposure to tobacco smoke by either of the parents. Exposure to pets: regular exposure to a cat or dog. Eczema/rhinitis of the child: a positive answer to the following question in any of the questionnaires: Did your child suffer from eczema/rhinitis during the past 6 (12) months? LRTI: lower respiratory tract infection: a serious lung or airways infection. At 4 yrs: a positive answer in the questionnaire returned at 42 or 48 months. *, ***: $p < 0.05$ or $< 0.001$ relative to those who never wheeze.
Table 2. Baseline resistance and reactance values and the change after bronchodilation in the groups of children with different wheezing phenotypes

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Never n=144</th>
<th>Early n=127</th>
<th>Persistent n=54</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean (95% CI)</td>
<td>mean (95% CI)</td>
<td>mean (95% CI)</td>
</tr>
<tr>
<td>R4 (hPa.s.L⁻¹)</td>
<td>10.3 (9.9, 10.7)</td>
<td>11.0 (10.5, 11.5)</td>
<td>11.9 (11.1, 12.7) ***, †</td>
</tr>
<tr>
<td>R6 (hPa.s.L⁻¹)</td>
<td>9.8 (9.5, 10.2)</td>
<td>10.5 (10.1, 11.0)</td>
<td>11.5 (10.7, 12.2) ***, ††</td>
</tr>
<tr>
<td>R8 (hPa.s.L⁻¹)</td>
<td>9.6 (9.2, 9.9)</td>
<td>10.1 (9.7, 10.6)</td>
<td>11.0 (10.3, 11.7) ***, †</td>
</tr>
<tr>
<td>X4 (hPa.s.L⁻¹)</td>
<td>-4.1 (-4.4, -3.9)</td>
<td>-4.5 (-4.8, -4.2)</td>
<td>-4.8 (-5.3, -4.3) *</td>
</tr>
<tr>
<td>X6 (hPa.s.L⁻¹)</td>
<td>-2.6 (-2.7, -2.4)</td>
<td>-3.0 (-3.2, -2.7)</td>
<td>-3.3 (-3.7, -2.9) **</td>
</tr>
<tr>
<td>X8 (hPa.s.L⁻¹)</td>
<td>-1.5 (-1.7, -1.4)</td>
<td>-2.0 (-2.3, -1.8) **</td>
<td>-2.3 (-2.8, -1.9) **</td>
</tr>
<tr>
<td>AX (hPa.L⁻¹)</td>
<td>19.9 (17.6, 22.1)</td>
<td>27.2 (23.4, 31.1) **</td>
<td>32.6 (25.8, 39.3) ***</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bronchodilation</th>
<th>n=139</th>
<th>n=121</th>
<th>n=53</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔR4 (hPa.s.L⁻¹)</td>
<td>-2.3 (-2.6, -2.0)</td>
<td>-2.6 (-3.1, -2.2)</td>
<td>-3.4 (-4.1, -2.7) *, †</td>
</tr>
<tr>
<td>ΔR6 (hPa.s.L⁻¹)</td>
<td>-2.1 (-2.4, -1.9)</td>
<td>-2.5 (-2.9, -2.2)</td>
<td>-3.2 (-3.8, -2.5) **</td>
</tr>
<tr>
<td>ΔR8 (hPa.s.L⁻¹)</td>
<td>-2.2 (-2.4, -1.9)</td>
<td>-2.4 (-2.8, -2.1)</td>
<td>-3.1 (-3.7, -2.5) **, †</td>
</tr>
<tr>
<td>ΔX4 (hPa.s.L⁻¹)</td>
<td>1.0 (0.9, 1.2)</td>
<td>1.2 (1.0, 1.5)</td>
<td>1.4 (1.0, 1.8)</td>
</tr>
<tr>
<td>ΔX6 (hPa.s.L⁻¹)</td>
<td>0.9 (0.8, 1.0)</td>
<td>1.1 (1.0, 1.3)</td>
<td>1.4 (1.0, 1.8) *</td>
</tr>
<tr>
<td>ΔX8 (hPa.s.L⁻¹)</td>
<td>0.9 (0.7, 1.0)</td>
<td>1.2 (1.0, 1.4) **</td>
<td>1.5 (1.1, 1.9) **</td>
</tr>
<tr>
<td>ΔAX (hPa.L⁻¹)</td>
<td>-9.8 (8.1)</td>
<td>-16.3 (-19.6, -13.0) **</td>
<td>-21.2 (-27.4, -15.1) ***</td>
</tr>
</tbody>
</table>

R4, R6, R8 and X4, X6, X8: resistance and reactance, respectively, at 4, 6 and 8 Hz. AX: area under the reactance curve. CI: confidence interval. *, **, ***: p< 0.05, 0.01 and 0.001, respectively, relative to the children who never wheeze. †, ††: p< 0.05 and 0.01, respectively, relative to the children with early-transient wheeze.
Table 3. Number of children in each group giving a significant bronchodilator response as determined by the 5th percentile for the children who never wheeze

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Early</th>
<th>Persistent</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔR4</td>
<td>&gt; 5.5 hPa.s.L⁻¹</td>
<td>6/139 (4%)</td>
<td>6/121 (5%)</td>
</tr>
<tr>
<td>ΔAX</td>
<td>&gt; 31.0 hPa.L⁻¹</td>
<td>6/139 (4%)</td>
<td>17/121 (14%)</td>
</tr>
<tr>
<td>ΔR4/R4</td>
<td>&gt; 43%</td>
<td>7/139 (5%)</td>
<td>6/121 (5%)</td>
</tr>
<tr>
<td>ΔAX/AX</td>
<td>&gt; 81%</td>
<td>6/139 (4%)</td>
<td>9/121 (7%)</td>
</tr>
</tbody>
</table>

Numbers in bold indicate a significant difference relative to the group of children who never wheeze (p<0.05, Pearson's chi-square test).
Figure 1. Short-term reproducibility of Zrs data obtained from 50 4-year-old children. Average values (and SD) of baseline resistance (Rrs) and reactance (Xrs) are depicted. The two baseline measurements were made 15 min apart.

*, **, ***: significantly different at the p<0.05, p<0.01 and p<0.001 level, respectively.
Figure 2. Average resistance (Rrs, top panel) and reactance (Xrs, bottom panel) in the groups of children with different wheezing phenotypes: those who never wheeze (n=144), those with early-transient wheeze (n=127) and those with persistent wheeze (n=54).
Figure 3. Baseline and post-bronchodilator values of resistance at 4 Hz (R4) and area under the Xrs curve (AX) in the groups who never wheeze, or who have early-transient wheeze or persistent wheeze. Average values and SEM are given. Significance of the difference between groups: *: p<0.05. **: p<0.01, ***: p<0.01. Full lines: comparisons between baseline values; broken line: comparison between post-bronchodilator values.
**Figure 4.** Relationship between baseline (closed symbols) and post-bronchodilator values (open symbols) of reactance at 4 Hz ($X_4$) and resistance at 4 Hz ($R_4$) in the groups of children who never wheeze, or who have early-transient wheeze or persistent wheeze. Average values and SEM are given.