Airway Hyperresponsiveness in young Children with Respiratory Symptoms: A Five-Year Follow-Up

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AIRWAY HYPERRESPONSIVENESS IN YOUNG CHILDREN WITH RESPIRATORY SYMPTOMS: A FIVE-YEAR FOLLOW-UP

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ABBREVIATIONS:
AHR: airway hyperresponsiveness,
DDA: doctor-diagnosed asthma,
EIB: exercise-induced bronchoconstriction,
FeNO: fractional concentration of nitric oxide,
FRC: functional respiratory capacity,
V’maxFRC: The maximal flow at functional residual capacity,
PD40V’maxFRC: the provocative dose of methacholine to cause a 40% decline in VmaxFRC
  sGaw: specific airway conductance
  Rrs5 ja Rrs20: respiratory resistance at 5 and 20 Hz
LOGDRS: logarithmic transformed dose-response slope
API: asthma predictive index

WORD COUNT: 3013
FIGURES: 2
TABLES: 4
INTRODUCTION

Lower respiratory symptoms with wheeze are common in early childhood (1). Most young children cease to wheeze before school age (2), but some early childhood risk factors may determine the lifelong respiratory outcome. Clinical characteristics associated with persistent wheeze include maternal smoking, parental asthma, severity of wheezing, atopy and elevated IgE (3,4). Increased airway hyperresponsiveness (AHR) in infancy has also been connected to persistent symptoms later in life (5-7).

The role of AHR in the pathogenesis of respiratory symptoms in young children is incompletely understood. Most reports have evaluated AHR in birth cohorts, and only one investigation focused on symptomatic infants (7). Some studies have provided evidence that AHR is present in all young children, at least those under one year of age, independently of the presence of respiratory symptoms (8-10). Contradictory results have also been reported. Increased AHR at the age of one month in children with atopic mothers was associated with asthma by the age of 7 years (5). Another study reported an association between increased AHR in neonates and decreased lung function, asthma and respiratory tract symptoms at 6 years of age (6). When AHR was assessed in children with wheeze before 2 years of age, a significantly higher reactivity to methacholine was found in children with persistent wheeze than in symptom-free children, but the level of AHR was not predictive of asthma 4 years later (7).

We aimed to determine whether AHR in symptomatic infants (6 to 24 months) could predict doctor-diagnosed asthma (DDA), defined as the need for regular control medication at the median age of 6 years. Secondary aims were to clarify whether AHR assessed in infancy persists and to identify other predisposing factors associated with persistence of lower respiratory symptoms at the median age of 6 years.
METHODS

Patients

Children born full term and aged 6-24 months, referred for lung function measurements, originally participating in a study evaluating the efficacy of montelukast (11), were recruited between September 2004 and April 2008. Study was prospective with observational design. Inclusion criteria included a history of persistent troublesome lower respiratory tract symptoms (wheeze, cough, dyspnea), at least one physician-diagnosed wheezing episode and successfully performed methacholine challenge test. Exclusion criteria were: a need for inhaled corticosteroids (ICS) within 8 weeks prior to the first visit; a cumulative life-time systemic prednisolone use for more than 3 days at a dose of 2 mg/kg, or an equipotent dose of another systemic corticosteroid or life-time ICS use more than 4 weeks; respiratory infection in the 14 days preceding the lung function measurement, or any obvious structural defect.

Out of 367 enrolled, 254 were not randomized because 1. did not fulfil the criteria (n=224), 2. technical problems (n=11), 3. sedation problems (n=9), 4. not willing to participate (n=10)(11).

After original study enrollment three more children performed the lung function measurements, fulfilled the inclusion criteria and are included in the present study. A total of 61 out of 116 children from the original study were able to participate in this follow-up study and performed all of the lung function measurements at median age of 6 years.

Lung function measurements

6 to 24 months of age
The measurements of lung function were performed by using commercial equipment (Body Masterscreen; Jaeger GmbH, Wurzburg, Germany) as described previously (12). Infant whole-body plethysmograph was applied to measure functional residual capacity (FRC) and specific airway conductance (sGaw) (12-14). The maximal flow at functional residual capacity ($V'_{\text{maxFRC}}$) was determined using the squeeze technique as reported elsewhere (12,15). Fractional concentration of exhaled nitric oxide (FeNO) was measured with a modification of the online single-breath technique (16,17). The methacholine challenge test was performed with a dosimetric protocol as described previously (12). The provocative dose of methacholine to cause a 40% decline in $V_{\text{maxFRC}}$ ($\text{PD}_{40}V_{\text{maxFRC}}$) was determined. A $\text{PD}_{40}V_{\text{maxFRC}}$ lower than 300 $\mu\text{g}$ was considered a positive test result (12).

Follow-up evaluation

Lung function tests were performed using impulse oscillometry (IOS). Regular asthma control medication was stopped at least 4 weeks before the lung function measurements. Respiratory system resistance at 5 Hz ($R_{\text{rs}5}$) and 20 Hz ($R_{\text{rs}20}$) as well as reactance at 5 Hz ($X_{\text{rs}5}$) were determined by IOS as described previously (18-20). AHR was determined by exercise challenge and methacholine challenge tests as described elsewhere (21).

Briefly, the exercise challenge test was performed as a standardized outdoor running test (18) lasting for 6 to 8 minutes at 85% to 90% of maximal heart rate. IOS was performed before and at 1, 5 and 10 minutes after exercise. A post-exercise increase of 35% in $R_{\text{rs}5}$ was considered a positive test result (18).

Methacholine challenge test was applied by a dosimetric bronchial provocation test modified to be appropriate for preschool children (12,21). First, the baseline $R_{\text{rs}5}$ was determined, and thereafter, increasing doses of methacholine were administered by an automatic, inhalation-synchronized
dosimeter (Spira Electro 2, Spira Respiratory Care Centre, Ltd., Hämeenlinna, Finland) connected to a calibrated nebulizer (Salter Labs 8900, Arvin, CA, USA). Rrs5 was measured 90 seconds after each methacholine inhalation. The procedure was continued until a 40% increase in Rrs5 was observed or the maximum dose of methacholine was administered. The provocative dose of methacholine causing a 40% increase in Rrs5 (PD40Rrs5) was determined from the dose-response curves (22,23). A PD40Rrs5 lower than 400 µg was considered a positive test result (24).

At both study points, the dose-response slope (DRS) was calculated by dividing the percentage change in the observed lung function parameter (Rrs5 or VmaxFRC) by the cumulative dose of methacholine at the last inhalation. This value was then log transformed to normalize the distribution (LOGDRS). The methacholine test results were analyzed both as categorical data (positive/negative) and as continuous data (PD40VmaxFRC, PD40Rrs5 and LOGDRS).

FeNO was measured with a stationary chemiluminescence-based device (NIOX, Aerocrine AB, Solna, Sweden) according to the American Thoracic Society recommendations (16).

Atopic status

Atopic status was defined as positive skin prick test reactivity to birch, timothy grass, meadow fescue, mugwort, Cladosporium herbarum, cat, dog, horse, cow, house dust mite, milk, egg, fish, wheat, shrimp and peanut. Positivity in skin prick tests was defined as a wheal of at least 3 mm diameter in reaction to at least one of the tested allergens. Blood samples at infancy were examined to assess eosinophil count and IgE level by the routine laboratory methods used in Helsinki University Central Hospital (25). Also children with doctor-diagnosed atopic eczema were considered as atopic.
Other characteristics of the study children

Individual and family histories of both respiratory and allergy symptoms, diagnoses, asthma medication, allergies and smoking status were assessed by a questionnaire at both stages of the study.

Asthma predictive index (API)

API was defined at the follow-up visit and was considered positive if a child fulfilled at least one major criterion or two minor criteria. Major criteria include parental asthma, doctor-diagnosed atopic dermatitis and sensitization to aeroallergen. Minor criteria include allergic sensitization to food, blood eosinophils ≥ 4% and wheezing unrelated to colds (26, 27).

Doctor-diagnosed asthma (DDA)

Doctor-diagnosed asthma was defined as need for regular asthma control medication at the follow-up assessment (median age of 6 years) (28).

Statistical methods

Normal distribution was tested with Shapiro-Wilks test. Because values were not normally distributed, non-parametric tests were used. Categorical data were analysed with Chi-square test and continuous data with Mann-Whitney U-test. Bivariate correlation was calculated by the Spearman correlation test. Effect size for Spearman coefficient (rho) is considered small if rho is ≤ 0.29, medium 0.3-0.49 and large ≥ 0.5. Possible explanatory factors were analysed with multivariate
logistic regression, where DDA was a dependent variable and gender, FeNO, number of wheezing episodes and infant methacholine PD40VmaxFRC were covariates. Data were analysed using SPSS 19.0 (SPSS, Inc, Chicago, IL, USA).

Ethics

The study was approved by the Ethics Committee of Helsinki University Central Hospital (81/E7/02 and 337/13/03/03/2008). Written informed consent was obtained from guardians or parents.
RESULTS

Tables 1 and 2 represent baseline characteristics of 61 children at infancy and at follow-up, respectively. No significant differences in patient characteristics were found between children who participated in the follow-up study and those lost to follow-up (Table 1). The median interval between the two stages of the study was 4.6 years (range 3.6-7.3 years).

AHR in infancy and associations with AHR and DDA at 6 years

In infancy (at age 6 to 24 months), all study children had recurrent lower airway symptoms including periodic wheezing and regular asthma control medication. Symptoms in infancy are listed in Table 1. At a median age of 6 years, 21 (34%) of the 61 children had doctor-diagnosed asthma (DDA). Children with DDA at 6 years had lower PD40VmaxFRC to methacholine, indicating increased AHR in infancy relative to children without DDA \( (p = 0.022, \text{Table 3}) \). In addition, infant methacholine LOGDRS was higher in children who had DDA at 6 years than in children without DDA \( (0.047 \text{vs} 0.025, p=0.033, \text{Figure 1}) \). Children with DDA at 6 years had also increased current AHR to methacholine compared with children without DDA \( (p = 0.029) \).

Methacholine LOGDRS at infancy and at 6 years of age were significantly associated with each other \( (p = 0.011, \text{rho} 0.324, \text{Figure 2}) \). Furthermore, children with positive methacholine challenge at 6 years had higher median infant LOGDRS than children with negative methacholine challenge at 6 years \( (0.031 \text{vs} 0.025, p = 0.047, \text{Figure 1}) \). Exercise-induced bronchoconstriction (EIB) was present in 8 children (13%) at 6 years. Children with EIB had higher methacholine LOGDRS in infancy than those without EIB \( (0.07 \text{vs} 0.03, p = 0.019, \text{Figure 1}) \).
API and AHR

In all, 49 children (80%) had positive API at follow-up. No associations between positive API and methacholine LOGDRS (p = 0.842) or methacholine positivity (p = 0.877) at age of 6 were found. In addition, no associations between API status and FeNO level or EIB at age 6 years existed. Methacholine LOGDRS in infancy did not differ significantly between API-positive and API-negative children (p = 0.301). API status was not associated with DDA at 6 years (p = 0.443), but API-positive children had more symptoms at 6 years than API-negative children (75% vs. 0%, p = 0.005).

AHR and lung function

Lung function (sGaw or VmaxFRC) at infancy was not associated with DDA or lung function at age 6 years. No association between infant AHR and lung function at 6 years was found.

FeNO measurements

Only a weak trend was observed between FeNO levels in infancy and early childhood (r = 0.558; p = 0.078). FeNO level in infancy was not associated with DDA or AHR at age of 6. Children with atopic status in infancy (SPT positivity and/or atopic eczema) had higher FeNO levels at age 6 years than those with non-atopic status (FeNO z-score 1.5 vs. 0.8, p = 0.006).
Infant atopic markers in association with preschool symptoms

Children with EIB had more often positive skin prick test result ($p = 0.002$) and higher IgE level ($p = 0.025$) in infancy than children without EIB, but these markers were not associated with DDA at the age of 6 years.

Multiple regression analysis

In multivariate logistic regression analysis, a higher PD40VmaxFRC to methacholine in infancy was associated with a lower risk for DDA at 6 years of age (OR 0.185, CI 0.04 – 0.926, $p = 0.040$, Table 4). Gender, number of wheezing episodes and FeNO level in infancy were not associated with DDA at 6 years.
DISCUSSION

We followed airway hyperresponsiveness in children with recurrent respiratory symptoms before 2 years of age from infancy until the age of 6 years. We showed that higher methacholine LOGDRS indicating increased AHR in symptomatic infants was associated with doctor-diagnosed asthma (DDA) and exercise-induced bronchoconstriction (EIB) at the age of 6 years. Furthermore, hyperresponsiveness to methacholine in infancy and at 6 years were associated with each other, suggesting persistence of AHR over early childhood.

All of our study children had recurrent lower respiratory tract symptoms, including wheezing, before the age of 2 years and were using regular asthma control medication. At the later study point (median age 6 years), 34% of these children were using regular asthma medication (defined as DDA). This is in line with the findings of several cohort studies (3,29) as well as with Delacourt et al. (7,30), who showed persistent wheezing at 5 years in 38% of children with infantile asthma. Earlier studies have also suggested that higher frequency of symptoms in infancy and persistent wheezing phenotype during the preschool years is connected to more severe asthma in later childhood (30).

Asthma predictive models, such as API, have been developed to aid asthma diagnostics at an early age (26). However, the usefulness of API to predict future asthma is only modest since its positive predictive value (PPV) for asthma at age 7 years is only reported to be 26% (31). Here, API-positive children experienced more symptoms than API-negative children at age 6 years, but there was no connection to DDA or AHR. Our study population is, however, small and the children represent a highly selected group with early asthmatic symptoms. This may have obscured the impact of API criteria.

Infant lung function and its predictive value for later asthma symptoms have been examined in several studies. In our study, VmaxFRC at infancy did not differentiate children with DDA from
those without DDA at 6 years. In a Tucson cohort, children with transient wheeze had decreased
VmaxFRC in infancy before the symptoms appeared, but infant VmaxFRC was not able to
differentiate persistent wheezers from non-wheezers (3). Delacourt et al. (7) showed a lower
VmaxFRC in infants with persistent wheeze at the age of 5 years than in children who stopped
wheezing. Although diminished lung function at infancy has been linked to later symptoms, it is
difficult to define cut-off levels and significant overlap in phenotypes exists regarding disease
progression. (7) In the present study, no significant differences in IOS lung function parameters
between children with DDA at 6 years and children with no DDA emerged. Some earlier studies
have shown diminished lung function in persistent wheezers compared with never-wheezers or
those who stopped wheezing (3,4,30).

Our study revealed an association between AHR to methacholine at infancy and DDA but not lung
function at age 6 years. Most of the knowledge on the connection between infant AHR and
persistence of symptoms and later lung function derives from cohort studies. As far as we are
aware, there is only one earlier study examining persistence of AHR and connection of AHR and
later symptoms in symptomatic young children (7). Delacourt et al. (7) reported altered lung
function at the age of 5 years in children with AHR and asthma at 16 months. Clarke et al. (32)
determined lung function and AHR with histamine in 73 healthy neonates. AHR was increased in
those female neonates who subsequently experienced wheezy episodes during the first year of life.
In an Australian birth cohort 243 children (6,33), an association was found between increased infant
AHR and decreased lung function and lower respiratory symptoms at the age of 6 years (6), but no
longer at 11 years (33).

We found persistency of infant AHR at six years in symptomatic children, in accord with findings
elsewhere (30). Both increased neonatal AHR and diminished lung function among high-risk
Danish neonates were connected to development of asthma by age 7 years (5). Our study evaluated
AHR with methacholine challenge test at both stages of the study, and additionally, an exercise
challenge test was performed at the later stage, i.e. at 6 years. The use of these direct and indirect AHR tests at 6 years enabled us to evaluate different mechanisms of AHR (34). Although a precise and clear definition for early childhood asthma is lacking, AHR shown in indirect tests such as an exercise challenge test, is thought to be more specific for paediatric asthma (35). We showed that EIB at age 6 years was associated with higher reactivity to methacholine in infancy, suggesting that even indirect AHR may be affected by susceptibility already present in infancy. Children with EIB had more symptoms during the last 12 months than children with no EIB, but the symptoms were not associated with infant AHR. AHR at different time points in life may manifest via various pathophysiological mechanisms, and according to the present findings, AHR in early life may be a predictor for later asthmatic symptoms. Our study suggests that children with multiple wheeze episodes and increased AHR to methacholine before 2 years of age may have permanent airway dysfunction with susceptibility to airway narrowing, possibly arising from structural changes with either increased airway muscle force or mechanical load of the airway (36). The study design and sample does not allow us to make conclusions about specific clinically meaningful levels of AHR that would indicate later asthma.

One of the shortcomings in this study is the selected patient population with lower respiratory tract symptoms and the lack of controls. However, this setting reflects the situation in day-to-day preschool asthma prognosis assessment. In addition, the sample size is relatively small and many children did not participate the follow-up, which is a common feature in these studies. To control for this effect, we analysed the demographics of participating and non-participating children and found no significant differences between the groups.

Interpretation of AHR test results in young children is ambiguous. We applied cut-off limits of AHR, which we have previously shown to be associated with current symptoms in infants (17), but there is no unequivocal cut-off level for the methacholine challenge test in predicting later asthma in young children. Therefore, the cut-off level in infants may need to be lower than earlier reported.
A provocative dose cut-off level of 400 µg in methacholine challenge in preschool children has been suggested to be associated with probable asthma (21,24).

In conclusion, AHR in infancy and AHR at 6 years of age were associated in children with recurrent wheezy symptoms in infancy. In addition, increased AHR in infancy was connected to DDA and EIB in early childhood. This suggests an early development of increased airway responsiveness in children with persistent lower respiratory tract symptoms.
REFERENCES


Figure 1.
Comparison of infant methacholine LOGDRS in children at 6 years a) with and without asthma b) with negative or positive methacholine test and c) with negative or positive exercise test.

Figure 2. Association of methacholine LOGDRS at infancy and at 6 years.
Table 1. Characteristics at infancy

<table>
<thead>
<tr>
<th></th>
<th>Infants who completed the study (n=61)</th>
<th>Lost in follow-up (n=55)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, months, median (range)</td>
<td>15.1 (6-24)</td>
<td>15.0 (6-24)</td>
<td>0.761</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>50 (82)</td>
<td>36 (66)</td>
<td>0.056</td>
</tr>
<tr>
<td>Parental smoking, n (%)</td>
<td>21 (34)</td>
<td>16 (29)</td>
<td>0.557</td>
</tr>
<tr>
<td>Maternal smoking, n (%)</td>
<td>14 (23)</td>
<td>13 (24)</td>
<td>0.930</td>
</tr>
<tr>
<td>Parental asthma, n (%)</td>
<td>28 (46)</td>
<td>21 (38)</td>
<td>0.454</td>
</tr>
<tr>
<td>Skin prick test positivity*, n (%)</td>
<td>17 (28)</td>
<td>15 (27)</td>
<td>0.899</td>
</tr>
<tr>
<td>All episodes, mean (range)</td>
<td>2.4 (1-6)</td>
<td>2.7 (1-7)</td>
<td>0.456</td>
</tr>
<tr>
<td>Wheezing episodes, mean (range)</td>
<td>2.2 (1-4)</td>
<td>2.2 (1-3)</td>
<td>0.635</td>
</tr>
<tr>
<td>Hospital admission, mean (range)</td>
<td>0.3 (0-2)</td>
<td>0.3 (0-1)</td>
<td>0.785</td>
</tr>
<tr>
<td>Atopic eczema, n (%)</td>
<td>21 (34)</td>
<td>25 (46)</td>
<td>0.708</td>
</tr>
<tr>
<td>Duration of symptoms, months, median (range)</td>
<td>8.5 (2-19)</td>
<td>7.4 (2-21)</td>
<td>0.364</td>
</tr>
<tr>
<td>VmaxFRC, z-score, median (range)</td>
<td>-1.3 (-3.8-1.2)</td>
<td>-1.0 (-3.5-1.2)</td>
<td>0.179</td>
</tr>
<tr>
<td>FeNO, ppb, median (range)</td>
<td>18 (0-134)</td>
<td>22.4 (0-59)</td>
<td>0.132</td>
</tr>
<tr>
<td>IgE, ku/l, median (range)</td>
<td>17.5 (1-2061)</td>
<td>17 (0-685)</td>
<td>0.258</td>
</tr>
<tr>
<td>PD40VmaxFRC, µg, median (range)</td>
<td>570 (50 - 3600)</td>
<td>480 (50-3600)</td>
<td>0.918</td>
</tr>
</tbody>
</table>

*prick tests not performed in one patient
Table 2. Characteristics at median age of 6 (n=61)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y), median (range)</td>
<td>6.0 (5.6 - 8.1)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>50 (82)</td>
</tr>
<tr>
<td>Parental smoking, n (%)</td>
<td>18 (30)</td>
</tr>
<tr>
<td>Maternal smoking, n (%)</td>
<td>10 (16)</td>
</tr>
<tr>
<td>Parental asthma, n (%)</td>
<td>28 (46)</td>
</tr>
<tr>
<td>Skin prick test positivity, n (%)</td>
<td>19 (31)</td>
</tr>
<tr>
<td>Current medication, n (%)</td>
<td>21 (34)</td>
</tr>
<tr>
<td>Seasonal medication, n (%)</td>
<td>10 (16)</td>
</tr>
<tr>
<td>Symptoms* during previous 12 months, n (%)</td>
<td>21 (34)</td>
</tr>
<tr>
<td>Wheezing during previous 12 months, n (%)</td>
<td>8 (13)</td>
</tr>
<tr>
<td>Rrs5 z-score, median (range)</td>
<td>0.33 (-1.78-3.53)</td>
</tr>
<tr>
<td>MPT positivity, n (%)</td>
<td>48 (79)</td>
</tr>
<tr>
<td>PD40VmaxFRC, µg, median (range)</td>
<td>190 (20-2060)</td>
</tr>
<tr>
<td>LOGDRS, median (range)</td>
<td>0.08 (0-0.52)</td>
</tr>
<tr>
<td>FeNO, z-score, median (range)</td>
<td>1.2 (-1.2-3.8)</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.28 (0.04-1.38)</td>
</tr>
</tbody>
</table>

*wheezing, cough, symptoms during exercise
MPT = methacholine provocation test
Table 3. Lung function, AHR, FeNO, atopic markers and infant symptoms according to doctor-diagnosed (DDA) asthma at age of 6 years.

<table>
<thead>
<tr>
<th></th>
<th>DDA (n=21)</th>
<th>No DDA (n=40)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRC, z-score, median (range)</td>
<td>1.2 (-1.8-4.8)</td>
<td>0.6 (-2.8-4.8)</td>
<td>0.374</td>
</tr>
<tr>
<td>sGaw, z-score, median (range)</td>
<td>-2.6 (-4.8-8.1)</td>
<td>-1.65 (-5.1-24)</td>
<td>0.611</td>
</tr>
<tr>
<td>V’maxFRC, z-score, median (range)</td>
<td>-1.4 (-3.1-1.0)</td>
<td>-1.1 (-3.8-1.2)</td>
<td>0.485</td>
</tr>
<tr>
<td>Infant PD40V’maxFRC, µg, median, range</td>
<td>330 (50-3600)</td>
<td>620 (50-3600)</td>
<td>0.083</td>
</tr>
<tr>
<td>MPT positivity at infancy, n (%)</td>
<td>20 (95)</td>
<td>28 (70)</td>
<td>0.022</td>
</tr>
<tr>
<td>Infant LOGDRS, median (range)</td>
<td>0.047 (0.01-0.19)</td>
<td>0.025 (0-0.21)</td>
<td>0.033</td>
</tr>
<tr>
<td>Infant FeNO</td>
<td>19 (2-56)</td>
<td>18 (0-134)</td>
<td>0.730</td>
</tr>
<tr>
<td>Infant eosinophils, median (range)</td>
<td>0.35 (0.1-0.9)</td>
<td>0.26 (0.01-1.16)</td>
<td>0.242</td>
</tr>
<tr>
<td>Infant IgE, median (range)</td>
<td>22 (11-452)</td>
<td>16 (1-2061)</td>
<td>0.053</td>
</tr>
<tr>
<td>Infant skin prick test positivity, n (%)</td>
<td>7 (33)</td>
<td>10 (25)</td>
<td>0.418</td>
</tr>
<tr>
<td>Episodes in infancy</td>
<td>2 (1-4)</td>
<td>2 (1-6)</td>
<td>0.057</td>
</tr>
<tr>
<td>Hospital admission in infancy</td>
<td>0 (0-1)</td>
<td>0 (0-2)</td>
<td>0.860</td>
</tr>
<tr>
<td>Rrs5, z-score, median (range)</td>
<td>0.52 (-1.78-3.53)</td>
<td>0.26 (-1.7-2.3)</td>
<td>0.366</td>
</tr>
<tr>
<td>Postexercise Rrs5 increase (%)</td>
<td>21 (-16-111)</td>
<td>18 (-12-83)</td>
<td>0.342</td>
</tr>
<tr>
<td>MPT positivity at age 6, n (%)</td>
<td>19 (90)</td>
<td>29 (73)</td>
<td>0.103</td>
</tr>
<tr>
<td>PD40Rrs5 at 6 years, µg, median (range)</td>
<td>150 (20-810)</td>
<td>230 (30-2060)</td>
<td>0.029</td>
</tr>
<tr>
<td>LOGDRS at age 6, median (range)</td>
<td>0.089 (0.01-0.52)</td>
<td>0.048 (0-0.37)</td>
<td>0.025</td>
</tr>
<tr>
<td>FeNO at 6 years, z-score, median (range)</td>
<td>1.4 (-1.2-3.7)</td>
<td>1.0 (-0.8-3.8)</td>
<td>0.304</td>
</tr>
</tbody>
</table>

MPT = methacholine provocation test
Table 4. Odds ratio from the multiple regression analysis in which the outcome parameter was DDA at the age of 6 years.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OR (95 % CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of wheezing episodes at infancy</td>
<td>0.819 (0.38 - 1.76)</td>
<td>0.608</td>
</tr>
<tr>
<td>Sex</td>
<td>2.946 (0.52 – 16.72)</td>
<td>0.223</td>
</tr>
<tr>
<td>PD40VmaxFRC to methacholine at infancy</td>
<td>0.185 (0.04 – 0.926)</td>
<td><strong>0.040</strong></td>
</tr>
<tr>
<td>FeNO at infancy</td>
<td>1.0 (0.97 – 1.0)</td>
<td>0.964</td>
</tr>
</tbody>
</table>
\[ p = 0.011 \]
\[ \text{rho} = 0.324 \]