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Article type : Regular Article

Allergic sensitisation did not affect bronchial hyper-responsiveness in children without respiratory tract symptoms

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Aeroallergen sensitisation and bronchial hyperresponsiveness

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Word count: 3046 words

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/APA.16129

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Abstract word count: 197 words

Key Notes word count: 70 words

References: 29

Figures: 2

Tables: 3

Conflicts of interest

The authors have no conflicts of interest to declare.

Funding

This study received funding from the Jalmari and Rauha Ahokas Foundation, Helsinki Allergy Foundation, Finnish Culture Foundation, and the Kerttu, and Kalle Viik Foundation.

Abbreviations: BHR; bronchial hyperresponsiveness, FEV1; forced expiratory volume in one second, FRT; free running test, MCT; methacholine challenge test, PD20; concentration of methacholine that caused a 20% fall of FEV1 after the methacholine test.

Abstract

Aim: The potential for immunotherapy to prevent asthma development has become a hot topic. This prompted us to revisit data from an early study that examined allergic sensitisation on bronchial hyperresponsiveness (BHR) in children with and without respiratory symptoms. . Unlike previous studies it used both indirect and direct test methods.

Methods: The study was conducted in Kuopio, Finland in 1994 and 247 children (55.1% boys) with a mean age 10.5 ± 1.7 years were recruited using a school survey: 165 with lower respiratory symptoms and 82 healthy controls. Each children underwent a six-minute free-running test and a methacholine test with a cumulative dose of $4900 \mu\text{g}$. All participants underwent skin-prick tests: 127 were sensitised and 120 were non-sensitised.

Results: There were no significant differences in lung function between the sensitised and non-sensitised children. However, sensitisation associated with BHR which was measured by both the methacholine test ($2400 \mu\text{g}$ versus $>4900 \mu\text{g}$, $p < 0.001$) and the free-running test (-3.5% versus -2.6% , $p = 0.042$). No such differences were observed among the healthy controls. Sensitisation was a predictor of allergic diseases, only multisensitisation to a minimum of four allergens increased the incidence of asthma.

Conclusion: Allergic sensitisation did not affect BHR in children without respiratory symptoms.

Keywords: allergic sensitisation, asthma, atopy, bronchial hyperresponsiveness, bronchial provocation test

Key Notes

- There is a lack of studies where the role of aeroallergen sensitisation to bronchial hyperresponsiveness (BHR) has been studied via both direct and indirect methods.
- We found that sensitised children have more BHR, both in direct and indirect methods. However, no such differences were observed in healthy controls.
- Our results indicate BHR appears after the onset of clinical symptoms. Preventing multiple sensitisation may decrease risk for developing BHR.

INTRODUCTION

Approximately 34%–51% of children living in the Western world have allergic sensitisation.¹⁻³ This prevalence is seemingly related to their standard of living² and has been strongly associated with rhinitis.³ Moreover, predisposition to produce specific immunoglobulin E antibodies to environmental allergens is linked to airway inflammation and causes bronchial hyperresponsiveness (BHR).⁴ Allergic sensitisation increases the prevalence of BHR in adults and children,^{5,6} and one study reported particular associations between sensitisation to animal allergens.⁵ Polysensitisation in early childhood predicts lower lung function in later life.⁷

Asthma is partly associated with T-helper 2-type immune responses in the airways,^{8,9} higher fractional expired nitrous oxide levels,¹⁰ and a higher degree of BHR.¹⁰ In a previous study, 34% of children with allergic rhinitis treated in a paediatric allergy unit in Turkey had BHR measured at PC20 $\leq$ 8000mg.¹¹ Furthermore, allergic sensitisation is a risk factor for asthma and the modified Asthma Predictive Index includes allergic sensitisation in its criteria.¹² Multiple sensitisation of allergens in early life increases risk of developing asthma at a later age.^{7,11,13}

Sensitisation to aeroallergens can also affect the severity of asthma.¹⁴ The Swedish BAMSE longitudinal birth cohort study collected blood samples from approximately 2,500 children aged 16-years reported that allergic sensitisation was associated with increased expired nitrous oxide levels regardless of whether the child had allergic rhinitis.¹⁵

Most studies have measured BHR using histamine or methacholine challenge tests (MCT). These drugs act directly on smooth muscle receptors, whereas indirect stimuli, such as exercise, act through an intermediate pathway.¹⁶ The effect of sensitisation on BHR may differ according to the employed assessment method,¹⁷ and most sensitisation studies only focused on direct bronchial challenge tests.

In our data, original papers were published on diagnostic methods for asthma in year 2002, the prevalence of asthma at aged 7 to 12 years, and of allergic sensitisation in relation to asthma in year 1996.¹⁸⁻²⁰ The present study revisited previously collected data to investigate the significance of allergic sensitisation on BHR and lower respiratory tract symptoms in children. We used both direct and indirect bronchial challenge test methods to evaluate BHR. The primary objectives were to determine whether sensitised and non-sensitised individuals exhibited any significant differences in BHR based on specific cut-offs and to explore if there were any differences in children without lower respiratory tract symptoms. The secondary objective was to

evaluate whether the degree of BHR was greater among sensitised children and to study the effect of different allergens and multi-sensitisation on BHR and clinical symptoms.

PATIENTS AND METHODS

Study population

We analysed Finnish data from 165 symptomatic and 82 non-symptomatic children, aged 7–12 years, which were collected as part of the children's asthma study published in *Acta Paediatrica* in 1996.¹⁹ The purpose of that study was to investigate allergic sensitisation and its effect on BHR at study population from 1994. The details of the study population and test methods have been published previously.¹⁸

Allergy test

Each child underwent a skin-prick test for birch, alder, mugwort, horse, cat, dog, cow, house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides. farinae*), and grasses (timothy, meadow fescue, meadow grass, cock's-foot, and ryegrass). However, due to the wide-scale cross-reactivity of grasses, only the results for timothy were analysed. The positive and negative controls were histamine dihydrochloride and physiological saline, respectively, with a wheal diameter ≥ 3 mm indicating a positive reaction.²⁰

Prerequisites of testing

Beta2-agonists and long-term beta2-agonists were not administered for 12 hours and 48 hours before the test, respectively. Further, antihistamines, cough medicines, and theophylline were not administered for 72 hours. The baseline forced expiratory volume in one second (FEV1) had to be at $\geq 70\%$ of the age-related and height-related reference values.²¹ The test was postponed by two weeks if a patient had an acute respiratory infection.

The six-minute free running test

During the six-minute free running test (FRT),¹⁸ the child's heart rate had to reach ≥ 170 beats/minute after running for six minutes. Spirometry was performed before the test and at 5, 10, 15, and 20 minutes after the test. The results were considered positive if the FEV1 fell by $\geq 15\%$, according to Finnish asthma guidelines.

Methacholine challenge test

The methacholine challenge test employed the dosimeter technique.¹⁸ Each child inhaled methacholine with physiological saline at increasing concentrations, with spirometry being performed before and after each cumulative increase. Cumulative concentration that caused a $\geq 20\%$ fall of the FEV1 (PD20) were calculated. Further, a cumulative methacholine dose was 4,900 μg and a PD20 ≤ 400 μg was considered positive.²² Finally, if no bronchoconstriction was observed, the result was coded as 4901 μg .

Grouping the children

The children were categorised into non-sensitised ($n = 120$) and sensitised ($n = 127$) groups, according to the skin-prick test results. They were considered sensitised if the wheal size was ≥ 3 mm for any of the 10 allergens (Figure 1).

Children were defined as symptomatic if they had displayed any lower respiratory tract symptoms, such as dry cough, or shortness of breath with wheezing in the past year, or if they had doctor-diagnosed asthma. Post-hoc analysis symptomatic children were divided into two groups: those only with the symptom of dry cough, and those with wheezing symptoms or doctor-diagnosed asthma. Asthma suspicion was based on a convincing history of asthma-like symptoms with the diagnosis being confirmed by a paediatric allergist.

Statistics

SPSS Version 22 (IBM Corp, New York, USA) was used for the statistical analysis. The Student's t-test was used for continuous data if the Shapiro–Wilk test score was ≥ 0.05 . Conversely, the Mann–Whitney U test was used if the Shapiro–Wilk test score was < 0.05 . For categorical data, the chi-square test and Fisher's exact test were used for numbers under five, and the Mann–Whitney U test was used for comparisons between groups.

Binary logistic regression analysis of BHR, as defined as having abnormal MCT and/or, FRT, was made using following covariates: gender; age, previous childhood asthma diagnosis; presence of allergic rhinitis and atopic dermatitis; study group; presence of perennial sensitisation or outdoor allergen sensitisation only; and sensitisation to birch, timothy, cat, dog, and house dust mite. A backward method by likelihood ratio was used in the analysis.

Ethics

The Ethics Committee of Kuopio University Hospital, Kuopio, Finland, approved the study. Written assent and consent for the study were collected from each patient and their parents, respectively.

RESULTS

Subject characteristics

The mean age of the children was 10.5 ± 1.7 years and their baseline forced expiratory volume in 1 second (FEV1) was $92.1 \pm 11.7\%$ of the predicted percentage. The proportion of children with doctor-diagnosed childhood asthma (26.0% versus 10.8%, $p = 0.002$), rhinitis (58% versus 21%, $p < 0.001$), and atopic eczema (48.0% versus 20.8%, $p < 0.001$) were significantly higher in the sensitised group compared the non-sensitised group. There were no other significant differences in the subject characteristics (Table 1).

Allergic sensitisation

The skin-prick test indicated that 127/247 (51.5%) children were sensitised to at least one allergen, of which 101 and 26 were from the symptomatic and control groups, respectively (Figure 1). Of those children 37/127 (29.1%) were sensitised to one, 23/127 (18.1%) to two, 22/127 (17.3%) to three, 23/127 (18.1%) to four, 12/127 (9.4%) to five (9.4%), 5/127 (3.9%) to six, 3/127 (2.4%) to seven, 2/127 (1.6%) to eight (1.6%), and 1/127 (0.8%) to nine of the 10 allergens. None of the children were sensitised to all 10 allergens.

Bronchial hyperresponsiveness

In total, 19/247 (7.7%) children experienced bronchoconstriction (defined as a $\geq 15\%$ fall of the FEV1 after the FRT), and 26/247 (10.5%) experienced bronchoconstriction after the $\leq 400 \mu\text{g}$ dose of methacholine. The median fall of FEV1 after the FRT was 3.1% (range: 39.5%–12.3%) in the 247 children, and the median cumulative dose of methacholine that caused a 20% fall in FEV1 was 4600 μg (range 54–4901 μg).

Differences were observed between sensitised and non-sensitised children exhibiting a $\geq 15\%$ fall of FEV1 after the FRT (11.8% versus 3.3%, $p = 0.015$), and having a $\text{PD}_{20} \leq 400 \mu\text{g}$ (17.3% versus 3.3%, $p < 0.001$). As shown in Figure 2A, similar findings were observed with the median fall of FEV1 after the FRT (-3.5% versus -2.6%, $p = 0.042$), and the median cumulative doses of methacholine (2400 μg versus 4901 μg , $p < 0.001$).

Among the 165 symptomatic participants, there were no significant differences between children having a $\geq 15\%$ fall of FEV1 after the FRT (13.9% versus 4.7%, $p = 0.059$). However, they exhibited $\text{PD}_{20} \leq 400 \mu\text{g}$ more often than non-sensitised children (20.8% versus 4.7%, $p = 0.004$). Although there were no significant differences between the groups in the median fall of FEV1 after the FRT (-3.7% versus -2.6%, $p = 0.063$), sensitised children exhibited a lower median

cumulative dose of methacholine (1700 µg versus 4901 µg, $p < 0.001$) compared to non-sensitised children (Figure 2B).

Sensitised children with doctor-diagnosed asthma or wheezing symptoms during the previous 12 months had more frequent bronchoconstriction (19.6% versus 0.0%, $p = 0.025$) and experienced more PD₂₀ ≤ 400 µg (33.9% versus 4.5%, $p = 0.008$) than non-sensitised children. As shown in Figure 2C, the median falls of FEV₁ after the FRT indicated no significant differences between the groups (-5.0% versus -2.9%, $p = 0.108$). Additionally, sensitised children with wheezing experienced bronchoconstriction with a lower median dose of methacholine (1400 µg versus 4901 µg, $p = 0.003$) than non-sensitised children as shown in Figure 2C.

There were no significant differences between children who only exhibited dry-cough symptoms at night only, with a ≥ 15% fall of FEV₁ after the FRT (7.5% versus 7.9%, $p = 1.00$) or having PD₂₀ ≤ 400 µg methacholine (4.4% versus 5.0%, $p = 1.00$). However, the median cumulative dose was lower for the sensitised group than non-sensitised group (3500 µg versus 4901 µg, $p = 0.039$), as shown in Figure 2D. Additionally, there was no difference in the median fall of FEV₁ after the FRT (-3.1% versus -2.3%, $p = 0.453$) (Figure 2D).

The 82 healthy controls demonstrated no significant differences between the sensitised and non-sensitised groups having bronchoconstriction after the FRT (3.8% versus 1.8%, $p = 0.547$), or having PD₂₀ ≤ 400 µg methacholine (3.8% versus 1.8%, $p = 0.547$), as shown in Table 2. When analysing the median fall of FEV₁ after the FRT (2.2% versus 2.1%, $p = 0.933$) and the median PD₂₀ (3050 µg versus 4901 µg, $p = 0.211$), no significant differences between the groups were revealed, as shown in Figure 2E.

It was observed that children with perennial sensitisation had more frequent a ≥ 15% falls of FEV₁ after the FRT (14.8% versus 4.3%, $p = 0.005$) and PD₂₀ ≤ 400 µg (21.5% versus 4.0%, $p < 0.001$) than children without perennial sensitisation. They also displayed a greater median fall of FEV₁ after the FRT (-3.6% versus -3.0%, $p = 0.006$), and had a lower cumulative dose in the methacholine challenge (760 µg versus 4901 µg, $p < 0.001$) than those without perennial sensitisation. Further, compared to children without outdoor allergen sensitisation, those with sensitisation exhibited more often ≥ 15% fall of FEV₁ after the FRT (15.6% versus 7.1%, $p = 0.032$), although this was not the case with PD₂₀ ≤ 400 µg (9.5% versus 7.5%, $p = 0.599$). Moreover, they exhibited no significant differences in median falls of FEV₁ after the FRT (-3.2% versus 2.4%, $p = 0.249$), or the median PD₂₀ in the methacholine challenge (4200 µg versus 4901 µg, $p = 0.064$).

The logistic regression analysis showed that a previous childhood asthma diagnosis (OR 3.80, 95% CI 1.58-9.12, $p = 0.003$), the number of sensitisations to aeroallergens tests (OR 1.66, 95% CI 1.34-2.06, $p < 0.001$) and sensitisations to perennial allergens only (OR 6.92, 95% CI 2.44-19.58, $p < 0.001$) were associated with BHR, as defined by having an abnormal MCT and/or FRT.

Lung function

Lung function was similar between sensitized and non-sensitized (FEV1 93.9% versus 91.4%, $p = 0.101$), and multisensitisation did not affect the results. Further, perennial or outdoor allergen sensitisation did not affect the FEV1 values.

Clinical manifestations of allergic sensitisation

Polysensitisation (a minimum of 2-3 allergens) increased the incidence atopic eczema compared to non-sensitised children ($p < 0.001$) and was associated with greater incidence of asthma. However, only children who were sensitised to at least four allergens had more frequently asthma compared to non-sensitised children (42.2% versus 10.8%, $p = 0.001$). Further, sensitised children exhibited significantly more rhinitis compared to non-sensitised children (74.8% versus 18.3%, $p < 0.001$), and children sensitised to a minimum of four allergens had more frequently rhinitis than children with monosensitisation (84.4% versus 62.2%, $p = 0.021$), as shown in Table 3.

Sensitisation to 1-3 allergens did not affect the results for BHR significantly.

However, children sensitised to at least four allergens displayed greater fall of FEV1 after the FRT (median -5.5% versus -2.6%, $p = 0.017$) and had a lower PD20 (median 900 μg versus 2400 μg , $p = 0.021$) in the methacholine challenge test than non-sensitised children, as shown in Table 3.

DISCUSSION

Our study produced three major findings. First, allergic sensitisation increased BHR as measured by direct and indirect bronchial challenge tests, although this was only true in children with respiratory symptoms. Second, allergic sensitisation did not affect lung function in our population. Third, multisensitisation to a minimum of four allergens increased the incidence of allergic diseases as compared to monosensitisation. The potential of immunotherapy for preventing asthma development has become a hot topic in recent years, underlining the relevance of analysing these excellent data that were collected 26 years ago.¹⁹

We observed significant differences between sensitised and non-sensitised children who demonstrated significant BHR in both the FRT and the MCT. Further, a clear difference was revealed between children with and without symptoms in the MCT. It should be noted that trends in the FRT differed according to sensitisation. Although, children with dry coughs displayed no differences in fall of FEV1 after the FRT, they exhibited a significant ($p < 0.05$) difference in the methacholine doses. In contrast, healthy controls displayed no differences in the methacholine doses and the FRTs as shown in Figures 2A–E. This observation underscores that symptoms, rather than biomarkers, could still be the optimum method of discerning asthma risk.

Only sensitisation to a minimum of four allergens caused increased BHR compared to monosensitisation. Further, these children tolerated a lower cumulative dose of methacholine than did monosensitised children (median 900 μg versus 2400 μg , $p = 0.021$), as shown in Table 3. It supports previous findings.^{5,6,7} Our study indicated that allergic sensitisation was associated with increased BHR as measured by the methacholine test and the FRT. In addition, we observed an abnormal fall of $\text{FEV1} \geq 15\%$ after the FRT and a PD20 after $\leq 400\mu\text{g}$ dose of methacholine. Further, sensitised children exhibited higher BHR compared to non-sensitised children, especially if they had experienced wheezing.

Interesting observation was that there were no differences in the fall of FEV1 among the 82 healthy controls after the FRT or the MCT. In this study, BHR occurred when children experienced lower respiratory tract symptoms. However, separating the effect of allergic rhinitis from aeroallergen sensitisation is very difficult. In another study from Turkey,¹¹ BHR measured by MCT showed that children with allergic rhinitis also often have BHR. It is possible that severe allergic rhinitis without clear asthma symptoms can increase the presence of BHR, compared our population with usually mild rhinitis symptoms those kinds of results were not observed.

Bronchial provocation tests in children without lower respiratory tract symptoms should not be performed for example before starting immunotherapy. Moreover, according to guidelines provided by the European Academy of Allergy and Immunology, asthma may be prevented by immunotherapy.²³

The allergen itself also played an important role, with perennial sensitisation increasing BHR in contrast to outdoor allergen sensitisation alone did not. These findings agree with those of other study.^{5,24,25,26} Further, sensitisation had no effect on lung function in this study, unlike multisensitisation in early childhood in recent studies.^{6,7}

The characteristics of the sensitised and non-sensitised groups were identical with regard to age, sex, and lung function. The sensitised children had more doctor-diagnosed rhinitis, atopic eczema, and asthma, which corresponded with the literature.^{12,14,26,27} Only 29% of the sensitised children were mono sensitised, which is partially explained by the cross-reactivity of allergens.

The clinical diseases associated with sensitisation to an aeroallergen are typically rhinitis and childhood asthma, as described in other studies.^{8,12,27} In our work, multisensitisation to 2-3 allergens did not increase the incidence of allergic asthma or rhinitis compared to monosensitisation. However, the prevalence of those conditions was higher in children who were sensitised to at least four allergens, especially if a child was sensitised to a perennial allergen. These findings are in agreement with the model by Custovic et al. in which sensitised children formed different clusters having variable associations with asthma.^{28,29} Similar findings have also been described in two other studies.^{6,13} Further, they support arguments against the traditional atopic march theory described in another study.²⁹

The children underwent both direct methacholine and indirect FRT bronchial challenge tests, which was a strength of our study. Additionally, having 82 healthy controls enabled us to analyse children with no respiratory symptoms and to investigate the timing of BHR compared to the onset of respiratory symptoms. The limitations were that our study did not include an analysis of the children's expired nitrous oxide levels. Another limitation can be considered the sample size: the control group could have been larger in our historical data. Also, separating BHR and allergic rhinitis is problematic, and many children in the control group had also rhinitis symptoms, usually during the pollen season. The diagnoses of allergic diseases were based on reporting by parents, which can also be considered a limitation.

CONCLUSION

In conclusion, our detailed analysis indicated that allergic sensitisation did not worsen lung function or increase BHR if the child did not exhibit respiratory symptoms. Moreover, our results suggest that BHR is not a slowly developing phenomenon when measured as a direct or especially indirect mechanism. Usually it appears after the onset of clinical symptoms - such as wheezing or a dry cough. Future studies are needed to examine whether early intervention preventing multiple sensitisation could decrease the risk of developing BHR.

CONFLICT OF INTEREST

Authors have no conflict of interest.

Accepted Article

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FIGURE LEGENDS

FIGURE 1 Flowchart of the study

FIGURE 2A Differences in bronchial hyperresponsiveness according to allergen sensitisation observed during the methacholine and free-running tests in all children; the Mann–Whitney U test was used for statistics. *** $P < 0.001$

FIGURE 2B Differences in bronchial hyperresponsiveness according allergen sensitisation observed during the methacholine and free running tests among symptomatic children; the Mann–Whitney U test was used for statistics. *** $P < 0.001$

FIGURE 2C Differences in bronchial hyperresponsiveness according to allergen sensitisation observed during the methacholine and free running tests among children with wheezing or doctor-diagnosed asthma; the Mann–Whitney U test was used for statistics. ** $P < 0.01$

FIGURE 2D Differences in bronchial hyperresponsiveness according to allergen sensitisation observed during the methacholine and free running tests among children with the symptom of dry cough only; the Mann–Whitney U test was used for statistics. * $P < 0.05$.

FIGURE 2E Differences in bronchial hyperresponsiveness according to allergen sensitisation observed during the methacholine and free running tests among healthy controls; the Mann–Whitney U test was used for statistics.

TABLE 1 Population characteristics of the 165 patients with respiratory symptoms and the 82 healthy controls, categorised as non-sensitised and sensitised children

Value	Non-sensitised children, n=120	Sensitised children, n=127	P-value
Age, mean (SD) years	10.4 (1.6)	10.6 (1.7)	0.325
Sex (male)	66 (55.0%)	70 (55.1%)	0.985
Asthma, n (%)	13 (10.8%)	33 (26.0%)	0.002
Atopic eczema, n (%)	25 (20.8%)	61 (48.0%)	<0.001
Rhinitis, n (%)	22 (18.3%)	95 (74.8 %)	<0.001
FVC % predicted mean (SD)	98.4 (13.6)	96.4 (11.3)	0.234
FEV1 % predicted mean (SD)	93.9 (12.6)	91.4 (9.6)	0.101
MEF50 % predicted mean (SD)	82.1 (17.5)	82.4 (20.6)	0.877
PEF % predicted mean (SD)	96.4 (16.6)	93.7 (17.8)	0.237
PD20FEV1 in µg, median (range)	4901 (180–4901)	2400 (54–4901)	<0.001
PD20FEV1 max 400 µg, n (%)	4 (3.3%)	22 (17.3%)	<0.001
Fall of FEV1 after FRT, median (range)	-3.5 (-49.5–8.2)	-2.6 (-42.8–12.3)	0.042
Minimum 15 % fall in FEV1 after FRT, n (%)	4 (3.3%)	15 (11.8%)	0.015

Data are presented as mean and SD or number and percentages. Chi-square test and the Mann Whitney U-test were used for statistical analysis. FEV1; Forced expiratory volume in 1 second FRT; Free running test, FVC; Forced vital capacity; MEF50; Maximal Expiratory Flow at 50% of Vital Flow Capacity, PD20FEV1; Concentration of methacholine caused 20% fall of FEV1 after the methacholine test PEF; Peak expiratory flow.

TABLE 2 Baseline characteristics and test results for 82 healthy controls categorised by their aeroallergen sensitisation

Value	Non-sensitised children, n=56	Sensitised children, n=26	P-value
Age, mean (SD) years	10.3 (1.6)	10.7 (1.7)	0.322
Sex (female), n (%)	24 (42.9%)	14 (53.8%)	0.353
Asthma, n (%)	0 (0.0%)	0 (0 %)	-
Atopic eczema, n (%)	10 (17.9%)	9 (34.6%)	0.094
Rhinitis, n (%)	4 (7.1%)	11 (42.3%)	<0.001
FVC, % predicted	96.6 (12.5)	92.4 (13.6)	0.209
FEV1 % predicted, mean (SD)	89.0 (12.3)	92.9 (9.1)	0.153
MEF50 % predicted, mean (SD)	88.2 (16.6)	83.6 (12.7)	0.241
PEF % predicted, mean (SD)	94.1 (13.6)	86.9 (13.4)	0.040
PD20FEV1, (ug), median (range)	4901 (320-4901)	3050 (100-4901)	0.211
PD20FEV1 max 400 µg, n (%)	1 (1.8%)	1 (3.8%)	0.547
Fall of FEV1 after FRT, median (range)	-2.1 % (-12.1- 11.8%)	-2.2% (-10.3-4.6 %)	0.933
Minimum 15% fall of FEV1 after FRT, n (%)	1 (1.8%)	1 (3.8%)	0.547

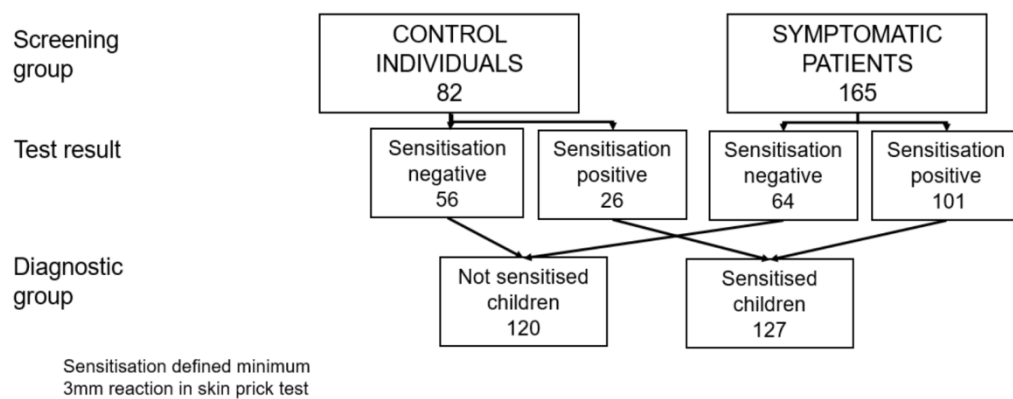
Data are presented as mean and SD, median and range or number and percentages. Chi-square test and the Mann Whitney U-test were used for statistical analysis. FEV1; Forced expiratory volume in 1 second FRT; Free running test, FVC; Forced vital capacity; MEF50; Maximal Expiratory Flow at 50% of Vital Flow Capacity, PD20FEV1; Concentration of methacholine caused 20% fall of FEV1 after the methacholine test PEF; Peak expiratory flow.

TABLE 3 Clinical manifestation and allergic sensitisation of all children based on a maximum 10 different sensitisations

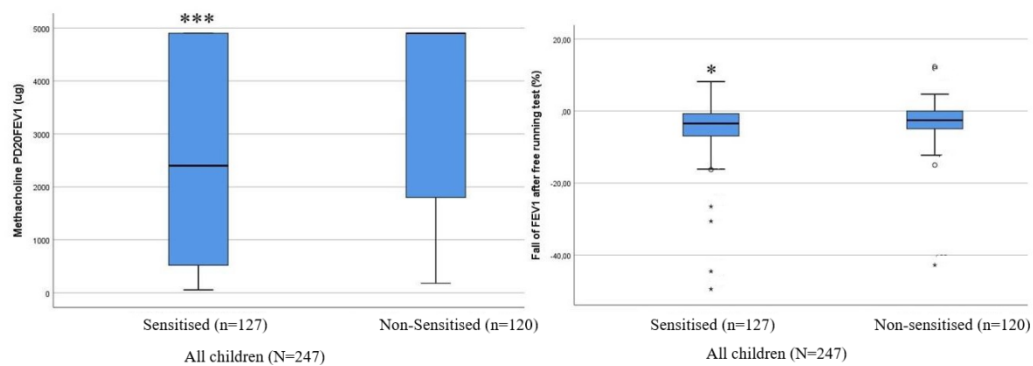
Number of sensitisations	Children in the group n (%)	Atopic eczema n (%)	Rhinitis n (%)	Asthma n (%)	PD20FEV1, ug	Fall of FEV1 in FRT (%), Median
0	120	25 (20.8%)	22 (18.3%)	13 (10.8 %)	4901 (180–4901)	-2.6 (-42.8–12.3)
1	37	13 (35.1%)	23 (62.2%)	7 (18.9%)	2400 (180–4901)*	-3.4 (-26.5–8.2)

2-3	45	22 (48.9%)	34 (75.6%)	7 (15.6%)	4901 (80–4901)	-2.8 (-44.6–4.6)
		***	***			
≥4	45	26 (57.7%)	38 (84.4%)	19 (42.2%)	900 (54–4901)	-5.5 (-49.5–6.8)
		***	***/*	***	***/*	*

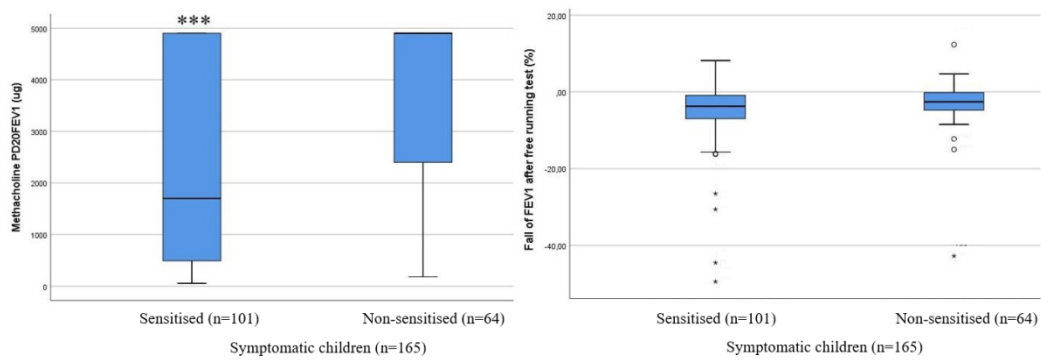
Sensitisation was defined as a minimum 3 mm reaction in the skin prick test. FEV1; forced expiratory volume in one second, FRT; free running test; PD20FEV1, cumulative methacholine dose causing a minimum 20 % fall in FEV1. *** p<0.001 versus non-sensitised children and * p<0.05 versus children with one sensitisation to aeroallergen. Chi-square test and Mann–Whitney U test were used for statistical analysis.



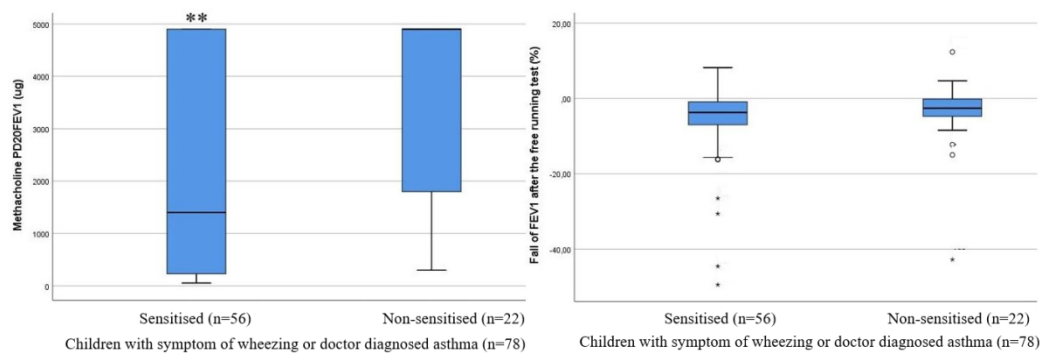
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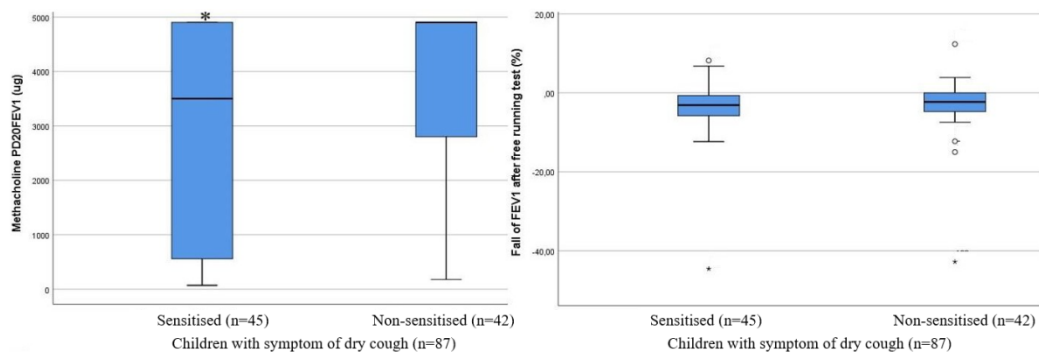
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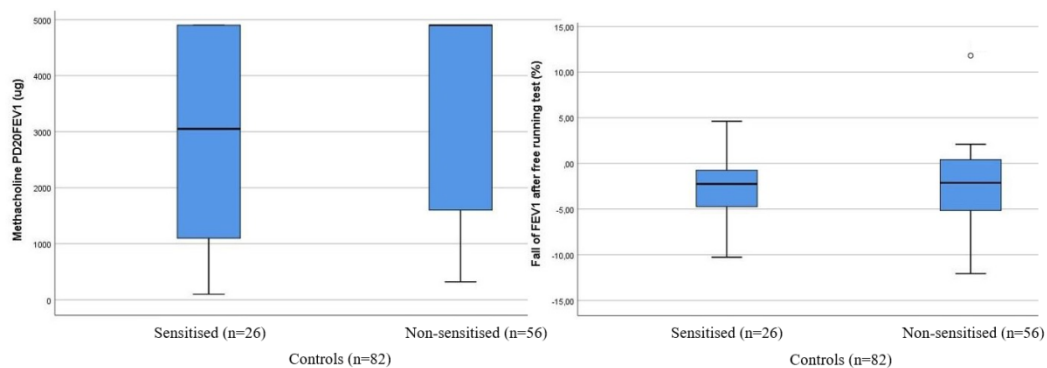
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