Determinants of blood eosinophilia in moderate and severe asthmatic patients during childhood: evidence from the SAMP cohort

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Determinants of blood eosinophilia in moderate and severe asthmatic patients during childhood: evidence from the SAMP cohort

Short Title: **Eosinophilia significance in SAMP cohort**

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Abstract

Background*:* Asthma is a heterogeneous disease in which the interaction of genetic and environmental factors plays a major role. The significance of blood eosinophil is unclear. The aim of the study was to determine the significance of blood eosinophil count in moderate to severe asthmatic children of preschool and school age.

Methods*:* This was a prospective cross-sectional study performed from 2011 to 2015 including children from the Severe Asthma Molecular Phenotype (SAMP) cohort at Trousseau Hospital (Paris, France). We included children with severe and moderate asthma, or severe and moderate recurrent wheeze, aged from 1 to 15 years at the time of exploration.

Results: We analyzed data from 402 children: 248 of preschool age and 154 of school age. Blood eosinophil count third quartile thresholds were 322 and 600 cells/µL for the preschool- and school-age groups, respectively. In multivariate analysis, a blood eosinophil count over this threshold was associated with elevated total IgE (OR=5.33; $P<0.01$), multiple hospitalizations for asthma attacks (OR=4.96; $P=0.03$), and a maternal history of asthma **(**OR=4.91; $P=0.01$) in preschool children; and with staphylococcal toxin-specific IgE (OR=2.75; $P=0.03$) in children of school age. Random forest analysis reinforced these results.

Conclusion: High blood eosinophil count is linked to both atopic features and control of asthma with different parameters associated with these features depending on age.

Keys words: asthma phenotype; children; eosinophil; severe asthma; staphylococcal toxin sensitization

Abbreviations:

EVW: Episodic viral wheeze

ICS: Inhaled corticosteroids

MTW: Multiple trigger wheeze

SAMP: Severe Asthma Molecular Phenotype

SPT: Skin prick tests

Introduction

Asthma is a heterogeneous disease in which genetic and environmental factors play a major role(1). It has been known since the end of the 1950s that the sputum eosinophil count is a predictor of the response to treatment with corticosteroids(2). In adults, the presence of eosinophils in asthma inflammation is an important factor in the disease pathophysiology and blood eosinophil count can be used as a non-invasive biomarker to identify patients with an improved response to some treatments(3,4). Several interesting studies have been carried out to determine the role of eosinophils in the management of asthma, but controversy persists. Some studies suggest that eosinophilic driven biomarkers (such as exhaled nitric oxide, sputum eosinophil count and blood eosinophil count) are associated with more severe asthma or with a poorer disease outcome in adults(5–7). One recent publication analyzing the response to mepolizumab in adults with severe eosinophilic asthma, reported that the blood eosinophil count was a better predictor of response than sputum eosinophil count(8). Furthermore, determining the sputum eosinophil count is a particularly complex and costly technique which is not available in all health centers. On the other hand, blood eosinophil count can be routinely measured in all clinical settings. Consequently, peripheral eosinophil assessment is a more practical option for determining the asthma phenotype in both adults and children. However, discrepancies about the significance of blood eosinophil count have been reported: some studies have observed that adult patients with high eosinophil count have more severe asthma(5,6), while others have described the opposite with fewer severe exacerbations reported(9), or even no correlation at all(10). Finally, the range of blood eosinophil count would appear to differ between children and adult asthmatic populations(11). The aim of this study was to determine the significance of high blood eosinophil count in preschool- and school-age children.

Methods

Design and setting

This was a prospective cross-sectional study performed from 2011 to 2015 from the SAMP cohort at Trousseau Hospital, Paris (France). All the children had been referred to the center by a secondary or primary physician due to persistence of recurrent wheeze despite long-term treatment. The Institutional Review Board of Saint Antoine Hospital, Paris, endorsed the protocol as an observational study. Written informed consent was obtained from the parents of the children included.

The children included in the present study met the following inclusion criteria: children with severe and moderate asthma or severe and moderate recurrent wheeze aged from 1 to 15 years at the time of exploration (12)

Health outcomes were collected in a computerized database using standardized questionnaires. Gender and age at inclusion were collected. The severity of asthma or recurrent wheeze of the entire population was assessed after at least 6 months of follow-up prior to inclusion in the study by an experienced pulmonologist paediatrician after repeated individual or group health education measures had been undertaken to improve adherence to a continuous anti-asthmatic treatment, and after advice by an environmental specialist to reduce exposure to indoor biological pollutants. Severe asthma was defined as controlled asthma with high doses of inhaled corticosteroids (ICS) (≥500 µg/day fluticasone propionate) and two other controller medications in school-age children; and as controlled symptoms with high doses of ICS (>200 µg/day fluticasone propionate) and leukotriene receptor antagonist in preschool-age patient.

The daily dosage of ICS was recorded. Children were classified as having either episodic viral wheeze (EVW) (wheezing only during colds and remaining asymptomatic between episodes) or multiple trigger wheeze (MTW) (wheezing during colds but symptomatic between episodes with wheezing activated by dust, grass, pets, tobacco smoke, exercise or cold air). Totally controlled asthma was defined as the absence of nocturnal or daily symptoms, exacerbation, short-acting β2-agonist use or activity limitation due to asthma, according to Global Initiative for Asthma(1). Partially control or uncontrolled asthma were defined as the presence of one or two of these parameters and uncontrolled asthma as the presence of three or four. The number of hospitalizations for an asthma attack in the year prior to inclusion was recorded. Both maternal and paternal asthma histories were collected. Allergic rhinitis and active atopic dermatitis were assessed by questions from the International Study of Asthma and Allergies in Childhood (ISAAC)(13), and IgE-mediated food allergy was defined by clinically relevant symptoms within 6 hours following food allergen consumption associated with an allergic sensitization to the same allergen. Total IgE (measured by ImmunoCAP, Thermofischer, Uppsala, Sweden) were collected. The following thresholds were used to define increased levels: elevated total IgE above or equal to the third quartile distribution of each studied population (total population, preschool-age group (children <6 years) and school-age group (children ≥6 years). Allergic sensitization was defined by positive skin prick tests (SPT) (mean weal diameter ≥3 mm; Stallergenes, Antony, France) and/or positive specific IgE levels (≥0.35 kIU/L, measured by ImmunoCAP, Thermofischer, Uppsala, Sweden) for cow's milk, egg, current inhaled allergens and staphylococcal toxins. Perennial sensitization was defined as house dust mite and/or cat or dog dander sensitization without associated seasonal allergen sensitization. Seasonal sensitization was defined as grass and/or birch pollens sensitization,

with no associated perennial sensitization. Perennial and seasonal co-sensitization was defined as the sensitization of at least one perennial and one seasonal allergen as described above. Sensitization to food allergen was defined as cow's milk and/or egg sensitization or peanut sensitization.

Data about habitation density (categorized as ≤9 m^2 per household member (high) or >9 m^2 per household member (low)); tobacco smoke exposure (based on smokers in the home, including mother, father, or other adult household members); and potential biologic allergens sources as molds at home (visible or moldy smell) were collected by means of a questionnaire validated in the PARIS neonatal cohort(14).

Blood eosinophilic count

Blood eosinophil count was measured by an automated Sysmex analyzer (Villepinte, France) outside systemic corticoid treatment and asthma exacerbations. Moreover, eosinophil count stability was assessed by at least two measures within the last 6 months of follow-up. High and low blood eosinophil counts were defined as above or equal to the third quartile distribution and under the third quartile distribution of blood eosinophil count, respectively, in each of the three groups (total population, preschool-age and school age groups).

Statistical analysis

The chi-square test and Fisher's exact test were used to compare the distribution of each variable (clinical and environmental) between the high and low blood eosinophil count groups. A logistic regression analysis was used to investigate the relationships between the binary outcome of interest (high blood eosinophil count) and multiple risk factors. Univariate and

multivariate models were constructed to better understand the presence of high blood eosinophil count. Risk factors associated with high blood eosinophil count in the univariate analysis (P<0.2) and parameters known to be associated with high blood eosinophil count in the literature (i.e., multiple hospitalizations for asthma attacks and uncontrolled asthma) were included in the multivariate analysis. The multivariate models were selected using the backward stepwise procedure based on Akaike Information Criteria.

A tree-based analysis was then performed to propose non-linear approaches to understand the presence of high blood eosinophil count. In a first step, classification and regression trees were considered to obtain a non-linear classifier able to distinguish between high and low blood eosinophil counts, the variables at the top of the tree being more predictive of high blood eosinophil count. In a second step, a random forest analysis was performed to provide another selection of important variables to predict high blood eosinophil count. This ensemble method uses a number of classification trees to improve the classification compared to a single tree. In addition to good predictive performance, random forests estimate the relevance (discriminating power) of each variable using importance measures (permutation-based mean decrease in accuracy). All analyses were two-sided, and a P-value ≤0.05 was considered statistically significant. Statistical analysis was performed with R version 3.5.0. The R package 'glm', 'Rpart' and 'randomForest' were used to perform the analyses.

Results

Four hundred and two children were included of whom 248 were of preschool age and 154 of school age. The population's characteristics are summarized in Table I. Elevated total IgE (above or equal to the third quartile distribution) was 344, 105, and 920 kIU/L in the total population, the preschool-age group and the school-age group, respectively.

High and low blood eosinophil count groups

Blood eosinophil count third quartile thresholds were 440, 322 and 600 cells per µL in the total population, the preschool-age group and the school-age group, respectively (Table II).

More features of allergy were apparent in the high blood eosinophil count group (Table II). This group had more allergic comorbidities, especially more allergic rhinitis ($P<.001$), more inhaled and food allergen sensitization, especially peanut sensitization ($P<.001$) and cow's milk and/or egg sensitization ($P<.001$), and more elevated total IgE ($P<.001$) (Figure 1). This group also had more children with MTW ($P\leq.001$). Blood eosinophil count was not significantly associated to asthma control and to ICS doses, in the total population, the preschool-age group and the school-age group (*P*-values: 0.547, 0.385 and 0.814 for asthma control; and 0.949, 0.554 and 0.596 for ICS doses, respectively), as presented in Figure 2. Similarly, Total IgE levels were not significantly associated to asthma control and to ICS doses, in the total population, the preschool-age group and the school-age group (*P*-values : 0.52, 0.072 and 0.769 for asthma control; and 0.679, 0.075 and 0.763 for ICS doses, respectively), as presented in Figure 3.

High blood eosinophil count risk factors

In multivariate analysis, a model was developed using an automatic stepwise procedure taking parameters with $P<0.2$ in univariate logistic regression analysis (Table II) and parameters known to be associated with high blood eosinophil count in the literature (i.e., multiple hospitalizations for asthma attack and uncontrolled asthma). After adjustment, this analysis led to a model indicating that the following parameters increased the risk of high blood eosinophil count in the entire population: older age (OR=1.01; $P<.01$), at least two hospitalizations for asthma attacks (OR=5.40; $P=0.01$), allergic rhinitis (0R=3.44; $P<.01$) and sensitization to peanut (OR=2.67; $P=0.04$) (Table III).

Similarly, in the preschool-age population, elevated total IgE (OR=5.33; $P<.01$), at least two hospitalizations for asthma attacks in the previous year (OR=4.96; $P=0.03$), and a maternal history of asthma (OR=4.91; $P=0.01$) were found to increase the risk of high blood eosinophil count (Table IV). The importance measure (permutation measure) obtained from the random forest analysis can be interpreted as a measure of discriminating power. These measures reinforced the previous results: the most important variables to explain high blood eosinophil count were elevated total IgE and at least two hospitalizations for asthma attacks. Maternal asthma history and cow's milk and/or egg sensitization were other determinants of high blood eosinophil count at preschool age.

In the school-age population, staphylococcal toxin-specific IgE sensitization (OR=2.75; $P=0.03$) was found to increase the risk of high blood eosinophil count after adjustment (Table V). The importance measures obtained from the random forest analysis also reinforced the previous results of determinants of high blood eosinophil count (sensitization to staphylococcal toxins) but also identified MTW and cow's milk and/or egg sensitization.

Discussion

The principal result of our study was that high blood eosinophil count was positively correlated with elevated total IgE and at least two hospitalizations for asthma attacks in the previous year, but also with a maternal history of asthma at preschool age, and to staphylococcal toxin-specific IgE sensitization at school age.

Increase in blood eosinophil count during childhood

It is well known that blood eosinophil count has different range levels during childhood in the general population: in the peripheral blood it varies by age group with higher upper threshold limits seen in infants and toddlers compared to adolescents and adults(15). We have previously described that eosinophil count is more frequently associated with severe asthma in school-age children than in preschool-age children(12).

High blood eosinophil count determinants and atopic features

The correlation between eosinophilia and total IgE level, especially in preschool cohorts, has often been associated with poor asthma prognosis during childhood(16). More recently, a prospective controlled trial showed that significantly higher levels of total serum IgE levels, blood eosinophil count and fractional exhaled nitric oxide were correlated with an atopic asthma group compared to a non-atopic asthma group(17). In the same manner, Park *et al*(18), in a cohort of preschool children with a follow-up of 2 years, showed that serum eosinophil percentage and total IgE were associated with an increased risk of allergic sensitization and allergic symptoms.

Both maternal and paternal histories of asthma are associated with an increased risk of asthma in the offspring with a stronger association for maternal asthma history(19). In the MAS cohort(20) a positive allergic family history was a strong predictor of asthma from childhood up to adulthood.

We found that staphylococcal toxin-specific IgE sensitization was the major determinant of high blood eosinophil count in children of school age. Staphylococcal colonization of the skin is commonly observed in subjects with atopic dermatitis and correlates with disease severity. In atopic dermatitis, results suggest that exotoxins incite a local super-antigen response, with clonal T-cell activation and massive cytokine release, which has been correlated with disease severity. Staphylococcus aureus colonization was more commonly observed in subjects with atopic dermatitis who had peripheral eosinophilia, elevated serum IgE levels, and/or a history of or active allergic rhinitis(21). Staphylococcal toxin-specific IgE has also been found in the serum of patients with chronic sinusitis with nasal polyps(22). Our results are in accordance with a phenotype of severe asthma at school age with high blood eosinophil count, elevated total IgE level and multiple sensitizations(23). As in atopic dermatitis, staphylococcal toxin-specific IgE significantly alter epithelial repair(24) and could initiate clonal T-cell activation and multiple sensitizations associated with eosinophilic inflammation and asthma severity.

We found that food allergy was associated with high blood eosinophil count in multivariate analysis in the whole population (peanut sensitization OR 2.67, P=0.04) and cow's milk and/or egg sensitization (in random forest analysis at preschool and at school age) but with fewer repetitive links than the other atopic determinants in the various statistical analyses.

Finally, the two atopic determinants of high blood eosinophil count are probably associated

with genetic traits of atopy in preschool children (maternal asthma history and elevated total IgE) and/or innate traits of atopy in children of school age (staphylococcal toxin-specific IgE) in relation with the long-term temporal trajectory of allergic diseases.

High blood eosinophil count determinants and asthma control

In our study**,** high blood eosinophil count in preschool-age children with moderate to severe recurrent wheezes was associated with more than two severe asthma exacerbations requiring hospitalization. This finding is in accordance with a special phenotype of very early onset of asthma and multiple sensitization at high risk of hospitalization as described both by Simpson and al.(25) in a British birth cohort and Herr and al.(26) in a French birth cohort. In adults, the association of severe asthma with blood eosinophil count and hospitalizations is a well-known finding(27). In a retrospective cohort study of 2,701 patients(28), those with uncontrolled asthma and high blood eosinophil count were four times more likely to be hospitalized and the associated costs were more than four times greater than for patients with controlled asthma without high blood eosinophil count.

In our study, a high blood eosinophil count at school age tended to be associated, though without reaching statistical significance, with MTW (OR=7.41, *P*=0.07). Moreover, MTW was the first determinant factor for high blood eosinophil count in random forest analysis at school age. The distinction between EVW and MTW is used to guide the management of preschool wheeze(29). In the literature, MTW is more often associated with asthma severity than EVW, especially in allergic children, and with persistence of asthma throughout childhood: severe disease was more frequent in children with MTW (31.8%) than in those with EVW (5.1%) in a cross-sectional survey of children of 7-12 years of age in Aberdeen city primary schools(30). In the

prospectively followed Trousseau Asthma Program cohort, we showed that remission was more frequently observed in children with EVW and that fewer remissions were observed in atopic MTW.

Finally, in the literature, an eosinophilic asthma phenotype(31) has been associated with disease severity(32) but consequently to uncontrolled asthma in 544 subjects: the eosinophilic phenotype, according to blood eosinophil count, was associated with uncontrolled asthma (OR=1.56; 95%CI[1.06 - 2.28]).

The strength of the present study is that it was performed in a large and well-defined population of children with severe asthma or severe recurrent wheeze. On the other hand, a main limitation lies in the fact that all the patients were recruited from one center. Nevertheless, while two-thirds of the asthmatic children were from Paris and the surrounding area (>10 million inhabitants), the remaining one-third live in regions throughout France, which limits this potential bias. Finally, another limitation is that the study was cross-sectional.

In conclusion, high blood eosinophil count in children with moderate and severe asthma is associated with different features depending on age. This finding suggests that blood eosinophil count could be a useful pharmacodynamic biomarker for a specific pathological pathway to better define the target of biologic drugs.

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290

291 **Key message**

292 In this study, we highlight that elevated blood eosinophil count in moderate to severe asthmatic
293 children has different significance according to age; mainly elevated IgE, multiple
294 hospitalizations and maternal asthma history for preschool-age children and staphylococcal
295 toxin-specific sensitization for children of school age. These results could help understand the
296 pathophysiology of asthma inflammation and lead to better management by targeted therapy.

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380 Table I: Population's characteristics according to age group

	Total population (N = 402)	Preschool-age group (N = 248)	School-age group (N = 154)
Demographic details			
Gender, male; n (%)	265 (66)	166 (67)	99 (64)
Age, months; mean (SD)	73.6 (57.4)	34.2 (19.0)	137 (38.7)
Exposure to tobacco; n (%)	136 (34)	91 (37)	45 (29)
Low socioeconomic categories of parents; n (%)	169 (42)	100 (40)	69 (45)
BMI, kg/m2; mean (SD)	17.4 (3.1)	16.4 (2.1)	19 (3.7)
Personal atopy			
Active atopic dermatitis; n (%)	82 (20)	44 (18)	38 (25)
Allergic rhinitis; n (%)	180 (45)	73 (29)	107 (69)
IgE-mediated food allergy, n (%)	60 (15)	29 (12)	31 (20)
Familial atopy			
Maternal asthma; n (%)	96 (24)	59 (24)	37 (24)
Paternal asthma; n (%)	79 (20)	44 (18)	35 (23)
Home environment			
Exposure to mold, n (%)	87 (22)	64 (26)	23 (15)
Exposure to tobacco, n (%)	136 (34)	91 (37)	45 (29)
Asthma history			
Age at wheeze onset months; mean (SD)	12.1 (18.9)	7.7 (12.1)	19.3 (24.7)
Single trigger wheeze; n (%)	248 (62)	179 (72)	69 (45)
Multiple trigger wheeze; n (%)	153 (38)	68 (27)	85 (55)
≥ 2 hospitalizations for asthma attacks; n (%)	51 (13)	41 (17)	10 (6)

Asthma severity			
Moderate; n (%)	74 (18)	45 (18)	29 (18)
Severe; n (%)	328 (82)	203 (82)	125 (81)
Severe asthma control			
Controlled with high-dose ICS; n (%)	58 (14)	42 (17)	16 (10)
Uncontrolled with high-dose ICS; n (%)	245 (61)	146 (59)	99 (64)
Blood inflammation			
Blood PN; mean (SD)	3923 (2563)	4292 (2830)	3329 (1925)
Blood PE; mean (SD)	328 (290)	266 (272)	430 (291)
Blood PB, mean (SD)	59 (305)	76 (386)	32 (41)
Blood Lymphocytes, mean (SD)	3722 (1910)	4411 (2055)	2612 (859)
Blood Monocytes, mean (SD)	661 (377)	725 (408)	558 (295)
IgG level; mean (SD)	8.0 (2.6)	6.9 (2.2)	9.8 (2.3)
IgA level; mean (SD)	1.0 (0.7)	0.6 (0.4)	1.5 (0.7)
IgM level; mean (SD)	1.0 (0.7)	1.0 (0.8)	1.1 (0.5)
Allergic sensitization			
Perennial sensitization only; n (%)	25 (6)	9 (4)	16 (10)
Seasonal sensitization only; n (%)	0	0	0
Perennial and seasonal co-sensitization; n (%)	150 (37)	39 (16)	111 (72)
Alternaria alternata sensitization; n (%)	36 (9)	1 (0.4)	35 (23)
Peanut sensitization; n (%)	48 (12)	13 (5)	35 (23)
Cow's milk and/or egg sensitization; n (%)	64 (16)	35 (14)	29 (19)
Staphylococcal toxin IgE sensitization; n (%)	76 (11)	21 (8)	55 (36)
Total IgE; mean (SD)	377 (867)	111 (229)	797 (1255)

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Blood PB, blood basophil cells; Blood PE, blood eosinophil cells; Blood PN, blood neutrophil cells; BMI, body mass index; ICS, inhaled corticosteroid; Allergic rhinitis and active atopic dermatitis were assessed by questions from the International Study of Asthma and Allergies in Childhood (ISAAC); Multiple trigger wheeze was defined as wheezing during colds but symptomatic between episodes with wheezing activated by dust, grass, pets, tobacco smoke, exercise or cold air; The severity of asthma or recurrent wheeze of the entire population was assessed after at least 6 months of follow up prior to inclusion in the study according to GINA; Uncontrolled asthma was defined as the presence of at least 3 criteria among: nocturnal or daily symptoms, exacerbation, short acting β2 agonist use or activity limitation due to asthma; Perennial sensitization was defined as house dust mite and/or cat or dog dander sensitization without seasonal allergen sensitization associated. Seasonal sensitization was defined as grass and/or birch pollens sensitization, with no perennial sensitization associated. Perennial and seasonal co-sensitization is defined as the sensitization of at least one perennial and one seasonal allergen as described above; Allergic sensitization was defined by positive skin prick tests and/or positive specific IgE levels.

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391 Table II. Population's characteristics according to blood eosinophil count (univariate analysis)

	Total population (N = 402)			Preschool-age group (N = 248)			School-age group (N = 154)		
	Low blood eosinophil count (<440 c/µL) (n=299)	High blood eosinophil count (≥ 440 c/µL) (n=103)	*P*-value	Low blood eosinophil count (<322 c/µL) (n=186)	High blood eosinophil count (≥322 c/µL) (n=62)	*P*-value	Low blood eosinophil count (<600 c/µL) (n=114)	High blood eosinophil count (≥ 600 c/µL) (n=40)	*P*-value
Gender, male; n (%)	196 (66)	69 (67)	0.22	121 (65)	45 (73)	0.35	71 (62)	28 (70)	0.60
Active atopic dermatitis; n (%)	49 (16)	33 (32)	**<.01**	29 (16)	15 (24)	0.18	25 (22)	13 (33)	0.26
Allergic rhinitis; n (%)	112 (37)	68 (66)	**<.001**	53 (28)	20 (32)	0.38	74 (65)	33 (83)	0.06
Multiple food allergy; n (%)	20 (7)	9 (9)	**0.02**	10 (5)	5 (8)	0.45	10 (9)	4 (10)	0.10
Alternaria sensitization; n (%)	19 (6)	17 (17)	**<.001**	0	1 (2)	0.11	23 (20)	12 (30)	0.06
Peanut IgE sensitization; n (%)	24 (8)	24 (23)	**<.001**	6 (3)	7 (11)	**0.04**	22 (19)	13 (33)	**0.04**
Cow's milk/egg IgE sensitization; n (%)	37 (12)	27 (26)	**<.001**	18 (10)	17 (27)	**<.01**	16 (14)	13 (33)	**<.01**
Perennial IgE sensitization; n (%)	69 (23)	74 (72)	**<.001**	15 (8)	21 (34)	**<.001**	68 (60)	39 (98)	**<.001**
Seasonal IgE sensitization; n (%)	30 (10)	37 (36)	**<.001**	1 (1)	4 (6)	**0.02**	40 (35)	22 (55)	**0.03**
Perennial and seasonal IgE co-sensitization; n (%)	29 (10)	33 (32)	**<.001**	1 (1)	2 (3)	0.24	37 (32)	22 (55)	**<.01**
Maternal asthma history; n (%)	67 (22)	29 (28)	0.37	37 (20)	22 (35)	**0.03**	30 (26)	7 (18)	0.36
Paternal asthma history; n (%)	60 (20)	19 (18)	0.66	35 (19)	9 (15)	0.55	25 (22)	10 (25)	0.86
Exposure to tobacco; n (%)	109 (36)	27 (26)	0.07	73 (39)	18 (29)	0.20	37 (32)	8 (20)	0.20
Exposure to mold, n (%)	64 (21)	23 (22)	0.95	43 (23)	21 (34)	0.13	14 (12)	9 (23)	0.19
High habitation density, n (%)	22 (7)	12 (12)	0.25	11 (6)	10 (16)	**0.03**	10 (9)	3 (8)	0.81

Episodic viral wheeze; n (%)	115 (38)	18 (17)	**<.001**	86 (46)	22 (35)	0.06	23 (20)	2 (5)	0.74
Multiple trigger wheeze; n (%)	98 (33)	55 (53)	**<.001**	45 (24)	23 (37)	0.06	61 (54)	24 (60)	0.74
High inhaled steroid treatment; n (%)	235 (79)	78 (76)	0.68	145 (78)	46 (74)	0.20	90 (79)	32 (80)	1
Uncontrolled asthma; n (%)	180 (60)	65 (63)	0.12	112 (60)	34 (55)	0.48	74 (65)	25 (63)	0.72
≥ 2 hospitalizations for severe exacerbation; n (%)	36 (12)	15 (15)	0.62	26 (14)	15 (24)	0.09	7 (6)	3 (8)	1
Elevated total IgE code; n (%)	44 (15)	55 (53)	**<.001**	30 (16)	31 (50)	**<.001**	25 (22)	14 (35)	0.23
Staphylococcal toxin IgE sensitization; n (%)	43 (14)	33 (32)	**<.001**	12 (6)	9 (15)	**<.001**	37 (32)	18 (45)	**<0.01**

Low blood eosinophil count group was defined as < the 3rd quartile distribution of blood eosinophil count in the entire population, in children from preschool age group and school age group; High blood eosinophil count group was defined as ≥ the 3rd quartile of blood eosinophil count distribution in the same 3 populations; Allergic rhinitis and active atopic dermatitis were assessed by questions from the International Study of Asthma and Allergies in Childhood (ISAAC); Allergic sensitization was defined by positive skin prick tests and/or positive specific IgE levels; Perennial sensitization was defined as house dust mite and/or cat or dog dander sensitization without seasonal allergen sensitization associated. Seasonal sensitization was defined as grass and/or birch pollens sensitization, with no perennial sensitization associated. Perennial and seasonal co-sensitization is defined as the sensitization of at least one perennial and one seasonal allergen as described above; High habitation density was defined as <9 m2 per household member; Multiple trigger wheeze was defined as wheezing during colds but symptomatic between episodes with wheezing activated by dust, grass, pets, tobacco smoke, exercise or cold air; Uncontrolled asthma was defined as the presence of at least 3 criteria among: nocturnal or daily symptoms, exacerbation, short acting β2 agonist use or activity limitation due to asthma. *P*-values in bold denote statistical significance ($P<0.05$)

403 Table III. Risk factors for high blood eosinophil count in the entire population: multivariate logistic regression analysis

	OR (CI [2.5% - 97.5%])	*P*-value
Age (months)	**1.01 (1.01 - 1.02)**	**<.01**
Gender (female)	0.61 (0.28 - 1.34)	0.22
Multiple trigger wheeze	3.02 (0.88 - 10.34)	0.08
≥ 2 hospitalizations for asthma attacks	**5.40 (1.54 - 18.91)**	**0.01**
Uncontrolled asthma	2.09 (0.16 - 27.07)	0.57
Atopic dermatitis	1.84 (0.79 - 4.28)	0.16
Allergic rhinitis	**3.44 (2.13 - 5.54)**	**<.01**
Multiple food allergy	0.38 (0.09 - 1.54)	0.18
Peanut IgE sensitization	**2.67 (1.03 - 6.94)**	**0.04**

404 Multiple trigger wheeze was defined as wheezing during colds but symptomatic between episodes with wheezing activated by dust, grass, pets,
405 tobacco smoke, exercise or cold air; Uncontrolled asthma was defined as the presence of at least 3 criteria among: nocturnal or daily symptoms,
406 exacerbation, short acting β2 agonist use or activity limitation due to asthma; Allergic rhinitis and active atopic dermatitis were assessed by
407 questions from the International Study of Asthma and Allergies in Childhood (ISAAC); Allergic sensitization was defined by positive skin prick
408 tests and/or positive specific IgE levels. Results in bold represent variable with statistical significance ($P<0.05$)

Table IV. Risk factors for high blood eosinophil count at preschool age: multivariate logistic regression analysis

	OR (CI [2.5% - 97.5%])	*P*-value
Gender (female)	0.72 (0.22 - 2.31)	0.57
≥ 2 hospitalizations for asthma attacks	**4.96 (1.20 - 20.49)**	**0.03**
Uncontrolled asthma	0.28 (0.02 - 4.64)	0.37
Maternal asthma history	**4.91 (1.56 - 15.47)**	**0.01**
Elevated total IgE	**5.33 (1.66 - 17.11)**	**<.01**

Uncontrolled asthma was defined as the presence of at least 3 criteria among: nocturnal or daily symptoms, exacerbation, short acting β2 agonist use or activity limitation due to asthma; Elevated total IgE was defined as ≥ the 3rd quartile distribution in the population. Results in bold represent variable with statistical significance (P<0.05)

Table V. Risk factors for high blood eosinophil count at school age: multivariate logistic regression analysis

	OR (CI [2.5% - 97.5%])	*P*-value
Gender (female)	0.66 (0.25 - 1.73)	0.39
≥ 2 hospitalizations for asthma attacks	0.84 (0.14 - 4.96)	0.84
Uncontrolled asthma	0.55 (0.03 - 11.26)	0.70
Exposure to tobacco	0.40 (0.14 - 1.18)	0.10
Exposure to mold	2.52 (0.84 - 7.60)	0.10
Multiple trigger wheeze	7.41 (0.87 - 63.22)	0.07
Staphylococcal toxin IgE sensitization	**2.75 (1.12 - 6.80)**	**0.03**

Uncontrolled asthma was defined as the presence of at least 3 criteria among: nocturnal or daily symptoms, exacerbation, short acting β2 agonist use or activity limitation due to asthma; Multiple trigger wheeze was defined as wheezing during colds but symptomatic between episodes with wheezing activated by dust, grass, pets, tobacco smoke, exercise or cold air; Allergic sensitization was defined by positive skin prick tests and/or positive specific IgE levels. Result in bold represents variable with statistical significance ($P<0.05$)

Figure legend

Figure 1: Total IgE values in entire population related to high blood eosinophil count and low blood eosinophil count groups

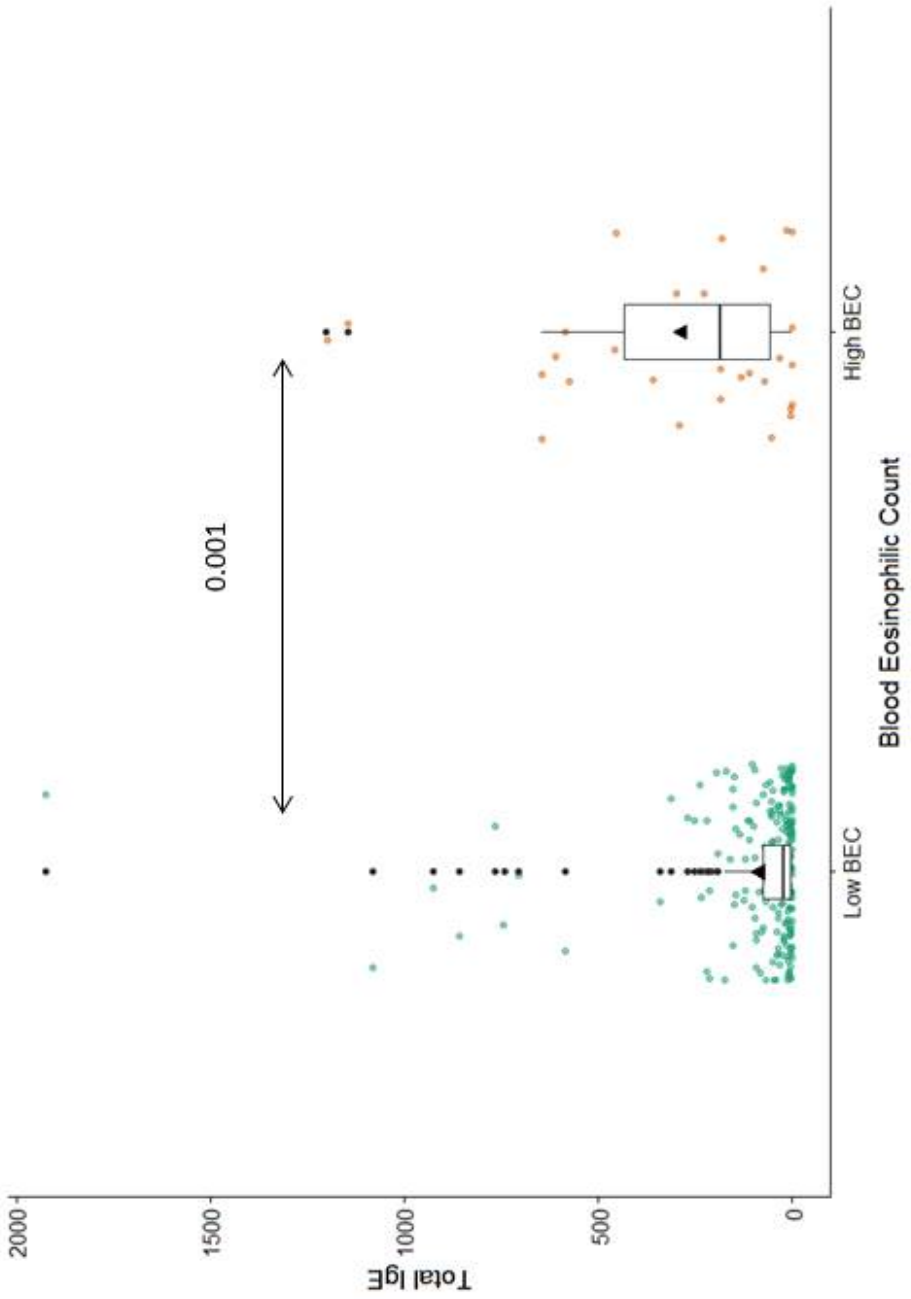
High blood eosinophil count and low blood eosinophil count groups was defined as ≥ and < the third quartile distribution of blood eosinophil count (≥400 cells per μL).

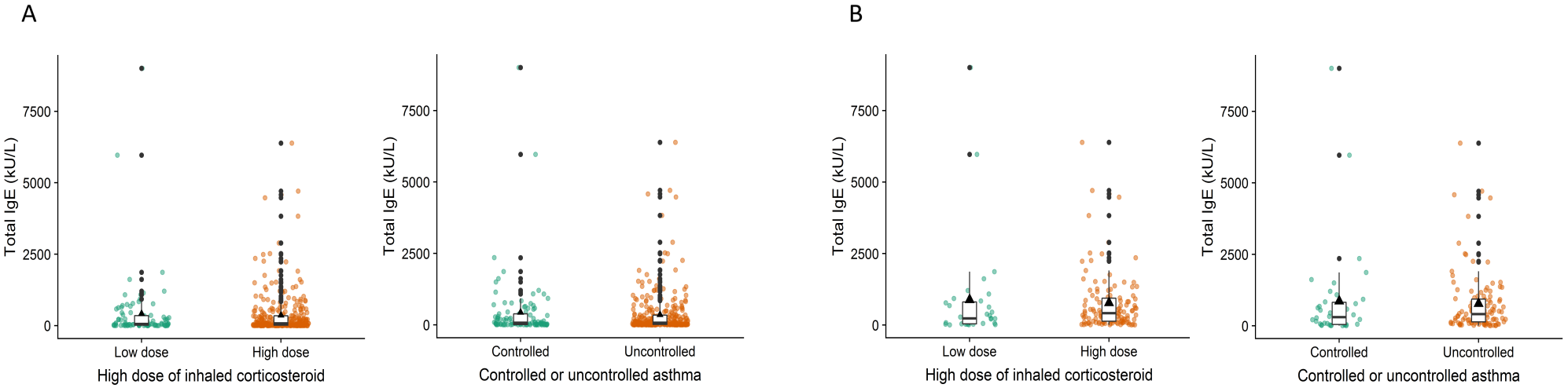
Figure 2: Blood eosinophil count according to inhaled corticosteroid doses and to asthma control

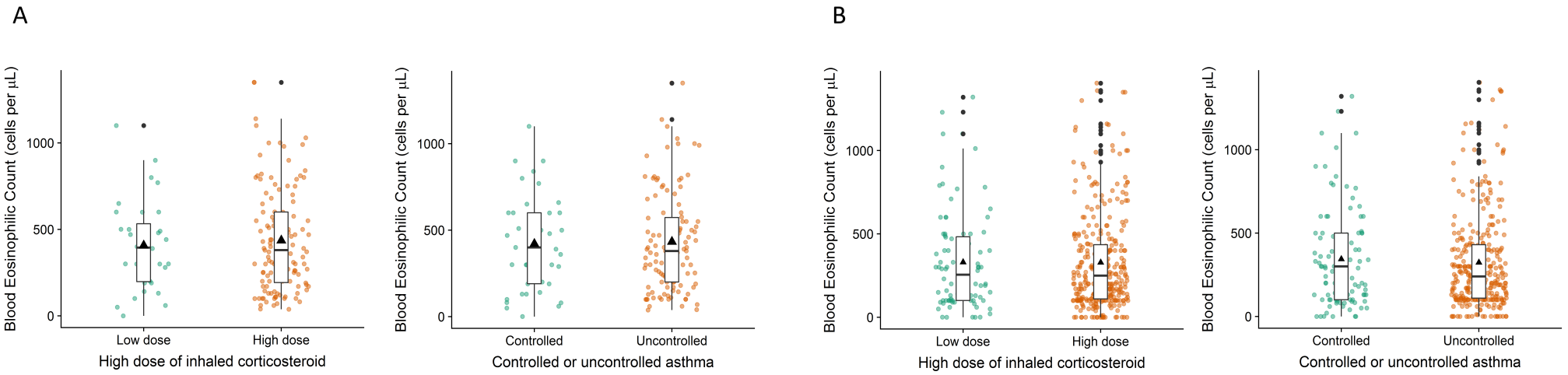
A. Blood eosinophil count according to inhaled corticosteroid doses. High doses of inhaled corticosteroids was defined as ≥500 μg/day fluticasone propionate. **B.** Blood eosinophil count according to asthma control. Totally controlled asthma was defined as the absence of nocturnal or daily symptoms, exacerbation, short-acting β2-agonist use or activity limitation due to asthma, according to Global Initiative for Asthma.

Figure 3: Total IgE values according to inhaled corticosteroid doses and to asthma control

A. Total IgE values according to inhaled corticosteroid doses. High doses of inhaled corticosteroids was defined as ≥500 μg/day fluticasone propionate. **B.** Total IgE values according to asthma control. Totally controlled asthma was defined as the absence of nocturnal or daily symptoms, exacerbation, short-acting β2-agonist use or activity limitation due to asthma, according to Global Initiative for Asthma.


Total IgE
2000
1500
1000
500
0
0.001
Low BEC
High BEC
Blood Eosinophilic Count


A
Total IgE (kU/L)
7500
5000
2500
0
Low dose
High dose
High dose of inhaled corticosteroid
Total IgE (kU/L)
7500
5000
2500
0
Controlled
Uncontrolled
Controlled or uncontrolled asthma
B
Total IgE (kU/L)
7500
5000
2500
0
Low dose
High dose
High dose of inhaled corticosteroid
Total IgE (kU/L)
7500
5000
2500
0
Controlled
Uncontrolled
Controlled or uncontrolled asthma


A
Blood Eosinophilic Count (cells per µL)
1000
500
0
Low dose
High dose
High dose of inhaled corticosteroid
Blood Eosinophilic Count (cells per µL)
1000
500
0
Controlled
Uncontrolled
Controlled or uncontrolled asthma
B
Blood Eosinophilic Count (cells per µL)
1000
500
0
Low dose
High dose
High dose of inhaled corticosteroid
Blood Eosinophilic Count (cells per µL)
1000
500
0
Controlled
Uncontrolled
Controlled or uncontrolled asthma