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► To cite this version:

Jocelyne Just, Sarah Saf, Tamazoust Guiddir, Nathalie Cotel, Flore Amat, et al.. Determinants of blood eosinophilia in moderate and severe asthmatic patients during childhood: evidence from the SAMP cohort. *Pediatric Allergy and Immunology*, 2021, 10.1111/pai.13507 . hal-03190188

HAL Id: hal-03190188

<https://hal.sorbonne-universite.fr/hal-03190188>

Submitted on 6 Apr 2021

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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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22 **Total word count: 2827 ; Abstract: 216; References: 32; Tables: 5; Figures: 3**

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Conflict of interest: Jocelyne JUST has received personal fees from Novartis, Thermofischer and Astra Zeneca and grant from Novartis. Sarah SAF, Tamazoust GUIDDIR, Nathalie COTTEL, Flore AMAT, Nathalie LAMBERT, Philippe SAINT-PIERRE and Mélisande BOURGOIN-HECK have no conflict of interest

Funding sources: Grant has been provided from ASTRA ZENECA for this publication

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/ μ L 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.

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

47

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

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50 **Abbreviations:**

51 EVW: Episodic viral wheeze

52 ICS: Inhaled corticosteroids

53 MTW: Multiple trigger wheeze

54 SAMP: Severe Asthma Molecular Phenotype

55 SPT: Skin prick tests

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56 Introduction

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

79 **Methods**

80 *Design and setting*

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

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

90 Health outcomes were collected in a computerized database using standardized questionnaires.
91 Gender and age at inclusion were collected. The severity of asthma or recurrent wheeze of the
92 entire population was assessed after at least 6 months of follow-up prior to inclusion in the
93 study by an experienced pulmonologist paediatrician after repeated individual or group health
94 education measures had been undertaken to improve adherence to a continuous anti-asthmatic
95 treatment, and after advice by an environmental specialist to reduce exposure to indoor
96 biological pollutants. Severe asthma was defined as controlled asthma with high doses of
97 inhaled corticosteroids (ICS) (≥ 500 $\mu\text{g/day}$ fluticasone propionate) and two other controller
98 medications in school-age children; and as controlled symptoms with high doses of ICS (>200
99 $\mu\text{g/day}$ fluticasone propionate) and leukotriene receptor antagonist in preschool-age patient.

100 The daily dosage of ICS was recorded. Children were classified as having either episodic viral
101 wheeze (EVW) (wheezing only during colds and remaining asymptomatic between episodes)
102 or multiple trigger wheeze (MTW) (wheezing during colds but symptomatic between episodes
103 with wheezing activated by dust, grass, pets, tobacco smoke, exercise or cold air). Totally
104 controlled asthma was defined as the absence of nocturnal or daily symptoms, exacerbation,
105 short-acting β 2-agonist use or activity limitation due to asthma, according to Global Initiative
106 for Asthma(1). Partially control or uncontrolled asthma were defined as the presence of one or
107 two of these parameters and uncontrolled asthma as the presence of three or four. The number
108 of hospitalizations for an asthma attack in the year prior to inclusion was recorded. Both
109 maternal and paternal asthma histories were collected. Allergic rhinitis and active atopic
110 dermatitis were assessed by questions from the International Study of Asthma and Allergies in
111 Childhood (ISAAC)(13), and IgE-mediated food allergy was defined by clinically relevant
112 symptoms within 6 hours following food allergen consumption associated with an allergic
113 sensitization to the same allergen. Total IgE (measured by ImmunoCAP, Thermofischer,
114 Uppsala, Sweden) were collected. The following thresholds were used to define increased
115 levels: elevated total IgE above or equal to the third quartile distribution of each studied
116 population (total population, preschool-age group (children <6 years) and school-age group
117 (children ≥ 6 years). Allergic sensitization was defined by positive skin prick tests (SPT) (mean
118 weal diameter ≥ 3 mm; Stallergenes, Antony, France) and/or positive specific IgE levels (≥ 0.35
119 kIU/L, measured by ImmunoCAP, Thermofischer, Uppsala, Sweden) for cow's milk, egg,
120 current inhaled allergens and staphylococcal toxins. Perennial sensitization was defined as
121 house dust mite and/or cat or dog dander sensitization without associated seasonal allergen
122 sensitization. Seasonal sensitization was defined as grass and/or birch pollens sensitization,

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

127 Data about habitation density (categorized as ≤ 9 m² per household member (high) or >9 m² per
128 household member (low)); tobacco smoke exposure (based on smokers in the home, including
129 mother, father, or other adult household members); and potential biologic allergens sources as
130 molds at home (visible or moldy smell) were collected by means of a questionnaire validated
131 in the PARIS neonatal cohort(14).

132 ***Blood eosinophilic count***

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

139 ***Statistical analysis***

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

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

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

162 **Results**

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

167 ***High and low blood eosinophil count groups***

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

170 More features of allergy were apparent in the high blood eosinophil count group (Table II). This
171 group had more allergic comorbidities, especially more allergic rhinitis ($P<.001$), more inhaled
172 and food allergen sensitization, especially peanut sensitization ($P<.001$) and cow's milk and/or
173 egg sensitization ($P<.001$), and more elevated total IgE ($P<.001$) (Figure 1). This group also
174 had more children with MTW ($P\leq.001$). Blood eosinophil count was not significantly
175 associated to asthma control and to ICS doses, in the total population, the preschool-age group
176 and the school-age group (P -values: 0.547, 0.385 and 0.814 for asthma control; and 0.949,
177 0.554 and 0.596 for ICS doses, respectively), as presented in Figure 2. Similarly, Total IgE
178 levels were not significantly associated to asthma control and to ICS doses, in the total
179 population, the preschool-age group and the school-age group (P -values : 0.52, 0.072 and 0.769
180 for asthma control; and 0.679, 0.075 and 0.763 for ICS doses, respectively), as presented in
181 Figure 3.

182 ***High blood eosinophil count risk factors***

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

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

200 In the school-age population, staphylococcal toxin-specific IgE sensitization (OR=2.75;
201 $P = 0.03$) was found to increase the risk of high blood eosinophil count after adjustment (Table
202 V). The importance measures obtained from the random forest analysis also reinforced the
203 previous results of determinants of high blood eosinophil count (sensitization to staphylococcal
204 toxins) but also identified MTW and cow's milk and/or egg sensitization.

205 **Discussion**

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

210 ***Increase in blood eosinophil count during childhood***

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

216 ***High blood eosinophil count determinants and atopic features***

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

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

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

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

246 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

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

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

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

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

286

287 **Acknowledgments:** The authors thank Rémi Couderc, Yannick Chantran, Marie-Ange Selva,
288 Pascal Poncet and Hélène Sénéchal for their help in the assessment of biological parameters in
289 this study.

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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For Peer Review

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/m ² ; 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)
Severe; n (%)	328 (82)	203 (82)
Severe asthma control		
Controlled with high-dose ICS; n (%)	58 (14)	42 (17)
Uncontrolled with high-dose ICS; n (%)	245 (61)	146 (59)
Blood inflammation		
Blood PN; mean (SD)	3923 (2563)	4292 (2830)
Blood PE; mean (SD)	328 (290)	266 (272)
Blood PB, mean (SD)	59 (305)	76 (386)
Blood Lymphocytes, mean (SD)	3722 (1910)	4411 (2055)
Blood Monocytes, mean (SD)	661 (377)	725 (408)
IgG level; mean (SD)	8.0 (2.6)	6.9 (2.2)
IgA level; mean (SD)	1.0 (0.7)	0.6 (0.4)
IgM level; mean (SD)	1.0 (0.7)	1.0 (0.8)
Allergic sensitization		
Perennial sensitization only; n (%)	25 (6)	9 (4)
Seasonal sensitization only; n (%)	0	0
Perennial and seasonal co-sensitization; n (%)	150 (37)	39 (16)
<i>Alternaria alternata</i> sensitization; n (%)	36 (9)	1 (0.4)
Peanut sensitization; n (%)	48 (12)	13 (5)
Cow's milk and/or egg sensitization; n (%)	64 (16)	35 (14)
Staphylococcal toxin IgE sensitization; n (%)	76 (11)	21 (8)
Total IgE; mean (SD)	377 (867)	111 (229)
		797 (1255)

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381 Blood PB, blood basophil cells; Blood PE, blood eosinophil cells; Blood PN, blood neutrophil cells; BMI, body mass index; ICS, inhaled
382 corticosteroid; Allergic rhinitis and active atopic dermatitis were assessed by questions from the International Study of Asthma and Allergies in
383 Childhood (ISAAC); Multiple trigger wheeze was defined as wheezing during colds but symptomatic between episodes with wheezing activated
384 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
385 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
386 among: nocturnal or daily symptoms, exacerbation, short acting β_2 agonist use or activity limitation due to asthma; Perennial sensitization was
387 defined as house dust mite and/or cat or dog dander sensitization without seasonal allergen sensitization associated. Seasonal sensitization was
388 defined as grass and/or birch pollens sensitization, with no perennial sensitization associated. Perennial and seasonal co-sensitization is defined as
389 the sensitization of at least one perennial and one seasonal allergen as described above; Allergic sensitization was defined by positive skin prick
390 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

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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

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

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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

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 β_2 agonist use or activity limitation due to asthma; 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. 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 β_2 agonist use or activity limitation due to asthma; Elevated total IgE was defined as $\geq$ 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 β_2 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 ($P<0.05$)

418 **Figure legend**

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

420 High blood eosinophil count and low blood eosinophil count groups was defined as $\geq$ and $<$ the third quartile distribution of blood eosinophil count
421 (≥ 400 cells per μL).

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

423 **A.** Blood eosinophil count according to inhaled corticosteroid doses. High doses of inhaled corticosteroids was defined as ≥ 500 $\mu\text{g/day}$ fluticasone
424 propionate. **B.** Blood eosinophil count according to asthma control. Totally controlled asthma was defined as the absence of nocturnal or daily
425 symptoms, exacerbation, short-acting β_2 -agonist use or activity limitation due to asthma, according to Global Initiative for Asthma.

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

427 **A.** Total IgE values according to inhaled corticosteroid doses. High doses of inhaled corticosteroids was defined as ≥ 500 $\mu\text{g/day}$ fluticasone
428 propionate. **B.** Total IgE values according to asthma control. Totally controlled asthma was defined as the absence of nocturnal or daily symptoms,
429 exacerbation, short-acting β_2 -agonist use or activity limitation due to asthma, according to Global Initiative for Asthma.