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IgE in childhood asthma: relevance of demographic characteristics and polysensitisation

M Silvestri,¹ A Pistorio,² E Battistini,¹ G A Rossi¹

¹Pulmonary and Allergy Disease Paediatric Unit, G Gaslini Institute, Genoa, Italy
²Epidemiology and Biostatistics Unit, Giannina Gaslini Research Institute, Genoa, Italy

Correspondence to
 Professor Giovanni A Rossi,
 Pulmonary and Allergy Disease Paediatric Unit,
 G Gaslini Institute, Largo G Gaslini 5, 16147 Genoa, Italy;
giovannirossi@ospedale-gaslini.ge.it

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ABSTRACT

Background Despite the therapeutic efficacy of the anti-IgE monoclonal antibody, the role of IgE in allergic asthma is still a matter of debate. This may be mostly relevant in childhood, where a wide range of total serum (s) IgE levels is often detected.

Aim To evaluate whether the relationships between total or allergen-specific sIgE levels and the clinical markers of allergic inflammation and the pulmonary function values might be affected by the demographic characteristics of the patients or by the presence of multiple sensitisations to allergens.

Methods 64 asthmatic children sensitised to house dust mites (HDM) were evaluated. The role of age, sex and multiple sensitisations was evaluated by multiple regression model (MRM) analysis.

Results Total and HDM-specific sIgE levels (Log) showed similar moderate-to-strong correlations with exhaled nitric oxide (FeNO) and blood eosinophilia (Log) ($p < 0.0001$) but not with forced vital capacity, forced expiratory volume in 1 s (FEV_1), % FEV_1 change after salbutamol. The positive associations between total sIgE levels and Log FeNO levels or Log blood eosinophilia were also detected by MRM analysis. Age brought a negative, although limited, contribution to FeNO levels and blood eosinophilia ($p < 0.043$). Positive similar associations were also detected between HDM-specific sIgE levels and FeNO levels or blood eosinophilia; however, no significant contribution of age or of other covariates was detected.

Conclusion In childhood allergic asthma, total and HDM-specific sIgE levels are tightly linked to markers of allergic inflammation but not to pulmonary functions. These relationships are weakly affected by age but not by sex or by the presence of multiple sensitisations.

One of the most important individual factors predisposing to development of asthma in childhood is a personal history of atopy,^{1 2} and in several studies, the presence of elevated levels of serum (s) IgE has been associated with childhood allergic asthma.^{3–5} However, a relevant question is to what extent serum may reflect tissue compartments. Indeed, IgE antibodies, continuously produced in the bone marrow by plasma cells, are released in blood where they have a relatively short half-life of 3 days, while a significant amount of IgE resides within the tissue compartments⁶ bound to the surface of a variety of cells with different life span.⁶ These include cells expressing the high-affinity IgE receptor (FcεRI), such as mast cells, basophils, eosinophils, monocytes, dendritic

What is already known on this topic

- ▶ One of the most important factors predisposing to childhood asthma is a personal history of atopy, that is, abnormal IgE production in response to allergens.
- ▶ The relationship between atopy and asthma is not straightforward: correlations between degree of allergic sensitisation and disease severity are often weak or not statistically significant.
- ▶ No study has evaluated whether the patient's demographic characteristics and the number of sensitisations to different classes of allergens may affect the pathogenetic role of the serum levels of total and allergen-specific IgE levels in childhood asthma.

What this study adds

- ▶ These data support the role not only of allergen-specific IgE but also of total IgE in serum in the pathogenesis of allergic inflammation in asthmatic children.
- ▶ These results may be clinically relevant to better understand the pathogenetic links between atopy and its clinical manifestations and for the diagnosis/treatment of atopic asthma.

cells and platelets and also cells expressing the low-affinity receptor, FcεRII (CD23), such as B lymphocytes, macrophages and airway smooth muscle cells.^{2 6}

Following allergen exposure, IgE-mediated reactions occur rapidly, activating the IgE receptor positive cells to release a vast array of preformed pro-inflammatory mediators and subsequently to synthesise and secrete cytokines, chemokines and growth factors into the surrounding milieu.^{2 6} In asthmatics, IgE-mediated cell activation results in eosinophilic inflammation, increased vascular permeability, increased mucous production and bronchoconstriction.

All these data support the concept of IgE as the hub of the effector arm of the allergic inflammatory cascade and, therefore, identify IgE as an ideal target in the development of therapies for asthma and other allergy-based inflammatory diseases.^{7 8}

Original article

However, the relationship between atopy and asthma is not straightforward, and correlations between degree of allergic sensitisation and disease severity are often weak or fail to achieve statistical significance, and even in large studies, results have been frequently mixed.^{9–12} This may be mostly relevant in the growing child, where a wide range of total sIgE levels is often detected, being influenced by a variety of factors, including the patient demographic characteristics and the number of positive tests to allergens.^{2 13–16}

With this background, a study was designed to evaluate whether, in asthmatic children, the patient's age and sex or the presence of multiple sensitisations might affect the relationships between total or allergen-specific sIgE levels and clinical markers of allergic inflammation or pulmonary function parameters.

MATERIALS AND METHODS

Participants

The study was performed in atopic asthmatic children, consecutively enrolled from a population referred to our outpatient clinic. During the period January 2007 to October 2008, 203 asthmatic children sensitised to house dust mites (HDM) (aged between 5 and 12 years) were referred to the outpatient clinic of Giannina Gaslini Research Institute. Among these, we included in the study 64 steroid-free children of both sexes who underwent nitric oxide (NO) level analysis and spirometry with reversibility test, in addition to standard blood analysis including the evaluation of total sIgE and HDM-specific sIgE levels and eosinophil counts. This sample population is representative of the children with these characteristics who are normally referred to the outpatient clinic of our institution. Inclusion criteria were: (1) a history of asthma with stable condition, defined and treated according to Global Initiative for Asthma guidelines¹⁷; (2) $\geq 12\%$ forced expiratory volume in 1 s (FEV₁) increase after salbutamol (200 µg) inhalation; (3) treatment only with short-acting β_2 -agonists, on an as-necessary basis, in the last 8 weeks; and (4) positivity to at least HDM tested in the skin prick test and confirmed by the presence of specific sIgE antibodies by solid-phase, chemiluminescent immunometric assays. Short-acting β_2 -agonists were avoided for 12 h before entering the study. Exclusion criteria were: (1) upper or lower respiratory infections in the 2 months preceding the study; (2) use of systemic or inhaled corticosteroids in the 3 months preceding the study; (3) systemic immunological or metabolic disease and (4) malignancies. Children sensitised to pollens were evaluated out of the pollen seasons. This was a retrospective study in which data from medical and clinical records were collected and analysed.

Diagnosis of allergic sensitisation

Sensitisation to the most common classes of aeroallergens was evaluated by skin-prick test (Lofarma, Milan, Italy) as previously described.¹⁰ Total and allergen-specific sIgE levels were determined by solid-phase, chemiluminescent immunometric assays (Immulite, Medical System; Genoa, Italy). Blood eosinophil count was performed by TECHNICON H6000 (Technicon Instrument, Tarrytown, New York, USA) and evaluated either as absolute number (cells/mm³) or as percentage of cells.¹⁰

Pulmonary functions

Forced vital capacity (FVC) and FEV₁ were measured in all participants by spirometry (Med Graphics, Pulmonary Function

System 1070 series 2; Med Graphics, St Paul, Minnesota, USA), according to the guidelines provided by the American Thoracic Society.¹⁸ On each occasion, at least three technically acceptable forced expiratory manoeuvres were obtained and the best values retained.¹⁰ After the "at baseline" evaluation, spirometry was repeated 15 min after two inhalations of a salbutamol metered dose inhaler (100 µg/actuation) via a spacer device, and "postbronchodilator" FVC and FEV₁ were recorded.

Detection of exhaled nitric oxide values

A chemiluminescence analyser (NiOX, Aerocrine AB, Solna, Sweden) was used and the manoeuvre was performed according to the American Thoracic Society recommendations.¹⁹

Statistical analysis

We checked the normal distribution of each variable using the Shapiro–Wilk W test. All variables in the study (with the exception of lung function parameters that were normally distributed) were log-transformed. All data were reported as mean and SE. Qualitative data were reported in terms of absolute frequencies and percentages. Student's unpaired t test was used to compare groups. Correlations were determined using Pearson's correlation coefficient with 95% CI. We labelled the strength of the association as follows: for absolute values of r, 0 to 0.19 is regarded as very weak, 0.2 to 0.39 as weak, 0.40 to 0.59 as moderate, 0.6 to 0.79 as strong and 0.8 to 1 as very strong correlation.²⁰ Multiple regression models were fitted using either Log exhaled nitric oxide (FeNO), Log eosinophil counts or percentage as dependent variables and sIgE (either total or HDM-specific), age, sex, number of positive specific IgE tests to allergens as independent variables. The results are presented as regression coefficients (β), and SE of β , the regression coefficient, represents the amount of change in the dependent variable per a unit change for each independent variable, adjusting for the role of all the other covariates included in the considered model. Because of the strong correlation between Log FeNO levels and blood eosinophilia (either counts or percentage), both variables were never included simultaneously in the same regression model. The level of statistical significance was set at $p < 0.05$. The needed sample size was calculated, assuming that the expected correlation coefficients would be considered acceptable if ranged around 0.40–0.45, the type I error=0.05, the power=0.80 and the t test on the correlation coefficient ($\rho=0$) would be two-sided. With these assumptions, the needed sample size ranges from 44 to 36. The needed sample size and power have been calculated according to Gatsonis C and Sampson AR formulations²¹ and using the Software NQuery Advisor (release 7.0).

Parents or guardians of the children gave their informed consent for evaluation and the anonymous publication of the information related to their children. Access to health records complied with the Italian legislation.

RESULTS

Demographic and clinical characteristics of the patients

Out of 64 atopic asthmatic children, 33 children were sensitised only to HDM (mono-sensitised) and 31 to HDM and to at least one other class of allergens, that is, pollens, pet danders or moulds (poly-sensitised). Baseline characteristics of the asthmatic population are listed in table 1.

Table 1 Demographic and clinical characteristics of the study population

Parameters	
Age (years (mean (SE)))	10.41 (0.26)
Sex (male-to-female ratio)	1.91
Frequency of monosensitisation (%)	51.56
Log blood eosinophil percentage (mean (SE))	0.78 (0.03)
Log blood eosinophil count (cells (N)/mm ³ (mean (SE)))	2.61 (0.03)
Baseline FVC (% predicted (mean (SE)))	95.55 (1.51)
Baseline FEV ₁ (% predicted (mean (SE)))	91.53 (1.27)
Baseline Log FeNO levels (ppb (mean (SE)))	1.23 (0.05)

FEV₁, forced expiratory volume in 1 s; FeNO, exhaled nitric oxide; FVC, forced vital capacity; ppb, parts per billion.

Correlations between total or HDM-specific sIgE levels and markers of allergic inflammation or pulmonary functions

Positive moderate-to-strong correlations were detected between Log total sIgE levels and Log FeNO concentrations ($r=0.544$ (95% CI 0.344 to 0.697), $p<0.0001$), Log blood eosinophil counts ($r=0.590$ (95% CI 0.403 to 0.730), $p<0.0001$) and percentages ($r=0.650$ (95% CI 0.481 to 0.772), $p<0.0001$) (figure 1A–C). In addition, Log HDM-specific IgE concentrations showed positive strong correlation with Log FeNO levels ($r=0.630$ (95% CI 0.454 to 0.758), $p<0.0001$) and moderate-to-strong correlation with Log eosinophil counts ($r=0.556$ (95% CI 0.360 to 0.706), $p<0.0001$) and percentages ($r=0.665$ (95% CI 0.502 to 0.783), $p<0.0001$).

Analysis by multiple linear regression models

Because of their possible role in affecting sIgE levels in childhood,^{2 13–16} the patient's demographic characteristics and the number of sensitisations to different allergens were forced as variables into the multiple linear regression models. The positive associations between Log total sIgE levels and Log FeNO levels were confirmed also by this analysis, showing that the degree of FeNO levels increases (0.400 Log units (2.51 ppb)) for each Log unit (10 kU/l) increase in total sIgE. Age, but not sex or number of allergic sensitisations, brought a negative, although limited, contribution to Log FeNO levels: 1 unit (ie, 1 year) increase in age was associated with a 0.040 Log units (1.10 ppb) decrease in FeNO levels. Similar results were detected when the associations between total sIgE levels and blood eosinophilia were evaluated, with a negative contribution of age but not of sex or of number of allergic sensitisations (table 2). Positive relationships were also demonstrated between Log HDM-specific sIgE and Log FeNO levels, or Log eosinophilia. However, no significant contribution of age or of other covariates was found (table 3).

DISCUSSION

Since elevated levels of total sIgE are closely associated with allergic disease and specifically with bronchial asthma,^{3 4 14 22} specific treatments have been developed to target IgE-mediated reactions, including humanised monoclonal antibodies against IgE or the IgE low-affinity receptor CD23.^{7 8} However, there is a considerable overlap between sIgE value ranges in healthy and allergic participants.¹³ In addition, involvement of environmental and also of genetic variables may greatly affect not only allergic disease expression but also IgE synthesis and release.^{12 23} Indeed, while the development of allergen-specific IgE levels has an absolute requirement for environmental exposure,^{1 2 23 24} twin studies have

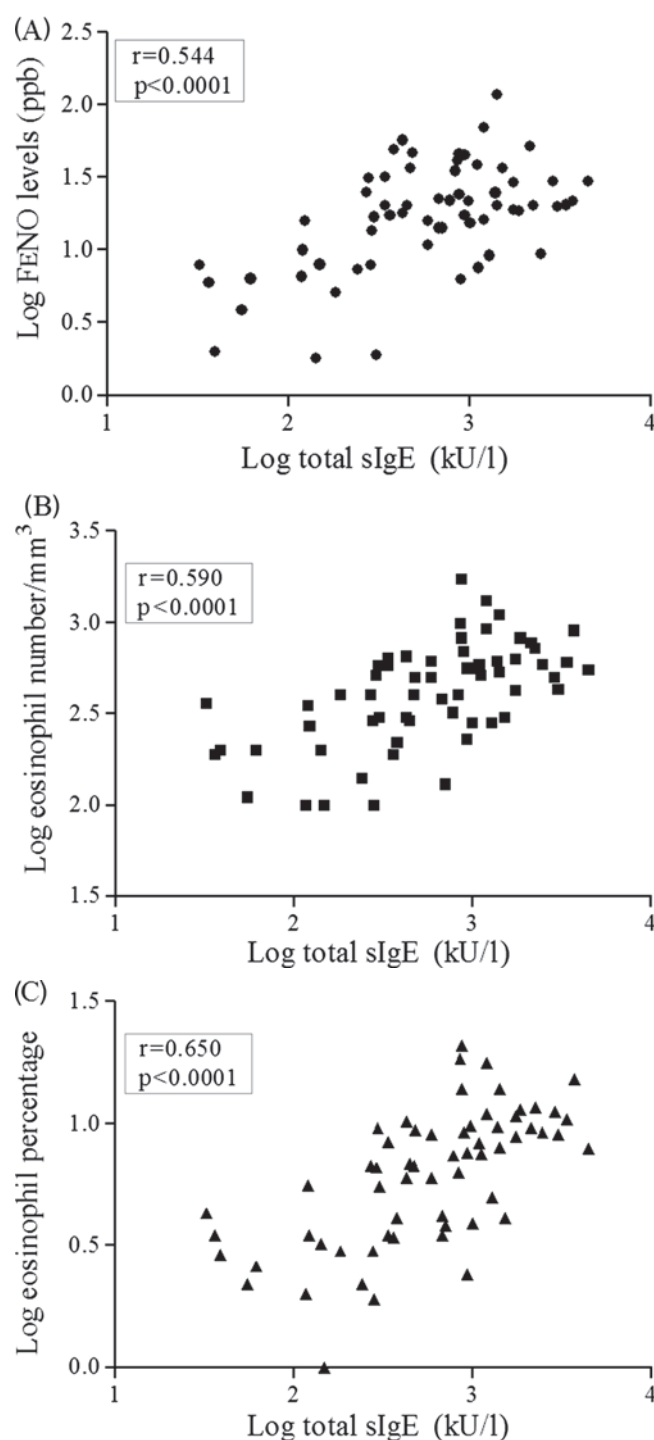


Figure 1 Correlations between total serum immunoglobulin (sIg) E concentrations and exhaled nitric oxide (FeNO) levels (A) or blood eosinophilia (B and C). IgE levels are reported on the abscissa as kU/l, whereas FeNO levels, expressed as ppb, or blood eosinophilia, expressed as absolute number (No. eosinophils/mm³) or as percentage are shown on the ordinate. Each variable was log-transformed before analysis.

shown that the heritability of total IgE levels can be estimated to be approximately 75–85%, with only a small portion of variance attributable to environmental factors.²³ Therefore, it is not surprising that the clinical value of total sIgE levels in detecting allergen-induced disease severity, specifically in childhood, could be under debate.^{2 5 25 26}

Table 2 Multiple linear regression models to evaluate the role of total sIgE levels and other predictors in the relationship with FeNO levels or blood eosinophilia

Dependent variable	Independent variables	β	SE (β)	p Value
Log FeNO levels (ppb)	Log total sIgE levels (kU/l)	0.400	0.077	<0.0001
	Age (years)	-0.040	0.019	0.043
	Sex: male	-0.084	0.084	0.323
	Number of positive specific IgE tests to allergens	0.0004	0.029	0.891
	Intercept	0.581	0.303	
Multiple R 0.590; R ² 0.348; p<0.0001				
Log eosinophil count (number of cells/mm ³)	Log total sIgE levels (kU/l)	0.349	0.055	<0.0001
	Age (years)	-0.029	0.014	0.038
	Sex: male	0.079	0.060	0.189
	Number of positive specific IgE tests to allergens	-0.005	0.021	0.820
	Intercept	1.900	0.215	
Multiple R 0.649; R ² 0.421; p<0.0001				
Log eosinophil percentage	Log total sIgE levels (kU/l)	0.379	0.050	<0.0001
	Age (years)	-0.034	0.013	0.008
	Sex: male	0.058	0.055	0.293
	Number of positive specific IgE tests to allergens	-0.001	0.019	0.943
	Intercept	0.059	0.197	
Multiple R 0.710; R ² 0.505; p<0.0001				

FeNO, exhaled nitric oxide; ppb, parts per billion; SIgE, serum IgE; β , regression coefficient.**Table 3** Multiple linear regression models to evaluate the role of HDM-specific sIgE levels and other predictors in the relationship with FeNO levels or blood eosinophilia

Dependent variable	Independent variables	β	SE (β)	p Value
Log FeNO levels (ppb)	Log HDM-specific sIgE levels (kU/l)	0.375	0.061	<0.001
	Age (years)	-0.025	0.018	0.168
	Sex: male	-0.020	0.081	0.807
	Number of positive specific IgE tests to allergens	0.025	0.028	0.372
	Intercept	0.901	0.243	
Multiple R 0.652; R ² 0.424; p<0.0001				
Log eosinophil count (number of cells/mm ³)	Log HDM-specific sIgE levels (kU/l)	0.274	0.048	<0.0001
	Age (years)	-0.016	0.014	0.250
	Sex: male	0.116	0.063	0.072
	Number of positive specific IgE tests to allergens	0.010	0.022	0.660
	Intercept	2.279	0.190	
Multiple R 0.612; R ² 0.375; p<0.0001				
Log eosinophil percentage	Log HDM-specific sIgE levels (kU/l)	0.321	0.042	<0.0001
	Age (years)	-0.020	0.012	0.101
	Sex: male	0.107	0.055	0.060
	Number of positive specific IgE tests to allergens	0.016	0.019	0.404
	Intercept	0.428	0.166	
Multiple R 0.720; R ² 0.518; p<0.0001				

FeNO, exhaled nitric oxide; ppb, parts per billion; HDM, house dust mites; SIgE, serum IgE; β , regression coefficient.

In agreement with a previous report from our group¹⁰ evaluating a group of “steroid-free” atopic asthmatic children sensitised to HDM, we demonstrated that total sIgE and HDM-specific sIgE levels showed similar, moderate-to-strong positive correlations with blood eosinophilia and FeNO values. The link between sIgE and markers of allergic inflammation may be easily explained. In sensitised individuals, exposure to allergens induces the activation of FcεRI-bearing mast cells and basophils through cross-linking of IgE molecules present on their surface. The engagement of IgE-occupied receptors initiates a series of molecular events causing the release of preformed and de novo synthesised allergic mediators able to induce eosinophil, locomotion, activation and survival.²⁷ The deep involvement of IgE-dependent mechanisms in

the eosinophilic inflammation is further supported by the demonstration of high levels of allergen-specific IgE in induced sputum of allergic asthmatic individuals, correlating with eosinophil cationic protein concentration.²⁸

We also found that total and allergen-specific sIgE levels were associated with FeNO values. In asthma, NO is thought to be produced by the inducible NO synthase (iNOS), an enzyme which can be rapidly activated by proinflammatory cytokines released by inflammatory cells.²⁹ The huge variety of mediators synthesised and released following activation of FcεRI+ cells by the allergen-IgE complexes include oxygen species and specifically NO.^{1 2 29} In addition, FcεRI ligation activates the NO-dependent pathway and induces a rapid upregulation of iNOS mRNA and subsequent release of NO

by variety of cells, including mast cells, alveolar macrophages and monocytes.^{30,31}

Despite the convincing association with markers of allergic inflammation, we could not demonstrate any correlation between total and HDM-specific IgE levels and pulmonary function test values. This previously reported discrepancy^{10,32–34} is thought to be an additional demonstration that, in asthma, airway inflammation is not strictly related to lung volume reduction or airflow limitation.^{35,36}

An additional variable is the role that in childhood individual factors may have in determining total sIgE levels and their relationships with FENO or eosinophilia.^{2,5} Total IgE level has been reported to increase steadily over the first 10 years of life, in parallel fashion among girls and boys.¹⁴ However, boys seem to have a greater predisposition than girls towards development of increased sIgE levels early in life¹⁵ and to reach a given median IgE level earlier.¹⁴ This observation may, at least partially, explain the higher prevalence of asthma in boys in the first decade of life.^{14,15} There is no evidence of any link between age and blood eosinophilia in childhood allergic asthma,²⁵ while the possibility that FENO levels may progressively increase with time in school-aged children has been reported.³⁷ Finally, polysensitisation has been associated with higher total sIgE²⁶ and FENO levels.³⁸

Because of their possible role in affecting sIgE levels, the patient's demographic characteristics and the number of sensitisations to different allergens were forced as variables into the multiple linear regression models. We first confirmed the associations between total and HDM-specific sIgE levels and markers of allergic inflammation. In addition, we found that age, but not sex or number of positive specific IgE tests to allergens, brought a negative, although modest, contribution to FENO levels and blood eosinophilia. Indeed, in our study population, 1 year increase in age was associated with a 0.040 Log units (1.10 ppb) decrease in FENO levels and with a 0.029 Log units (1.069 cells/mm³) in blood eosinophil counts.

In conclusion, the data here reported suggest that the similar role of allergen-specific and total sIgE in allergic inflammation is not different in boys and girls and is not affected by the presence of polysensitisation and only modestly by the chronological age of the patients. This information, which may be useful in the management of this disorder, has, however, important limitations. The population evaluated in the present study included a homogeneous group of participants, with little age spread, with mild asthma, not on regular inhaled steroid. Therefore, the transfer value of these findings may be considered somehow limited and clearly needs further evaluations in different patient populations.

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Competing interests Declared. GAR served as advisor for Novartis. MS, AP, EB: none to declare.

Ethics approval This study was conducted with the approval of the parents or guardians of the children who gave their informed consent for evaluation and the anonymous publication of the information related to their children. Access to health records complied with the Italian legislation.

Provenance and peer review Not commissioned; externally peer reviewed.

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