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IgE mediated sensitisation to aeroallergens in an asthmatic cohort: relationship with inflammatory phenotypes and disease severity

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Disclosure
None.

SUMMARY

Background: Atopy is known to play an important role in the asthmatic disease. The main objective of this study was to evaluate the frequency of sensitisation to common aeroallergens in a cohort of asthmatics with different inflammatory phenotypes and disease severity. **Methods:** We have conducted a retrospective cross-sectional study including 772 asthmatics recruited between 2003 and 2014 in our Asthma Clinic. The patients were defined as asthmatics on the basis of respiratory symptoms together with a positive methacholine test (PC20M) < 16 mg/ml and/or a reversibility to short-acting β2-agonists (salbutamol) ≥ 12% and 200 ml. Sensitisation to house dust mites, grass and birch pollens, cats, dogs and moulds was assessed by RAST and a specific immunoglobulin E (IgE) > 0.35 kU/l was considered as significant. Inflammatory phenotypes were subdivided between pauci-granulocytic (n = 309) (40%), eosinophilic (n = 311) (40%), neutrophilic (N = 134) (17%) and mixed-granulocytic (N = 18) (3%) asthmatics. Severe asthmatics (n = 118) were defined according to the American Thoracic Society (ATS 2000) criteria and compared with mild-to-moderate asthmatics (N = 654). **Results:** The eosinophilic phenotype was associated with higher levels of total serum IgE compared with neutrophilic and pauci-granulocytic asthma ($p < 0.001$ for both). Sensitisation rate to dogs and cats was higher in eosinophilic asthmatics (31% and 37%, respectively, $p < 0.01$ both) compared with neutrophilic (18% and 23% respectively) and pauci-granulocytic asthmatics (20% and 24%, respectively), while sensitisation rate to house dust mites and moulds were rather similar between the groups (ranging from 33% to 40% and from 10% to 16%, respectively). Severe asthmatics had slightly increased total serum IgE compared with mild-to-moderate asthmatics ($p < 0.05$) without any difference in the sensitisation rate to common aeroallergens. **Conclusion:** Eosinophilic asthma exhibits higher total serum IgE and sensitisation rate towards animal dander while clinical severity, though also associated with higher total IgE, did not preferentially relate to any type of common aeroallergens.

What's known
Immunoglobulin E (IgE)-mediated sensitisation to aeroallergens is an established risk factor for asthma. It is now recognised that asthma features different airways inflammatory phenotypes.

What's new
Here, we provide original data describing how IgE-mediated sensitisation to aeroallergens relates to inflammatory phenotypes in asthma.

Introduction

Atopy is defined as the propensity to raise specific immunoglobulin E (IgE) to common aeroallergens (1). Allergen-specific IgE binds to its cognate receptors and is able to trigger a series of cellular events like presentation of antigen by dendritic cells, degranulation of mast cells and basophils leading to the release of mediators playing an important role in the disease symptoms (2). Prevalence in allergic diseases is increasing worldwide and environmental factors such as exposure to allergens or air pollution largely contribute to their development (3). A European survey (ECRHS, 1994) estimates that the prevalence of atopy reaches 34% in the general population with sensitisation to house dust mite (21%), grass pollen (19%) and cats (8%) being the most frequent (4). The population-based proportion of asthma cases which are attributable to atopy was found to range from one-third to one half according to the country and the region (5).

Demographic factors like age, sex and tobacco status have been shown to influence serum IgE levels (6). According to ECRHS sensitisation to moulds and, to a lesser extent to *Dermatophagoides pteronyssinus* (*Dpt*), was a powerful risk factor for

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severe asthma in adults whereas it was not the case for sensitisation to cats or pollen (7).

It is now accepted that asthma features different airway inflammatory profiles, which has supported the concept of inflammatory phenotype (8). The relationship between serum IgE and sensitisation to aeroallergens and airways inflammatory profile in asthmatics has not been extensively studied (9). There are epidemiological data showing that, in adult asthmatics, sensitisation to moulds, furry animals and food allergens (10) is associated with raised fraction of exhaled nitric oxide (FeNO). Other studies conducted in children showed a preferential association between levels of FeNO and sensitisation towards indoor, but not outdoor, aeroallergens (11). In addition, level of domestic exposure to indoor aeroallergens in sensitised patients was shown to further enhance the FeNO levels (12). The purpose of our study was to examine the relationship between the level of serum total IgE and the sensitisation to aeroallergens according to sputum cellular phenotypes in a large asthma cohort seen at a university hospital. In addition, we sought to determine if there was any influence of age, sex and tobacco status as well as asthma severity on serum total and specific IgE.

Methods

Study design

We have conducted a retrospective study on 772 asthmatic patients recruited from the Asthma Clinic (CHU of Liège, Sart-Tilman) between 2003 and 2014. Patients included in the study were those who had successful sputum induction and serum IgE measurements. Age of the patients ranged from 18 to 85 years. Among them, there were 654 mild-to-moderate asthmatics and 118 severe asthmatics, which were included in the Severe Belgian Asthma Registry (13). A patient was considered as asthmatic if he had chronic respiratory symptoms such as wheeze, breathlessness or cough together with a positive methacholine test (< 16 mg/ml) and/or a FEV1 reversibility to short-acting β2-agonists (salbutamol) ≥ 12% and 200 ml. Severe asthmatics were defined according to the American Thoracic Society (ATS) criteria (14). In brief, patients were uncontrolled despite usage of high doses of inhaled corticosteroids (ICS) (fluticasone > 880 μg/day) or oral corticosteroids as maintenance treatment.

Serum total and specific immunoglobulin E (IgE) were measured with the Radioallergosorbent test (RAST) (Phadia, Groot-Bijgaarden, Belgium). The positive threshold was > 0.35 kU/l. Atopy was defined by at least one positive RAST (> 0.35 kU/l) towards the following aeroallergens: house dust mite, cat, dog, moulds, grass and birch pollen. The study was approved by the Ethics Committee of CHU Liege.

Sputum induction and processing

After premedication with 400 μg, inhaled salbutamol administered by MDI (+Spacer), sputum was

Table 1 Patient demographic characteristics and disease severity according to sputum cellular phenotype

	Eosinophilic (*N* = 311) (40%)	Neutrophilic (*N* = 134) (17%)	Pauci-granulocytic (*N* = 309) (40%)	Mixed-granulocytic (*N* = 18) (3%)
Age (years)				
<40 (*N* = 262)	101 (38.5)	31 (12)	126 (48)	4 (1.5)
40–60 (*N* = 338)	141 (42)	65 (19)*	127 (38)***	5 (1)
> 60 (*N* = 172)	69 (40)	38 (22)**	56 (33)**	9 (5)*
Sex				
Male (*N* = 312)	148 (47)	42 (13)	115 (37)	7 (3)
Female (*N* = 460)	164 (36)†	92 (20)‡	193 (42)	11 (2)
Tobacco status				
Ex-smokers (*N* = 204)	88 (43)	37 (18)	73 (36)	6 (3)
Current smokers (*N* = 164)	66 (40)	24 (15)	73 (45.5)	1 (0.5)
Non-smokers (*N* = 398)	157 (39.5)	73 (18.5)	157 (39)	11 (3)
Severity of asthma				
Mild-to-moderate (*N* = 654)	256 (39)	79 (12)	304 (46)	15 (3)
Severe (*N* = 118)	54 (46)	25 (21)§	36 (30)¶	3 (3)

Values within parentheses are expressed as percentages. *p < 0.05 vs. < 40 years, **p < 0.01 vs. < 40 years, ***p < 0.001 vs. < 40 years; ‡p < 0.05 vs. male; §p < 0.05 vs. mild-to-moderate.

Table 2 Comparison of serum total IgE and specific IgE to common aeroallergens according to the age of the patients

	< 40 years (*N* = 262)	40–60 years (*N* = 338)	> 60 years (*N* = 172)
Serum IgE (kU/l)	178 (2–17,183)**,‡	102 (1–9235)	81 (1–5767)
≤ 113	94 (35%)	173 (53%)	100 (58%)
> 113	177 (65%)***,§	155 (47%)	72 (42%)
House dust mite (kU/l)	1.58 (0–161)***,§	0.49 (0–2029)§	0.23 (0–61)
≤ 0.35	163 (60%)	198 (61%)	110 (64%)
> 0.35	107 (40%)	130 (39%)	62 (36%)
Cat (kU/l)	0.78 (0–111)***,§	0.3 (0–111)‡	0.17 (0–98)
≤ 0.35	145 (54%)	250 (76%)	151 (88%)
> 0.35	125 (46%)***,§	78 (24%)‡	21 (12%)
Dog (kU/l)	0.51 (0–111)***,§	0.27 (0–111)§	0.16 (0–28)
≤ 0.35	170 (63%)	254 (78%)	159 (92%)
> 0.35	100 (37%)***,§	70 (22%)§	13 (8%)
Moulds (kU/l)	0.23 (0–62)*,§	0.18 (0–83)	0.16 (0–17)
≤ 0.35	223 (83%)	286 (88%)	151 (89%)
> 0.35	46 (17%)	39 (12%)	19 (11%)
Birch pollen (kU/l)	0.51 (0–199)**,§	0.28 (0–111)	0.21 (0–111)
≤ 0.35	175 (65%)	270 (82%)	146 (86%)
> 0.35	93 (35%)***,§	58 (18%)	24 (14%)
Grass pollen (kU/l)	1.23 (0–801)***,§	0.32 (0–111)§	0.18 (0–38)
≤ 0.35	123 (46%)	244 (74%)	147 (86%)
> 0.35	148 (54%)***,§	84 (26%)	24 (14%)

*p < 0.05, **p < 0.01, ***p < 0.001 vs. 40–60 years. †p < 0.05, ‡p < 0.01, §p < 0.001 vs. > 60 years.

Table 3 Comparison of serum total IgE and specific IgE to common aeroallergens between males and females

	Male (*N* = 312)	Female (*N* = 460)
Serum IgE (kU/l)	155 (1–13,754)***	98 (1–17,183)
≤ 113	124 (40%)	243 (53%)
> 113	188 (60%)***	216 (47%)
House dust mite (kU/l)	0.87 (0–2029)**	0.49 (0–363)
≤ 0.35	196 (63%)	275 (60%)
> 0.35	116 (37%)	183 (40%)
Cat (kU/l)	0.36 (0–101)	0.38 (0–101)
≤ 0.35	219 (70%)	327 (71%)
> 0.35	92 (30%)	132 (29%)
Dog (kU/l)	0.29 (0–111)	0.3 (0–111)
≤ 0.35	234 (76%)	349 (76%)
> 0.35	75 (24%)	108 (24%)
Moulds (kU/l)	0.2 (0-83)	0.19 (0-46)
≤ 0.35	260 (85%)	400 (87%)
> 0.35	46 (15%)	58 (13%)
Birch pollen (kU/l)	0.39 (0–111)†	0.29 (0–199)
≤ 0.35	229 (74%)	362 (79%)
> 0.35	80 (26%)	95 (21%)
Grass pollen (kU/l)	0.57 (0–801)*	0.4 (0–469)
≤ 0.35	191 (61%)	323 (70%)
> 0.35	120 (39%)*	136 (30%)

*p < 0.05, **p < 0.01, ***p < 0.001 vs. Females. †p = 0.08.

Table 4 Comparison of serum total IgE and specific IgE to common aeroallergens according to the tobacco status

	Ex-smokers (*N* = 204)	Current smokers (*N* = 164)	Non-smoker (*N* = 398)
Serum IgE (kU/l)	110 (2–17,183)	126 (1–8105)	120 (1–13,754)
≤ 113	102 (50%)	73 (45%)	188 (47%)
> 113	102 (50%)	90 (55%)	210 (53%)
House dust mite (kU/l)	0.4 (0–2029)**	0.51 (0–129)*	0.83 (0–363)
≤ 0.35	123 (61%)	108 (66%)	235 (59%)
> 0.35	80 (39%)	56 (34%)	162 (41%)
Cat (kU/l)	0.28 (0–111)**	0.35 (0–111)	0.43 (0–111)
≤ 0.35	158 (77%)	120 (73%)	263 (66%)
> 0.35	46 (23%)**	44 (27%)	133 (34%)
Dog (kU/l)	0.23 (0–111)**	0.28 (0–111)	0.34 (0–111)
≤ 0.35	170 (83%)	126 (77%)	282 (72%)
> 0.35	34 (17%)**	38 (23%)	110 (28%)
Moulds (kU/l)	0.19 (0–84)	0.17 (0–15)	0.2 (0–62)
≤ 0.35	178 (88%)	144 (89%)	332 (84%)
> 0.35	25 (12%)	18 (11%)	61 (16%)
Birch pollen (kU/l)	0.25 (0–111)**	0.24 (0–89)*	0.43 (0–199)
≤ 0.35	171 (85%)	135 (83%)	279 (71%)
> 0.35	31 (15%)***	28 (17%)*	116 (29%)
Grass pollen (kU/l)	0.3 (0–111)***	0.38 (0–74)	0.6 (0–801)
≤ 0.35	154 (75%)	114 (70%)	241 (61%)
> 0.35	50 (25%)***	50 (30%)*	155 (39%)

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. non-smoker.

induced by inhalation of hypertonic saline (NaCl 5%) when FEV1 postsalbutamol was ≥ 65% predicted and isotonic saline (NaCl 0.9%) when FEV1 was < 65% predicted. Saline was combined with additional salbutamol delivered by an ultrasonic nebuliser: Somerset, PA, USA with an output set at 0.9 ml/min as previously described (15). Each subject inhaled the aerosol for three consecutive periods of 5 min and for a total time of 15 min.

The whole sputum was collected, weighted and homogenised by adding three volumes of phosphate-buffered saline (PBS), vortexed for 30 s and centrifuged at 800 ***g*** for 10 min at 4 °C. Supernatant was separated from cell pellet. We added DTT (dithiotreitol) to the cells which were agitated for 20 min. Cells were washed once more with PBS and resuspended in 1 ml. Squamous cells, total cell counts and cell viability checked by trypan blue exclusion were performed with a manual haemocytometer as described previously (16). When squamous cells were > 80%, the sample was considered inappropriate (17). Ninety per cent of the samples used for our study had squamous cell count ranging from 0% to 50%. A patient was defined as eosinophilic if he had a sputum eosinophil count ≥ 3%. He was defined as neutrophilic if the sputum neutrophil count was ≥ 76%. Mixed-granulocytic patients were defined by a sputum eosinophil count ≥ 3% and a sputum neutrophil count ≥ 76% and pauci-granulocytic were those with a sputum eosinophil count < 3% and a sputum neutrophil count < 76% (18).

Statistical analysis

Results were expressed as mean ± SD or median (range) according to the distribution of the data. For the qualitative analysis, comparisons between groups were performed using a chi-square test or a Fisher's exact *t*-test to compare the proportion between groups. For quantitative analysis, we used a Kruskal–Wallis test for a multiple group comparison followed by a Dunn's test and a Mann–Whitney test to compare between two groups. A $p < 0.05$ was considered as statistically significant.

Results

Patient demographic and functional characteristics

Mild-to-moderate and severe asthmatics had similar age (46 ± 17 years for mild-to-moderate vs. 49 ± 14 years for severe asthmatics). The female sex was dominant both in mild-to-moderate asthmatics (58%) and in severe asthmatics (69%), but the

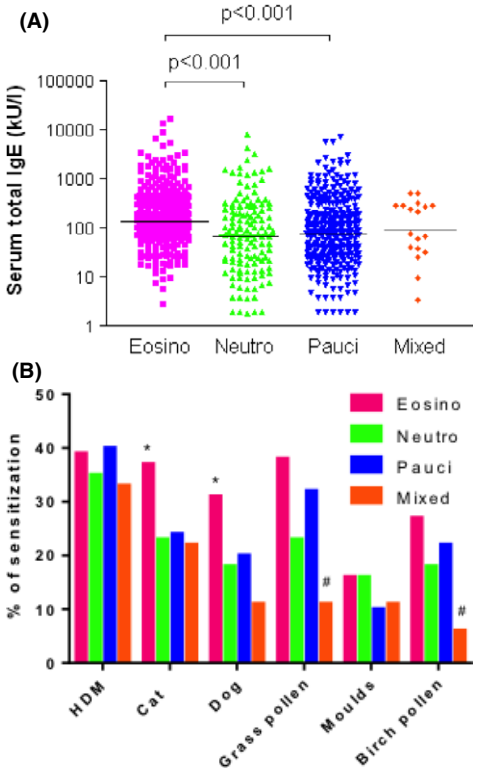

Figure 1 Comparison of serum total IgE (A) and sensitisation to aeroallergens (B) according to sputum cellular phenotypes. *$p < 0.01$ vs. neutrophilic and pauci-granulocytic, #$p < 0.05$ vs. eosinophilic

proportion of female was higher in severe asthma than in mild-to-moderate asthma ($p < 0.05$). The number of never smokers was also lower in severe than in mild-to-moderate asthma (41% vs. 54%) ($p < 0.001$). The fraction of atopic patients was quite similar between groups (61% in severe asthma vs. 55% in mild-to-moderate asthma). All severe asthmatics were receiving high dose of inhaled corticoids (ICS) (> 1000 μg equivalent fluticasone/day) and long acting β2 agonists, while only 57% of mild-to-moderate asthmatics were receiving ICS at an average dosage of 268 μg fluticasone/day. Sixteen per cent of severe asthmatics, but none of mild-to-moderate asthmatics, were receiving maintenance treatment with oral corticoids. None of the severe asthmatics were receiving omalizumab at the time of the blood and sputum sampling. FEV1 was clearly decreased in severe asthmatics (65 ± 21% predicted) compared with mild-to-moderate asthmatics (86 ± 19% predicted; $p < 0.001$). The distribution of patients according to sputum cellular phenotype and age, sex, tobacco status and disease severity are given in Table 1. Males were more eosinophilic than females ($p < 0.01$) while the neutrophilic phenotype was more common in females ($p < 0.05$). The proportion of neutrophilic asthma increased with the age and the disease severity while the proportion of pauci-granulocytic asthma showed changes going in the opposite direction. In contrast, the proportion of eosinophilic asthma was not influenced by age or disease severity, but was the dominant phenotype in severe asthmatics (see Table 1).

Serum total IgE/specific IgE according to demographic and treatment features

There was a clear decrease in serum total IgE with the age ($p < 0.01$). Sensitisation rate towards dogs, cats, grass and birch pollen declined as age progressed while the rate of sensitisation towards house dust mite and moulds remained stable across the different age groups (Table 2). Male had higher serum total IgE and a higher rate of sensitisation to grass pollen (39%) than female (30%) ($p < 0.05$; Table 3).

There was no difference in total serum IgE according to the smoking status. Ex-smokers were characterised by a reduced sensitisation rate to cats ($p < 0.01$) dogs ($p < 0.01$), and grass and birch pollen ($p < 0.001$ for both) compared with never smokers. Current smokers also showed reduced sensitisation to grass and birch pollen ($p < 0.05$ for both) compared with never smokers (Table 4). When mild-to-moderate asthmatics were split according to the use of ICS, serum total IgE was increased in those taking ICS [75 kU/l (2–1783) vs. 119 kU/l (0.1–9235), $p < 0.001$] together with the sensitisation rate towards moulds compared to those without ICS (18% vs. 11%, $p < 0.05$).

Serum total IgE/specific IgE according to sputum cellular phenotypes

The distribution of the patients according to sputum cellular phenotypes is 40% of eosinophilic, 17% of neutrophilic, 40% of pauci-granulocytic and 3% of mixed-granulocytic asthma. Serum total IgE was higher in eosinophilic asthmatics [186 (0.1–17,183)] when compared with neutrophilic [76 (2–8105)] and pauci-granulocytic [91 (2–7338)] asthmatics ($p < 0.001$ for both) (Figure 1A). Sensitisation rate to dogs and cats were higher in eosinophilic asthmatics (31% and 37%, respectively, $p < 0.01$ for both) compared with neutrophilic (18% and 23% respectively) and pauci-granulocytic asthmatics (20% and 24% respectively) while sensitisation to house dust mites and moulds were rather similar between the groups (ranging from 33% to 40% and from 10% to 16%, respectively). Sensitisation to grass and birch pollen was higher in eosinophilic asthmatics (38% and 27%, respectively, $p < 0.05$%) when compared with mixed-granulocytic (11% and 6%, respectively) (Table 5) (Figure 1B). We specifically analysed the patients

Table 5 Comparison of serum total IgE and specific IgE to common aeroallergens according to sputum cellular phenotypes

	Eosinophilic (*N* = 311)	Neutrophilic (*N* = 134)	Pauci-granulocytic (*N* = 309)	Mixed-granulocytic (*N* = 18)
Serum IgE (kU/l)	186 (1–17,183)***,§	76 (2–8105)	91 (2–7338)	96 (4–529)
≤ 113	106 (34%)	80 (60%)	172 (56%)	9 (50%)
> 113	205 (66%)***	54 (40%)	136 (44%)	9 (50%)
House dust mite (kU/l)	1.23 (0–111)**,†	0.35 (0–363)	0.62 (0–2029)	0.38 (0–58)
≤ 0.35	189 (61%)	86 (65%)	184 (60%)	12 (67%)
> 0.35	121 (39%)	47 (35%)	125 (40%)	6 (33%)
Cat (kU/l)	0.53 (0–111)***,†	0.25 (0–111)	0.3 (0–111)	0.24 (0–11)
≤ 0.35	195 (63%)	103 (77%)	234 (76%)	14 (78%)
> 0.35	115 (37%)**,‡	30 (23%)	75 (24%)	4 (22%)
Dog (kU/l)	0.42 (0–111)***,‡	0.22 (0–111)	0.25 (0–101)	0.19 (0–3)
≤ 0.35	213 (69%)	107 (82%)	247 (80%)	16 (89%)
> 0.35	96 (31%)**,‡	23 (18%)	62 (20%)	2 (11%)
Moulds (kU/l)	0.2 (0–83)	0.19 (0–40)	0.19 (0–62)	0.18 (0–5)
≤ 0.35	259 (84%)	112 (84%)	273 (90%)	16 (89%)
> 0.35	49 (16%)	21 (16%)	32 (10%)	2 (11%)
Birch pollen (kU/l)	0.39 (0–199)*	0.23 (0–69)	0.32 (0–111)	0.15 (0–6)
≤ 0.35	226 (73%)	108 (82%)	240 (78%)	17 (94%)
> 0.35	83 (27%)¶	23 (18%)	68 (22%)	1 (6%)
Grass pollen (kU/l)	0.6 (0–801)**	0.32 (0–39)	0.44 (0–111)	0.16 (0–4)
≤ 0.35	192 (62%)	97 (72%)	209 (68%)	16 (89%)
> 0.35	118 (38%)¶	37 (28%)	99 (32%)	2 (11%)

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. neutrophilic; †$p < 0.05$, ‡$p < 0.01$, §$p < 0.001$ vs. pauci-granulocytic; ¶$p < 0.05$, ††$p < 0.01$, ‡‡$p < 0.001$ vs. mixed-granulocytic.

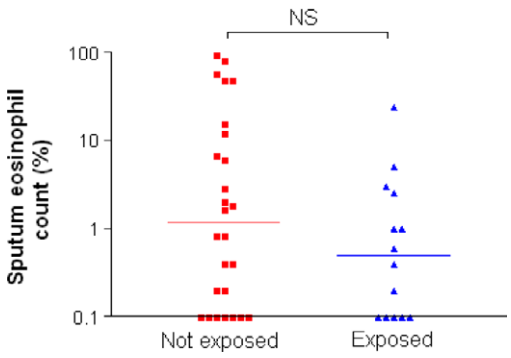

Figure 2 Comparison of sputum eosinophils (%) between asthmatics not exposed or exposed to pollens in patients exclusively sensitised to grass/birch pollens. Season of exposure for birch was from 1 March to 30 April and for grass from 1 May to 30 June. Daily dose of inhaled ICS and levels of specific IgE towards pollens were similar between the two group

mono-sensitised to birch and/or grass pollen and compared the sputum eosinophil counts between those sampled during ($N = 14$) vs. outside ($N = 26$) the critical season. There was no difference regarding sputum eosinophils between asthmatics taken during or outside the pollen season [0.5 (%) (0–24) vs. 1.2 (0–93), respectively] neither regarding the dose of ICS received by the patients at the time of sputum induction [400 μg/day (0–2000) vs. 400 μg/day (0–4200), respectively] (Figure 2). Likewise, total serum IgE and specific IgE towards pollens were not significantly different between the groups [Total IgE kU/l: 110 (7–2824) vs. 70 (6–580), birch IgE kU/l: 1.2 (0–79) vs. 2.77 (0–69), grass IgE kU/l: 1.19 (0–243) vs. 4.32 (0.1–25)].

Serum total IgE/specific IgE according to clinical disease severity

Severe asthmatics displayed higher total serum IgE compared to mild-to-moderate asthmatics ($p < 0.05$) (Figure 3A and Table 6). However, severe asthmatics had not greater sensitisation rate to any common aeroallergens compared to mild-to-moderate asthma (Figure 3B). Specific IgE levels towards birch pollen were even lower in severe asthmatics compared to mild-to-moderate asthmatics ($p < 0.05$, Table 6).

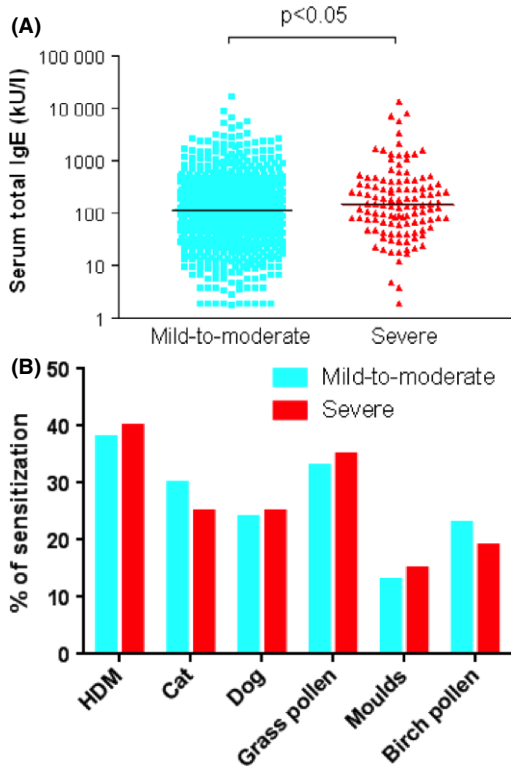

Figure 3 Comparison of serum total IgE (A) and sensitisation to aeroallergens (B) in mild-to-moderate vs. severe asthmatics

Overall, there was no correlation between ACQ and total serum IgE ($r = 0.15$; $p = 0.15$). There were no differences in ACQ between patients not sensitised ($N = 352$), sensitised to pollens only ($N = 40$), to perennial allergens ($N = 140$) only or to perennial allergens combined with pollens (240) (Figure 4).

It is worth noting that there was a clear increase in sensitisation rate towards house dust mites, cats and grass pollen in our asthmatic cohort compared with a general population according to previously published figures from a Belgium city involved in the European Community Health Survey (4) (Table 6).

Some severe allergic asthmatics may benefit from a treatment with omalizumab, a monoclonal antibody directed towards IgE. In our series of severe asthmatics, 28% met the three following criteria that would place them as potential candidate for omalizumab: total serum IgE ranging between 30 and 700 kU/l, at least one sensitisation to a perennial allergen and FEV1 (% predicted values) <80%.

Discussion

Here, we report for the first time an association between the pattern of airway inflammation and the IgE mediated sensitisation in a large cohort of asthmatics. Our study shows that eosinophilic asthmatics exhibit higher serum total IgE and sensitisation rate towards cats and dogs. Severe asthmatics were characterised by a slight increase in serum total IgE when compared to mild-to-moderate asthmatics without significant difference in the rate of sensitisation to common aeroallergens.

The relationship between total serum IgE and sputum eosinophils confirms here, in a very large cohort of well-characterised asthmatics, our previous finding established on a limited series (16). Although significant for the whole cohort, it is interesting to note that this relationship is less convincing in older patients in whom serum IgE levels decline, whereas the proportion of eosinophilic asthma remains fairly stable throughout the age classes. The particular association between cats and dogs and eosinophilic asthma is an original finding. It is likely to reflect the Th2 driven airway inflammatory process as the consequence of continuous exposure to these perennial allergens. Our finding is in keeping with a very recent study conducted in a Korean population seeking medical opinion for lower airway respiratory symptoms (19). In the latter study, positive skin prick tests to cats and dogs were a risk factor for sputum eosinophilia. In contrast to what we observed with animal dander, and perhaps surprisingly, we did not find such a relationship between the rate of house dust mite and mould sensitisation and eosinophilic inflammation although quantitative assessment of specific IgE towards house dust mites show greater values in eosinophilic asthmatics. Our finding is also perfectly in line with the recent report of Patelis et al. who showed that sensitisation to furry animals, but not to mites, was a strong independent factor for elevated FeNO, a biomarker of sputum eosinophils (10). The reason for this is unclear. We could however speculate that sensitised subjects are more prone to take preventive measures to reduce humidity and dust in their home than to separate from their pets. We did not find raised sputum eosinophilia in patients specifically and exclusively sensitised to pollens and sampled during the season as opposed to their counterparts sampled outside the season. Our finding fits the lack of correlation between FeNO and sensitisation to pollens observed in another cross-sectional study (10). However, it does not exclude that prolonged seasonal exposure to pollen in sensitised asthmatics would have resulted in increased sputum eosinophilia, but the retrospective and cross-sectional design of our study is a clear limitation and does not allow confirming this link.

Overall, neutrophilic phenotype shows total IgE and an IgE sensitisation profile comparable to that seen in pauci-granulocytic asthma. The sensitisation

Table 6 Comparison of serum total IgE and specific IgE to common aeroallergens between mild-to-moderate and severe asthmatics

	Reference population in Belgium (City of Antwerp (4), %)	Mild-to-moderate asthma (*N* = 654)	Severe asthma (*N* = 118)
Serum IgE (kU/l)		110 (1–17,183)	162 (2–13,754)*
≤ 113		317 (49%)	50 (43%)
> 113		337 (51%)	67 (57%)
House dust mite (kU/l)		0.65 (0–2029)	0.47 (0–101)
≤ 0.35		402 (62%)	69 (60%)
> 0.35	22–27%	252 (38%)	47 (40%)
Cat (kU/l)		0.39 (0–111)	0.29 (0–101)
≤ 0.35		458 (70%)	88 (75%)
> 0.35	9%	195 (30%)	29 (25%)
Dog (kU/l)		0.3 (0–111)	0.29 (0–111)
≤ 0.35		497 (76%)	86 (75%)
> 0.35		155 (24%)	28 (25%)
Moulds (kU/l)		0.19 (0–83)	0.18 (0–31)
≤ 0.35		560 (87%)	100 (85%)
> 0.35		87 (13%)	17 (15%)
Birch pollen (kU/l)		0.35 (0–199)	0.25 (0–111)*
≤ 0.35		499 (77%)	92 (81%)
> 0.35		153 (23%)	22 (19%)
Grass pollen (kU/l)		0.45 (0.1–469)	0.39 (0.1–801)
≤ 0.35		437 (67%)	77 (65%)
> 0.35	16–17%	215 (33%)	41 (35%)

Results are expressed as median (minimum–maximum). *$p < 0.05$ vs. mild-to-moderate asthma. †$p = 0.06$ vs. mild-to-moderate asthma.

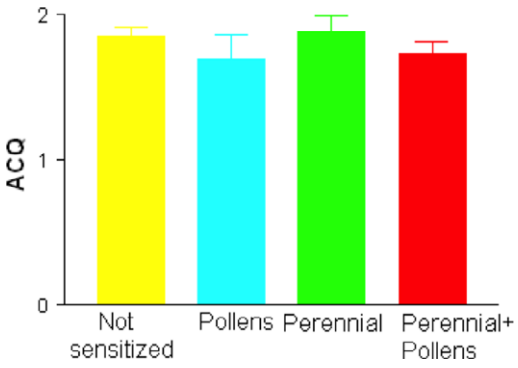

Figure 4 ACQ comparison between asthmatics not sensitised, sensitised to pollen only, sensitised to perennial allergens only or sensitised to perennial allergens combined with pollens. An ACQ above 1.5 denotes uncontrolled asthma

rate in non-eosinophilic phenotype was, however, clearly higher than in a general population suggesting that atopy is a risk factor for asthma whichever the airway inflammatory phenotype. Likewise, total serum IgE in neutrophilic and pauci-granulocytic asthma, though often within the normal range, is at least twice as high as the level reported in a general population (21) and three times higher than in a population excluding those with nasal allergic symptoms (22).

In our cross-sectional study, IgE was associated with some demographic features including age, sex and tobacco consumption. There was a clear decrease in total serum IgE with advancing age which is in line with longitudinal studies (23). Accordingly, there was a reduction in the sensitisation rate for all types of aeroallergens as age progresses except for house dust mite, the sensitisation to which remained fairly constant over the age categories. The reason for a decrease in IgE with age is not known but could reflect a facet of immune senescence. As aforementioned sputum eosinophilia, although linked to serum IgE in the whole cohort, did not show reduction with age. This suggests that mechanisms favouring eosinophilic airway inflammation may change during the life and become less dependent on IgE mediated pathway in the oldest patients. Our finding of greater total serum IgE in male sex is confirmatory of a previous study at population level (24). However, in contrast to Kerkhof et al., our data

show equal sensitisation rate to house dust mites and birch pollen in male and female asthmatics. In our series, only the sensitisation rate to grass pollens was found to be higher in males. Overall, the influence of sexual hormone on IgE sensitisation remains unclear and is certainly an area worth being further explored (25).

We also found that ex-smokers were characterised by a reduced sensitisation rate to cats, dogs, grass and birch pollens when compared to non-smokers while current smokers showed reduced sensitisation to grass and birch pollens. More than 15 years ago, Jarvis et al. showed in a population study, that current smokers were at increased risk of sensitisation to house dust mite but at decreased risk of sensitisation to grass and cats allergens (26). It is worth noting that if smoking is associated with less frequent sensitisation towards common aeroallergen, the total serum IgE is very similar in smokers as compared with never smokers. This may suggest that smoking is triggering IgE production independently of sensitisation to common aeroallergens.

We did not find association between asthma severity and any type of sensitisation. A previous cross-sectional study from ECRHS has shown that sensitisation to airborne moulds cladosporium and alternaria assessed by skin prick test was associated with more severe asthma (7). Here, we could not confirm this preferential association between severity and sensitisation rate to moulds allergens but we did not specifically select the type of moulds as RAST were performed for a mixture including aspergillus, alternaria, cladosporium and penicillium. Furthermore, we determined sensitisation by measuring serum specific IgE rather than assessing local allergenic reaction, which may be considered as less physiological than the skin prick tests. However, we found that total serum IgE was greater in severe than in mild-to-moderate asthmatics. Therefore, our data suggest that it is the overall production of IgE rather than the targeting of a specific aeroallergen that matters in determining asthma severity. To the best of our knowledge, the link between the level of serum IgE and asthma severity had not been described in a large unselected asthma population so far. Supporting our observation, the role of IgE in severe asthma has now been established by the demonstration of the efficacy of omalizumab, a monoclonal antibody directed to IgE, in treatment of severe atopic (27) and non-atopic asthma (28). Omalizumab has mainly been validated in those allergic patients sensitised to a perennial aeroallergens with IgE ranging from 30 to 700 kU/l. In our series of severe asthmatics, only 28% satisfied the main criteria for considering treatment with omalizumab.

We conclude that eosinophilic asthma is associated with raised serum IgE and, in particular, with a greater sensitisation rate to domestic animal dander while severe asthma display raised total serum IgE without increase in sensitisation rate to any common aeroallergen.

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