

ORIGINAL ARTICLE

Severe childhood asthma and allergy to furry animals: Refined assessment using molecular-based allergy diagnostics

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Keywords

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Abstract

Background: Allergy to cats and dogs and polysensitization towards these animals are associated with severe childhood asthma. Molecular-based allergy diagnostics offers new opportunities for improved characterization and has been suggested to be particularly useful in patients with polysensitization and/or severe asthma. The aim was to use extract- and molecular-based allergy diagnostics to compare patterns of IgE sensitization towards aeroallergens in children with problematic severe and controlled asthma.

Methods: Children with a positive ImmunoCAP towards any furry animal (cat, dog or horse) were recruited from a Nationwide Swedish study on severe childhood asthma. Severe ($n = 37$, age 13 years) and controlled ($n = 28$, age 14 years) asthmatics underwent assessment of allergic sensitization by ImmunoCap (kU_A/l) and immuno-solid-phase allergen chip (ISAC). In addition, Asthma Control Test, spirometry and a methacholine challenge were performed.

Results: Children with severe asthma had lower asthma control ($p < 0.001$) and FEV₁ ($p = 0.001$) and more bronchial hyper-responsiveness ($p = 0.008$) in spite of high doses of inhaled steroids ($\geq 800 \mu\text{g}$ budesonide). Children with severe asthma displayed higher levels of IgE antibodies towards cat (17 vs. 3.9, $p = 0.027$), dog (3.8 vs. 1.2, $p = 0.012$) and horse (7.4 vs. 0.7, $p = 0.014$). Sensitization towards Can f 2 (22% vs. 0%, $p = 0.009$) and Equ c 1 (51% vs. 25%, $p = 0.03$) was more common in severe asthma. IgE levels towards Equ c 1 correlated with asthma control ($r = -0.41$, $p = 0.04$).

Conclusion: Children with severe allergic asthma had higher sIgE levels to cat, dog and horse. Molecular-based allergy diagnostics revealed a more complex molecular spreading of allergen components in children with the most severe disease.

A small proportion of children with asthma suffer from chronic symptoms and severe exacerbations despite treatment with high doses of currently available asthma medications (1). The term problematic severe asthma has been established to classify these children, and 80% of children with such severe asthma are atopic (2). These patients require extensive health-care resources and represent a continuous clinical challenge to the paediatrician.

Allergy to cats and/or dogs is considered to be a major risk factor of development of asthma and rhinitis (3), and

sensitization towards these allergens is associated with a reduced quality of life (4) as well as increased bronchial hyper-responsiveness and airway inflammation (5). Furthermore, polysensitization, comorbidity and/or cross-reactivity towards cats, dogs and other mammalian animals are prevalent (6) and are associated with more severe allergic disease (4).

The prevalence of pet keeping and allergy towards furry animals (7) as well as the prevalence of allergic airway disease is increasing (8), and exposure to allergens from these animals is ubiquitous (9); accordingly, the clinician should evaluate all

patients with persistent asthma for sensitization towards cat and dog allergens (10, 11). The management of a patient with asthma and sensitization towards furry animals includes a judgment of the current exposure to the specific allergens, the impact of the exposure on the morbidity and providing sound treatment advice related to the patient with respect to the allergen exposure, that is, giving advice on whether or not to keep a domestic animal. Further, the clinician would like to identify which animal is actually causing symptoms, and whether some of the positive test results could be without clinical relevance.

Traditionally, allergic sensitization is determined using allergen extracts made from animal dander and/or by measurement of specific IgE in serum or skin prick testing. One disadvantage with these methods is that they only provide information about the possible sensitizing allergen source, but no information about sensitization towards specific allergens is provided. Thus, it is not possible to differentiate between the primary source of sensitization and cross-reactivity between allergens from different allergen sources (12).

Thus, there is an urgent need to implement novel biomarkers into clinical practice to increase our understanding of the morbidity, particularly in children with problematic severe allergic asthma. During the last few years, an important step forward in the diagnosis of allergy has been made with the introduction of molecular-based allergy diagnostics (12, 13), which offers new opportunities for improved characterization and management. Molecular-based allergy diagnostics has been suggested to be particularly useful in patients with polysensitization (14, 15) and/or severe asthma (16).

In the current study, we investigated school-age children with allergy towards furry animals and different manifestations of

persistent asthma. We utilized extract-based and molecular-based allergy diagnostics, and the aim was to compare patterns of IgE sensitization towards perennial and seasonal aeroallergens in children with problematic severe and controlled asthma.

Methods

In this cross-sectional, explorative case-control study, children aged 6–18 years with problematic severe asthma were compared with age-matched peers with controlled asthma. These two conditions were defined in accordance with guidelines established by the Global Initiative for Asthma (17), Table 1. Written informed consent was obtained from all participants and their guardians. The study was approved by the regional board of ethics at Karolinska Institutet (Dnr 2006/1324-31/1 and 2008/378).

Subjects

The children recruited were participating in the Swedish National Study on children with severe asthma. Details of recruitment and inclusion are presented elsewhere (2). Briefly, allergists throughout Sweden were invited to identify and refer children with problematic severe asthma and age-matched peers (± 12 months) with controlled asthma for this investigation. The referred children were invited to participate and, following reassessment of their medical histories, medication and adherence to prescribed treatment, final selection of the participants was performed according to the inclusion criteria, Table 1. In the current project, children who were allergic to at least one furry animal (cat, dog or horse) were selected for further analysis.

Table 1 Inclusion criteria. Criteria for inclusion of children suffering from problematic severe and controlled persistent asthma

Problematic severe asthma	Controlled persistent asthma
Major criteria (all required)	Major criteria (all required)
A diagnosis of asthma by a paediatric allergist	A diagnosis of asthma by a paediatric allergist
Allergic to furry animals	Allergic to furry animals
Daily high-dose administration of ICS (≥ 800 μ g budesonide or ≥ 400 μ g fluticasone/mometasone per day) in combination with LABA and/or LTRA*	Daily low- to medium-dose administration of ICS (≥ 100 – ≤ 400 μ g budesonide or ≥ 50 – ≤ 200 μ g fluticasone per day). Use of either LABA or LTRA was acceptable.
Minor criteria observed within the preceding 12-month period (minimum of at least one required)	Minor criteria observed within the preceding 12-month period (all required)
At least one emergency hospitalisation	No hospitalisation
At least two emergency out-patient visits	No emergency out-patient visits
At least one oral treatment with corticosteroid	No oral corticosteroid treatment
At least twelve exacerbations of asthmatic symptoms per year or symptoms present continuously for at least 3 months	Less than five exacerbations of symptoms**
Symptoms that limited daily activities (including sport or leisure activities) more than twice a week for at least 3 consecutive months	Occasional symptoms related to strenuous exercise only, otherwise no symptoms
Nocturnal symptoms more than twice a week for at least 3 consecutive months	No nocturnal symptoms

ICS, inhaled corticosteroid; LABA, long-acting β -2 agonist; LTRA, leukotriene receptor antagonist.

*High-dose administration of ICS for at least 6 months during the preceding year; previous use of LABA or LTRA only was considered acceptable if this treatment was discontinued due to inefficacy or the occurrence of unacceptable side effects.

**An increase in the ICS dosage for a maximum of 2 wks in connection with asthma exacerbations was considered acceptable.

Procedures

Asthma control was estimated according to the Asthma Control Test (18). A total of 25 points can be achieved with optimal asthma control, and a score of 19 or less suggests poorly controlled asthma.

Exhaled nitric oxide, pulmonary function and assessment of bronchial hyper-responsiveness

A NIOX™ analyzer (Aerocrine AB, Solna, Sweden) was used to measure the fraction of exhaled nitric oxide in exhaled air (FENO), in accordance with international guidelines (19). Spirometry was performed using a Vitalograph® 2120 (Vitalograph®, Ennis, Ireland), in accordance with recommendations from the European Respiratory Society using the reference values reported by Polgar (20). Bronchial hyper-responsiveness to methacholine was assessed utilizing a Spira nebuliser (Spira Respiratory Care Centre, Hämeenlinna, Finland), and the slope of the dose–response curve (DRS_{methacholine}) was calculated (21).

Serologic analysis and Number of Eosinophils in blood

Following application of local anaesthesia (EMLA cream, Astra Zeneca, Sweden), samples of venous blood were collected and an eosinophil count was performed. The serum concentrations of IgE (kU/l) and specific IgE (kU_A/l) towards cat, dog, horse, birch and grass were analysed by ImmunoCAP™ (Thermo Fisher Scientific, Uppsala, Sweden). Allergy towards furry animals was defined as a level of antibodies towards cat, dog or horse ≥ 0.35 kU_A/l. Further, all serum samples were analysed using an experimental research ISAC prototype (Thermo Fisher Scientific, Uppsala, Sweden). The chip contained allergens derived from cat (Fel d 1, Fel d 2 and Fel d 4), dog (Can f 1, Can f 2, Can f 3, Can f 5), horse (Equ c 1, Equ c 3), birch (Bet v 1, Bet v 2, Bet v 4) and grass (Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7, Phl p 11, Phl p 12). A positive ISAC test result was defined by a level of ≥ 0.30 ISAC independent units (ISU).

Statistical analyses

The Mann–Whitney test was used to analyse between group differences in levels of skewed variables (IgE antibodies, FENO and DRS_{methacholine}), whereas the Student's t-test was used to analyse differences in normally distributed variables (age, Asthma Control Test, FEV1 and eosinophils). Correlations were assessed using the Pearson test or the Spearman test, and a chi-square test was used to test associations between two categorical variables. All values were analysed with the IBM SPSS Statistics version 20.0 (Chicago, IL, USA).

Results

Blood samples were available from 54 children with severe asthma and 39 children with controlled asthma. Of these, 69% (n = 37) of the severe asthmatics and 72% (n = 28) of the controlled asthmatics were sensitized to one or more furry animal (p = 0.73) and included in this analysis. Children with

severe asthma had inferior asthma control (p < 0.001), reduced pulmonary function (p = 0.001), higher number of eosinophils in blood (p = 0.007) and increased bronchial hyper-responsiveness (p = 0.008) compared with children with controlled asthma, Table 2.

Sensitization towards furry animals assessed by extract-based tests

The frequency of sensitization towards each furry animal was similar among the groups of children (Table 3). However, children with problematic severe asthma displayed higher levels of IgE antibodies towards cat (17 kU_A/l [5.0–54] vs. 3.9 kU_A/l [0.7–33], p = 0.027), dog (3.8 kU_A/l [1.1–22] vs. 1.2 kU_A/l [0.5–4.2], p = 0.012) and horse (7.4 kU_A/l [0–32] vs. 0.7 kU_A/l [0–4.8], p = 0.014) compared with children with controlled asthma. Multisensitization towards more than one animal occurred equally common in both asthma groups, 84% vs. 82%, p = 0.86; however, there was a trend towards more frequent multisensitization to all three animals in the severe asthma group, 70% vs. 46%, p = 0.052.

Molecular-based diagnostics of allergy towards furry animals

Sensitization towards individual allergen components of all three animals was common in both groups, Table 3. When comparing IgE antibody concentrations towards each component from cat and dog, no significant differences were found (results not shown). All children sensitized to cat were also sensitized to Fel d 1, and there were similar correlations between the levels of IgE antibodies towards cat extract and Fel d 1 (r = 0.93, p < 0.001) and the sum of all Fel d antibodies

Table 2 Clinical characteristics of the included children

Sensitized to any furry animal	Severe asthma n = 37	Controlled asthma n = 28	p-value
Age	12.9 (3)	13.9 (3)	0.22
Females, %	41	39	0.92
Inhaled steroids (µg), medians (IQR)	800 (800–800)	200 (125–400)	NA
Asthma Control Test (max 25)	17 (3.4)	22.9 (1.5)	<0.001
FEV1 (%)	78 (19)	91 (10)	0.001
Exhaled nitric oxide (p.p.b), medians, (IQR)	30 (15–42)	19 (14–32)	0.199
Eosinophils (10 ⁹ × l ⁻¹)	0.5 (0.5)	0.3 (0.2)	0.007
IgE, kU/l, medians (IQR)	690 (210–1550)	443 (142–1085)	0.225
Bronchial hyper-reactivity (DRS), medians (IQR)	50 (13–95)	5 (0.4–45)	0.008

All values given in means (SD), unless otherwise stated.

µg, microgram; IQR, inter-quartile range; FEV1, forced expiratory volume in one second; P.P.B, parts per billion; DRS, dose–response slope; NA, not applicable.

Table 3 The frequencies(%) of allergic sensitization towards furry animals using extract- and molecular-based allergy diagnostics

Variable	Classification	Severe asthma n = 37	Controlled asthma n = 28	p-value
Cat	Cat extract	89	86	0.67
Fel d 1	Uteroglobin/ secretoglobulin	89	86	0.67
Fel d 2	Serum albumin	3	4	0.84
Fel d 4	Lipocalin	38	21	0.16
Dog	Dog extract	92	86	0.43
Can f 1	Lipocalin	30	21	0.45
Can f 2	Lipocalin	22	0	0.009
Can f 3	Serum albumin	8	7	0.89
Can f 5	Arginine esterase/ prostatic kallikrein	38	18	0.08
Horse	Horse extract	70	57	0.27
Equ c 1	Lipocalin	51	25	0.03
Equ c 3	Serum albumin	5	4	0.71
Any Lipocalin		54	46	0.52
≥3 Lipocalin		30	4	0.007

($r = 0.94$, $p < 0.001$). All children sensitized to Fel d 2 and Fel d 4 were also sensitized to Fel d 1, but not vice versa.

Forty-three (43)% of the children sensitized to dog had antibodies towards Can f 1, Can f 2, Can f 3 or Can f 5. Six children were mono-sensitized to Can f 1, and six children were mono-sensitized to Can f 5. Sensitization to Can f 2 occurred in 8 of the 37 children with severe asthma as compared to none in the controlled asthma group, and no child was mono-sensitized towards Can f 2. Five children were sensitized towards Can f 3; all of them were also sensitized towards Can f 1. The Spearman correlation coefficient between dog extract and the sum of Can f components was 0.71 ($p < 0.001$).

Sixty per cent of the children sensitized to horse had antibodies towards Equ c 1. Sensitization towards Equ c 1 occurred more frequently among children with severe asthma, ($p = 0.03$), Table 2. There were equal correlations between antibodies towards horse extract and Equ c 1 and the sum of Equ c antibodies ($r = 0.77$, $p < 0.001$ for both). The levels of antibodies towards Equ c 1 were increased in children with severe asthma ($p = 0.017$) and following log transformation, an inverse correlation with the score on the Asthma Control Test was found, $r = -0.41$, $p = 0.036$.

Cross-reacting lipocalins

A similar proportion of children were sensitized to any lipocalin in both groups, Table 3, whereas multisensitization to three or more lipocalin components occurred more frequently in severe asthma ($p = 0.007$).

Sensitization to grass and birch pollen allergens assessed by extract- and molecular-based allergy diagnostics

The frequency of sensitization towards birch and/or grass pollen was equally common in the two groups of children

Table 4 The frequencies(%) of sensitization towards birch and grass pollens using extract-based methods and molecular-based allergy diagnostics

Variable	Classification	Severe asthma n = 37	Controlled asthma n = 28	p-value
Birch or grass	Extract	81	82	0.97
Birch and grass	Extract	54	52	0.86
Birch	Extract	65	79	0.23
Bet v 1	PR-10	68	79	0.33
Bet v 2	Profilin	14	7	0.41
Bet v 4	Polcalcin	0	0	N A
Grass	Extract	70	56	0.23
Phl p 1	Group 1	46	57	0.37
Phl p 2	Group 2	11	18	0.42
Phl p 4*	Berberine bridge enzyme	49	36	0.30
Phl p 5	Group 5	30	32	0.84
Phl p 6	Group 6	16	14	0.83
Phl p 7	Polcalcin	0	0	N A
Phl p 11	Trypsin inhibitor	8	14	0.43
Phl p 12	Profilin	11	7	0.61
Phl p 1 or Phl p 5		54	64	0.41

*nPhl p 4 is a glycosylated protein and part of the IgE response may be due to asymptomatic IgE ab binding to cross-reacting carbohydrate determinants (CCD).

(Table 4), and there was no difference in the levels of IgE antibodies towards grass ($p = 0.29$) and birch ($p = 0.92$).

Further, there was no difference in sensitization to individual birch and grass components. Only a few individuals ($n = 7$) were sensitized to Bet v 2, and nobody was sensitized to Bet v 4. There were equal correlations between the concentration of IgE antibodies to birch and Bet v 1 and the sum of all Bet v components ($r = 0.98$, $p < 0.001$ for both).

Phl p 1, Phl p 4 and Phl p 5 were the most common grass pollen components. No children were sensitized to Phl p 7. The Spearman correlation coefficient between the timothy extract and the sum of all Phl p components was 0.90 ($p < 0.001$).

Discussion

In this study, we have investigated children with allergy towards furry animals by comparing the sensitization patterns towards common inhalant allergens in children with problematic severe and controlled asthma. We have utilized extract- and molecular-based allergy diagnostics and interpreted the patterns of sensitization in relation to the clinical presentation the patient. The main findings were that children with problematic severe asthma had higher levels of IgE antibodies towards cat dog and horse compared with children with controlled asthma and were more likely to be triple sensitized to the furry animals. Further, multisensitization towards lipocalin occurred more commonly in severe asthmatics, and finally, sensitization towards Equ c 1, a lipocalin and the major horse allergen was associated with more severe disease. The

clinical relevance of these findings is that high levels of specific IgE antibodies and multisensitization towards furry animals points to more severe airway disease.

In a previous work from this cohort (4), we analysed all children included in the Swedish Nationwide Study on severe childhood asthma ($n = 96$) and found that multisensitization towards allergen components from furry animals (secretoglobulin, kallikrein and lipocalin) was associated with severe asthma. In the current analysis, we have extended and refined those results by exploring the pattern of sensitization exclusively in children allergic to furry animals and found that it is, in fact, the lipocalins that seem to be associated with the most severe disease in this cohort.

Lipocalins represent the largest mammalian allergen family, and more than 50% of the allergens that have been identified from furry animals are lipocalins (22). In general, they share sequence identities of only 20–30%, but their tertiary structures are highly similar. Mammalian lipocalin allergens are found in dander, saliva and urine, and they are sticky, become easily airborne, disperse effectively and are widely present in indoor environments (22). Lipocalins are a group of proteins that are either involved as ligand transport proteins, have enzymatic activity and/or participate in cell homeostasis. As opposed to the secretoglobulin (Fel d 1), lipocalins do not seem to activate dendritic cells and initiate the process of allergic inflammation (23). It has been speculated that the existence of endogenous human lipocalins could be responsible for the generally low T-cell reactivity towards lipocalins (22). Thus, it is probable that multisensitization towards lipocalins is not actually causing the severe disease, but is merely a marker of extensive exposure leading to multisensitization and severe disease, a phenomena that could be referred to as molecular spreading. The association between molecular spreading and clinical disease was introduced by Hatzler et al. (24) who observed that grass-sensitized children without clinical symptoms were sensitized to fewer allergen molecules compared with children with clinical disease.

The association between high levels of IgE antibodies towards furry animals and disease severity is a novel and clinically relevant finding in line with previous reports where higher levels of IgE antibodies have been linked to an increased probability of clinically relevant disease (25). The lack of difference observed for grass and birch pollen allergens could be explained by that these are seasonal allergens, thereby having less impact on the asthma morbidity in these children.

The strong correlation between Fel d 1 and cat dander extract supports previous evidence that Fel d 1 is the most clinically relevant cat allergen (26), although recent publications describe the possible clinical relevance of novel cat allergens such as Fel d 7 and Fel d 8 (27). Approximately 40% of children positive to dog extract displayed antibodies towards any of the dog components included in this chip, which is an indication that other dog components such as Can f 4 (28) and Can f 6 (29) might also be of clinical relevance.

The current study adds to the relatively sparse evidence that uses molecular allergology to describe patterns of allergic sensitization in children allergic to furry animals. A particular strength of this study is that the molecular assessment is interpreted in the light of a detailed clinical assessment. Pet allergy is a complex area; many of the patients are polysensitized to several pets, the clinical history is often inconclusive, and the cross-reactivities between, for example, cat, dog and horse are not fully clarified at the molecular level (13). The physician's advice on whether or not to keep a household pet has a huge impact on the child's well-being (30) and should be based upon the most updated available knowledge.

In summary, the main findings of this analysis are that higher IgE levels to cat, dog and horse and a more complex molecular spreading of allergen components for these animals were observed for children with severe allergic asthma. Molecular-based allergy diagnostics refines the severity assessment of asthmatic children with allergy towards furry animals.

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