ORIGINAL ARTICLE

Can Molecular Allergology Be a Useful Approach Towards Pet Allergy?

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Abstract

Introduction: The increasing presence of pets in domestic households, coupled with the significant levels of pet allergens found in places without animals (*eg* schools, nurseries, workplaces) has contributed to an increase in the frequency of allergies to these animals in industrialized countries. This study aimed to assess the prevalence of allergic sensitization to pets using the test ImmunoCAP ISAC (ThermoFisher Scientific, Massachusetts, USA).

Methods: Retrospective study of patients, in the age range of 0-18 years, who were followed at our pediatric allergology outpatient center and tested with ImmunoCAP ISAC between April 2015 and September 2019. Epidemiological, clinical, and laboratory variables related to pet allergies were assessed.

Results: A total of 101 individuals were included in the study, with a median age of 8 years and 4 months, of whom 62/101 (61.3%) were male. It was found that 42/101 (41.6%) had sensitization to at least one major animal-specific allergen, and in this group, 24/42 (54.8%), 12/42 (28.6%), and 7/42 (16.7%) were monosensitized, bisensitized, and polysensitized, respectively. A major sensitization to dogs was detected in 25/101 (24.8%) patients. The allergen Can f 1 was the most detected (17/25, 68%) followed by Can f 5 (10/25, 40%, six of whom were female). Cat sensitization was observed in 37/101 (36.6%) patients, with Fel d 1 being the most detected molecular allergen (33/37, 89.2%). All cases sensitized to Fel d 4 (n = 5) were also sensitized to Fel d 1. Half of the cases sensitized to Fel d 2 (4/8, 50%) were not sensitized to Fel d 1, and 3/4 (75%) of them were sensitized to Bos d 6 in the context of clinically relevant cow milk allergy. All cases sensitized to Equ c 1 (n = 4) were also sensitized to Fel d 4. Asthma was found in 29/101 (28.7%) of the children and the majority were sensitized to pets (21/29, 72.4%). All the patients polysensitized to major animal-specific allergens were also asthmatic (7/7, 100%). It was noted that all patients polysensitized to other aeroallergens (mites, pollens, and fungi) were also sensitized to pets, the majority being asthmatic.

Discussion: Monosensitization to a major animal-specific allergen was more frequent than polysensitization. Fel d 1 and Can f 1 were the most frequent molecular allergens involved. The majority of the asthmatic patients in our study were sensitized to pets, and all of the patients polysensitized to major animal-specific allergens were also asthmatic, indicating that pet sensitization was a representation of a more complex and serious phenotype of the allergic disease. Furthermore, the majority of the patients polysensitized to other aeroallergens (mites, pollens, and fungi) were also sensitized to pets and were asthmatic as well.

Keywords: Adolescent; Allergens/adverse effects; Asthma/etiology; Child; Hypersensitivity/diagnosis; Hypersensitivity/etiology; Infant; Pets/immunology

Keypoints

What is known:

- Molecular-based allergy diagnosis allows healthcare professionals to understand the primary sensitizing allergen source and distinguish co-sensitization from cross-sensitization, which is particularly important when immunotherapy is intended to implement the optimal treatment.
- Female patients sensitized to Can f 5 can present an allergic reaction to human seminal fluid due to cross-reactivity, an important fact to be considered in the case of those with this sensitization. It is also relevant to highlight that the majority of patients sensitized to Can f 5 can tolerate neutered or female dogs.

What is added:

- Despite the fact that serum albumins are considered to be an uncommon cause of allergic sensitization and given a small sample of patients sensitized to Fel d 2, the study confirms the association between high levels of IgE to Fel d 2 and atopic dermatitis.
- The majority of the asthmatic patients were sensitized to pets, and all of the patients polysensitized to major animal-specific allergens were also asthmatic, indicating that pet sensitization represents a more complex and serious phenotype of the allergic disease. Furthermore, the majority of those polysensitized to other aeroallergens (mites, pollens, and fungi) were also sensitized to pets and asthmatic.

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Introduction

The increasing presence of cats and dogs in homes, associated with significant levels of dog and cat allergens found in areas devoid of these animals (*eg* homes, schools, nurseries, workplaces), has contributed to an increase in the frequency of allergies to these animals in industrialized countries.[1] Mammalian furry animals are an important source of indoor allergens. They are considered risk factors for the development of allergic rhinitis and asthma. Although the most advisable measure would be to avoid the animal, this is often impossible and associated with a major emotional impact.[2]

Animal allergens are present in urine, saliva, and dander, which stick to animal hair and dander and are dispersed indoors. These allergens also adhere to human clothes and are easily transported to public places, which explains their presence in locations where an animal has not been present. Exposure measurement studies have shown their presence in schools, day-care centers, public transport, and non-pet owners households.[3]

A diagnosis of an allergy to dogs or cats is based on medical history which should be taken in coordination with physical examination and is confirmed using the prick-test. An important breakthrough in the diagnosis of allergy to furry animals has been made with the introduction of molecular-based allergy diagnosis which offers new opportunities for improved characterization of this pathology.[2]

Some cat, dog, and horse allergens have been described, and most of them are presented in Table 1. Lipocalins are synthesized in salivary glands and are dispersed into the environment by saliva and dander. They are the most important allergen protein, and most of them are major allergens characterized by a common three-dimensional structure and a low sequence identity. Serum albumins are highly cross-reactive molecules and are minor allergens abundant in saliva and dander[4] which can be important in the context of clinically relevant allergy to cow milk.[5]

Literature shows that most patients sensitized to dogs have antibodies to Can f 1.[3] Can f 5, a prostatic kallikrein protein isolated from the urine of male dogs, is considered a major allergen in the same way as lipocalins.[4] No patterns of cross-reactivity to allergens from other furry animals have been identified except for human prostate-specific antigen, which is a major allergen in seminal plasma, suggesting that sensitization to Can f 5 could be relevant to seminal fluid allergy.[6]

Major cat allergens are Fel d 1 and Fel d 4, although the clinical significance of sensitization to Fel d 4 is still unknown. Fel d 1 is associated with hormone production and acts as uteroglobin. It is found mainly in saliva but can also be found in sebaceous glands of cat skin and urine.[2] A monosensitization to Fel d 2 seems to be very rare. The occurrence of specific immunoglobulin (Ig) E to Fel d 2 without sensitization to Fel d 1 could be a marker of cross-reactivity, and the primary sensitization source should be searched for.[5]

Evaluation of specific IgE through microarray technique ImmunoCAP ISAC (ThermoFisher Scientific, Massachusetts, USA) might be useful to define the primary allergenic source, particularly if specific immunotherapy is intended. Co-sensitization must be distinguished from cross-sensitization. It is important to emphasize that IgE-cross-reactivity may not always imply clinical cross-reactivity. Exposure to furry animals can lead to different sensitization patterns with different clinical implications. The dose of exposure is also important.[4,7]

Therefore, this study aimed to investigate the pattern of sensitization to domestic animals using the ImmunoCAP ISAC method.

Methods

The sample in this retrospective study comprised all patients in the age range of 0-18 years who were followed at our pediatric allergology consultation center, from April 2015 to September 2019, and underwent a specific serum IgE antibodies test using the 112 component ImmunoCAP ISAC allergen microarray immunoassay. The amount of ISU/L ≥ 0.3 were considered positive. The present study focused on the group of animal-derived molecules: cat (Fel d 1, Fel d 2, Fel d 4), dog (Can f 1, Can f 2, Can f 3, Can f 5), horse (Equ c 1, Equ c 3), and cow (Bos d 6). Epidemiological, clinical, and laboratory variables were assessed as well (Table 1).

Results

A total of 101 individuals were included in this study, with a median age of 8 years and 4 months, of whom 62/101 (61.3%) were male. It was found that 42/101 (41.6%) had sensitization to at least one major animal-specific allergen (Table 2).

A total of 42 patients, including 23 (54%) males, were sensitized to at least one major animal-specific allergen and had a mean age of 8 years and 8 months. In this sample, 24/42 (54.8%), 12/42 (28.6%), and 7/42 (16.7%) patients were monosensitized, bi-sensitized, and polysensitized, respectively (Table 3). Sensitization to

Table 1. Known allergens of cat, dog, and horse

Allergen source	Allergen	Biochemical name	Allergen category	Tested in ISAC
Dog (*Canis familiaris*)	Can f 1	Lipocalin	Major	Yes
	Can f 2	Lipocalin	Major	Yes
	Can f 3	Serum albumin	Minor	Yes
	Can f 4	Lipocalin	Major	No*
	Can f 5	Kallikrein	Major	Yes
	Can f 6	Lipocalin	Major	No*
Domestic horse	Equ c 1	Lipocalin	Major	Yes
	Equ c 2	Lipocalin	Minor	No
(*Equus caballus*)	Equ c 3	Serum albumin	Minor	Yes
	Equ c 4	Latherin	Minor	No
Cat	Fel d 1	Uteroglobin	Major	Yes
	Fel d 2	Serum albumin	Minor	Yes
(*Felis domesticus*)	Fel d 3	Cystatin	Minor	No
	Fel d 4	Lipocalin	Major	Yes
	Fel d 5	Immunoglobulin A	Minor	No
	Fel d 6	Immunoglobulin M	Minor	No
	Fel d 7	Lipocalin	Major	No
	Fel d 8	Latherin-like protein	Minor	No

*Animal-derived molecules added to ImmunoCAP ISAC tests performed from 2020 onwards

Table 2. Demographic and clinical data of the study sample

Variable	n (%)
Total participants	101
Gender	101 (100)
Female	39 (38.6)
Male	62 (61.4)
Age (years) (median ± median absolute deviation)	8.3 ± 3.5
Sensitized to at least one specific major animal allergen	42 (41.6)
Non-sensitized	59 (58.4)
Asthmatics	29 (28.7)
Sensitized to at least one specific major animal allergen	21 (72.4)
Non-sensitized to animals	8 (27.6)
Simultaneous sensitization to pollens, mites, and fungi	10 (9.9)
Sensitized to at least one specific major animal allergen	8 (80.0)
Non-sensitized to animals	2 (20.0)

Table 3. Results of the sensitization to pets

Variable	n (%)
Total participants sensitized to at least one specific major animal allergen	42
Gender	42 (100)
Female	19 (45.2)
Male	23 (54.8)
Age (years) (median ± median absolute deviation)	8.7 ± 7.7
Sensitization	
Monosensitized	23 (54.8)
Bisensitized	12 (28.6)
Polysensitized	7 (16.7)
Symptomatic due to contact with pets	25 (59.5)
Indoor	5 (11.9)
Outdoor	6 (14.3)
Occasionally (two or more times in a month)	13 (31.0)
Total eviction	1 (0.02)
Had eczema	35 (90.0)

pets was observed in 42 children, and 25 children were symptomatic. An association between symptomatic patients and frequency of contact with pets could not be established (Tables 3 and 4).

As can be observed in Fig. 1, this study detected sensitization to dogs in 25/101 (24.8%) patients, with Can f 1 being the most frequent molecular allergen detected in 17/25 (68%) patients, followed by Can f 5 in 10/25 (40%) patients, six of whom were female.

As illustrated in Fig. 2, 37/101 (36.6%) patients presented sensitization to cats, with Fel d 1 being the most frequently detected molecular allergen (33/37, 89.2%). All cases sensitized to Fel d 4 (n = 5) were also sensitized to Fel d 1, and all cases sensitized to Equ c 1 (n = 4) were sensitized to Fel d 4 as well (not illustrated).

It was observed that 34/42 (81%) patients with sensitization to at least one major animal-specific allergen had eczema. Furthermore, all of those who were

Table 4. ImmunoCAP ISAC results and clinic

	ISAC results	Number of individuals	
		Had asthma	
Levels of molecular allergens detected*	< 0.3 = Undetectable	0	0
	0.3-0.9 = Low	2	1
	1-14.9 = Moderate to high	28	11
	≥ 15 = Very high	12	9
	Total	42	21

* ISAC standardized units (ISU-E).

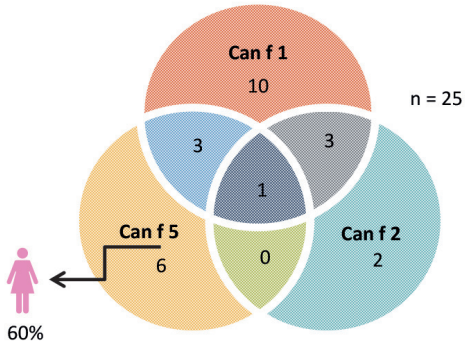

Figure 1. Results of sensitization to dogs. A major sensitization to dogs was detected in 25/101 (24.8%) patients. Can f 1 was the most detected allergen (17/25, 68%), followed by Can f 5 (10/25 patients, 40%, six of whom were female).

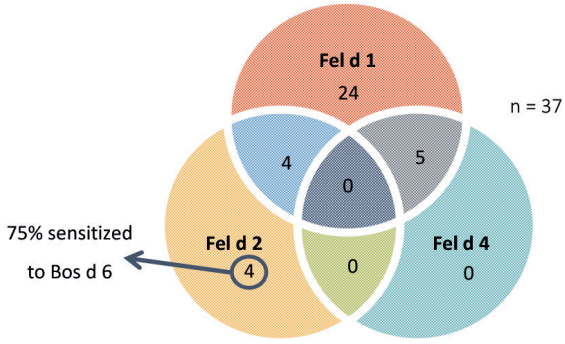

Figure 1. Results of sensitization to cats. Sensitization to cats was detected in 37/101 (36.6%) patients. Fel d 1 was the most detected molecular allergen (33/37, 89.2%) and all cases sensitized to Fel d 4 (n = 5) were also sensitized to Fel d 1. Half of the cases sensitized to Fel d 2 (4/8, 50%) were not sensitized to Fel d 1, most (3/4, 75%) of whom presented sensitization to Bos d 6 as well.

sensitized to Fel d 2 had eczema (8/8, 100%), half of them were not sensitized to Fel d 1, of whom the majority presented sensitization to Bos d 6 (3/4, 75%), in a context of clinically relevant allergy to cow milk (Fig. 2).

Asthma was found in 29/101 (28.7%) children, of whom 21/29 (72.4%) were sensitized to pets, and 16/21 (76.2%) presented with symptomatology before the age of five. It was also possible to conclude that all of the individuals polysensitized to major animal-specific allergen were asthmatic as well (7/7, 100%). Almost all (28/29, 96.6%) asthmatic patients sensitized to pets showed moderate to very high IgE levels detected in ImmunoCAP ISAC results (Table 4).

Simultaneous sensitization to mites, pollens, and fungi occurred in 10/101 (9.9%) of the population 8/10 (80%) of whom were sensitized to pets and 5/8 (62.5%) were asthmatic (Table 2).

Discussion

Although molecular-based allergy diagnosis is a complex area of study, it provides novel and relevant information, and will soon become a standard tool in the allergist armamentarium.[8] Thanks to the ImmunoCAP ISAC method, it was observed that monosensitization to major animal-specific allergens was more common than polysensitization and that Fel d 1 and Can f 1 were the molecular allergens more frequently identified in our population, which was in line with the literature.[8]

Female patients sensitized to Can f 5 may present allergic reactions to human seminal fluid due to cross-reactivity. This is an important finding which may warrant an explanation for those with this sensitization. It can also be highlighted that the majority of patients sensitized to Can f 5 can tolerate neutered or female dogs.[9]

Serum albumins are considered to be an uncommon cause of allergic sensitization. It is described an association between high levels of IgE to Fel d 2 and atopic dermatitis.[10] In our study all the patients who were sensitized to Fel d 2 had eczema. Additionally, the occurrence of specific IgE to Fel d 2 without sensitization to Fel d 1 could be a marker of cross-reactivity to another animal and not a primary sensitization to cats.[5] Many different syndromes and associations due to cross-reactivity between aeroallergens and food allergens of animal origin have been described. It is important to underline the impact of cross-reactivity between aeroallergens and food allergens with or without clinical relevance.[11] In this study, 75% of the patients sensitized to Fel d 2 and non-sensitized to Fel d 1 showed sensitization to Bos d 6. All of these patients were younger than 6 years old and allergic to cow milk.[12] In this context, Bos d 6 and Fel d 1 could be the primary sensitizer and a marker of cross-reactivity, respectively.[4]

ImmunoCAP ISAC provides an *in vitro* quantitative measurement of IgE in human serum which explains how IgE antibody developments can be detected at an early stage, indicating that sensitization has been developed even before clinical symptoms. Higher ISAC standardized units (ISU-E) indicate a higher degree of sensitization, which means higher levels of specific IgE antibodies to common inhalant allergens.[8,13] In this

study, almost all asthmatic patients sensitized to pets had moderate to very high levels of IgE antibody.

This study confirms that sensitization to pets is a representation of a more complex and serious phenotype of allergic diseases. In fact, the majority of the asthmatic patients in this study were sensitized to pets, and all of those polysensitized to major animal-specific allergens were asthmatic as well. Moreover, the majority of the population who were polysensitized to aeroallergens (including pets, mites, pollens, and fungi) had asthma too. These results match closely with those reported in the literature, in which multiple sensitizations towards lipocalins, kallikrein, and uteroglobin components have been associated with more serious cases, especially those with asthma.[7,14-17]

Author Contribuitions

JPO participated in the study conception or design. JPO participated in acquisition of data. JPO participated in the analysis or interpretation of data. IPR, TM, MC and MQ participated in the drafting of the manuscript. MS, TM, MC and MQ participated in the critical revision of the manuscript. All authors approved the final manuscript and are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflicts of Interest

The authors declare that there were no conflicts of interest in conducting this work.

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Protection of human and animal subjects

The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki 2013).

Provenance and peer review

Not commissioned; externally peer reviewed

Confidentiality of data

The authors declare that they have followed the protocols of their work centre on the publication of patient data.

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Pode a Alergologia Molecular Ser uma Abordagem Útil na Alergia a Animais de Estimação?

Introdução: A presença crescente de cães e gatos nos lares, associada a níveis significativos dos respetivos alergénios em locais sem presença animal (escolas, infantários, locais de trabalho), tem contribuído para um aumento da prevalência de alergia a estes animais nos países industrializados.

O objetivo deste estudo foi avaliar a prevalência de sensibilização alergénica a animais domésticos através do método *ImmunoCAP* ISAC.

Métodos: Estudo retrospetivo de doentes dos 0-18 anos de idade, seguidos na Consulta de Pediatria-Alergologia que realizaram *ImmunoCAP* ISAC entre abril de 2015 e setembro de 2019. Dados recolhidos por consulta dos processos clínicos, tendo sido analisadas variáveis epidemiológicas, clínicas e laboratoriais.

Resultados: Foram incluídos 101 doentes no estudo, com mediana de idade de 8 anos e 4 meses, 62/101 (61,3%) do género masculino, 42/101 (41,6%) sensibilizados a pelo menos um alergénio específico major animal, dos quais 23/42 (54,8%) monossensibilizados, 12/42 (28,6%) bissensibilizados e 7/42 (16,7%) polissensibilizados.

Foi detetada sensibilização *major* ao cão em 25/101 (24,8%) doentes, sendo que o alergénio molecular mais frequentemente detetado foi Can f 1 (17/25, 68%), seguido de Can f 5 (10/25, 40%, 6 dos quais do género feminino).

No que toca ao gato 37/101 (36,6%) doentes demonstraram sensibilização a este, sendo o alergénio molecular mais detetado o Feld 1 (33/37, 89,2%). Todos os casos sensibilizados a Fel d 4 (n=5) também estão a Fel d 1. Metade dos sensibilizados a Fel d 2 (n=8) não estão sensibilizados a Fel d 1, sendo que destes, 3 (75%), apresentam sensibilização a Bos d 6, em contexto de alergia a proteínas do leite de vaca. Todos os sensibilizados a Equ C 1 (n=4) estão sensibilizados a Fel d 4.

Eram asmáticos 29/101 (28,7%) dos doentes e a maioria sensibilizada a animais de estimação (21/29, 72,4%), sendo de referir que 100% (7/7) dos polissensibilizados a alergénicos específicos *major* animal são asmáticos.

Também constatamos que os doentes com sensibilização simultânea a outros aeroalergénios (ácaros, pólenes e fungos) também se encontravam sensibilizados a animais domésticos, sendo a maioria asmática.

Discussão: Verificamos que a monossensibilização a alergénio específico *major* animal foi mais comum que a polissensibilização. os alergénios moleculares mais frequentemente envolvidos foram o Fel d 1 e o Can f 1.

A maioria dos doentes asmáticos do nosso estudo estava sensibilizada a animais domésticos e todos os doentes polissensibilizados a alergénio específico *major* animal eram também asmáticos. Tal indica que a sensibilização a animal doméstico confere um fenótipo mais complexo e grave da doença alérgica. Além disso, a maioria dos doentes com sensibilização simultânea a outros aeroalergénios (ácaros, pólenes e fungos) também se encontravam sensibilizados a animais domésticos e eram asmáticos.

Palavras-Chave: Adolescente; Alérgenos/efeitos adversos; Animais de Estimação; Asma/etiologia; Criança; Hipersensibilidade/diagnóstico; Hipersensibilidade/etiologia; Lactente