

Analysis of Sensitization Profiles in Central European Allergy Patients Focused on Animal Allergen Molecules

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Keywords

Sensitization profile · Molecular diagnostics · Animal allergen · Animal allergy · Specific IgE

Abstract

Introduction: Frequently observed multiple sensitizations to several animals highlights the importance of a molecular diagnosis, distinguishing between sensitizations specific to single species and sensitizations due to cross-reactivity. **Objective:** The aim of our study was to assess the usefulness of a molecular diagnosis in the description of sensitization profiles in allergy patients living in Central Europe, with a particular focus on animal-derived molecules. **Methods:** The molecular diagnosis was performed using the ImmunoCAP ISAC microarray. Results of 1,255 allergy patients were subjected to statistical analysis. **Results:** The highest sensitization rates were observed for uteroglobin Fel d 1 (31.8%) and kallikrein Can f 5 (16.4%), followed by animal lipocalins Can f 1 (13.9%), Equ c 1 (6.2%), Fel d 4 (5.3%), Can f 2 (4.2%), and Mus m 1 (4.1%). Sensitization rates to serum albumins Fel d 2, Can f 3, Equ c 3, and Bos d 6 were very low, with the highest being 3.2% to Fel d 2. Detailed subanalysis confirmed the dominant role of Fel d 1 or Can f 5 and/or Can f 1 in cat- or dog-sensitized

patients, respectively. Further analysis focused on lipocalins and albumins confirmed a high rate of cosensitizations within both groups. **Conclusion:** The sensitization to animal allergen molecules is very frequent in Central Europe. The most common is sensitization to species-specific cat uteroglobin Fel d 1 and dog kallikrein Can f 5, followed by sensitizations to animal lipocalins. Our data suggest that commonly observed multiple sensitizations detected by extract approach can be explained not only by true cosensitization, but also by cross-reactivity, mainly in the frame of lipocalins. Cross-reactive serum albumins are minor sensitizers and are probably not important from this point of view.

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Introduction

Pets, in particular cats and dogs, are an important source of indoor allergens [1]. In addition to residential environments, exposure to pet allergens can occur in occupational and leisure environments (i.e., animal facili-

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Table 1. Characteristics of animal allergens involved in the study

Allergen	Protein family	MW, kDa	Species specificity/potential cross-reactivity patterns based on high-sequence homology
<i>Cat</i>			
Fel d 1	Secretoglobulin	38	Species-specific
Fel d 2	Serum albumin	69	Cross-reactive with Can f 3 (dog), Equ c 3 (horse), Bos d 6 (bovine)
Fel d 4	Lipocalin	22	Cross-reactive with Equ c 1 (horse), Can f 6 (dog), Mus m 1 (mouse)
<i>Dog</i>			
Can f 1	Lipocalin	23–25	Cross-reactive with Fel d 7 and human tear lipocalin
Can f 2	Lipocalin	19	Limited patient-dependent cross-reactivity with Fel d 4
Can f 3	Serum albumin	69	Cross-reactive with Fel d 2 (cat), Equ c 3 (horse), Bos d 6 (bovine)
Can f 5	Kallikrein	28	Species-specific, homology with human prostate-specific antigen
<i>Horse</i>			
Equ c 1	Lipocalin	25	Cross-reactive with Fel d 4 (cat), Can f 6 (dog), Mus m 1 (mouse)
Equ c 3	Serum albumin	67	Cross-reactive with Fel d 2 (cat), Can f 3 (dog), Bos d 6 (bovine)
<i>Mouse</i>			
Mus m 1	Lipocalin	17–19	Fel d 4 (cat), Can f 6 (dog), Equ c 1 (horse)
<i>Bovine</i>			
Bos d 6	Serum albumin	66	Fel d 2 (cat), Can f 3 (dog), Equ c 3 (horse) and human serum albumin

Adapted from Konradsen et al. [1], Dávila et al. [3], and Matricardi et al. [8].

ties, stables, pet shops, and farms). Besides these places, the allergen quantities derived from animals might also be clinically significant at public places, i.e., schools, on public transport, etc., due to passive transfer [2]. The prevalence of allergy to pets is increasing, and allergy to cats and dogs is considered an important risk factor for the development of asthma and rhinitis [1].

All patients with allergic airway or skin disease should be evaluated for sensitization to animal allergens. Standard diagnostic tests (skin prick test and specific IgE measurement) are still based on animal (cat, dog, horse, mouse) diagnostic extracts. Unfortunately, these tests very often result in multiple positivities, and correct interpretation is often challenging. Nowadays, especially in these polysensitized patients (detected by extract approach), it is strongly recommended to perform a molecular diagnosis [3]. This method helps to distinguish between sensitizations specific to single species and sensitizations due to cross-reactivity. Sensitization to animals can be induced not only by species-specific allergens, but also by a cross-reaction mechanism involving some families of allergenic proteins. In this context, an examination of specific IgE to a broader spectrum of animal allergens by using a molecular diagnosis might be very useful to assess the possibility of cross-reactions between the allergens of different animals [4]. Moreover, it

might even be useful for predicting clinical symptoms and their severity [5–7].

A number of pet allergens have been described so far. Major mammalian allergens come from three protein families – the lipocalin, secretoglobulin, and kallikrein families. Serum albumins are considered minor allergens [8]. While cat uteroglobin Fel d 1 and dog kallikrein Can f 5 do not display significant cross-reactivity with other animal allergens and are conclusively species specific, serum albumins and some lipocalins often display cross-reactivity within relevant protein families and likely play a role as cross-reacting allergens in individuals sensitized to several types of animal dander [9–12].

The aim of our study was to assess the usefulness of molecular diagnosis in the description of sensitization profiles focused on animal-derived molecules (Table 1). Specifically, we studied sensitization profiles of allergy patients living in the western part of the Czech Republic. It is necessary to bear in mind that in our study we focused only on sensitization rates and not their clinical relevance.

Methods

As in our previous study [13], we retrospectively analyzed sera from 1,766 patients who had been examined based on suspicion of allergy at the out-patient service of the Department of Immu-

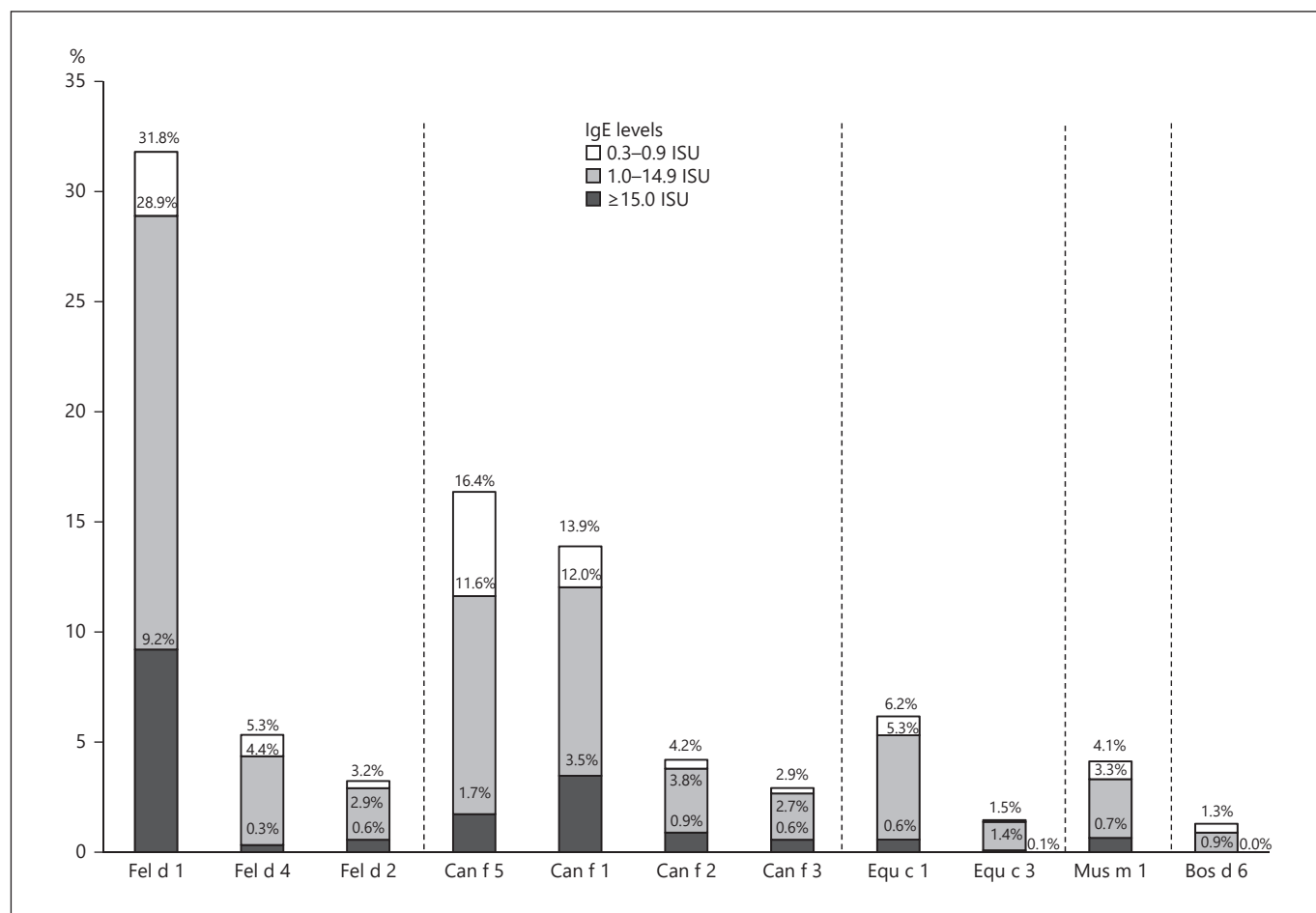


Fig. 1. Sensitization rates to animal-derived molecules.

nology and Allergy of the University Hospital in Pilsen, Czech Republic. The detection of specific IgE to multiple allergen molecules was performed using the 112 component ImmunoCAP ISAC allergen microarray immunoassay (Thermo Fisher Scientific, Uppsala, Sweden). The inclusion criterion was the detection of sensitization to at least one allergen component derived from any allergen source included in the ImmunoCAP ISAC biochip, as these patients were considered atopic. Results ≥ 0.3 ISU/L were considered to be positive. A total of 511 patients without any detectable sensitization were excluded from the study, while 1,255 patients sensitized to at least one allergen component were included. All of them had at least one of the following allergic diseases: chronic rhinitis (73%), bronchial asthma (41%), atopic dermatitis (34%), urticaria or edema (19%), and/or anaphylaxis (11%). Patient ages ranged from 1 to 68 years, with a mean age of 25 years. The sex ratio was 45.3% men to 54.7% women. The results of all these 1,255 allergy patients were subjected to a detailed statistical analysis, 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), mouse (Mus m 1), and cow (Bos d 6).

Results

The results of the analysis describing the sensitization patterns to animal molecules in the group of 1,255 allergy patients in our region are listed below. All percentages were calculated using the whole group of 1,255 patients. Sensitization to at least one animal molecule was detected in 43.6% of patients.

Analysis of Cat-Derived Molecules

Sensitization to at least one cat molecule (Fel d 1, Fel d 2, Fel d 4) was observed in 34.2% of our allergy patients. Sensitization rates to Fel d 1, Fel d 2, and Fel d 4 were 31.8, 3.2, and 5.3% (Fig. 1). The most common monosensitization in the frame of cat-derived molecules was to Fel d 1 in 26.7% of patients, while monosensitization to Fel d 4 and Fel d 2 was observed only in 1.6 and 0.9% of patients. The most frequent cosensitization was cosensitization of

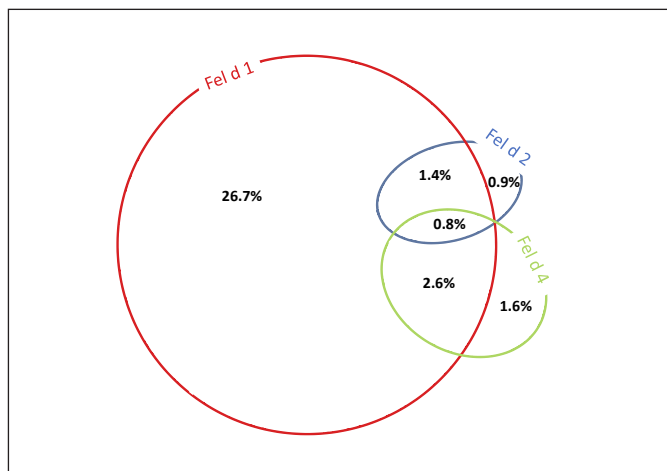


Fig. 2. Venn diagram depicting mono- and cosensitizations to cat-derived molecules. Sensitizations with a frequency of less than 0.3% are not shown.

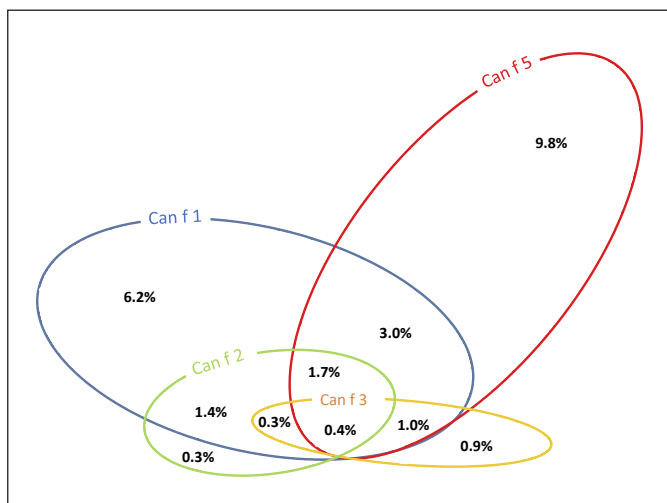


Fig. 3. Venn diagram depicting mono- and cosensitizations to dog-derived molecules. Sensitizations with a frequency of less than 0.3% are not shown.

Fel d 1 with Fel d 4 in 2.6%, followed by cosensitization of Fel d 1 with Fel d 2 in 1.4% of patients. Cosensitization of all three molecules was relatively rare, observed only in 0.8% of cases (Fig. 2).

Analysis of Dog-Derived Molecules

Sensitization to at least one dog molecule (Can f 1, Can f 2, Can f 3, Can f 4, Can f 5) was observed in 25.3% of patients. The sensitization rates to Can f 1, Can f 2, Can f 3, and Can f 4 were 13.9, 4.2, 2.9, and 16.4% (Fig. 1). The most common monosensitization in the frame of dog-derived

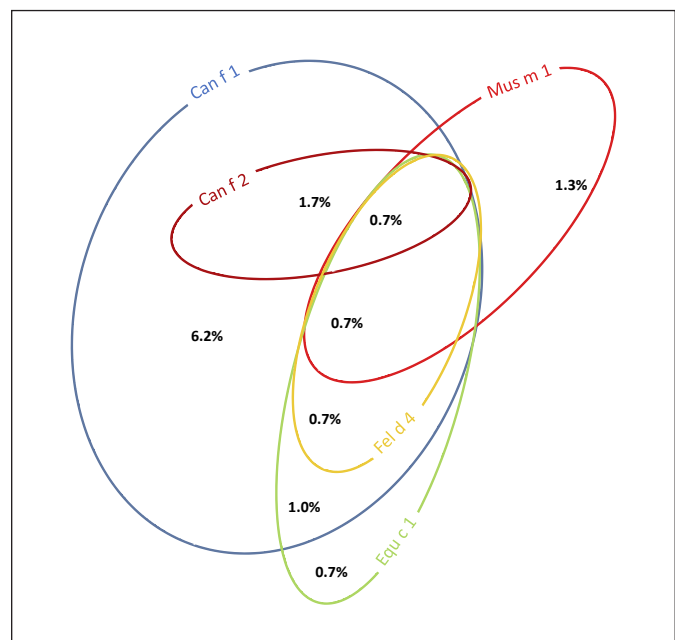


Fig. 4. Venn diagram depicting mono- and cosensitizations to animal lipocalins. Sensitizations with a frequency of less than 0.7% are not shown.

molecules was to Can f 5 in 9.8% of patients. Monosensitization to Can f 1, Can f 2, and Can f 3 was observed in 6.2, 0.3, and 0.9% of patients. Nearly 8% of dog-sensitized patients were sensitized to several dog-derived molecules, most frequently to Can f 1 with Can f 5 in 3% of patients (Fig. 3).

Analysis of Other Animal-Derived Molecules

Sensitization to at least one horse, mouse, and/or cow molecule (Equ c 1, Equ c 3, Mus m 1, Bos d 6) was observed in 9.6% of patients. The sensitization rates to horse Equ c 1 and Equ c 3, to mouse Mus m 1, and to cow Bos d 6 were 6.2, 1.5, 4.1, and 1.3% (Fig. 1). Almost all patients (8.7%) sensitized to these horse-, mouse-, and cow-derived molecules were cosensitized with a cat- and/or dog-derived molecule.

Analysis Focused on Lipocalins and Serum Albumins

The frequency of sensitization to at least one animal lipocalin (Can f 1, Can f 2, Fel d 4, Equ c 1, Mus m 1) was 18.0%. Although cosensitization of more molecules was commonly observed within this family, monosensitizations to single lipocalins were also detected, most frequently in the case of Can f 1 (Fig. 4).

The frequency of sensitization to at least one serum albumin (Can f 3, Fel d 2, Equ c 3, Bos d 6) was 4.3%. Co-

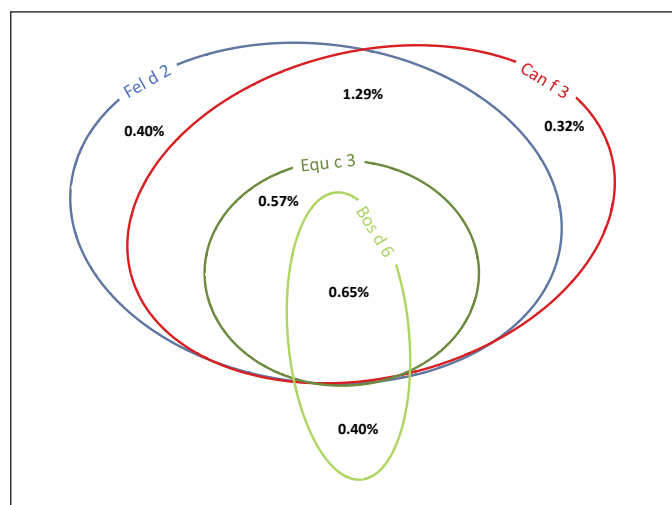


Fig. 5. Venn diagram depicting mono- and cosensitizations to serum albumins. Sensitizations with a frequency of less than 0.3% are not shown.

sensitization of more molecules clearly prevailed within this allergen family, while monosensitizations to serum albumins were rare (Fig. 5).

Discussion

Although the sensitization rate to animal-derived molecules is lower than the sensitization rate to pollen-derived molecules in our group of patients [14], especially relatively high sensitization rates to cat and dog underline their clinical importance in the Central European region. In contrast to some previous studies that showed a lower prevalence of cat allergy than house dust mite allergy [15], we showed approximately the same global sensitization rate for both these allergen sources; sensitization to at least one house dust mite-specific molecule was observed in 32.7% of our patients [13]. The explanation for this finding may be that the climate for mites is not optimal in Central Europe and so the sensitization rate is lower than in other regions. Furthermore, the accumulation of cat and dog allergens in workplaces, schools, and homes (without an animal) is a reflection of the overall prevalence of pet ownership in the community, which is rather high in our region. Recent European statistical data show that the share of households owning at least 1 dog or 1 cat is 41 and 21%, respectively, in the Czech Republic; in other European countries, it ranges from 14 to 42% for dogs and from 11 to 47% in the case of cat ownership [16].

The generally high prevalence of sensitization to cat supports the theory that this sensitization is not dependent on having a cat in the home. The allergen particles are sticky and are carried into other houses as well as public buildings, schools, and work places, etc., and avoiding cats at home in most communities would not decrease the prevalence of sensitization to cats [17]. A recent meta-analysis of pooled results from 11 prospective European birth cohorts concluded that there is no clear evidence for a protective or “harmful” effect of cat ownership on sensitization to animal dander [18]. It appears that there is a significant number of children and young adults living in homes with a cat, who despite being sensitized have no symptoms. This sensitization in childhood may anyway predict the risk of symptoms in adulthood [19].

The role of the major cat-derived molecule Fel d 1 in cat allergy is reported to be very dominant and it corresponds to our data. The vast majority of our cat-sensitized patients were sensitized to this species-specific molecule; moreover, monosensitization to this component was the most frequent in these patients. The importance of another cat-derived molecule, Fel d 4, has been recently demonstrated by its cross-reactivity with dog-, horse-, mouse-, and rat-derived lipocalins [10]. Although a relatively high sensitization rate (63%) to Fel d 4 in Australian cat-allergic patients has been previously described [20], we cannot confirm this finding as in our patients the sensitization rate to Fel d 4 was substantially lower and cosensitization with Fel d 1 was present in the majority of cases. Regardless of the different designs of studies, similar sensitization patterns were also recently observed in other European regions [21, 22]. A Swedish study showed that adults sensitized to Fel d 4 and 2 were more likely to be sensitized to Fel d 1, but not vice versa, which is similar to our study. In a German study, all patients sensitized to Fel d 4 and Fel d 2 where cosensitized with Fel d 1. In the light of these findings, Fel d 1 seems to be the most relevant cat-derived molecule from a diagnostic point of view. On the other hand, from a clinical point of view, although Fel d 1 is the most prevalent cat allergen, cosensitization with Fel d 4 seems to be more associated with asthma [6]. This suggests that sensitization to Fel d 1 may represent the original sensitization to a cat, and sensitization to Fel d 4 a further evolved immune response with a higher probability of allergic disease [6]. This hypothesis is based on the concept of “molecular spreading” described for grass-pollen components [23] and mites [24]. Unfortunately, we could not confirm this theory as our study was focused only on sensitization rates and not on their clinical relevance. Nevertheless, the published data

stress the importance of a molecular diagnosis using a broader spectrum of cat-derived molecules not only in detecting possible sensitizations to other animals by cross-reacting mechanisms [4], but also in predicting clinical symptoms and their severity [6, 7].

Dog allergens, like cat allergens, belong to the allergens with possible passive transfer to homes without an animal. Dog sensitization is definitely more complex and involves more molecules in comparison to cat allergy, where Fel d 1 clearly dominates. The major dog allergens are Can f 1 and Can f 5 [25], which was also confirmed by our study. The majority of our dog-sensitized patients were sensitized to Can f 5 and/or Can f 1, sometimes in cosensitization with other molecules, especially with Can f 2. Sensitization to Can f 2 was almost exclusively seen as cosensitization to Can f 1. Similar sensitization patterns were also observed in a recent Swedish study, where the most common overlap was between Can f 5 and Can f 1/Can f 2 (Can f 1 and Can f 2 were combined in that study given their similarity in structure) rather than with Can f 3, and only a minority of subjects were simultaneously sensitized to all dog components [21], as was also shown in our study. Similarly, as in the case of cat allergy, it has been shown that sensitization to more than one molecule from the same animal represents a greater risk of asthma [6]. In dog-sensitized Swedish children, cosensitization to Can f 5 and Can f 1/Can f 2 conferred the greatest risk for asthma [6].

Furthermore, more complex sensitization profiles in dog-sensitized patients may be one of the causes of poor clinical efficacy of dog immunotherapy based on conventional allergenic extracts [26]. It has been shown that the quality of such extracts is frequently problematic, and generally dog extracts vary greatly in terms of allergenic content [27]. From this point of view, in accordance with our data, it is necessary to point out the quantification of both Can f 1 and Can f 5 in dog therapeutic and diagnostic extracts.

Sensitizations to other animal-derived molecules (horse, mouse, cow) were not common in our group. Sensitizations to horse lipocalin Equ c 1 and mouse lipocalin Mus m 1 were relatively the most frequent. Sensitization to both these molecules was almost exclusively observed in cosensitization with cat and dog, supporting cross-reactivity within the lipocalin protein family [10, 11, 25]. Initially, lipocalins were considered as species-specific markers, as they only display little sequence identity (between 20 and 30%) and the existence of cross-reactivity among them is supposed to be unlikely. However, further studies showed that some lipocalins have much higher sequence identities, which can be as high as 67% [8]. Rep-

resentatives of this highly cross-reactive group are mainly Fel d 4, Can f 6, Equ c 1, and Mus m 1 [10, 11]. Our data support this cross-reactive mechanism, as we confirmed a high degree of cosensitizations between lipocalins; for example, all patients sensitized to Fel d 4 were cosensitized to Equ c 1, Can f 1, and frequently also to Mus m 1. Sensitization rates to serum albumins were generally very low in our group, as was the case in a Swedish study, where sensitization to serum albumins was also uncommon and not associated with asthma [6].

Conclusion

Sensitization to animal-derived allergen molecules is very common in the Central European region. Sensitizations to species-specific cat uteroglobin Fel d 1 and dog kallikrein Can f 5 are dominant, followed by sensitizations to animal lipocalins. The sensitization rate to serum albumins is very low. Our data suggest that commonly observed multiple sensitizations to animal allergens (cat, dog, horse, mouse) detected by an extract approach can be explained not only by true cosensitization, but also by cross-reactivity in the frame of lipocalins. Cross-reactive serum albumins are minor sensitizers and are probably not important from this point of view.

Statement of Ethics

The study was approved by the Ethics Committee of the Faculty Hospital in Pilsen. Data were anonymized before the analysis.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

M.V. participated in study design, data analysis, and manuscript writing. P.P. participated in study design, data analysis, and manuscript writing. T.V. participated in laboratory analyses, anonymization of the data, and critical revision of the manuscript. P.V. participated in data analysis and critical revision of the manuscript.

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