

Component-resolved allergy diagnosis: a new era?*

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Samenvatting

Componentgeoriënteerde allergiediagnostiek vormt ongetwijfeld een diagnostische aanwinst. De laatste jaren is immers in toenemende mate gebleken dat de techniek niet alleen een betrouwbaar instrument vormt voor de diagnosestelling van IgE-gemedieerde allergieën, maar ook de mogelijkheid biedt tot het opstellen van individuele prognostische sensibilisatieprofielen, het discrimineren tussen klinisch relevante en irrelevante specifieke IgE (sIgE) antistoffen en optimaliseren van behandelingsstrategieën zoals diëten en allergeenspecifieke immunotherapieën. Bovendien heeft de techniek ook al bijgedragen tot de identificatie van geografisch- en leeftijdsafhankelijke sensibilisatiepatronen. Of componentgeoriënteerde allergiediagnostiek verder zal doorbreken dankzij de ontwikkeling van nieuwe technologieën zoals microarrays blijft onduidelijk. Vast staat dat de techniek, om tot de dagelijkse praktijk te kunnen doordringen, verder voldoende klinisch gevalideerd dient te worden. Ook mag men zich vandaag nog niet laten verleiden om componentgeoriënteerde allergiediagnostiek als gouden standaard te beschouwen en de klassieke sIgE doseringen te verlaten.

Zoals uit het overzicht zal blijken, blijkt steeds meer dat er met betrekking tot verschillende allergieën duidelijke geografische verschillen bestaan. Daarom zal ook onze eigen ervaring beschreven worden omdat dit het meest relevant lijkt voor de Belgische praktijkvoering.

Summary

During the last decades a new *in vitro* technique has entered the field of allergy diagnosis, that is, component-resolved allergy diagnosis (CRD). In contrast to traditional specific IgE (sIgE) assays, CRD does not rely upon whole extract preparations from native allergens but on quantification of sIgE antibodies to single protein components, purified from natural sources or obtained by recombinant techniques. At present, it emerges that CRD can improve management of the allergic patient as it allows (to some extent) to discriminate between clinically significant and irrelevant sIgE result and to establish sensitization patterns with particular prognostic outcomes. Nevertheless, further clinical validation is mandatory before the technique can enter mainstream application. By no means can CRD currently be considered as a substitute to traditional sIgE assays.

Introduction

IgE-mediated allergies constitute an important and increasing health problem with significant morbidity and potential mortality. Correct diagnosis with identification of all offending allergens is a prerequisite for appropriate and potentially lifesaving preventive measures or initiation of allergen-specific immunotherapy. At present, physicians generally rely upon a thorough history, quantification of allergen-specific IgE (sIgE) antibodies and skin tests to confirm their clinical diagnosis. However, since none of these tests demonstrates an absolute predictive value, diagnosis is not always straightforward.

This “state of the art lecture” focuses on the principles and applications of CRD by traditional singleplexed assays as well as by microarray technology. Particular focus is put on our personnel experience with CRD, as it is clear that sensitization patterns can exhibit geographical differences with distinct clinical and prognostic characteristics.

Historical perspective and principles of CRD

For over a century *in vivo* and *in vitro* diagnosis of IgE-mediated allergy has been established with (protein) extracts prepared from putative natural allergens. Since these extracts can contain variable amounts of sometimes instable genuine and cross-reactive allergenic or non-allergenic components, correct interpretation of results is not always straightforward. For example, it has been repeatedly shown that ubiquitous structures such as profilins and cross-reactive carbohydrate determinants (CCD) present on glycoproteins of plants and hymenoptera venom frequently elicit false-positive outcomes of the serologic diagnosis of allergic diseases with a broad spectrum of cross-reactivity involving pollen, *Hevea* latex, plant food, and venoms extending beyond clinical relevance, as these antibodies rarely give rise to clinically relevant allergy [1-6].

Recent advances in biochemistry and molecular biology allowed the identification, cloning, and recombinant production of relevant allergen proteins and epitope-emulating peptides, enabling detection and quantification of IgE antibodies to these proteins or sequential epitopes, that is, CRD. In contrast to traditional sIgE assays, CRD does not rely upon whole extract preparations

but on quantification of sIgE antibodies to single components, purified from natural sources or obtained by recombinant techniques. There are various databases on allergen components and protein families, such as the official “International Union of Immunological Societies (IUIS)” allergen nomenclature database that is available from <http://www.allergen.org> and the “Allfam” allergen database grouping allergens into protein (sub) families (<http://www.meduniwien.ac.at/allergens/allfam>). Table 1 displays the most relevant specific (unique) and cross-reactive plant and animal allergen components for our regions. In the IUIS nomenclature the first 3 letters of an allergen component refer to the genus followed by the first (or first two) letters of the species and a Roman numeral to indicate the component in the chronological order of purification. Thus, the first allergen component identified in house dust mite (*Dermatophagoides pteronyssinus*) is designated as Der p 1. The prefix “n” denotes a purified component whereas “r” denotes a recombinant protein.

The principles, potential, and pitfalls of CRD have recently been comprehensively described elsewhere [7-10]. Briefly, CRD involves specific (unique) marker components and substructures to investigate the genuine sensitization of patients towards a given allergen as well as cross-reactive determinants or components, such as CCD or panallergens that point to cross-sensitization to several even unrelated allergen sources. As will be exemplified below, these “gatekeeper” tests not only allow to establish individual primary and cross-reactive sensitization profiles (which parts of allergen(s) are recognized by an individual patient), but can also add to discriminate between genuine allergy and sensitization. Furthermore, CRD enables an individual risk assessment of severity and possibly prediction of persistence, facilitates the selection of patients for allergen-specific immunotherapy, and may eventually help in monitoring the immunological effects of a treatment. Today, CRD techniques can be performed using traditional singleplexed methods and a multiplexed microarray assays (for review [11-13]).

Clinical applications

Inhalant allergens

The most common inhalant allergens in our regions are house dust mite (*Dermatophagoides pteronyssinus*), pollen (grasses, trees and weeds), epithelia from cat and dogs and cockroach. Diagnosis of these inhalant allergies is generally straightforward and relies upon a thorough history documented by quantification of sIgE and/or skin prick tests using extracts prepared from putative natural allergens. In our opinion, the major indication for CRD in inhalant allergy is facilitating and optimizing selection of patients that need grass or tree pollen-specific immunotherapy (hyposensitization) in which traditional tests fail to discriminate between genuine co-sensitization (needing immunotherapy with both allergens) and cross-sensitization (immunotherapy to be restricted to sensitizing allergen). In these cases the selection of patients for immunotherapy with birch (*Betula verrucosa*) and grass pollen allergens could include the major birch pollen allergen Bet v 1 [14;15] and a combination of the two major timothy grass (*Phleum pratense*) pollen allergens, Phl p 1 and Phl p 5 [16-18]. In an own survey including 206 patients inhalant allergy documented by a skin test, 93% of patients with birch pollen allergy (n=129) was sensitized to Bet v 1, whereas 89% of grass pollen allergic patients (n=126) was sensitized to Phl p 1 and/or Phl p 5 (fig 1a). Jahn-Schmid *et al* [19] demonstrated a high correlation between conventional singleplexed CAP/RAST methods a microarray technology to detect sIgE to recombinant birch and timothy grass pollen components. Finally, Gadermaier *et al* [20], in their population observed that almost 90% of patients with mugwort (*Artemisia vulgaris*) allergy reacted to Art v 1 and/or Art v 3.

Theoretically CRD can also be applied for other inhalant allergens such as house dust mite [21] and epithelia from cat [22] and dog [23] and cockroach [24;25]. However, this will be rather rarely indicated in daily practice, as diagnosis of these allergies is readily established by traditional sIgE quantification and/or skin testing. From the survey mentioned above, it emerged that 73% of patients with a house dust mite allergy (n=101) was sensitized to Der p 1, Der p 2 or Der p 10. Eighty-one percent of the patients with cat (*Felis domesticus*) allergy (n=93) demonstrated IgE reactivity to Fel d 1, Fel d 2 or Fel d 4, whereas 45% of dog (*Canis familiaris*)

allergic patients (n=66) was sensitized to Can f 1, Can f or Can f 4 (fig 1b). Alternatively, Fel d 1 sensitization has been detected years for clinical cat allergy [26] and whereas high titres of anti Fel d 1 antibodies have been demonstrated to constitute a risk factor for asthma in cat allergic patients [27].

Food allergens

Plant-derived

Diagnosis of food allergy, particularly from plant-derived food, constitutes one of the major applications of CRD. First, it has repetitively been shown that sensitization to ubiquitous structures such as profilins and cross-reactive carbohydrate determinants (CCD) present on glycoproteins of plants can severely affect the specificity of traditional sIgE results [1-6]. In our region, such anti-profilin and anti-CCD sIgE antibodies are found in about 25-30% of patients with a grass pollen allergy [3] and display a very broad spectrum of cross-reactivity involving pollen, latex, fruit, vegetables, nuts and seeds. Second, CRD enables to establish individual sensitization profiles with prognostic implications. For example, a sensitization to prolamins (e.g. nonspecific lipid transfer proteins (nsLTP), 2S albumins and α -amylase/trypsin inhibitors), cupins (e.g. vicilins and legumins) is frequently associated with more severe potentially life-threatening allergy, whereas cross-reactivity to labile Bet v 1 homologues generally results in a bothersome oral allergy syndrome (OAS) (fig 1). This “state of the art” review will focus on peanut (*Arachis hypogaea*: Ara h) and hazelnut (*Corylus avellana*: Cor a).

For peanut it has emerged that sensitization to the 2S albumin Ara h 2 and Ara h 6 is related to a more severe prognosis [28-30]. Also sensitization to the vicilin Ara h 1, legumin Ara h 3 and nsLTP Ara h 9 is associated to more severe reactions [29;31]. In contrast, a unique sensitization to Ara h 8, the Bet v 1 homologue in peanut, is generally characterized by an OAS but systemic reactions can occur [32]. A positive sIgE peanut result without sIgE reactivity to Ara h 1, 2, 3, 8 and 9 could relate to a sensitization to CCD without overt peanut allergy. Recently, it has been demonstrated that peanut allergy can exhibit clear geographic sensitization patterns with

sensitization to Ara h 1, 2 and 3 being prevalent in Northern Europe and the USA, whereas in Southern Europe the majority of patients is sensitized to the nsLTP Ara h 9 [33;34]. In our series 44 of 51 (86%) patients with severe peanut allergy demonstrated sensitization to Ara h 1 and/or 2 and/or 3 and 3 patients (6%) were monosensitized to Ara h 8 (unpublished data) and in 4 (8%) no sensitization to the components currently spotted on the micro-array (Ara h 1, 2, 3, 8) was demonstrable.

Several studies, including 2 of our own research group, have revealed that also hazelnut allergy exhibits different sensitization patterns with distinct clinical characteristics. As in peanut allergy, sensitization to the labile Cor a 1.04 (the Bet v 1 homologue in hazelnut) is generally associated with a mild OAS and is mostly observed in adults from birch endemic regions [35]. In contrast, the more severe, systemic reactions are generally unrelated to an underlying birch pollen allergy and rely upon a sensitization to the hazelnut nsLTP (Cor a 8) [35] or the 11S legumin-like seed storage protein Cor a 9 [35]. In an own survey 65% of preschool children (median 2 years, 0-6) and half of school children (7-18 years) were sensitized to Cor a 9. This sensitization was invariably associated with severe symptoms [36]. Moreover, sensitization to this potential dangerous component was already demonstrable in 60 of infants (<12 months) with atopic dermatitis that demonstrated a positive sIgE for hazelnut during routine screening [37]. Another important observation from these 2 surveys is that using the currently available microarray, 40% of the pollen-unrelated hazelnut allergic patients and infants with atopic dermatitis failed to demonstrate sIgE reactivity to Cor a 1.01 from hazel pollen, Cor a 1.04, Cor a 8, or profilins and 14% of the patients with systemic reactions seem solely sensitized to Cor a 1.04. This, probably reflects that the panel of purified and recombinant proteins spotted on the current microarray does not cover the entire hazelnut IgE response and that additional components such as the vicilin Cor a 11 [38] and 2S albumin Cor a 14 [39] might be necessary to establish correct diagnosis in some cases.

Unfortunately, CRD does not always enable to discriminate between clinical relevant and irrelevant sIgE results. For example, on several occasions we have demonstrated that the technique does not allow to establish the clinical relevance of positive results for Bet v 1 homologues such as Mal d 1 from apple (*Malus domestica*) [40] and Cor a 1 from hazelnut

[36]. In such cases only functional tests such as skin tests and basophil activation tests can elucidate on the clinical value of a positive sIgE result.

Animal derived

In contrast to sIgE results to plant-derived food sIgE assays for animal-derived food seem more reliable with better predictive value. Consequently, the benefit of CRD will not relate to optimize diagnosis, but rather in the identification of prognostic and cross-reactive sensitization patterns. It has been demonstrated that sensitization to ovomucoid (Gal d 1), the major allergen from hen's egg, is prognostic unfavorable with a risk for persistent and more severe egg allergy [41-44]. It has been demonstrated that children with a sIgE Gal d 1 result < 1.2 kUA/L (normal 0.35 kUA/L) have 97% chance to tolerate heated egg. In contrast, children with a sIgE Gal d 1 $\geq$ 10.2 kUA/L have 88% to react upon eating heated egg [43].

With respect to cow's milk, the situation appears to be different and it has been shown that sensitization to thermoresistant linear epitopes from α -lactalbumin (Bos d 4), β -lactoglobulin (Bos d 5) en casein (Bos d 8) is prognostically worse than a sensitization to thermolabile conformational determinants [45-47].

Invertebrate tropomyosins [48;49] and parvalbumins [50;51] constitute 2 important protein superfamilies that are responsible for a significant number of patients with allergy to crustaceans and fish, respectively. Sensitization to these stable allergen components is generally associated with severe allergies and significant cross-reactivity within the species [48;52;53]. However, absence of these sensitizations does not preclude allergy to crustaceans or fish. For example, we reported on a series of patients with allergy to giant fresh water shrimps (erroneously called scampi) that generally failed to demonstrated IgE reactivity to recombinant tropomyosin Pen a 1 from the shrimp species *Penaeus aztecus* [54] and described a patient with selective allergy to pangasius and tilapia that was not sensitized to parvalbumin from cod fish (Gad c 1) [55] .

Hymenoptera venom

Venom allergy constitutes a potentially life-threatening health condition and prospective studies have disclosed that up to 50% of allergic patients will react when re-stung. In such patients, correct diagnosis with unequivocal entomological identification of the offending insect is a prerequisite for effective management. In general, physicians will start their diagnostic approach by quantification of venom sIgE antibodies to document their clinical suspicion [56]. However, it has repeatedly been demonstrated that the predictive value of these venom sIgE antibodies is far from optimal and that up to 40% of patients with a single venom allergy can demonstrate double positive sIgE results for wasp and bee venom. Again, as for plant-food allergens, sensitization to CCD has been identified as an important confounder [3;57-60]. Although skin testing and basophil activation tests can help to correctly identify the culprit insect in these cases, it has become apparent that CRD with venom components constitutes a simple, reliable and safe instrument that can add to correct diagnosis and selection of venom immunotherapy. Since these recombinant venom components are expressed by *Escherichia coli* (*E. coli*), they lack the glycan moieties and allow to circumvent the CCD issue that is responsible for the majority of false-positive venom sIgE results [58;61]. In an own series with 25 wasp allergic patients with double-positive venom sIgE results, CRD enabled to establish correct diagnosis in 24 cases, by demonstrating a positive sIgE to rVes v 5 (a major allergen component from wasp: *Vespula vulgaris*) and negative sIgE to rApi m 1 from honey bee (*Apis mellifera*) (unpublished data). Patients with a double positive sIgE result to rVes v 5 and rApi m 1 should be considered as co-sensitized and are potential candidates for hyposensitization with both venoms [58]. It is likely that identification and cloning of other wasp and bee venom components will optimize CRD for hymenoptera venom allergy [60].

Latex

Several studies have focused on CRD in IgE-mediated allergy to latex from *Hevea brasiliensis*. From these studies it emerged that CRD constitutes a reliable diagnostic instrument that enables to discriminate between clinically relevant and irrelevant IgE results [62;63][6;64-66] that generally result from sensitization to CCD and profilin (Hev b 8). Alternatively, CRD in latex allergy creates the opportunity to better predict a future “latex-fruit” syndrome that is generally related to a sensitization to Hev b 2, 5 or 6. Finally, as already exemplified for peanut

and hazelnut), it appears that the panel of purified and recombinant proteins spotted on the current microarray (Heb v 1, 3, 5, 6 and 8) does not cover the entire latex IgE response and that additional components Hev b 9 and Hev b 11 are needed to establish correct diagnosis in some cases.

Conclusions and perspectives

It is obvious that allergy diagnosis can benefit from a component resolved approach. First, the technique can help to elucidate equivocal sIgE results obtained by traditional extract-based assays. CRD is particularly helpful in discriminating between clinically relevant and irrelevant sIgE that result from sensitization to profilins and CCD. Second, CRD allows to establish particular sensitization profiles with prognostic information about severity and persistence for the individual patient. Component resolved allergy diagnosis, by creating the opportunity to study whether allergies demonstrate age-related and/or geographical characteristics, might disclose sensitization pathways. However, it is clear that CRD needs further validation before it can enter mainstream use and that the technique should gain from the identification and cloning of additional components and/or epitopes.

Table 1a: Important plant allergen components (adapted from [10]; not exhaustive)			
Common name	Latin name	Component	Protein family
Legumes, fruits, grains, nuts and seeds			
Peanut	<i>Arachis hypogaeae</i>	Ara h 1	7S albumin (vicilin)
		Ara h 2	2S albumin
		Ara h 3	11S albumin (legumin)
		<u>Ara h 5</u>	Profilin
		Ara h 6	
		<u>Ara h 8</u>	PR10 (Bet v 1 homologue)
		Ara h 9	nsLTP
Soy	<i>Glycine max</i>	<u>Gly m 4</u>	PR10 (Bet v 1 homologue)
		Gly m 5	7S albumin (vicilin)
		Gly m 6	11S albumin (legumin)
Wheat	<i>Triticum aestivum</i>	Tri aA_TI	α -amylase/trypsin inhibitor
		Tri 14	nsLTP
		Tri 19	Ω -5-gliadine
Apple	<i>Malus domestica</i>	<u>Mal d 1</u>	PR10 (Bet v 1 homologue)
		<u>Mal d 3</u>	nsLTP
Hazelnut	<i>Corylus avellana</i>	<u>Cor a 1</u>	PR10 (Bet v 1 homologue)
		Cor a 8	nsLTP
		Cor a 9	11S albumin (legumine)
Kiwi	<i>Actinidia deliciosa</i>	Act d 1	Thiolprotease
		Act d 2	Thaumatococin-like protein
		Act d 5	Kiwifruit allergen

		<u>Act d 8</u>	PR10 (Bet v 1 homologue)
Peach	<i>Prunus persica</i>	<u>Pru p 1</u> Pru p 3	PR10 (Bet v 1 homologue) nsLTP
Walnut	<i>Juglans regia</i>	Jug r 1 Jug r 2 Jug r 3 Jug r 4	2S albumin 7S globulin (vicilin) nsLTP 11S albumin (legumine)
Carrot	<i>Daucus carota</i>	<u>Dau c 1</u>	PR10 (Bet v 1 homologue)
Pollen			
Birch	<i>Betula verrucosa</i>	Bet v 1 <u>Bet v 2</u>	PR10 Profilin
Timothy grass	<i>Phleum pratense</i>	Phl p 1 <u>Phl p 4</u> Phl p 5 Phl p 6 <u>Phl p 12</u>	Group 1 grass allergen (β -expansin) Group 4 grass allergen (berberin) Group 5 grass allergen (ribonuclease?) Group 6 grass allergen Profilin
Latex			
Latex	<i>Hevea brasiliensis</i>	Hev b 1 Hev b 3 Hev b 5 Hev b 6 <u>Hev b 8</u> Hev b 11	Rubber elongation factor Small rubber particle protein Acid protein Hevein Profilin Chitinase
Components put in bold : primary sensitization, components underlined: sensitization mostly via cross-reactivity, components in bold italics : sensitization primary or via cross-reactivity			

Table 1b: Important animal allergen components (adapted from [10]; not exhaustive)			
Common name	Latin name	Component	Protein family
Animal food			
Egg white (henn)	<i>Gallus domesticus</i>	Gal d 1 Gal d 2 Gal d 3 Gal d 4 Gal d 5	Ovomucoid Ovalbumin Ovotransferrin Lysozyme Serum albumin (α -livetin)
Milk (cow)	<i>Bos domesticus</i>	Bos d 4 Bos d 5 Bos d 6 Bos d 8	α -lactalbumin β -lactoglobulin Serum albumin Casein
Crustaceans (shrimp)	<i>Penaeus aztecus</i>	Pen a 1	Tropomyosin
Fish (cod)	<i>Gadus callarias</i>	Gad c 1	Parvalbumin
Mites			
House dust mite	<i>Dermatophagoides pteronyssinus</i>	Der p 1 Der p 2 Der p 10	Cysteine protease NPC2 family Tropomyosin
Epithelia			
Cat	<i>Felis domesticus</i>	Fel d 1 <u>Fel d 2</u> Fel d 4	Uteroglobulin Serum albumin Lipocalcin
Dog	<i>Canis familiaris</i>	Can f 1 Can f 2 <u>Can f 3</u>	Lipocalcin Lipocalcin Serum albumin

		<i>Can f 5</i>	Prostate kallikrein
Hymenoptera			
Bee	<i>Apis mellifera</i>	Api m 1	Phospholipase A2
		<i>Api m 2</i>	Hyaluronidase
		Api m 4	Mellitin
Wasp	<i>Vespula vulgaris</i>	Ves v 1	Phospholipase A1
		<i>Ves v 2</i>	Hyaluronidase
		Ves v 5	Antigen 5
Components put in bold : primary sensitization, components underlined: sensitization mostly via cross-reactivity, components in <i>bold italics</i> : sensitization primary or via cross-reactivity			

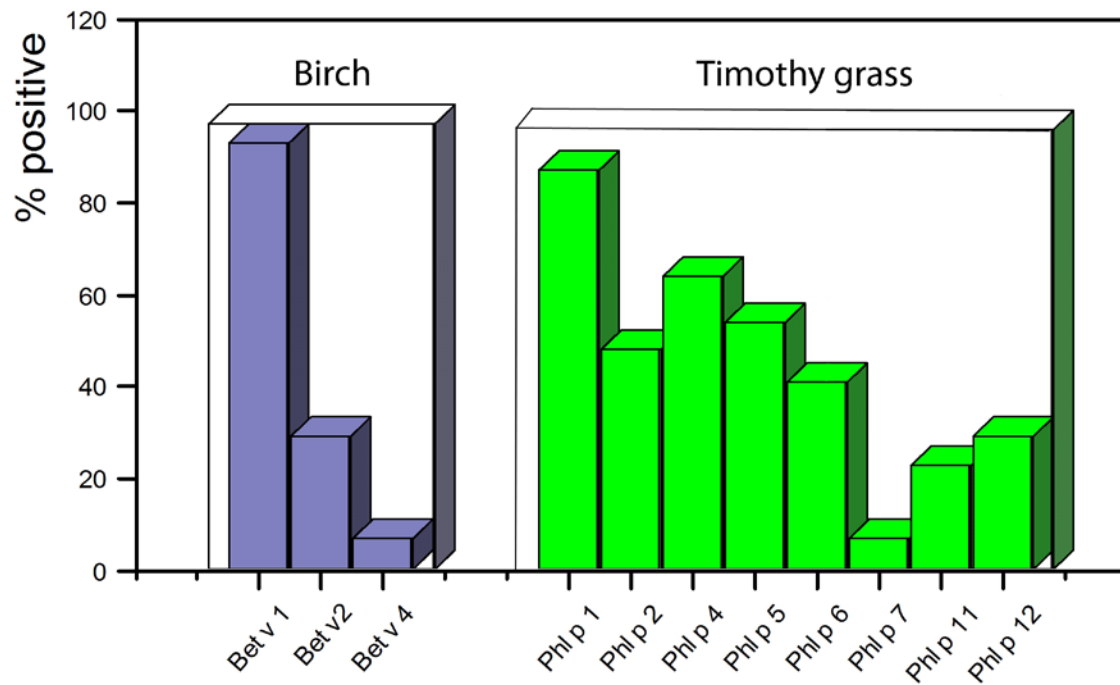


Figure 1: sensitization patterns in patients with allergy to birch (*Betula verrucosa*; n=129) and/or Timothy grass (*Phleum pratense*; n=126) documented by skin prick test. Results are expressed as percentages of positive results.

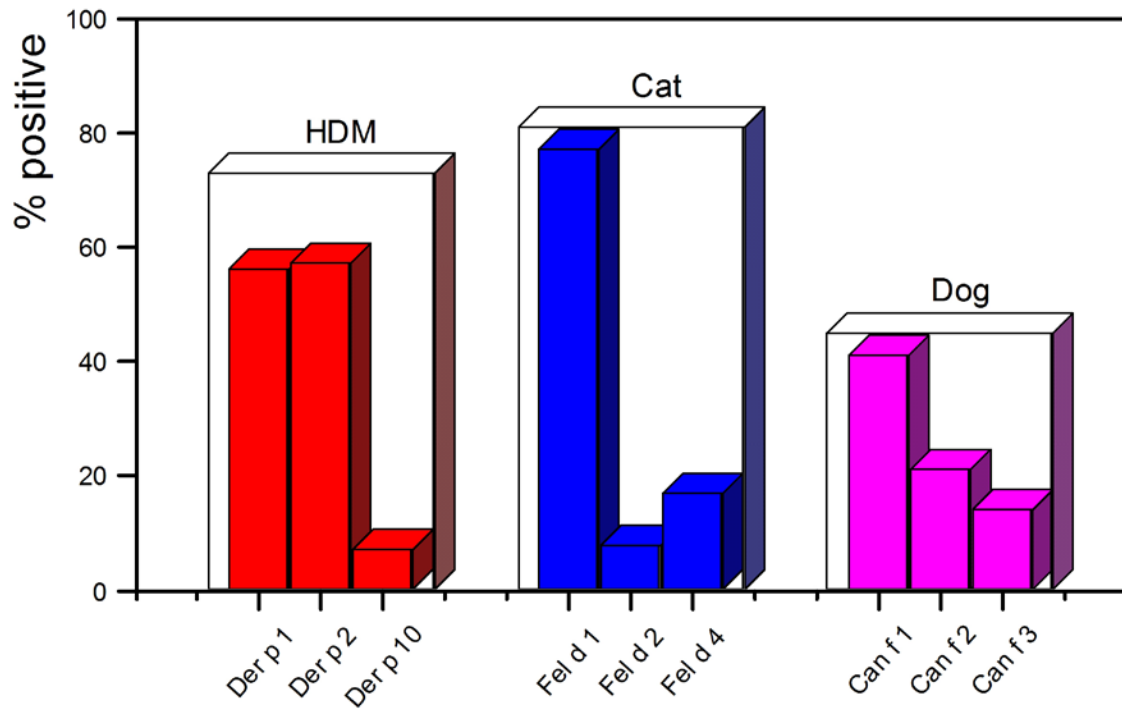


Figure 1: sensitization patterns in patients with allergy to house dust mite (*Dermatophagoides pteronyssinus*; n=101), cat (*Felis domesticus*; n=93) and dog (*Canis familiaris*; n=66) documented by skin prick test. Results are expressed as percentages of positive results.

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