Review paper

Dermatophagoides pteronyssinus proteins and their role in the diagnostics and management of house dust mite allergy: exploring allergenic components

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Abstract

Component-resolved diagnostics enables the detection of allergen-specific immunoglobulins E (asIgE) to house dust mite (HDM) proteins, which may help clinicians to face the difficulties in diagnostics of HDM allergy. Currently, almost 40 proteins of *Dermatophagoides* species, that are able to bind asIgE, have been identified, and this number will certainly increase. The association between sensitisation to particular molecules and allergy symptoms is extensively studied. This investigation enables us to identify HDM molecules that promote respiratory, skin, or food allergies, and it could help us predict the possible course of allergic diseases. The individual repertoire of asIgE improves our understanding of immunological response, which underlies allergy symptoms, and helps to form individual therapeutic regimens. This review provides clinicians with information on *Dermatophagoides pteronyssinus* proteins available in commercial arrays, and their role in the diagnostics and management of HDM allergy.

Key words: component-resolved diagnostics, house dust mites, sensitisation.

Introduction

As house dust mites (HDM) are present in almost all environments, they are one of the most common triggers of allergy [1]. Patients sensitised to HDM most frequently develop allergic rhinitis (AR), allergic asthma (AA), and atopic dermatitis (AD) [2]. The estimated prevalence of sensitisation to HDM allergens ranges between 1% and 20% of the global population and can reach up to 50% in atopic individuals [2, 3]. The pattern of sensitisation to various species of HDM varies around the world, which signalises the need for further research across various populations [2–5]. Four species of mites, commonly known as HDM, which are present in regions with different humidity and temperature, have been identified: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Bloomia tropicalis*, and *Euroglyphus maynei* [6].

The diagnostics of HDM allergy consists of several steps. The first, and the most important one, is anamnesis. The symptoms after exposure to an allergen source reported by a patient cannot be omitted in the diagnostic process. Next, skin prick tests (SPT) or measurement of allergen-specific immunoglobulins E (asIgE) with HDM extracts should be performed. Nevertheless, in the case of HDM allergy, testing with extracts may not provide enough information to establish the proper diagnosis. Component-resolved diagnosis (CRD) revolutionised the diagnostics of HDM allergy. The CRD tests enable us to identify allergen specific immunoglobulins E against allergen molecules, which are ingredients of an allergen source. Thanks to CRD, it is possible to create an individual profile of sensitisation and suit the therapeutic regimen to the profile of sensitisation.

When the connection between symptoms and exposure to HDM is clear, avoidance of the allergen should be advised. The patient should also be prescribed the drugs against allergy symptoms. In poorly controlled cases of AA or atopic dermatitis caused by sensitisation to HDM, biological treatment should be considered [6]. Recently, a therapeutic strategy concordant with the individual profile of sensitisation to HDM has been promoted [7, 8]. Allergic people sensitised to allergens from group 1 and 2 HDM allergens are known to be

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good candidates for allergen immunotherapy (AIT) [7]. That is why the need for more detailed recommendations based on individual profiles of sensitisation emerges [8]. The characteristics of HDM proteins that trigger sensitisation, and consequently better understanding of associations between these proteins and their influence on immunological response, will enable us to individualise the therapeutic regimen.

Aim

This review aims to provide a clinician who takes care of patients allergic to house dust mites with information on *Dermatophagoides pteronyssinus* proteins available in commercial arrays (Table 1) and their role in the diagnostics and management of HDM allergy.

Table 1. Summary of *Dermatophagoides pteronyssinus* allergens currently available at commercial arrays

Molecule	Protein family[a]	Availability on tests: ALEX2® ■ ImmunoCap™ ● ImmunoCap™ISAC™ ✚ Polycheck® House Dust Mites Recombinants ◆	Clinical relevance of sensitisation
Der p 1	Cysteine protease	■● ✚ ◆	Major allergen of HDM[b]. Risk factor of developing AA[c]. Its proteolytic activity suggests a role in initiating sensitisation, potentially explaining higher sensitivity in children than adults. The greatest structural similarities were observed to Der f 1 and Eur m 1, which corresponds to clinically significant cross-reactions. Several cases of possible allergy symptoms have been described after eating fruits such as figs, papaya, pineapple, and kiwi. A marker of efficacy of immunotherapy with HDM extracts.
Der p 2	Niemann-Pick protein type C2	■● ✚ ◆	Major allergen of HDM. The highest structural similarity was reported for Der f 2 and Eur m 2. Not clear whether sensitisation increases the risk of AA versus AR[d] only. A marker of efficacy of immunotherapy with HDM extracts.
Der p 5	Group 5/21 allergen	■	More frequently detected in patients with atopic dermatitis. May be underrepresented in HDM[d] extracts (false negative results when testing with extracts only; uncertain effectiveness of immunotherapy).
Der p 7	Bacterial permeability-increasing like protein	■	More frequently detected in patients with AR and AA. May be underrepresented in HDM extracts (false negative results when testing with extracts only; uncertain effectiveness of immunotherapy).
Der p 10	Tropomyosin	■● ✚ ◆	Risk factor of developing shrimp, shellfish and edible insect allergy. Risk factor of anaphylaxis, e.g. after inhalation of fumes or oral exposure to shellfish. Possible cross-reactions with mugwort tropomyosin.
Der p 11	Paramyosin	■	More frequently detected in patients with atopic dermatitis.
Der p 20	Arginine kinase	■	More frequently detected in patients with atopic dermatitis or AA.
Der p 21	Group 5/21 allergen	■	More frequently detected in patients with atopic dermatitis. More frequently detected in patients with AA, levels of asIgE[e] correlate with severity of symptoms.
Der p 23	Peritrophin-like protein domain	■● ✚ ◆	Major allergen of HDM. May be underrepresented in HDM extracts (false negative results when testing with extracts only). Risk factor of developing AA. Capable of activating basophils in patients with HDM allergy at concentrations 10 times lower than Der p 1. Research is necessary to provide evidence of the accuracy of immunotherapy in people allergic only to Der p 23 (the lack or too little Der p 23 in vaccines used in immunotherapy may result in its lack or low effectiveness).

[a]https://www.allergen.org/; access from 26th April 2024; [b]House dust mites; [c]Allergic asthma; [d]Allergic rhinitis; [e]Allergen-specific immunoglobulins E; ■ Molecules available at ALEX2® (https://www.macroarraydx.com/products/alex and https://a.storyblok.com/f/164899/x/7fa6fde2a6/20230626_web_madx_alex_allergenliste_210x250mm_en.pdf; access from 26th April 2024); ● ✚ Molecules available at ImmunoCAPTM and ImmunoCAPTM ISACTM (https://www.abacusdx.com/media/PU_Product%20Catalogue%202022.pdf; access from 30th April 2024); ◆ Molecules available at Polycheck® House Dust Mites Recombinants (https://www.polycheck.pl/oferta/alergologia/panele-molekularne-specjalne/polycheck-rekombinanty-roztocze; access from 26h April 2024).

HDM allergens

The two main species of HDM in regions with moderate climate are *Dermatophagoides pteronyssinus* (*D. pteronyssinus*) and *Dermatophagoides farinae* (*D. farinae*). In the database http://allergen.org/, 34 allergenic proteins of *D. pteronyssinus* and 37 allergenic proteins of *D. farinae* have been listed to date (9.12.2023). The proteins of both species exhibit high structural similarity, which may result in cross-reactions between allergens from one species and asIgE in serum of patients sensitised to another HDM species [9]. Thus, 40 groups of *D. pteronyssinus* proteins and *D. farinae* proteins that are able to bind asIgE have been identified, based on their structural similarity and function [8]. Three HDM proteins (Der p 27, Der p 40, and Der f 40) were added in the last 2 years, so our knowledge about potential elicitors of HDM allergy is growing. Such dynamic discoveries of new HDM proteins require constant attention from the clinicians, as these discoveries provide us with information on the development of allergy to HDM, may lead to rapid diagnostic and therapeutic updates.

Regarding the number of patients with symptoms of allergy to a certain source, who are sensitised to particular proteins, the proteins were divided into major and minor allergens. A protein is defined as a major allergen when more than 50% of allergic patients are sensitised to it. Studies from all around the world proved that at least 55% of HDM allergic patients are sensitised to Der p 1 [1, 10]. In the case of proteins from group 2, the sensitisation rate is 87% in symptomatic patients in North Eastern Poland [10] and 80% in allergic adults from Canada, Europe, South Africa, and the USA [1].

For a long time, only proteins from groups 1 and 2 were identified as major HDM allergens. Recently, another protein with established clinical significance, Der p 23, emerged as major HDM allergen [1, 8, 10]. So far, in the case of HDM, Der p 1, Der p 2, and Der p 23 are recognised as major allergens [1]. The prevalence of sensitisation to Der p 4, Der p 5, Der p 7, Der p 10, Der p 11, Der p 20, and Der p 21 is below 50%, so these proteins are classified as minor HDM allergens [6].

Major HDM allergens

Der p 1

This molecule of 24 kDa weight was identified in 1988, and to date 24 isoforms of this protein have been described [11, 12]. This protein, present in faeces of mites, is a cysteine protease able to damage epithelial and surfactant proteins in lung tissue [6]. Such activity promotes the entrance of other HDM proteins through airway epithelium and may result in sensitisation to them. Group 1 of HDM proteins also paves the way for different proteins due to activation of mechanisms that lead to secretion of IL-33 [13]. Furthermore, Der p 1 also seems to play an important role in turning precursors of other HDM proteases, such as Der p 3, 6, and 9, into their active forms [14]. The proteolytic activity of Der p 1 suggests its initiatory role in sensitisation and may explain more prevalent sensitisation to this molecule in children compared to adults [15]. The presence of asIgE against Der p 1 is connected with higher risk of AA [16]. As shown in a study conducted among 158 allergic children, children sensitised to Der p 1 and Der p 23 before the age of 5 years are at risk of developing AA at a school age [17]. Monosensitisation to Der p 1 is a rare condition, and it occurs in about 3% of HDM allergic patients [1]. It was shown that Der p 1 shares sequences of high similarity and identity with Der f 1 and Eur m 1, so the risk of cross reactions between these proteins is high [18, 19]. Surprisingly, patients sensitised to Der p 1 may experience allergy symptoms after eating foods containing cysteine proteases, such as fig, papaya, pineapple, and kiwi [20]. In a 10-year-old girl with AR, who reacted immediately after eating a fresh fig with oral allergy symptoms, urticaria, angioedema, and dyspnoea, *in vivo* and *in vitro* tests were positive for raw fig, *D. pteronyssinus*, and *D. farinae* [20]. The authors of this case report suggested that in this patient anaphylactic reaction occurred due to cross-reactivity between ficin, a cysteine protease from fig, and Der p 1 [20]. Ficin was described as a cysteine protease from food with the highest amino acid identity with Der p 1 (38%) [21]. Although low sequence and structural similarity between Der p 1 and cysteine proteases from fruits (about 30%) was observed, proteins from this group can share asIgE binding epitopes [21, 22]. In another study, among 341 patients with allergy symptoms, only one person sensitised to Der p 1 reported allergic reaction after eating a fig (levels of asIgE to Der p 1 and ficin were 1.04 FIU and 0.55 FIU, respectively) [21]. None of the participants with asIgE to Der p 1 reacted after eating other fruit containing cysteine proteases (pineapple, kiwi, papaya) [21]. Even though avoidance of eating kiwi, papaya, pineapple, and mulberry was proposed, herein we would like to highlight that results of CRD should be interpreted according to the clinical history, to prevent the patient from an unnecessary elimination diet [20].

Der p 2

One year after the discovery of Der p 1, another protein of *D. pteronyssinus*, Der p 2, was purified using immunoaffinity chromatography [23]. This protein of 15 kDa, found mainly in faecal particles of HDM, represents the NPC intracellular cholesterol transporter 2 (NPC2) family, also known as Niemann-Pick protein type C2 family [18]. Although NPC2 proteins lack enzymatic activity, they can bind lipopolysaccharides [24]. The highest structural similarities among proteins from Group 2 was observed between Der p 2, Der f 2, and Eur m 2, and it corresponds with clinically relevant cross reactions [18]. Interestingly, cross-reactivity between Der p 2 and Tyr p 2 from a storage mite *Tyrophagus putrescentiae*, despite quite low

sequence identity, was highlighted [18]. Nonetheless, no significant cross reactivity was observed between Der p 2 and other proteins from Group 2 allergens, such as Blo t 2, Gly d 2, and Lep d 2 [18]. The allergenicity of proteins from this group can be explained by activation of the Toll-like receptor 4 (TLR4), which occurs due to their homology to myeloid differentiation factor-2, which is a part of TLR4 signalling complex [25]. Furthermore, Der p 2 initiates the inflammatory process through nuclear factor-κB and mitogen activated protein kinases [26]. In various regions around the world, sensitisation to Der p 2 is more frequent than to Der p 1 [1, 10]. Moreover, the prevalence of sensitisation to major HDM allergens (Der p 1, Der p 2 and Der p 23) was higher in patients with AA than in those with AR only [27]. On the other hand, there are data showing that the presence of asIgE against Der p 2 in a child's serum does not indicate higher risk of AA [28]. Fifteen isoforms of Der p 2 have been identified [12]. In a study assessing sensitisation to *D. pteronyssinus* allergens among allergic patients in Northeastern Poland, the authors suggested that detection of asIgE against recombinant isoform rDer p 2.0101 is comparable to nDer p 2 [10].

Der p 23

Identified in 2013, this small molecule soon attracted the attention of researchers because it was defined as the third major allergen of *D. pteronyssinus* [29]. Like the two other major *D. pteronyssinus* allergens, Der p 23 is present in faecal pellets of HDM, and thus exposition to this protein occurs via the respiratory tract [29]. It belongs to the peritrophin-like proteins, weighs 14 kDa, and up to now only one isoform of Der p 23 is known [12]. Some data suggest that due to homology with chitin-binding peritrophins, Der p 23 may directly activate innate immune response [6, 29]. Furthermore, Der p 23 is regarded as a particularly allergenic molecule, able to activate basophils in HDM-allergic patients in concentrations 10 times lower than Der p 1 [29]. Although the exact role of Der p 23 in induction and development of allergy is not yet established, its clinical relevance has already been shown. Resch *et al.* indicated that asIgE levels against 4 HDM molecules, including Der p 23, were higher in HDM-sensitised children who developed AA, in comparison to those who did not [17]. Similar findings were presented by Potapova *et al.*, who claimed that levels of asIgE against Der p 1 and Der p 23 correlated with AA [30]. Furthermore, Posa *et al.* recognised sensitisation to both Der p 1 and Der p 23 in early childhood as a risk factor of AA at school age [28]. As shown in a study by Jiménez-Feijoo *et al.*, sensitisation to Der p 23 may also correlate with more severe symptoms of AA [27]. These findings are in line with observations made among HDM-allergic patients in Northeastern Poland. Firstly, co-sensitisation to all three major HDM allergens was more common among Polish patients with AA than in those with AR only. The authors of the study also assessed forced expiratory volume in 1 s (FEV_1) in asthmatic patients sensitised to HDM and presented an inverse correlation between this lung function parameter and sensitisation to Der p 23. They also proved, through measurement of exhaled nitric oxide, that the airway inflammation was more severe in patients with asIgE against Der p 23 [10]. Nevertheless, Villalta *et al.* signalised that in an Italian study group, monosensitisation to Der p 23 was associated with AR, but not with AA [31]. Out of 4% of HDM-allergic patients from all around the world who were sensitised to only one HDM molecule, more than a half (2.3%) were monosensitised to Der p 23 [1]. In Northeastern Poland the rate of monosensitisation to Der p 23 among HDM-allergic individuals was higher and reached almost 5% [10]. All these findings indicate why it is important to include detection of asIgE against Der p 23 into the diagnostic routine. This molecule is a fantastic example of how CRD can explain diagnostic dilemmas and help to recommend a therapy adequate for the patient. Because HDM extracts lack some molecules, including Der p 23, patients with symptoms after exposure to HDM and negative skin prick tests with HDM extracts can be diagnosed with HDM allergy based on the results of CRD arrays [6]. For a long time, it has been thought that immunotherapy with HDM extracts is effective in the case of sensitisation to Der p 1 and Der p 2, while patients monosensitised to Der p 23 do not seem to benefit from this form of treatment [27]. Lack or lower effectiveness of immunotherapy also may be caused by the fact that Der p 23 is underrepresented in HDM extracts [32, 33]. Interestingly, a study by Rodríguez-Domínguez *et al.* (2020) demonstrated an increase in immunoglobulins G (IgG) production after administering a mite extract vaccine specifically targeting Der p 23 [7]. This finding suggests that a vaccine containing Der p 23 may trigger an immune response, potentially leading to therapeutic effects. The discussion on accuracy of immunotherapy in Der p 23 sensitised individuals is still open, and, undoubtedly, further research on the role of Der p 23 in allergic diseases, as well as possible interventions changing their course in patients sensitised to this molecule, is indispensable.

Minor HDM allergens

Der p 5

Built of 132 amino acids, Der p 5 belongs to Group 5 of mites' allergens and is secreted by epithelial cells of mites' gut [34, 35]. It weighs 14 kDa and was first described in 1994 [34]. The molecular function of Der p 5 is to activate protein homodimerisation, and it takes part in protein self-association [36, 37]. This protein, with two identified isoforms, is classified as a mid-tier allergen. Depending on the study group, it sensitises up to 40% of patients and is more often recognised by asthmatic patients than by those with AR only [6, 34].

Among 481 HDM-sensitised individuals in Lithuania, only 13 (2.7%) subjects had asIgE against non-major *D. pteronyssinus*-specific allergens (Der p 5, Der p 7, Der p 21) and were simultaneously negative for HDM major allergens [38]. Furthermore, almost 1.5% of Lithuanian patients were monosensitised to either Der p 5 or Der p 21, although in previous studies no monosensitisation to Der p 5 was reported [38, 39]. It has also been shown that sensitisation to Der p 5 was more common in patients with atopic dermatitis [40]. These findings are in line with the results of a study on atopic individuals in Tenerife, where atopic dermatitis was more frequently observed than AR or AA among patients sensitised to Der p 5 (42%, 23%, and 38%, respectively) [41]. This study also revealed that individuals with AD had higher titres of asIgE against 8 HDM molecules, including Der p 5, than those with AR only [41]. The sensitisation to Der p 5 might not only indicate clinical manifestation of allergy, but can also differentiate sensitisation to HDM, storage mites, and tropical mites in patients exposed to all three groups [35]. Polyclonal rabbit anti-Der p 5 IgG identified only *D. pteronyssinus* and *D. farinae* allergens, whereas no reaction with allergens from storage mites nor *Bloomia tropicalis* was observed [35]. Despite quite a conserved amino-acidic sequence (43% of homology) between Der p 5 and Blo t 5 from *Bloomia tropicalis*, in several studies little cross-reactivity between these two mite species was reported [42]. Nevertheless, in a Lithuanian study group, more than 80% of individuals sensitised to Blo t 5 also had asIgE for Der p 5 [38]. Similarly to Der p 23, Der p 5 might be underrepresented in extracts [32].

Der p 7

Der p 7 is built of 198 amino acids and it naturally occurs as a mixture of three particles with molecular weight ranging from 26 to 31 kDa [43, 44]. There is a remote structural similarity between Der p 7 and human bactericidal permeability increasing protein (BPI)/lipopolysaccharide-binding protein (LBP) which may interact with Toll-like receptors (TLRs) after binding lipopolysaccharide and other bacterially derived lipid ligands. The structure of the dust mite allergen Der p 7 exhibits resemblances to innate immune proteins. Additionally, it interacts with polymyxin B weakly through a binding site that is comparable to the one used by these immune proteins. The results imply that the biological reactivity of the group 7 mite allergens may contribute to their allergenicity [45]. Commonly recognised as a mid-tier allergen, with a sensitisation rate of 30% among European HDM-allergic individuals, Der p 7 has emerged as a protein of clinical relevance [1]. Muddaluru *et al.* discovered that in South Africa, 56% of the study group were sensitised to Der p 7; thus, in this region Der p 7 can be described as a major HDM allergen [1]. Patients with AR seem to be more frequently sensitised to Der p 7 than asymptomatic individuals (more than 40% and about 5%, respectively), as reported by Zidarn *et al.* [46]. The authors of another study also noted that sensitisation to Der p 7 was more frequent in patients with AR and AA in comparison to asymptomatic patients [47]. The above studies indicate a relationship between exposure to Der p 7 and respiratory symptoms in people with dust mite allergy. Therefore, Der p 7 is an important allergen associated with house dust mite allergy and should be included in vaccines used for allergen-specific immunotherapy in house dust mite allergy [47]. Nevertheless, the amount of Der p 7 in HDM extracts is not standardised. Recognition and establishment of asIgE binding epitopes in the protein, provided by Curin *et al.*, may be a key step to produce an effective vaccine [47]. Further research on the production of molecular vaccines for allergen immunotherapy, containing proteins that are low-abundant in HDM extracts (for example Der p 5, Der p 7, Der p 21, and Der p 23), should be performed. Once such vaccines are available, more patients with allergy symptoms sensitised to HDM could benefit from immunotherapy [47].

Der p 10

This protein, which weights 36 kDa and is built out of 284 amino acids, belongs to the tropomyosin family, found in the muscles of invertebrates [19, 48]. Because Der p 10 is a muscular protein, its amount in routinely used extracts, which contain mainly proteins from the faeces of mites, is low. As tropomyosins are panallergens with high structural similarity, they are responsible for cross-reactivity between mites, insects, crustaceans, molluscs, and nematodes [6, 19]. Tropomyosins are resistant to heat and are a known trigger of anaphylaxis, including food-dependent exercise-induced anaphylaxis [6, 49]. People who become sensitised to tropomyosins in an occupational environment are prone to severe allergy symptoms after inhalation of cooking fumes or first oral exposure to shellfish, which could be explained by the high thermal stability of tropomyosins [6]. Although tropomyosins are major shellfish allergens, they are minor HDM allergens: about one in ten individuals sensitised to HDM in Europe has asIgE against Der p 10 [50]. Despite being a minor HDM allergen, Der p 10 is a protein of clinical relevance. It has been suggested that in some regions primary sensitisation to mites' tropomyosin might lead to shellfish sensitisation and allergy [51]. The inhibition of asIgE binding to shrimp tropomyosin, Pen a 1, with HDM and cockroach extracts reported in a group of Orthodox Jews sensitised to shrimp (who do not eat shellfish because of religious rules) implied that sensitisation to shrimp occurred due to cross-reactions between tropomyosins from shrimp and HDM and/or cockroach. In other words, it indicated that an individual may develop food sensitisation without prior consumption of a particular allergen [52]. It has also been proven that the coincidence of sensitisation to Der p 1, Der p 2, and Der p 10 increases the odds of shrimp allergy de-

velopment [53]. Furthermore, patients allergic to both HDM and crustaceans have higher levels of asIgE against Der p 10 than against Der p 1 and Der p 2 [54]. Based on these observations, sensitisation to Der p 10 can be treated as a risk factor for developing allergy to shellfish and shrimp. Indolfi *et al.* proposed regular follow-up for sensitisation to Der p 10 and other tropomyosins in asthmatic patients sensitised exclusively to Der p 1 and Der p 2 [55]. Through assessment of asIgE to Der p 10 and Pen a 1 before, during, and after completion of HDM AIT, a research group from Croatia showed that AIT with HDM extracts did not trigger clinically significant sensitisation to tropomyosins in 56 patients allergic to HDM [56]. Sensitisation to tropomyosins was reported in 5 HDM-allergic subjects who underwent subcutaneous HDM AIT, and in this group 3 individuals were sensitised to tropomyosins before starting the AIT. In the study group, only one subject experienced oral allergy symptoms after eating shrimp or squid before starting HDM AIT, and they gained long-term tolerance to seafood after completing AIT with HDM extracts (confirmed by food challenge), which corresponded with a decrease in asIgE to tropomyosins [56]. On the other hand, severe cases of life-threatening anaphylaxis upon eating snail a few months after initiation of HDM AIT in children allergic to snail and HDM were described [57]. Thus, the measurement of asIgE to HDM and shrimp tropomyosins (Der p 10 and Pen a 1) was indicated as valuable tool to qualify patients with seafood allergy to subcutaneous AIT with HDM extract [56]. Sensitisation to Der p 10 is also a risk factor of food allergy to edible insects, such as *Tenebrio molitor*, *Acheta domesticus*, and *Locusta* spp., due to cross-reactions between proteins of the species [58]. In 2014, Verhoeckx *et al.* proved asIgE binding to mealworm in 6 out of 7 HDM and crustacean allergic patients, as well as positive basophil activation test with mealworm extract in all (n = 5) patients [59]. Although shrimp allergic patients are more prone to allergy to edible insects than HDM-allergic patients, we should be aware of possible symptoms after consumption of larvae in subjects with HDM allergy [60]. The above-mentioned findings imply the importance of diagnosing sensitisation to Der p 10 in the search for the cause of food allergy, including anaphylaxis, or prediction of the allergy course. The authors of another study asked if *Sarcoptes scabiei* (*S. scabiei*) tropomyosin, Sar s 10, could be applied in diagnosis of infestation with this parasite. They concluded that conserved homology of HDM allergens from group 10, reaching 97% between Der p 10, Der f 10, and Sar s 10, limits the role of Sar s 10 in immunodiagnosis of scabies infestation [61]. Surprisingly, a study group from China observed that HDM-sensitised patients with AR more frequently made appointments with doctors in mugwort pollen season, and then revealed that synthetic mugwort tropomyosin activated basophils primed with asIgE to HDM, which resulted in T helper cell 1 and T helper cell 17 activation. The cross-reactivity between HDM and mugwort tropomyosins' was proven in inhibition tests: mugwort tropomyosin blocked binding between asIgE and Der p 10, depending on the dose. The avoidance of exposition to mugwort in the pollen season might be worth considering in patients allergic to HDM with AR who are sensitised to shrimp. The authors highlighted the need to investigate whether mugwort AIT would be beneficial for patients with AR sensitised to HDM [62]. On the other hand, sensitisation to Der p 10 was less frequent in patients with symptoms from airways (10%) than in patients with atopic dermatitis (25% in a German cohort and 67% in an Austrian cohort), which indicates a possible association between the route of exposition and clinical manifestation of the allergy [63].

Der p 11

This protein with weight of 103 kDa represents the paramyosin family, and it is present in the muscles of mites. Research on homology between Der p 11 and other paramyosins revealed the highest similarity with paramyosins from mites (HDM, tropical mites, e.g. *Bloomia tropicalis*, and itchy mites, e.g. *S. scabiei*), ticks, lice, and insects, which may be suggestive of possible cross-reactions [19, 63]. Der p 11 has the most similar amino acid sequence with paramyosins from *D, farinae* and *S. scabiei* (97% and 94%, respectively) [63]. Nonetheless, the asIgE response to Sar s 11, *S. scabiei* paramyosin, was characteristic for subjects with ordinary scabies, and not for those allergic to HDM. The authors of the study recognised recombinant Sar s 11 as a potential marker in immunodiagnosis of scabies, and proposed its use to control the infestation in endemic regions [61]. The frequency of sensitisation to Der p 11 among HDM-allergic subjects in Zimbabwe (44%) was higher than in European countries (7–16%). Results from Germany and Austria indicated that the prevalence of sensitisation to Der p 11 among patients with atopic dermatitis was 55% and 67%, respectively, and 5% in Austrian patients with respiratory symptoms [64]. This may imply that the sensitisation to allergens present in mite bodies occurs mainly via skin (Der p 10, Der p 11) or gut (Der p 10 due to cross-reactivity with food allergens), whereas HDM proteins found in faecal particles (Der p 5, Der p 7, Der p 20 and Der p 21) trigger sensitisation through airways. However, no correlation between atopic dermatitis and Der p 11 recognition was found in an Italian study group [31]. Further research on Der p 11 and clinical significance of this protein in various populations is needed. Thanks to increasing availability of multiparametric assays, the collection of data on profiles of sensitisation will be less demanding.

Der p 20

This protein weighs 40 kDa and it is recognised by asIgE in 7–44% of HDM-allergic individuals [8]. Next to tropomyosins, arginine kinases, to which belongs Der p 20,

are other invertebrate panallergens [65]. In 2001 the first arginine kinase Plo i 1, derived from the Indian meal moth *Plodia interpunctella,* was described [66]. Nonetheless, the immunological characteristic of the protein was provided 20 years later [67]. To date, 22 allergens from this family have been described [68]. A sequence identity of > 70% was observed between Der p 20 and arginine kinases from other mites, including S. scabiei [67]. Their highly conserved amino acid sequence results in common cross-reactions. It was shown that subjects affected with scabies and those with past scabies infections have high asIgE levels to Der p 20 and Der p 4. The authors of the study also suggested to distinguish ongoing or past scabies infection with HDM allergy based on the asIgE repertoire: high levels of asIgE to Der p 1 and Der p 2 indicate HDM allergy, whereas low levels of asIgE to major HDM allergens with high levels of asIgE to Sar s 4, Sar s 14.3, and Sar s 20 indicate scabies [69]. A study conducted among almost 400 HDM-allergic individuals revealed a connection between recognition of Der p 20 by asIgE and atopic dermatitis [40]. Furthermore, Villalta *et al.* implied that the presence and levels of asIgE to Der p 20 correlated with higher risk of AA [31]. In another study, subjects sensitised to Der p 20 more often reported symptoms of AA and overall breathing difficulties, and the difference reached statistical significance. The authors excluded the influence of other allergen sources and proposed that Der p 20 could be a marker of lower respiratory symptoms in HDM allergic individuals. Interestingly, in contrast to other HDM proteins causing respiratory symptoms, Der p 20 is localised mainly in the bodies of mites [67].

Der p 21

This mid-tier allergen, responsible for sensitisation in approximately 30% of HDM allergic patients, is present in HDM gut epithelium and was found in faecal particles [6, 70]. The molecular weight of Der p 21 is about 14 kDa. Thermal stability and capacity to refold after cooling to room temperature characterise Der p 21. Because stable proteins are more likely to survive in the environment in a folded form, they exhibit significant allergenicity. The high allergenicity of Der p 21 was observed in basophil activation test: Der p 21 could induce maximal histamine release in concentrations 10 to 100 times lower than Der p 1 [70]. This member of lipid binding proteins is able to activate Toll-like receptor 2, and consequently to stimulate production of interleukin 8 [6, 71]. Although Der p 21 shares about 40% of amino acid sequence with Der p 5, and both of these proteins have similarly localised asIgE conformational epitopes, no cross reactivity was observed among these molecules [70, 72]. What may contribute to the lack of cross-reactivity between Der p 21 and Der p 5 is the fact that they do not share asIgE binding epitopes, recognised in asIgE inhibition tests [71]. Nevertheless, both of these molecules are able to activate Toll-like receptor 2 response [71, 73]. Walsemann *et al.* indicated that asIgE reactivity to Der p 5, Der p 20, and Der p 21 was more common in individuals with atopic dermatitis among HDM-allergic patients. The same study group revealed that sensitisation to Der p 5 and Der p 21 was more frequently associated with AA than with AR [40]. Moreover, Villalta *et al.* observed a statistically significant correlation between asIgE-mediated response to Der p 21 and severity of AA symptoms. Italian HDM-allergic patients who were sensitised to Der p 21 were at three-fold higher risk of developing moderate or severe AA in comparison to individuals who were negative to this molecule [31]. As HDM extracts lack Der p 21, the implementation of Der p 21 into commercially available arrays could help to predict the clinical course of allergic disease in an individual [74].

The diagnostics of HDM allergy

The key point of allergy diagnostics is proving a relationship between exposition to the allergen source and allergy symptoms. While in food allergy the association is usually clear, in the case of HDM allergy, the trigger of symptoms might be difficult for the patient to identify. Consequently, the suspicion of HDM allergy is based on appropriate anamnesis. Once the clinical history suggests HDM allergy, the asIgE-mediated character of the reaction has to be verified. Skin prick tests with HDM extracts are most commonly performed to prove sensitisation. In routine practice, a patient undergoes SPT with *D. pteronyssinus* and *D. farinae* extracts. However, not every patient will be successfully diagnosed with HDM allergy only with HDM extracts. As mentioned before, some proteins are underrepresented in HDM extracts. The most spectacular example is Der p 23, a major HDM allergen, which is present in extracts only in tiny amounts [10, 27]. Currently, another tool could help us to establish the diagnosis in a patient with allergy symptoms and negative results of SPT: the CRD. The CRD provides a more detailed analysis of individual sensitisation pattern. It enables us to find asIgE against molecules, which form parts of an allergen. Thus, a symptomatic patient with a negative result of SPT against HDM extract may truly benefit from the CRD. Sensitisation to particular HDM molecules may help us predict the course of allergy, as described above. When a patient is sensitised to a wider range of HDM molecules and/or they have higher titres of asIgE against HDM molecules, they are more prone to develop AA rather than AR only [1]. It is noteworthy that HDM-allergic subjects with AA are sensitised to a broader spectrum of HDM proteins and they have higher levels of asIgE to the molecules than patients who develop AR only [1]. That is why it could be particularly beneficial to detect asIgE against several molecules simultaneously, using multiparametric arrays, such as ALEX2®, which provides detection of asIgE against the

broadest spectrum of HDM molecules among commercially available tests [75].

The treatment of HDM allergy

Avoiding exposure to the allergen is the main recommendation in all allergic diseases. In the case of HDM allergy, it can be achieved by regular use of vacuum cleaners with HEPA filters, washing bedding in hot water or freezing it, removal of carpets, and humidity control. A patient can also be prescribed medicines to relieve allergy symptoms, such as antihistamines or nasal corticosteroids. Nevertheless, these do not influence the course of the allergic disease. The most specific form of therapy is AIT with extracts. A preventive effect of AIT on AA development has been widely described [76]. This shielding result of AIT may be explained, among others, by the shift in immunological response from dominant T helper 2 pathway to T helper 1 pathway [77]. Available data suggest that both subcutaneous and sublingual immunotherapy with HDM extracts enhance the level and recognition of protective IgG, which can compete with asIgE for binding of the allergen [78, 79]. AIT is a cost-effective form of treatment, which results in reduction of airway hyperreactivity, symptom severity, and use of control and rescue medication, as well as number of respiratory tract infections in patients with AR and/or AA [80–82]. Thanks to AIT, patients with AA achieve better control of the disease and are less prone to experience AA exacerbations. The preventive role of AIT in the progression of AA has also been suggested [82]. These positive effects of AIT can be observed several years after the end of the therapy [82, 83]. Despite high appraisal for AIT, this therapy is not free from drawbacks. What raises many concerns is the standardisation of vaccines only to Der p 1 and Der p 2, which means that the amount of other HDM proteins in vaccines remains unknown. Because of underrepresentation of some allergens, like Der p 5, Der p 7, or Der p 23, in HDM extracts, subjects sensitised to these proteins may not benefit from AIT. It has been shown that AIT with HDM extracts resulted in an increase of protective IgG levels to Der p 1 and Der p 2 and an insignificant change in levels of IgG to other HDM proteins [84]. The authors of the study also observed that only patients sensitised to Der p 1 and Der p 2 reported significant reduction of nasal symptoms in the course of AIT [85]. These outcomes suggest that detailed analysis of individual sensitisation profiles with CRD arrays can help to select candidates who will truly benefit from AIT. We already have appropriate diagnostic tools, and the goal now is to provide allergic patients with broader sensitisation profiles with therapy well-suited for them.

Conclusions

As a ubiquitous allergen, HDM poses a real threat of developing allergic diseases in sensitised patients. Due to the usually unclear relationship between exposure to HDM and onset of allergy symptoms, reliable anamnesis is key to appropriate diagnosis. Available *in vivo* and *in vitro* tests provide information about sensitisation to HDM. The introduction of CRD into routine clinical practice broadens clinicians' horizons on allergology. This innovative diagnostic tool enables detection of cross-reactions, facilitates the establishment of proper diagnosis, and permits therapeutic recommendations to be suited to the profile of a patient.

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Ethical approval

Not applicable.

Conflict of interest

E.M. is employee of EMMA MDT Sp z o.o. company. For the remaining authors, none are declared.

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