

Short Communication

The diagnostic performance of allergen-molecules in comparison to allergen-extracts

Ivon J.M. van der Linden^{1,*}, Monique J.M. de Groot¹,
Neletta C.C.M. de Jong¹, Zahide Bozkurt¹ and
Christa M. Cobbaert²

¹Laboratory of Clinical Chemistry and Hematology,
Amphia Hospital, Breda, The Netherlands

²Laboratory of Clinical Chemistry, University Medical
Center Leiden, Leiden, The Netherlands

Abstract

Background: This study evaluated the diagnostic performance of naturally purified allergen-molecules compared to that of allergen-extracts for house dust mite, cat dander epithelium and dog dander.

Methods: In vitro tests for allergen-specific IgE were performed on the IMMULITE® 2000 in serum samples from 66 allergic patients.

Results: House dust mite: specificity for the allergen-extract (D1) and the allergen-molecules (nDer p 1, nDer f 1, nDer p 2 and nDer f 2) is comparable. The allergen-extract has a significantly higher sensitivity (100%) and total agreement (TA) (93%) relative to sensitivity (57%–70%) and TA (76%–81%) of the individual allergen-molecules. Cat dander epithelium: sensitivity (90%), specificity (96%) and TA (94%) of the allergen-molecule (nFel d 1) are comparable to those of the allergen-extract (E1). Dog dander: The allergen-molecule (nCan d 1) and allergen-extract (E5) have comparable specificity and TA. The allergen-extract has a lower sensitivity (52%) than the allergen-molecule (71%), although not significant ($p=0.125$).

Conclusions: There is no diagnostic benefit of using allergen-molecules instead of allergen-extracts for initial allergy screening on cat dander epithelium and dog dander. However, use of these allergen-molecules might contribute to better standardization of the specific IgE tests. The studied allergen-molecules for house dust mite are of minor diagnostic value, because of loss of sensitivity.

Keywords: allergen-extracts; allergen-molecules; cat; dog; house dust mite.

In today's world, type I allergic reactions are becoming more and more widespread, affecting up to 20% of the population (1). Diagnosing an allergy is based on medical history and clinical symptoms. However, in vivo and in vitro tests for allergen-specific IgE (sIgE) are essential in confirming clinical suspicion and choosing and starting treatment.

In vivo and in vitro diagnostics of allergies are currently based on natural extracts from allergenic sources. These allergen-extracts are heterogeneous in composition, containing dominant allergens, allergens only present in little numbers, potent allergens, cross-reactive allergens of less impact and non-allergenic components (2–4). Standardization of allergy tests would greatly improve if the specific allergen-molecule responsible for the allergic reaction could be identified. Recently, allergen-molecules have been introduced, which can be naturally purified (n), recombinant (r) or synthetic (s) allergens (3, 5–7). In the present study we evaluated the diagnostic performance of naturally purified allergen-molecules for house dust mite (nDer p 1, nDer p 2, nDer f 1 and nDer f 2), cat dander epithelium (nFel d 1) and dog dander (nCan d 1) in relation to the diagnostic performance of allergen-extracts for house dust mite (D1), cat dander epithelium (E1) and dog dander (E5).

The study population has been described previously (8) and consisted of 118 Caucasian adolescents and adults (≥ 15 years of age) who were referred to the allergist for a suspected allergy. For the present study, a selection of 66 individuals was made, based on the amount of serum sample available for analyses. The selection consisted of 18 males and 48 females with a mean age of 36.4 years (standard deviation=16.2). A patient was diagnosed as study diagnosis-positive when both (i) typical clinical symptoms of inhalant allergy with a positive anamnesis or family history and (ii) a positive skin test for inhalant allergy were present. Controls were study diagnosis-negative for an inhalant allergy against house dust mite, cat dander epithelium and dog dander. All participants gave written informed consent.

Allergen extracts for skin testing [intracutaneous test (ICT) and skin prick test (SPT)] were purchased from ALK-Abello BV (Nieuwegein, The Netherlands). A positive ICT was defined as a wheal diameter $\geq 70\%$ of the histamine control diameter. A positive SPT was defined as a wheal diameter ≥ 3 mm (8). Allergen-extracts and allergen-molecules used for in vitro testing were obtained from Siemens

*Corresponding author: Ivon J.M. van der Linden, Laboratory of Clinical Chemistry and Hematology, Amphia Hospital, Location MG, Molengracht 21, 4818 CK Breda, The Netherlands
Phone: +31-76-5954231, Fax: +31-76-5953807,
E-mail: yvanderlinden@amphia.nl

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Healthcare Diagnostics (Breda, The Netherlands). Allergen-extracts included: D1, house dust mite (*Dermatophagoides pteronyssinus*); E1, cat dander epithelium; and E5 dog dander. Naturally purified allergen-molecules included: nDer p 1, house dust mite (*Dermatophagoides pteronyssinus*); nDer p 2, house dust mite (*Dermatophagoides pteronyssinus*); nDer f 1, house dust mite (*Dermatophagoides farinae*); nDer f 2, house dust mite (*Dermatophagoides farinae*); nFel d 1, cat dander epithelium (*Felis domesticus*); nCan d 1, dog dander (*Canis familiaris*); and nPen m 1, tropomyosin purified from the Black Tiger Shrimp (*Penaeus monodon*) (9, 10). A positive result for the quantitative allergen-specific IgE tests was defined as a concentration ≥ 0.35 kU/L, which is equivalent to class one or higher by the standard scoring system. Because the patient samples were collected in a previous study (8) and stored at -70°C for several years, new and former determined sIgE values were compared in a selection of patient samples to test the quality. No decline of sIgE was observed.

House dust mite: There were 54 patients tested for house dust mite allergy of whom 31 were study diagnosis-negative and 23 were study diagnosis-positive. The sensitivity and TA of the allergen-extract D1 are significantly higher than the sensitivity and TA of each of the individual allergen-molecules nDer p 1, nDer p 2, nDer f 1 and nDer f 2 (Table 1). The specificity of the allergen-extract D1 and the allergen-molecules is comparable (Table 1). Combining the allergen-molecules does not improve sensitivity, specificity and TA (Table 1).

In an attempt to explain the difference in sensitivity between the house dust mite allergen-extract and the allergen-molecules for house dust mite, sIgE against the allergen-

molecule tropomyosin (nPen m 1) was determined in the 54 patients tested for house dust mite. Results are given for patients positive for the allergen-extract and negative for the allergen-molecules (Table 2).

Cat dander epithelium: There were 47 patients tested for an allergy against cat dander epithelium; 27 were study diagnosis-negative and 20 were study diagnosis-positive. The sensitivity, specificity and TA of the allergen-molecule (nFel d 1) are comparable to those of the allergen-extract (E1) (Table 1).

Dog dander: There were 50 patients tested for dog dander allergy of whom 29 were study diagnosis-negative and 21 were study diagnosis-positive. The allergen-molecule (nCan d 1) and allergen-extract (E5) have comparable specificity and TA. The allergen-extract has a lower sensitivity (52%) than the allergen-molecule (71%), although this did not reach significance ($p=0.125$) (Table 1).

Allergen-molecules should increase the specificity of in vitro tests for sIgE by reducing cross-reactivity. In our study however, the specificity of the allergen-molecules for house dust mite, cat epithelium dander and dog dander was not significantly better than the specificity of the allergen-extracts. This could be explained by the quality of the allergen-extracts that were used or by the limited size of our study population in which cross-reactivity may just not be present. In addition, our study population is biased since it consists of individuals referred to the allergist for a suspected allergy.

In contrast with a study by Weghofer et al., who reported that the combination of nDer p 1 and nDer p 2 can diagnose $\geq 97\%$ of the *D. pteronyssinus* allergic patients in Europe (6), our data indicate that the sensitivity of the allergen-

Table 1 Sensitivity, specificity and TA with their 95% confidence intervals for the different allergen-extracts and allergen-molecules.

Allergen	Sensitivity (95% CI)	p-Value	Specificity (95% CI)	p-Value	TA (95% CI)	p-Value
House dust mite						
D1	100% (85%–100%)	–	87% (70%–96%)	–	93% (82%–98%)	–
nDer p 1	57% (34%–77%)	0.002	90% (74%–98%)	1.000	76% (62%–87%)	0.001
nDer p 2	65% (43%–84%)	0.008	90% (74%–98%)	1.000	80% (66%–89%)	0.004
nDer f 1	61% (39%–80%)	0.004	90% (74%–98%)	1.000	78% (64%–88%)	0.002
nDer f 2	70% (47%–87%)	0.016	90% (74%–98%)	1.000	81% (69%–91%)	0.008
nDer p 1/p 2/f 1/f 2	70% (47%–87%)	0.016	90% (74%–98%)	1.000	81% (69%–91%)	0.008
Cat dander/epithelium						
E1	85% (62%–97%)	–	93% (76%–99%)	–	89% (77%–96%)	–
nFel d 1	90% (68%–99%)	1.000	96% (81%–100%)	1.000	94% (82%–99%)	1.000
Dog dander						
E5	52% (30%–74%)	–	97% (82%–100%)	–	78% (64%–88%)	–
nCan d 1	71% (48%–89%)	0.125	93% (77%–99%)	1.000	84% (71%–93%)	0.125

In vitro tests for the various sIgE were performed on The IMMULITE® 2000 (Siemens Healthcare Diagnostics, Breda, The Netherlands). A biotin-labeled allergen and 50 μL of patient sample are incubated with streptavidin-coated beads for 30 min. After a spin wash cycle, monoclonal anti-IgE antibodies labeled with alkaline phosphatase are incubated with the allergen-specific IgE captured on the bead for 30 min again. After the second spin wash cycle a chemiluminescent substrate is added and incubated for 5 min with shaking. Subsequently, chemiluminescence is measured. Sensitivity, specificity and total agreement (TA) with 95% confidence intervals for binominal data were calculated for all allergen-extracts and allergen-molecules in relation to the study diagnosis (<http://statpages.org/confint.html>). TA was calculated: (total number of results-number of discrepancies) $\times 100$ /total number of results. Differences in sensitivity, specificity or TA between the allergen-extracts and allergen-molecules were tested for significance using the McNemar test.

Table 2 Quantitative results for the allergen-molecule tropomyosin in patients positive for the house dust mite allergen-extract and negative for the house dust mite allergen-molecules.

Patient	Study diagnosis	D1, kU/L	nDer p 1, kU/L	nDer p 2, kU/L	nDer f 1, kU/L	nDer f 2, kU/L	nPen m 1, kU/L
1	Positive	1.36	<0.10	<0.10	<0.10	<0.10	<0.10
3	Negative	0.71	<0.10	<0.10	<0.10	<0.10	<0.10
4	Positive	0.86	<0.10	<0.10	<0.10	<0.10	0.33
5	Positive	0.39	<0.10	<0.10	<0.10	<0.10	<0.10
7	Positive	3.04	<0.10	<0.10	<0.10	<0.10	<0.10
8	Positive	1.29	0.30	0.20	<0.10	<0.10	<0.10
9	Positive	1.37	<0.10	<0.10	<0.10	<0.10	<0.10
10	Positive	0.93	<0.10	<0.10	<0.10	<0.10	<0.10

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molecules for house dust mite (nDer p 1, nDer p 2, nDer f 1 and nDer f 2) is significantly inferior to the sensitivity of the allergen-extract for house dust mite (D1). The presence of sIgE directed against tropomyosin (nPen m 1), that may cause clinical cross-reactivity between house dust mite and various shellfish (11), could not explain this discrepancy (Table 2). This implies that the allergen-molecules nDer p 1, nDer p 2, nDer f 1 and nDer f 2 are not the only and/or major allergen-molecules present in the allergen-extract for house dust mite. Because of the expected increase in false-negative results when the allergen-molecules for house dust mite would be used for initial allergy testing, screening should be performed with the more sensitive allergen-extract for house dust mite, until other contributing components are available for diagnostic use.

Identification of the specific component(s) responsible for an allergic reaction remains challenging. It might however contribute to standardization of specific IgE tests, which are not uniform at the moment, leading to different laboratory results. In addition, information on the responsible allergic component might help in predicting the existence, severity and/or chance of developing clinical signs and symptoms (7). Finally, identification of the allergen-molecule that elicits the allergy could play an important role in allergen-specific immunotherapy (ASIT). Hyposensitization with the specific allergen-molecule that elicits the allergic reaction, instead of a crude allergen-extract, could enhance efficacy of the treatment and avoid sensitization against other allergen-molecules (5, 12).

In conclusion, in the present study the diagnostic performance of the allergen-molecules and allergen-extracts for cat dander epithelium and dog dander is comparable. The diagnostic performance of the house dust mite allergen-molecules is inferior to that of the house dust mite allergen-extract. Since the size of our study population is limited, conclusions should be drawn carefully and more studies are warranted to confirm our results.

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Conflict of interest statement

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References

1. Jackson M. Allergy: the making of a modern plague. *Clin Exp Allergy* 2001;31:1665–71.
2. Larenas-Linnemann D, Cox LS. Immunotherapy and Allergy Diagnostics Committee of the American Academy of Allergy, Asthma and Immunology. European allergen extracts units and potency: review of available information. *Ann Allergy Asthma Immunol* 2008;100:137–45.
3. Mari A, Alessandri C, Bernardi ML, Ferrara R, Scala E, Zennaro D. Microarrayed allergen molecules for the diagnosis of allergic diseases. *Curr Allergy Asthma Rep* 2010;10:357–64.
4. Brunetto B, Tinghino R, Braschi MC, Antonicelli L, Pini C, Iacovacci P. Characterization and comparison of commercially available mite extracts for in vivo diagnosis. *Allergy* 2010;65:184–90.
5. Grönlund H, Saarne T, Gafvelin G, van Hage M. The major cat allergen, Fel d1, in diagnosis and therapy. *Int Arch Allergy Immunol* 2010;151:265–74.

6. Weghofer M, Thomas WR, Kronqvist M, Mari A, Purohit A, Pauli G, et al. Variability of IgE reactivity profiles among European mite allergic patients. *Eur J Clin Invest* 2008;38:959–65.
7. Sastre J. Molecular diagnosis in allergy. *Clin Exp Allergy* 2010;40:1442–60.
8. Cobbaert CM, Jonker G. Allergy testing on the IMMULITE 2000 Random-Access immunoanalyzer – a clinical evaluation study. *Clin Chem Lab Med* 2005;43:772–81.
9. Banik U, Maranon M, Achilles M, Lopez M, Jaggi K, Ferrara R, et al. Native mite allergenic molecules nDer p 1, nDer p 2, nDer f1 and nDer f 2: novel tools for mite component-resolved allergy diagnosis. *Allergy* 2007;62:149.
10. Maranon M, Banik U, Maldonado S, Lopez M, Jaggi K, Quarantino D, et al. Native cat and dog allergenic molecules nFel d 1 and nCan f 1: new tools for component-resolved allergy diagnosis. *Allergy* 2007;62:150.
11. Lopata AL, O’Hehir RE, Lehrer SB. Shellfish allergy. *Clin Exp Allergy* 2010;40:850–8.
12. Niederberger V, Valenta R. Molecular approaches for new vaccines against allergy. *Expert Rev Vaccines* 2006;5:103–10.