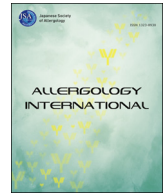




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Original article

IgE Abs to Der p 1 and Der p 2 as diagnostic markers of house dust mite allergy as defined by a bronchoprovocation test



Takafumi Minami ^{a,*}, Yuma Fukutomi ^{a,*}, Jonas Lidholm ^b, Hiroshi Yasueda ^a, Akemi Saito ^a, Kiyoshi Sekiya ^a, Takahiro Tsuburai ^a, Yuji Maeda ^a, Akio Mori ^a, Masami Taniguchi ^a, Maki Hasegawa ^a, Kazuo Akiyama ^a

^a Clinical Research Center for Allergy and Rheumatology, Sagamihara National Hospital, Kanagawa, Japan

^b Thermo Fisher Scientific, Uppsala, Sweden

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HDMs, house dust mites;

DP, *Dermatophagoides pteronyssinus*;

DF, *Dermatophagoides farinae*;

IAR, immediate asthmatic response;

BP, bronchoprovocation; Ab, antibody;

Allergen PC20, allergen concentration

producing a 20% fall in FEV₁;

CRD, component-resolved diagnostics; nDer

p 1, native Der p 1; rDer p 2, recombinant

Der p 2; FEV₁, forced expiratory volume in

1 s; PC, provocation concentration;

ROC, receiver operating characteristic;

AUC, area under the ROC curve;

CI, confidence interval

ABSTRACT

Background: Limited information is available regarding the clinical usefulness of measuring the levels of IgE to allergen components from house dust mites (HDMs) in the diagnosis of genuine HDM allergy.

Methods: To evaluate the diagnostic usefulness of measuring levels of serum IgE antibodies (Abs) to allergen components from *Dermatophagoides pteronyssinus* (DP) as a predictor of immediate asthmatic response (IAR) to bronchoprovocation, we studied 55 DP-sensitized asthmatic patients who underwent a bronchoprovocation test using crude DP extract. The levels of IgE Abs to crude DP, nDer p 1, rDer p 2, and rDer p 10 in patients who showed IAR ($n = 41$) were compared with those in patients who showed no IAR ($n = 14$).

Results: While the frequencies of positivity for IgE Abs to nDer p 1 and rDer p 2 among the entire study population were 89 and 86%, respectively, all patients with IAR tested positive for both of them with high IgE concentrations. The areas under the receiver operating characteristic curves for IgE to nDer p 1 and rDer p 2 as predictors of IAR were 0.913 and 0.906, respectively. The specificity of IgE to nDer p 1 and rDer p 2 was higher than IgE to crude DP even at low cut-off points.

Conclusions: IgE to nDer p 1 and/or rDer p 2 was highly predictive of allergen-induced IAR. These findings validate the clinical usefulness of measuring the levels of IgE to nDer p 1 and rDer p 2 as a diagnostic tool for genuine HDM allergy.

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Introduction

House dust mites (HDMs) are among the most important allergen sources worldwide.^{1,2} While many different species of HDM have been reported to occur in indoor environments, *Dermatophagoides*

pteronyssinus (DP) and *Dermatophagoides farinae* (DF) are the most important species in the development of HDM allergy. Epidemiological studies have shown a high correlation of exposure and sensitization to HDMs with the development of bronchial asthma.^{3–5} On the other hand, it is well recognized that not all the patients who are sensitized to HDMs develop clinical symptoms of HDM allergy. Additionally, the clinical relevance of sensitization to crude DP extract is not always clear, particularly for multisensitized patients. This is sometimes a problem in clinical settings when allergen-specific immunotherapy using DP extract is considered.

* Corresponding author. Clinical Research Center for Allergy and Rheumatology, Sagamihara National Hospital, 18-1 Sakuradai, Minami-ku, Sagamihara, Kanagawa 252-0392, Japan.

E-mail address: y-fukutomi@sagamihara-hosp.gr.jp (Y. Fukutomi).

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A positive case history is an important element in the diagnosis of IgE-mediated allergy. However, regarding HDM allergy, the reliability of case history is not always high⁶; namely, self-reported hypersensitivity symptoms after house dust exposure may not necessarily identify HDM as the actual trigger of symptoms. One possible reason for this is that most patients are not able to recognize the presence of HDMs in their indoor environment. Additionally, HDMs usually coexist with other arthropods, such as storage mites,^{7,8} booklice,^{9,10} or cockroaches, pet dander, or mold spores.¹¹ It is generally considered that an immediate asthmatic response (IAR) after the bronchoprovocation (BP) using allergen aerosols is a more reliable marker for the diagnosis of clinically relevant allergen-specific reactivity in patients with asthma than a positive case history in sensitized individuals.^{12,13}

To date, measurement of serum IgE antibodies (Abs) to crude extract and/or the skin prick test using crude extract has been performed as a standard test for allergy diagnosis in combination with case history. However, studies have shown that some allergen components from crude DP extract, such as tropomyosin,^{14,15} arginine kinase,¹⁶ and glutathione S-transferase,¹⁷ can contribute to cross-reactivity between crude DP extract and extracts from other invertebrates. Therefore, sensitization to crude DP extract does not always indicate genuine HDM sensitization. Currently, allergen components purified from their native sources or produced as recombinant proteins have been introduced into the battery of tests available for the diagnosis of allergic diseases,^{18–21} which is generally called component-resolved diagnostics (CRD),^{22–26} or recently called molecular-based allergy diagnosis.²⁷ Such techniques and diagnostics may solve these problems; however, there have been few CRD studies on HDM allergy.^{28–30}

In the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature database,³¹ 17 allergenic proteins from DP have been registered. Among these proteins, it is possible to measure the levels of IgE Abs to native Der p 1 (nDer p 1), recombinant Der p 2 (rDer p 2), and rDer p 10 using the commercially available ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden). Der p 1 and Der p 2 are the major allergens classified into Group 1 allergens (Der p 1, Der f 1) and Group 2 allergens (Der p 2, Der f 2), respectively. The Group 1 allergens Der p 1 and Der f 1 are allergenic proteins of 24 kDa sharing 80% amino acid sequence identity and a biochemical function as cysteine proteases.³² The Group 2 allergens (Der p 2 and Der f 2) also show high cross-reactivity with each other.³³ Owing to the strong cross-reactivity between major allergens from DP and DF, sensitization to Der p 1 and/or Der p 2 is considered as a marker of sensitization to mites of the genus *Dermatophagoides*. Evaluating the sensitization to Der p 1 and/or Der p 2 is considered important for the selection of patients suitable for allergen immunotherapy using HDM extract.²⁹ Der p 10 (tropomyosin) is a minor allergen in HDMs, but it is highly responsible for cross-reactivity between different HDM species and other invertebrates.

The aim of this study was to evaluate the clinical usefulness of measuring the levels of IgE Abs to commercially available allergen components from DP in the diagnosis of clinically relevant HDM allergy among asthmatic patients. We considered IAR after BP as a reliable indicator of genuine HDM allergy, and compared challenge outcome with the levels of IgE Abs to nDer p 1, rDer p 2, and rDer p 10.

Methods

Patients

We studied 55 adult asthma patients sensitized to crude DP who visited the Allergy Department of Sagami National Hospital and underwent BP using DP aerosol between 2000 and 2009.

All the patients studied showed positive results in the skin test and/or the test for serum specific IgE Abs to DP (ImmunoCAP[®], Allergen d1). Indications for the BP using allergen aerosol for each patient were determined by their physicians according to the clinical necessity. In most cases, patients underwent BP to confirm the airway reactivity to DP before starting allergen immunotherapy using DP extract. Patients being treated with systemic corticosteroids, previously treated with allergen immunotherapy using any allergen extract, or with an upper or lower tract respiratory infection in the previous month were excluded from the study. All the patients recruited to the study were considered as having mild to moderate persistent asthma on the basis of the Global Initiative for Asthma 2012 guidelines.³⁴ Written informed consent was obtained from all the patients included in the study. The ethics committee of Sagami National Hospital approved the study protocol.

Bronchoprovocation test

The BP with aqueous DP extracts (HollisterStier, Washington, USA), which was the same as extract used for skin test, was performed by the 2-min tidal breathing method.³⁵ Forced expiratory volume in 1 s (FEV₁) was determined and recorded using the same spirometer in all the subjects. All medications were withheld for at least 24 h before the start of the study until the end of the study period. The patients inhaled a solution in a DeVilbiss nebulizer (DeVilbiss, Somerset, Pa) operated using compressed air at a flow rate of 5 L/min. The nose was clipped and aerosols were inhaled through a mouthpiece during tidal breathing. After the measurement of baseline FEV₁, patients inhaled an allergen diluent for 2 min. When no change in FEV₁ was observed, allergen inhalation was performed. The diluted allergen extract was inhaled for 2 min, and FEV₁ was measured 10 min after each inhalation. The allergen was administered at increasing concentrations (10, 100, 500, and 1000 AU/ml). The inhalation test was stopped when an IAR occurred, which was defined as a decrease in FEV₁ of at least 20% from baseline within 20 min after allergen inhalation.

Serological analysis

Serum samples were obtained from patients before the BP and were stored at –20 °C until assay. The levels of serum specific IgE Abs to crude DP, nDer p 1, rDer p 2, and rDer p 10 were determined using a commercially available ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden; allergen codes, d1, d202, d203, and d205). The sum of the levels of IgE Abs to nDer p 1 and that to rDer p 2 were also calculated and described as “nDer p 1 + rDer p 2” in this manuscript. A level of IgE Abs ≥ 0.35 kU_A/L was regarded as positive according to the manufacturer's recommendation. The levels of IgE Abs lower than 0.35 kU_A/L detection limit were assigned a value of 0.34 kU_A/L in the statistical analysis.

Statistical analyses

The patients were divided into two groups according to the development of IAR, and the levels of specific IgE Abs were compared between the groups. Receiver operating characteristic (ROC) curves as a predictor of IAR positivity were plotted for the levels of IgE Abs to crude DP, nDer p 1, rDer p 2, and rDer p 10 separately, and the area under each ROC curve (AUC) was calculated. The quantitative relationship between the logarithm of levels of IgE Abs and challenge outcome was analyzed using logistic regression.³⁶ Fitted predicted probability curves were plotted using the results from the logistic regression analysis. To explore the diagnostic value of the levels of IgE Abs to allergens at different cut-off points, sensitivity and specificity were calculated from two-by-

two tables at the cut-off points of 0.35, 0.70, 3.5, and 17.5 kU_A/L. These cut-off points were determined in accordance with the classification recommended by the manufacturer, namely, class 0, less than 0.35 kU_A/L; class 1, 0.35 – <0.7 kU_A/L; class 2, 0.70 – <3.5 kU_A/L; class 3, 3.50 – <17.5 kU_A/L; class 4, 17.5 – <50 kU_A/L; class 5, 50 – ≤100 kU_A/L; and class 6, >100 kU_A/L.

The collected data were analyzed using SPSS ver. 21.0 (IBM, Tokyo, Japan). Spearman's rank correlation test was performed to determine the coefficient of correlation between different IgE test results. The statistical significance of differences between the continuous variables studied was analyzed by the Mann–Whitney *U*-test. The chi-square test was used to determine whether distributions of categorical variables significantly differed from each other. A *p* value of 0.05 or less was considered statistically significant.

Results

All patients except one were positive for IgE Abs to crude DP. One single patient tested negative for IgE to crude DP but positive in the skin test. The frequencies of positivity for IgE Abs to allergen components from DP were as follows: nDer p 1, 89%; rDer p 2, 86%; and rDer p 10, 6%.

Forty-one patients showed IAR and 14 showed no IAR in the BP. Table 1 shows the characteristics of patients with and without IAR. Age and total serum IgE level differed significantly between these groups. Fig. 1 shows levels of IgE Abs to crude DP, nDer p 1, rDer p 2, and rDer p 10 in sera from IAR-positive and IAR-negative patients. All the IAR-positive patients were positive for IgE Abs to crude DP, nDer p 1, and rDer p 2. The levels of IgE Abs to crude DP, nDer p 1 and rDer p 2, but not to rDer p 10, were significantly higher in the IAR-positive patients than in the IAR-negative patients.

The levels of IgE Abs to nDer p 1 and rDer p 2 correlated closely with those to crude DP (nDer p 1–crude DP; $R = 0.99$, $p < 0.001$, rDer p 2–crude DP; $R = 0.97$, $p < 0.001$) (Fig. 2A, B). All patients whose level of IgE Abs to crude DP ≥ 3.5 kU_A/L ($n = 43$) were positive for IgE Abs to both nDer p 1 and rDer p 2. In contrast, among those with levels of IgE Abs to crude DP < 3.5 kU_A/L ($n = 12$), 6 (50%) tested negative to both nDer p 1 and rDer p 2. None of these 6 patients showed a positive IAR. There was no significant correlation between the levels of IgE Abs to rDer p 10 and crude DP ($R = 0.17$, $p = 0.225$) (Fig. 2C). However, there was a strong correlation between the levels of IgE Abs to nDer p 1 and rDer p 2 ($R = 0.95$, $p < 0.001$) (Fig. 2D). As shown in Fig. 2A and B, the levels of IgE Abs were higher to crude DP extract than to either of nDer p 1 and rDer p 2 in most cases. However, the sum of IgE levels to nDer p 1 and rDer p 2 was similar to the level of IgE Abs to crude DP extract (Fig. 2E), demonstrating that both Der p 1 and Der p 2 are major allergenic determinants in HDM.

ROC analysis was performed to evaluate the ability of IgE Abs to crude DP and pure DP components to predict IAR (Fig. 3A). The

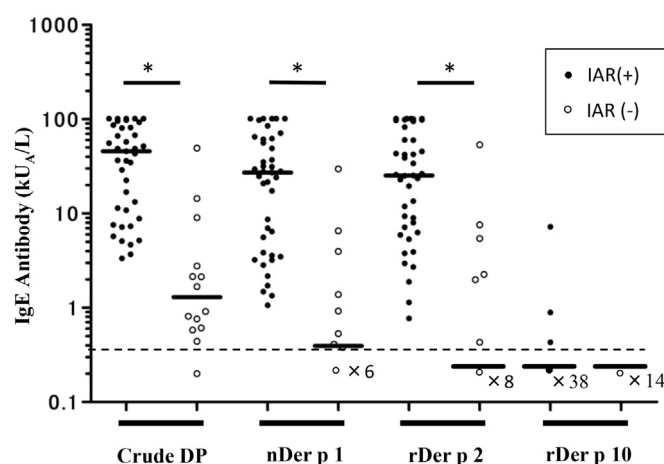


Fig. 1. Levels of IgE Abs to crude DP, nDer p 1, rDer p 2, and rDer p 10 in sera from IAR-positive and IAR-negative patients. * $p < 0.01$. The number of patients showing negative results in each set of measurements is indicated below the dotted cut-off line (0.35 kU_A/L).

AUCs for the levels of IgE Abs to nDer p 1 and rDer p 2 were 0.913 (95% confidence interval (CI), 0.817–1.000) and 0.906 (95% CI, 0.801–1.000), respectively, comparable to that for DP (0.920; 95% CI, 0.828–1.000).

Fig. 3B shows the relationship between the levels of IgE Abs and IAR positivity predicted using a logistic regression model. The curves show ascending slopes in which an increasing IgE concentration value corresponds to a higher probability of IAR positivity. Table 2 shows diagnostic values of IgE Abs to crude DP, nDer p 1, and rDer p 2 at different cut-off points. IgE Abs to crude DP showed good diagnostic values at the cut-off point of 3.5 kU_A/L. However, with the cut-off point at 0.7 kU_A/L, the specificity of IgE to crude DP was low (29%). IgE to nDer p 1 and rDer p 2 showed good diagnostic values even at cut-off points of 0.35 and 0.70 kU_A/L, demonstrating higher specificity than crude DP.

Discussion

The aim of this study was to analyze the diagnostic efficiency of levels of IgE Abs to nDer p 1 and rDer p 2 as predictors of IAR after BP. ROC analysis showed comparable AUCs for IgE Abs to nDer p 1, rDer p 2, and crude DP while the pure allergen components displayed higher diagnostic specificity than the natural allergen extract. These findings support the clinical usefulness of measuring the levels of IgE Abs to Der p 1 and Der p 2 as a diagnostic tool for genuine HDM allergy.

Sensitization to an allergen does not always indicate a clinically relevant relationship between allergen exposure and the occurrence of allergen-specific allergic symptoms. In this study, we considered the results of BP using allergen aerosol as an indicator of a clinically relevant relationship between allergen exposure and allergic symptoms, and examined the diagnostic performance of IgE Abs to allergen components using IAR positivity as an outcome variable. To the best of our knowledge, this is the first report on the association between the levels of IgE Abs to HDM allergen components and allergen-specific airway reactivity determined by the BP test. Although BP is not a routine procedure, it is a highly relevant test in the diagnosis of respiratory allergy and useful in the case of uncommon, newly identified, or occupationally encountered allergens.³⁷ When allergen immunotherapy for the treatment of allergic asthma is considered, the selection of allergens may be guided by positive results in BP. This is considered to be the most

Table 1
Characteristics of patients with and without IAR after bronchoprovocation with DP aerosol.

	IAR-positive ($n = 41$)	IAR-negative ($n = 14$)	<i>p</i> -Value
Age (yr)	31 (26–39)	46 (32–58)	0.02
Gender, <i>n</i> . (%)			0.78
Men	13 (32)	5 (36)	
Women	28 (68)	9 (64)	
Total IgE (kU _A /L)	482.17 (222.47–827.04)	149.31 (77.00–418.17)	0.04
FEV ₁ (L)	2.60 (2.42–2.91)	2.46 (1.85–2.83)	0.29
FEV ₁ (% predicted)	81.3 (76.7–86.5)	79.3 (72.6–89.8)	0.06

DP, *Dermatophagoides pteronyssinus*; FEV₁, forced expiratory volume in 1 s. Results are expressed as medians with interquartile range or as absolute numbers with percentages.

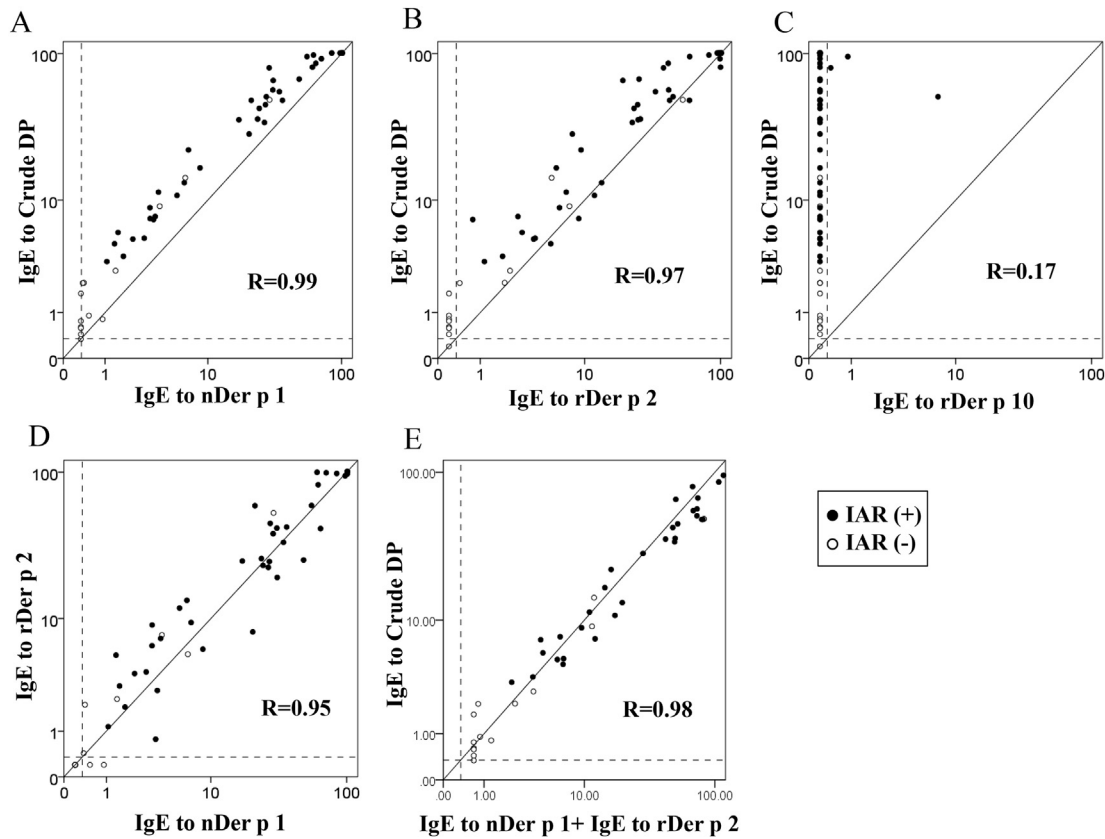


Fig. 2. Correlations between the levels of IgE Abs to nDer p 1 and crude DP (A), rDer p 2 and crude DP (B), rDer p 10 and crude DP (C), nDer p 1 and rDer p 2 (D), and nDer p 1 + rDer p 2 (sum of the levels of IgE Abs) and crude DP (E). Closed circles, IAR-positive patients; open circles, IAR-negative patients. The coefficients of correlation between variables are also shown in each figure.

specific of all procedures for determining allergen-specific airway reactivity.

Der p 1 and Der p 2 are major DP allergens and sensitization to Der p 1 and/or Der p 2 is considered to be a hallmark of genuine *Dermatophagoides* sensitization.^{29,38} In contrast, an IgE response to crude DP but not to Der p 1 or Der p 2 can occur in patients with a primary sensitization to another allergen source, not necessarily

associated with HDM allergy. In this study, half ($n = 6$) of the patients with low levels of IgE Abs to crude DP (<3.5 kU_A/L) tested negative to both nDer p 1 and rDer p 2. As these patients displayed no IAR to HDM, we consider measurements of IgE Abs to nDer p 1 and rDer p 2 to be particularly relevant and informative for patients showing low levels of IgE Abs to crude DP. While the identities of the determinants causing the positive crude DP test results in these

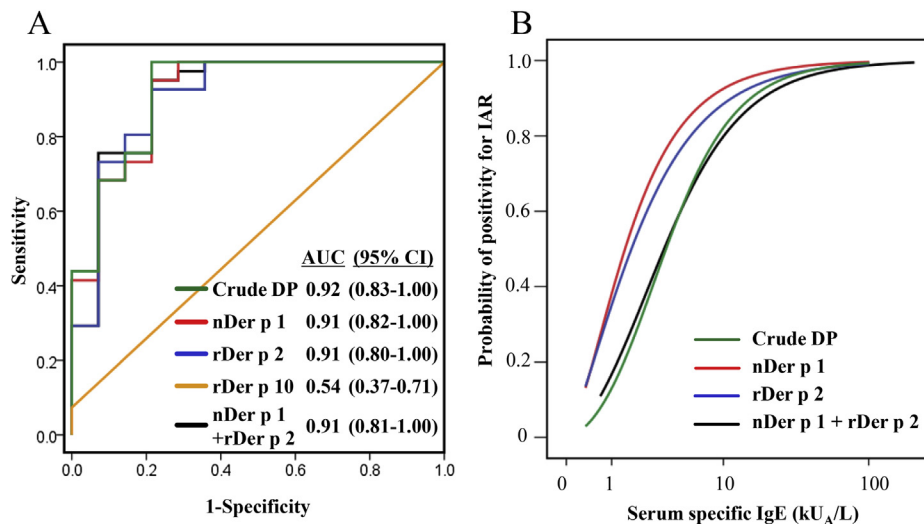


Fig. 3. Receiver operating characteristic curves (A) and fitted predicted probability curves (B) showing the relationship of the levels of IgE Abs to crude DP and allergen components from DP with IAR positivity after bronchoprovocation. AUC, area under the curve; CI, confidence interval.

Table 2

Diagnostic values of IgE to crude DP and allergen components at different cut-off points as predictor of IAR.

	Sensitivity (%)	Specificity (%)
Crude DP (kU _A /L)		
0.35 [†]	100	7
0.70	100	29
3.50	98	79
17.5	66	93
nDer p 1 (kU _A /L)		
0.35	100	43
0.70	100	64
3.50	78	79
17.5	61	93
rDer p 2 (kU _A /L)		
0.35	100	57
0.70	100	64
3.50	88	79
17.5	61	93

[†] Interpretation of values in this line is limited because only one patient showed IgE <0.35 kU_A/L to crude DP.

patients are presently unknown, Der p 10 can safely be excluded based on the measurements performed in this study. We also measured IgE Abs to cockroach (i6), moth (i8), *Acarus siro* (d70), *Lepidoglyphus destructor* (d71), and *Tyrophagus putrescentiae* (d72) in these patients to explore possible cross-reactivity between DP and these arthropods. However, only two of the patients showed positive results to any of these allergen sources (IgE to i6, i8, d70, d71 and d72 in one patient, 0.42, 2.80, <0.35, <0.35 and 0.36, and in the other patient, 1.03, 2.78, 1.17, 0.49 and 1.40 kU_A/L, respectively).

One previous study has evaluated the performance of IgE to microarrayed Der p 1 and/or Der p 2 as a predictor of HDM allergy determined on the basis of allergen-specific histories.³⁰ The AUC of IgE Abs to microarrayed DP-allergen components was 0.808, which was lower than that of IgE Abs to crude DP as determined using ImmunoCAP system (AUC, 0.911) although the difference between these AUCs did not reach statistical significance. Additionally, only 69% of the patients with HDM allergy were positive for IgE Abs to microarrayed DP-allergen components. However, in our study, AUC of IgE Abs to crude DP and DP-allergen components were not different, and all of the IAR-positive patients were sensitized to Der p 1 and Der p 2. One reason for the difference in findings between these studies was supposed to be related to the difference in the definition of HDM allergy. The reliability and specificity of HDM allergy diagnosis can be increased by basing the diagnosis on both the positive reaction after BP using a DP extract and case histories. Another reason for this was that the measurements of IgE Abs in the two studies are not fully comparable as the present study was conducted entirely using ImmunoCAP, which is a more sensitive method than microarrays.²⁷

The main limitation of this study is related to the recruitment of subjects. The indication of the BP for each patient was determined according to clinical relevance, rather than predefined inclusion criteria, which may be related to the generality of our findings and applicability to other patient populations. Another limitation was that we have not evaluated the association between the levels of IgE Abs and the threshold of allergen concentration in the BP test, that is the allergen concentration producing a 20% fall in FEV₁ (allergen PC20). A study has shown that IgE to crude DP is the main determinant of allergen PD20 after BP using crude DP extract.³⁹ However, we were unable to accurately calculate allergen PC20 in our study, because the stepup of allergen concentrations in the BP test in our study was not 2-fold, which is recommended in the literature.⁴⁰

In conclusion, this study showed that IgE to nDer p 1 and/or rDer p 2 was highly predictive of IAR after BP using DP aerosol. The

outcome of the BP test using crude DP was largely dependent on the levels of specific IgE Abs to these DP-allergen components. Measurement of IgE Abs to nDer p 1 and/or rDer p 2 is a useful tool and these tests may replace crude DP for the diagnosis of genuine HDM allergy, providing for higher diagnostic specificity.

Conflict of interest

JL is an employee of Thermo Fisher Scientific. The rest of the authors have no conflict of interest.

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