



Comparison of two different assays and the predictive value of allergen components in house dust mite allergy

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Aim: In house dust mite (HDM) allergy diagnostics, the IMMULITE, ImmunoCAP and assays for allergen components (nDer p 1 and rDer p 2) are available. **Methods:** Serum sIgE levels were compared and the predictive values for the detection of an early asthmatic response (EAR) were calculated with receiver operating characteristics and a log-logistic regression model. **Results:** sIgE levels of IMMULITE and ImmunoCAP were similar (*Dermatophagoides pteronyssinus* [*D. pter.*] 47.3 ± 35.7 and 42.9 ± 34.4 kU.l⁻¹; $p = 0.23$). ImmunoCAP sIgEs exhibited similar accuracy in detecting an EAR, area under the curves (AUCs): *D. pter.* (0.76); *Dermatophagoides farinae* (0.79); nDer p 1 (0.69); and rDer p 2 (0.72). At low sIgE concentrations (3.5 kU.l⁻¹), rDer p 2 was more specific and better predicted an EAR (probability rDer p 2: 62%; *D. pter.*: 19%).

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Allergy testing is an important part of the diagnostic process in children suffering from allergic diseases [1]. For seasonal allergies, the patient's history and a simple skin prick test are often sufficient for an exact diagnosis. Recombinant allergens have tremendously improved our knowledge of and diagnostic accuracy for food allergies [2]. Diagnostics for perennial allergies are still challenging because the clinical symptoms are less clear and are often more chronic, such as a blocked nose and chronic cough in rhinitis and asthma. Current guidelines recommend nasal or bronchial challenges when specific IgE (sIgE) test results do not agree with the patient's history, especially when specific immunotherapy (SIT) is indicated [1].

The IMMULITE and ImmunoCAP tests are available for allergy diagnostics. In a subgroup of 35 patients allergic to house dust mites (HDMs), the IMMULITE results showed higher sIgE concentrations than the ImmunoCAP results. Both tests had very similar sensitivity and specificity and an almost perfect agreement as measured by Cohen's kappa coefficient [3]. We recently reported that the IMMULITE sIgE levels for *Dermatophagoides pteronyssinus* (*D. pter.*) and *Dermatophagoides farinae* (*D. far.*) significantly predicted a positive reaction in bronchial allergen provocation challenges (BAP) with an accuracy of 88% [4]. Similar results were shown in a small group of pediatric patients for the ImmunoCAP [5].

HDM (*D. pter.* and *D. far.*) are distributed worldwide, although *D. pter.* prefers more humid climates and *D. far.* is more common in drier areas. *D. pter.* contain high concentrations of group 1 and 2 allergens, including purified natural (nDer p 1) and recombinant (rDer p 2) allergens [6]. In a European population from Austria, France, Italy and Sweden, nDer p 1 and rDer p 2 were sufficient for the diagnosis of $\geq 97\%$ of *D. pter.* allergic patients [7]. The sources of *D. pter.* and *D. far.* are whole body cultures, whereas nDer p 1 and rDer p 2 represent well-defined components. Thus, allergen components might be an alternative to crude allergens for the diagnosis of allergic asthma and the indication of SIT [7].

In addition to allergen sIgE, total IgE has been shown to be a predictor in BAPs [4,5,8]. However, the optimal cut-off IgE levels were different in the studies, which limited the diagnostic use of total IgE. The ratio of sIgE to total IgE compared to sIgE alone showed inconsistent results for predictions of the outcomes of oral challenges with food allergies [9–11]. In SIT, the sIgE to total IgE ratio significantly predicted successful clinical responses [12–14]. Less is known about the predictive value of this ratio in bronchial challenges.

Pittner *et al.* distinguished between two groups of patients who were sensitized to mite allergens [15]. The majority of the patients were sensitized to the major Der p allergens. A smaller group had a broad sensitization profile, including tropomyosin. The authors hypothesized that tropomyosin might be useful for identifying patients who were less suitable for SIT.

In the present study, we compared the results and predictive values of HDM sIgEs measured by IMMULITE and ImmunoCAP. Furthermore, the predictive value of the allergen components nDer p 1 and rDer p 2 was compared with the crude extracts *D. pter.* and *D. far.*, allergen component rDer p 10 (tropomyosin) was measured and the results were presented in the context of HDM allergies.

Materials & methods

Subjects & selection

50 outpatients seeking treatment from the Department of Allergy, Pulmonology and Cystic Fibrosis at our hospital between August 2010 and May 2014 were included in the study. The measurements were part of the study.

During the study, the patients underwent specific BAPs with HDM before SIT and after 1 and 2 years of SIT. At each appointment, a full blood count was measured and serum samples were obtained from the subjects and stored at -80°C for further analysis.

The inclusion criteria comprised allergic asthma based on a previous physician's diagnosis, age between 5 and 18 years, a positive skin prick test for HDM (*D. pter.* and *D. far.*, Allergopharma KG, Reinbek, Germany) with a wheal size ≥ 3 mm and a predicted baseline forced expiratory volume for 1 s (FEV₁) $\geq 75\%$.

Short-acting β -agonists were withdrawn 8 h before the challenge and inhaled corticosteroids, leukotriene receptor antagonists and long acting β -agonists were withdrawn a minimum of 24 h before the challenge. The patients had no airway infections and their parents provided written informed consent. The study was approved by the medical ethics committee of Goethe University Hospital, Frankfurt am Main, Germany.

sIgE antibodies

Samples were analyzed for specific antibodies against *D. far.* and *D. pter.* IgE using a two-sided chemiluminescent assay (IMMULITE, Siemens Healthcare, Erlangen, Germany). The analysis was performed according to the manufacturer's instructions.

The samples were analyzed by Thermo Scientific in Freiburg, Germany, for specific antibodies against a-IgE, *D. pter.*, *D. far.*, nDer p 1, rDer p 2 and rDer p 10 (tropomyosin) using the Pharmacia ImmunoCAP System.

Pulmonary function tests

A baseline pulmonary function test was performed using the MasterScreen spirometer (CareFusion, Hoechst, Germany). The following parameters were recorded: forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁) and Tiffeneau index (FEV₁%FVC).

Specific allergen challenge

BAP with a mite allergen (*D. pter.*, 5000 standardized biological units/ml [SBU], Allergopharma KG) was performed using the APS dosimeter technique (CareFusion). The incremental challenge protocol consistently followed the previously described algorithm [4]. The dose of inhaled allergen was doubled beginning with the lowest dose of ten SBUs, according to the following pattern from step one to step five: 10, 20, 40, 80, 160 and 320 SBU. Thus, the entire protocol delivered cumulative doses of 10, 30, 70, 150, 310 and 630 SBU. The individual allergen dose that caused a 20% drop in FEV₁ in the EAR (PD₂₀FEV₁ allergen) was calculated using a logarithmic interpolation between the doses before and after the 20% drop in FEV₁.

Statistical analysis

GraphPad Prism 5.01 (GraphPad Software, CA, USA), BiAS for Windows™ version 11 (epsilon-Verlag, Darmstadt, Germany), R Core Team (2014) (R Foundation for Statistical Computing, Vienna, Austria) and Microsoft

Table 1. Patient characteristics.

Subjects	(n)	50
Male/female	(n)	34/16
Age at first challenge	(yr, mean $\pm$ SD)	9.6 $\pm$ 2.7
FEV ₁ at first challenge	(%pred, mean $\pm$ SD)	98.4 $\pm$ 13.7
FVC at first challenge	(%pred, mean $\pm$ SD)	93.3 $\pm$ 9.9

FEV₁: Forced expiratory volume in 1s; FVC: Forced vital capacity.

Table 2. Specific challenge and radio-allergo-sorbent-test measurements.

PD ₂₀ FEV ₁ mite	(SBU, median, IQR)	66 (27.5; 150.0)
IMMULITE (n = 115)		
Spec. IgE <i>D. pter.</i>	(kU.l ⁻¹ , median, IQR)	37.9 (13.6; 85.1)*
Spec. IgE <i>D. far.</i>	(kU.l ⁻¹ , median, IQR)	42.3 (22.0; 91.7) [†]
ImmunoCAP (n = 119)		
Total IgE	(kU.l ⁻¹ , median, IQR)	309 (146.0; 804.0)
Spec. IgE <i>D. pter.</i>	(kU.l ⁻¹ , median, IQR)	32.4 (16.3; 74.1)*
Spec. IgE <i>D. far.</i>	(kU.l ⁻¹ , median, IQR)	37.5 (19.6; 85.8) [†]
Spec. IgE nDer p 1	(kU.l ⁻¹ , median, IQR)	18.0 (2.6; 40.3)
Spec. IgE rDer p 2	(kU.l ⁻¹ , median, IQR)	22.4 (10.5; 64.8)
Spec. <i>D. pter.</i> : total IgE ratio	(median, IQR)	0.09 (0.04; 0.16)

*p = 0.23,

[†]p = 0.29RAST: Radio-allergo-sorbent-test; PD₂₀FEV₁ mite: Provocation dose of mite allergen causing a 20% drop in FEV₁; SBU: Standardized biological unit; IQR: Interquartile range.

Excel 2010 were used for the statistical analysis. The parameters were tested for normal distributions using the Kolmogorov-Smirnov test. The correlations were calculated using Spearman's rank correlation test. Repeatability was evaluated using the method described by Bland and Altman. To define the sensitivity and specificity of a predictor for the detection of an EAR or a late asthmatic response, a receiver operating characteristic (ROC) curve was plotted. Cut-off levels were optimized using the Youden index (sensitivity + specificity-1). The accuracy was measured by the area under the curve (AUC) analysis. The individual probability of the occurrence of an EAR was calculated by log-logistic regression models. In the stepwise regression analysis, the addition of the components nDer p 1 and rDer p 2 to *D. pter.* and *D. far.* were tested. The p-values ≤ 0.05 were considered statistically significant.

Results

Patient characteristics & sIgE measurements

All 50 patients underwent a total of 120 BAPs with HDM. At the first challenge, the mean age was 9.6 years, the mean FEV₁ was 98.4% predicted and the mean FVC was 93.3% predicted. In 102 BAPs (85%) a significant EAR occurred ($\geq 20\%$ drop in FEV₁). In all challenges, the median PD₂₀FEV₁ mite allergen was 66 SBUs (Table 1). The sIgE levels for *D. far.* and *D. pter.* IgE were measured by the IMMULITE test in 115 aliquots and the total IgE (aIgE) and sIgE levels for *D. far.*, *D. pter.*, nDer p 1, rDer p 2 and rDer p 10 were measured by the ImmunoCAP test in 119 aliquots. Then, the nDer p 1: total IgE ratio was calculated. The sIgE measurements are shown in Table 2.

Comparison of IMMULITE with ImmunoCAP

The sIgEs for *D. pter.* and *D. far.* obtained with the IMMULITE test were higher than the sIgEs obtained with the ImmunoCAP test, although the difference was not significant (p = 0.23 and p = 0.29, respectively) (Figure 1). For *D. pter.* and *D. far.*, the IMMULITE and ImmunoCAP results had a strong correlation (r = 0.91 and r = 0.90; p < 0.001). The agreement between the tests was expressed according to the method of Bland and Altman as a 95% CI and as percentages of the values [16]. For *D. pter.*, the 95% CI ranged from -22.2 to 33.2 kU.l⁻¹ (mean 5.5 kU.l⁻¹; SD: 14.1) and 31 of the 114 (27.2%) ¹⁰log transformed values were outside of the $\pm 20\%$ limit of

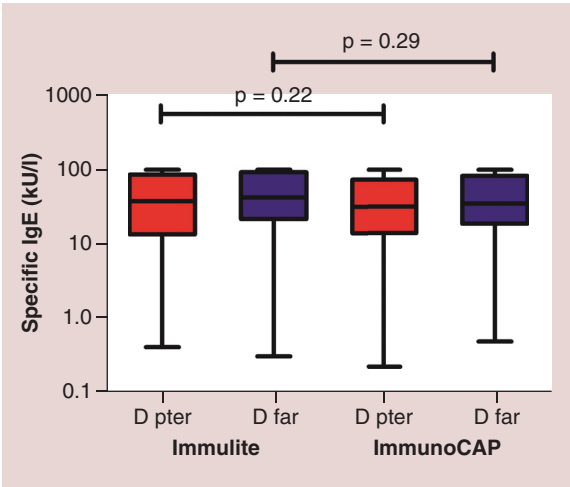


Figure 1. Comparison of IMMULITE and ImmunoCAP on a log scale. The median, the interquartile range, and minimum and maximum mite-specific IgE values are presented. No significant differences were observed between the tests.

Table 3. Spearman's rank correlation Association with the PD ₂₀ FEV ₁ mite.			
		Correlation coefficient (r)	p-value
IMMULITE (n = 115)			
Spec. IgE <i>D. pter.</i>	(kU.l ⁻¹)	-0.43	<0.001
Spec. IgE <i>D. far.</i>	(kU.l ⁻¹)	-0.46	<0.001
ImmunoCAP (n = 119)			
Total IgE	(kU.l ⁻¹)	-0.26	0.09
Spec. IgE <i>D. pter.</i>	(kU.l ⁻¹)	-0.36	<0.001
Spec. IgE <i>D. far.</i>	(kU.l ⁻¹)	-0.53	<0.001
Spec. IgE nDer p1	(kU.l ⁻¹)	-0.20	0.03
Spec. IgE rDer p2	(kU.l ⁻¹)	-0.53	<0.001
Spec. <i>D. pter.</i> total a-IgE ratio		-0.20	0.03

agreement. For *D. far.*, the 95% CI ranged from -21.1 to 29.5 kU.l⁻¹ (mean 4.2 kU.l⁻¹; SD: 12.9) and 21 of the 114 (18.4%) ¹⁰log transformed values were outside of the ± 20% limit of agreement.

In positive allergen challenges (PD₂₀ <310 SBUs), the IMMULITE and ImmunoCAP tests for *D. pter.* and *D. far.* were positive in all measurements (≥0.35 kU.l⁻¹). The ImmunoCAP for nDer p 1 was negative in seven measurements and for rDer p 2 was negative in six measurements (<0.35 kU.l⁻¹).

Correlations & ROC analysis to detect an EAR

We correlated the different IgEs with the PD₂₀FEV₁ mite allergen in BAP. With the exception of total IgE, all sIgEs correlated significantly with the PD₂₀FEV₁ mite allergen (Table 3).

In the ROC analysis, the total IgE and all sIgEs (except the *D. pter.*: total IgE ratio) significantly predicted an EAR in the bronchial challenge with HDM. The sIgEs for *D. pter.* and *D. far.* had the best accuracies (Table 4 & Figures 2 & 3). The AUCs of ImmunoCAP for *D. far.* and nDer p 1 were significantly different (p < 0.05).

Log-logistic regression models

The IMMULITE ¹⁰log *D. pter.* and ¹⁰log *D. far.* results significantly predicted the individual probability of an EAR with an accuracy of 86.1 and 87.8%, respectively (p < 0.001). The ImmunoCAP ¹⁰log *D. pter.*, ¹⁰log *D. far.*, ¹⁰log nDer p 1 and ¹⁰log rDer p 2 results significantly predicted the individual probability of an EAR with an accuracy of 89.2, 87.2, 85.0 and 84.9%, respectively (p < 0.001). The ¹⁰log *D. pter.* equation used to predict the individual probability of an EAR was $p = 1 \cdot (1 + \exp[-(-0.57 + 1.87 \cdot ^{10}\log D. pter.)])^{-1}$ (ImmunoCAP, intercept = 0.57; β = -1.87, AUC: 0.89). At 3.5 kU.l⁻¹, the probability of an EAR was estimated to be 19% for *D. pter.*, 69% for nDer p 1 and 62% for rDer p 2. A 90% probability of an EAR for *D. pter.* was estimated at 96 kU.l⁻¹, for nDer p 1 at 93 kU.l⁻¹ and for rDer p 2 at 92 kU.l⁻¹ (Figures 3 & 4). In the stepwise logistic regression model, the

Table 4. Receiver operating characteristic analysis to detect an early asthmatic response.

		Optimal cut-point	Sensitivity (95% CI)	Specificity (95% CI)	AUC	p-value
IMMULITE (n = 115)						
Spec. IgE <i>D. pter.</i>	(kU.l ⁻¹)	32.9	0.62 (0.51–0.72)	0.78 (0.52–0.94)	0.73	<0.001
Spec. IgE <i>D. far.</i>	(kU.l ⁻¹)	42.4	0.57 (0.46–0.67)	0.89 (0.65–0.99)	0.79	<0.001
ImmunoCAP (n = 119)						
Total IgE	(kU.l ⁻¹)	211.5	0.63 (0.53–0.73)	0.67 (0.41–0.87)	0.66	0.03
Spec. IgE <i>D. pter.</i>	(kU.l ⁻¹)	32.5	0.55 (0.45–0.65)	0.83 (0.59–0.96)	0.76	<0.001
Spec. IgE <i>D. far.</i>	(kU.l ⁻¹)	26.8	0.69 (0.59–0.78)	0.83 (0.59–0.96)	0.79 [§]	<0.001
Spec. IgE nDer p1	(kU.l ⁻¹)	9.8	0.67 (0.57–0.76)	0.67 (0.41–0.87)	0.69 [§]	0.01
Spec. IgE rDer p2	(kU.l ⁻¹)	16.8	0.68 (0.58–0.77)	0.72 (0.47–0.90)	0.72	<0.005
Spec. <i>D. pter.</i> total a-IgE ratio		0.06	0.68 (0.58–0.77)	0.55 (0.30–0.78)	0.62	0.11

[§]Difference p < 0.05.

AUC: Area under curve; EAR: Early asthmatic response; ROC: Receiver operating characteristic.

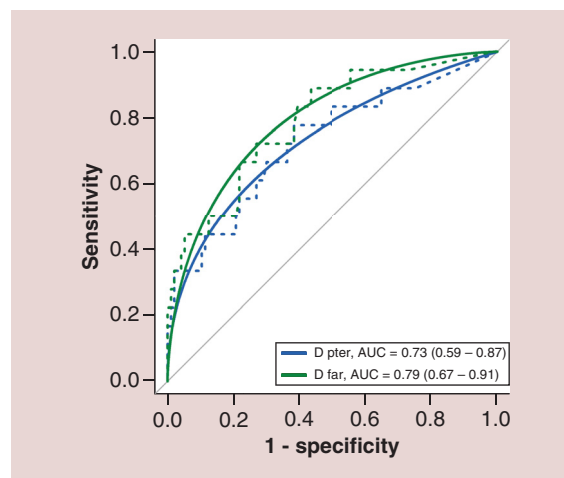


Figure 2. Receiver operation characteristic curves (IMMULITE). A specific IgE of 32.9 kU.l⁻¹ against *D. pter.* and 42.4 kU.l⁻¹ against *D. far.* were the optimal cut-off values to identify an early asthmatic response.

combination of ¹⁰log *D. pter.* and ¹⁰log *D. far.* did not improve the prediction of an EAR (88.0%), similar to the combination of ¹⁰log *D. pter.* or ¹⁰log *D. far.* with ¹⁰log nDer p 1 or ¹⁰log rDer p 2.

Allergen component Der p 10

Allergen component Der p 10 was positive in three patients (two patients were class 2 positive in two and three consecutive measurements and one patient was class 5 and 4 positive in two consecutive measurements). (Figure 5).

Discussion

IMMULITE and ImmunoCAP are widespread systems in routine allergy diagnostics. IMMULITE is commonly agreed to measure higher sIgE levels compared with ImmunoCAP [3,17,18]. In 50 children and adolescents seeking treatment from a pediatric allergy practice, weighted linear regression lines for *D. far.* indicated that the values were not significantly larger than the values obtained for IMMULITE [17]. In 35 patients with mite allergies, IMMULITE yielded higher median sIgE concentrations, and the Spearman analysis showed strong correlations between IMMULITE and ImmunoCAP (overall HDM allergens $r = 0.912$) [3]. In our study, the IMMULITE results were slightly higher than the ImmunoCAP results, although the difference was not significant. The excellent

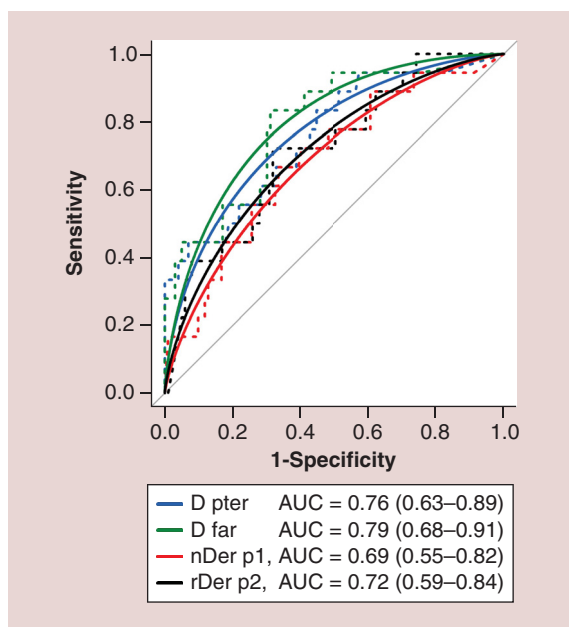


Figure 3. Receiver operation characteristic curves (ImmunoCAP). A specific IgE of 32.5 kU.l⁻¹ against *D. pter.*, 26.8 kU.l⁻¹ against *D. far.*, 9.8 kU.l⁻¹ against nDer p 1, and 16.8 kU.l⁻¹ against rDer p 2 were the optimal cut-off points to identify an early asthmatic response.

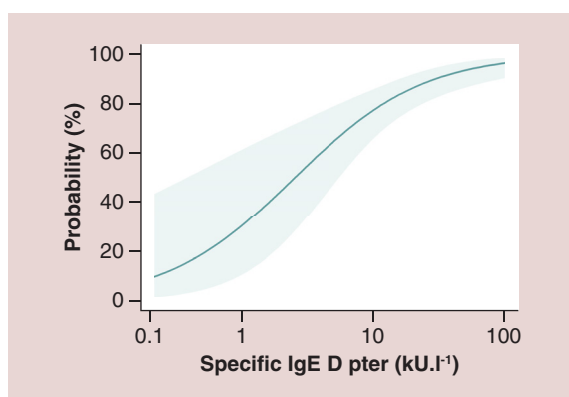


Figure 4. Probability of an early asthmatic response, ImmunoCAP *D. pter.* For *D. pter.* the probability of an EAR was estimated as 19% at 3.5 kU.l⁻¹ and 90% at 96 kU.l⁻¹.

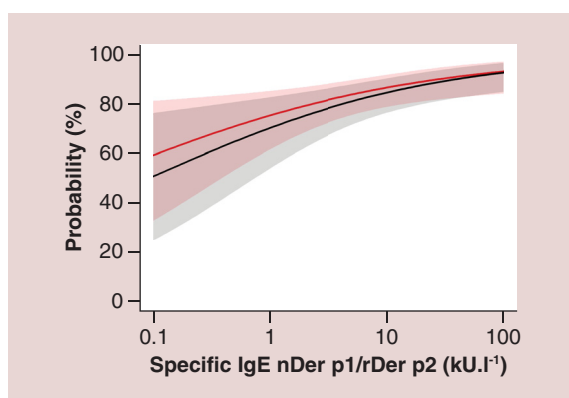


Figure 5. Probability of an early asthmatic response, ImmunoCAP nDer p 1 and rDer p 2. For nDer p 1, the probability of an EAR was estimated as 69% at 3.5 kU.l⁻¹ and 90% at 93 kU.l⁻¹. For rDer p 2, the probability of an EAR was estimated as 62% at 3.5 kU.l⁻¹ and 90% at 92 kU.l⁻¹.

correlation agreed with our results. In Bland and Altman plots the accepted deviation between two assays was $\pm 20\%$ [19]. In the study of Wang *et al.* both tests had large limits of agreement, with 38% of the values falling outside of the $\pm 20\%$ limit [17]. We could not confirm these results because the 95% limits of agreement were between approximately -21 and 30 kU.l⁻¹ and only 18.4% (*D. far.*) and 27.2% (*D. pter.*) of the patients exceeded the $\pm 20\%$ limits.

The presence of allergen-sIgE alone does not necessarily prove an existing allergy [20]. Therefore, nasal, conjunctival, bronchial and oral challenges remain the gold standards for the diagnosis of allergic diseases. The basophil activation test was shown to be able to distinguish *in vitro* the allergens causing clinical symptoms from those simply sensitizing, but its accessibility is still limited [21]. We investigated the predictive value of sIgEs using a well established bronchial provocation model. Both IMMULITE and ImmunoCAP *D. pter.* and *D. far.* equally predicted a positive reaction. The optimal cut-off levels between sensitivity and specificity were similar for *D. pter.*, whereas the level for *D. far.* was slightly higher for IMMULITE and lower for ImmunoCAP.

For IMMULITE, the cutoffs were higher and the accuracy was slightly lower compared to our previous study [22]. In the previous study, all data were collected before SIT, whereas the present data were measured before and during the course of SIT. Because the procedure and the challenge protocol were equal, the SIT itself may have influenced the results. In the log-logistic regression analysis, the accuracy of the individual probability of an EAR was equal for *D. far.* in both studies (88%). In a former study, the optimal cutoffs for the ImmunoCAP test were much lower ($\geq 5 \text{ kU.l}^{-1}$) [5]. However, the number of patients in that study was small.

HDM allergen components may be a more accurate test for the diagnosis of a mite allergy. One previous study evaluated the performance of IgE to nDer p 1 and/or rDer p 2 as a predictor of HDM allergies measured by BAPs [23]. The accuracy of both recombinant allergens was equal to crude *D. pter.*, although the recombinant allergens were more specific (but less sensitive) at low concentrations ($< 3.5 \text{ kU.l}^{-1}$). In our study, the accuracies of nDer p 1 and rDer p 2 were similar to *D. pter.* and *D. far.* in the ROC analysis. However, at the optimal cut-point, the allergen components showed a better balance of sensitivity and specificity. In the regression model, allergen components at low and middle concentrations better predicted the probability of positive allergen challenges. This outcome was mainly due to the better specificity.

In the stepwise regression model, the addition of nDer p 1 and rDer p 2 did not improve the diagnostic value of *D. pter.* or *D. far.* Because *D. pter.* contains high nDer p 1 and rDer p 2 concentrations [6], the difference may be too small to obtain any additional information. Indeed, 91% of people with skin prick-positive mite allergies were positive for nDer p 1 and 86% were positive for rDer p 2 [15], which reflected the close relationship between the tests. *In vitro*, the IgE-binding capacity of nDer p 1 in nondenaturing ELISA assays was considerably lower than the IgE-binding capacity of rDer p 2 [15]. Our results confirmed these observations, because nDer p 1 had the weakest accuracy in predicting a positive bronchial challenge.

Total IgE predicts an EAR following BAP. In a group of adult patients allergic to grass pollen, the predictive accuracy of total IgE was 73%, which was highly significant [8]. In a small group of children with HDM and grass pollen allergies, the accuracy was 77% [5]. In our large retro- and prospective studies with children allergic to HDM, the accuracy of total IgE was 60%, which was similar to the present data [4]. Despite consistently significant accuracies, the optimal cut-off values of the total IgEs differed between the studies. This discrepancy is a limiting factor in the use of total IgE as a predictor of an EAR.

Next, we evaluated whether the sIgE to total IgE ratio was a valuable marker to predict an EAR in the BAP. The sensitivity and specificity of the AUC of the ratio were higher than the measurements for serum sIgE and total IgE alone for the prediction of a clinical response to SIT [12]. However, in our study, the specific *D. pter.* to total IgE ratio did not significantly predict a positive reaction. The total IgE had a greater deviation than the sIgE, which explained the less precise accuracy of the ratio. This result agreed with the findings of studies conducted in peanut and hazelnut allergic children. In these studies, the specific to total IgE ratios did not improve the prediction, whereas the sIgE levels were the best single predictors for allergies [2,11].

In our population, only three of 50 patients (6%) were rDer p 10-positive. In contrast, rDer p 10 was recognized in 15.2% of pediatric and adult Austrian patients allergic to HDMs [24]. The allergic reactivity of rDer p 10 was very low compared to rDer p 1 and rDer p 2 [24]. Allergen component Der p 10 allows the identification of patients with a broad sensitization to HDM and these polysensitized patients may be less suitable for SIT [15,24]. In our pediatric group, measurement of rDer p 10 was of little value compared to a more complex IgE repertoire for HDM in adult patients.

Shellfish (shrimp and snail) allergies have been related to mite sensitization, which is mainly mediated by the muscle protein tropomyosin (rDer p 10). Der p 10-positive patients might have a higher risk during SIT, because four children with food allergies to snails and mite-induced asthma experienced life-threatening reactions after inadvertent ingestion of snails several months after starting SIT against mites [25]. Van Ree *et al.* investigated the sera of 17 patients receiving SIT for an HDM allergy. Serum samples were taken at the start of SIT and 14–20 months later. The average response to snail sIgE showed a significant increase and a reaction to tropomyosin

was demonstrated in two-thirds of the serum samples. The authors concluded that induction of sIgE during mite SIT might occasionally cause a food allergy [26]. However, this finding was not confirmed by a recent study, because mite SIT did not induce clinically relevant sensitization to tropomyosin. In contrast, the authors hypothesized that the treatment might even improve food allergy symptoms [27]. In our small group of three tropomyosin-positive patients, we did not observe a severe reaction to food or an increase in specific rDer p 10 during SIT.

Our study had limitations. Because we measured the sIgEs at different SIT steps, the SIT might have influenced the results, especially the cut-off values in the ROC analysis. For the main objectives of the study, the comparison of IMMULITE and ImmunoCAP and the comparison of *D. pter.* and *D. far.* with recombinant allergens for SIT did not influence the data because the measurements were taken from the same samples. The strength of the design was that the predictive value of sIgE was demonstrated both before and during SIT.

Conclusion

IMMULITE and ImmunoCAP are similar allergy diagnostics for HDM allergies. IMMULITE estimated slightly higher sIgE levels, although the levels were not significantly different from the levels obtained with ImmunoCAP. For ImmunoCAP, the allergen components nDer p 1 and rDer p 2 were comparable to *D. pter.* and *D. far.* and better predicted the probability of an EAR at the low and middle concentrations.

Summary points

- Diagnostics for perennial allergies still face challenges and current guidelines recommend nasal or bronchial challenge when specific IgE (sIgE) test results do not agree with the patient's history.
- Among allergy diagnostics, IMMULITE showed higher sIgE levels than ImmunoCAP.
- Natural (nDer p 1) and recombinant (rDer p 2) allergens might be more accurate for the diagnosis of allergic asthma.
- The predictive values of IMMULITE and ImmunoCAP sIgE levels for *Dermatophagoides farinae* (*D. far.*) and *Dermatophagoides pteronyssinus* (*D. pter.*) and of ImmunoCAP sIgE levels for nDer p 1 and rDer p 2 were investigated in 120 bronchial allergen challenges.
- The sIgE levels were higher for IMMULITE than for the ImmunoCAP test, although the difference was not significant.
- IMMULITE and ImmunoCAP sIgE levels for *D. far.* and *D. pter.* and ImmunoCAP sIgE levels for rDer p 2 equally predicted an early asthmatic response (EAR) to bronchial allergen challenges.
- ImmunoCAP nDer p 1 and rDer p 2 better predicted the probability of an EAR at low and middle concentrations. The 90% probability of an EAR was similar for *D. pter.* (cut-off 96 kU.l⁻¹), nDer p 1 (cut-off 93 kU.l⁻¹) and rDer p 2 (cut-off 92 kU.l⁻¹).

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Ethical consent

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Informed consent disclosure

The authors state that they have obtained verbal and written informed consent from the patient/patients for the inclusion of their medical and treatment history within this case report.

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