

Bronchial allergen challenges in children – safety and predictors

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Abstract

Background: In allergic asthma, the diagnosis of house dust mite (HDM) allergy is mainly based on the patient's history, allergy testing by the skin prick test (SPT) or the levels of allergen-specific IgE. We retrospectively analysed data from 350 bronchial provocations with HDM and related it to the following parameters: specific IgE, bronchial hyperresponsiveness (BHR) to methacholine testing (MCT) and exhaled NO (eNO).

Methods: Approximately 350 patients (5–18 yr of age) with allergic asthma and a positive SPT to HDMs were included. To define the sensitivity and specificity for the detection method of an early asthmatic response (EAR), a receiver-operating characteristic (ROC) curve was plotted. The accuracy was measured by the area under the ROC curve (AUC). A logistic regression model was used to predict the individual probability of a positive challenge. The results of the regression model were validated in a prospective group of $n = 75$ patients.

Results: The following cut-off values showed the best combination of sensitivity and specificity: specific IgE *Dermatophagoides farinae* 19.6 kU/l (AUC, 0.88), PD₂₀FEV₁ 0.13 mg methacholine (AUC, 0.73) and eNO 20.1 ppb (AUC, 0.71). The following equation predicted the individual probability of a positive challenge in the retrospective and prospective group: $p = 1/[1 + \exp\{-(-1.78 + 2.46 \cdot 10^3 \log D_{far} - 1.25 \cdot 10^3 \log PD_{20} \text{ metha})\}]^{-1}$, (AUC = 0.88).

Conclusions: The value of using the specific IgE and MCT as predictors was confirmed in a large number of patients. We also showed, for the first time, that the eNO predicted the EAR. The logistic regression model is repeatable with a good accuracy.

Specific bronchial provocations are predominantly used in asthma research and in the testing of antiallergic and antiasthmatic agents (1). In clinical practice, bronchial allergen provocations are time-consuming, and safety concerns exist. Therefore, in allergic asthma, the diagnosis of an allergy, for example to house dust mites (HDMs), is mainly based on the patient's history, allergy testing by a skin prick test (SPT) or the levels of allergen-specific IgE, the results of a pulmonary function test (PFT), and, if applicable, exhaled nitric oxide (eNO). However, the presence of allergen-specific IgE alone (sensitization) does not necessarily imply the existence of allergic symptoms, and there are a considerable number of individuals who are sensitized but who are not clinically allergic (2). Even in patients who are allergic to cats, the predictive value of the clinical history to detect cat allergy was low (3). Possible reasons for the modest degree of accuracy

might include multiple sensitizations and the occurrence of several different allergens together (3). Allergen challenges allow for the demonstration of both the presence of allergen-specific IgE and the causal role of the allergen (4). In allergic rhinitis, a specific nasal allergen provocation is recommended if the results of the SPT and the allergen-specific IgE test are negative (or if the tests are not feasible) or discordant from the clinical history (4).

For food allergies in children, diagnostic cut-off values for SPT and allergen-specific IgE test results have improved the diagnosis of food allergy and have thereby reduced the need to perform oral food challenges (5). In addition, food allergies are an independent trigger of fractional exhaled nitric oxide and sputum eosinophils in children with asthma (6). In bronchial allergen challenges, attempts have been made to predict the early or late asthmatic response (EAR, LAR). Bronchial

hyperresponsiveness (BHR) to methacholine, the wheal size in SPT, the allergen-specific IgE, the baseline PFT and sputum eosinophils have been shown to correlate with and predict asthmatic reactions in adults (3,7–9). In children, the wheal size in the SPT, allergen-specific and total IgE, as well as clinical signs of atopic diseases, were the strongest predictors of response to allergen challenge (10,11). Increased bronchial NO output was found to be associated with the risk and magnitude of exercise-induced bronchoconstriction in atopic children (12). To the best of our knowledge, the predictive value of the eNO in allergen challenges has not yet been published.

In the absence of abundant paediatric case series, we retrospectively analysed the data of 350 bronchial provocations with HDMs and related it to the following predictive parameters: allergen-specific and total IgE, BHR in methacholine challenge testing and eNO. In a prospective survey, the predictors were validated and the safety of the method was investigated.

Material and methods

Subjects and selection

We retrospectively screened specific bronchial allergen challenges with HDMs performed from 2005 to 2010 in the Department of Allergy, Pulmonology and Cystic Fibrosis at our hospital. The data from the retrospective patients were compared with a prospective group challenged in 2011. Inclusion criteria comprised allergic asthma based on a previous physician's diagnosis, age 5–18 yr, a positive SPT to *Dermatophagoides farinae* and/or *pteronyssinus* (*D. far.*, *D. pter.*, Allergopharma KG, Reinbek, Germany) with a wheal size of ≥ 3 mm, and a predicted baseline forced expiratory volume in 1 s (FEV₁) of $\geq 75\%$.

In our department, bronchial allergen challenges are part of the routine diagnostic procedure in patients with HDM allergies, to prove or to exclude the clinical relevance of the disease-related allergen, particularly before specific immunotherapy (SIT). Therefore, the indication for the challenges was mainly based on the general indications for SIT. Of the 350 retrospective patients, only a small part ($n = 42$) participated in a study that investigated the success of SIT in children (M. Rosewich, S. Arendt, J. Schulze, R. Schubert, B. Bohle, S. Zielen. Measuring Allergen-Specific Bronchial Hyperactivity after SIT to detect Responder and Non-Responder States, as presented at the EAACI 2012). Because of our retrospective approach, the parameters specific IgE, PD₂₀ methacholine and eNO were not available in all patients.

The main intention of the prospective part of the study was the investigation of safety aspects, mainly the LAR. At the same time, parts of the prospective group ($n = 30$) served as a baseline group in an ongoing study investigating the effect of SIT on the LAR.

Short acting beta-agonists were withdrawn 8 h before the methacholine testing (MCT), and inhaled corticosteroids (ICS), leukotriene receptor antagonists (LTRA) and long acting beta-agonists were withdrawn a minimum of 24 h before the MCT. Patients were clinically free of airway

infections and provided written informed consent. The study (NCT00677209) was approved by the medical ethics committee of the Goethe University Hospital, Frankfurt/Main and was registered at clinical trial.gov.

Specific IgE antibodies

Samples were analysed for specific antibodies against *D. far.* and *D. pter.* IgE using a two-sided chemiluminescent assay (Immulate, DPC). Analysis was performed according to the manufacturer's instructions.

Pulmonary function tests

Baseline PFT was performed using the MasterScreen spirometer (CareFusion, Hoechberg, Germany). The following parameters were recorded: forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁) and Tiffeneau index (FEV₁%FVC).

Methacholine challenge testing

MCT was performed using of the Aerosol Provocation System (APS, MedicAid-dosimeter; CareFusion). The APS dosimeter technique (CareFusion) allows computer-controlled production of aerosols, using a jet-type nebulizer (Sidestream, MedicAid) powered by compressed air. The integrated pressure calibration procedure associated with the compressor ensures a highly constant and reproducible nebuliser output. APS was calibrated to produce a continuous output of 240 mg/min. Several studies have shown particle size in terms of mass median aerodynamic diameter of around 3.2 μ m and an average of the fine particle fraction $<5 \mu$ m of 49.7%.

MCT was performed as described previously (13). The doses of inhaled methacholine with a concentration of 16 mg/ml were increased according to the following pattern, from step 1 to 5: 0.01, 0.1, 0.4, 0.8 and 1.6 mg. Thus, the entire protocol delivered cumulative doses of 0.01, 0.11, 0.51, 1.31 and 2.91 mg. Two minutes after each inhalation, spirometry was performed. The individual provocation dose (PD) causing a 20% drop in FEV₁ (PD₂₀FEV₁) was calculated by logarithmic interpolation using an integrated program.

Specific allergen challenge

Specific bronchial challenge with mite allergen was performed using the APS dosimeter technique (CareFusion). Before each challenge, lyophilized mite allergen (Allergopharma KG) was resolved in 5 ml of 0.9% saline as a solution of 5000 SBU/ml (standardized biological unit).

The incremental challenge protocol consistently followed the same algorithm as described previously (14). The dose of inhaled allergen was doubled beginning with the lowest dose of 10 SBU, according to the following pattern from step 1 to step 5: 10, 20, 40, 80 and 160 SBU. Thus, the entire protocol delivered cumulative doses of 10, 30, 70, 150 and 310 SBU. Ten minutes after each step-up, FEV₁ was measured. Inhalation was stopped if FEV₁ dropped $\geq 20\%$ compared to the baseline values. The individual allergen dose that caused a

Table 1 Patient's characteristics

	Retrospective total	Prospective total	p
Subjects (n)	350	75	
Male/female (n)	234/116	57/18	
Age (yr, mean $\pm$ s.d.)	8.7 $\pm$ 2.7	9.0 $\pm$ 2.7	0.39
FVC (%pred, mean $\pm$ s.d.)	92.4 $\pm$ 12.6	94.3 $\pm$ 9	0.11
FEV ₁ (%pred, mean $\pm$ s.d.)	100.4 $\pm$ 13.4	100.2 $\pm$ 12.2	0.92
Max fall FEV ₁ during EAR (%pred, mean $\pm$ s.d.)	25.6 $\pm$ 11.9	27.8 $\pm$ 12.2	0.19
PD ₂₀ FEV ₁ mite (n = 290/69) (SBU, mean $\pm$ s.d.)	73.1 $\pm$ 73.5	76.0 $\pm$ 77.9	0.87
Total IgE (n = 291/75) (kU/l, mean $\pm$ s.d.)	575.5 $\pm$ 691.0	683.7 $\pm$ 954.5	0.90
Spec. IgE <i>D. far.</i> (n = 297/75) (kU/l, mean $\pm$ s.d.)	55.5 $\pm$ 38.3	60.1 $\pm$ 35.7	0.55
Spec. IgE <i>D. pter.</i> (n = 297/75) (kU/l, mean $\pm$ s.d.)	56.3 $\pm$ 38.2	53.9 $\pm$ 35.6	0.52
PD ₂₀ FEV ₁ metha (n = 217/71) (mg, mean $\pm$ s.d.)	0.40 $\pm$ 0.63	0.34 $\pm$ 0.5	0.65
eNO (n = 334/62) (ppb, mean $\pm$ s.d.)	34.5 $\pm$ 28.5	30.7 $\pm$ 27.6	0.28
LAR (n = 114/71) (n,%)	53 (46%)	65 (91.5%)	

FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; EAR, early asthmatic response; PD₂₀FEV₁ mite, provocation dose of mite allergen causing a 20% drop in FEV₁; *D. far.*, *Dermatophagoides farinae*; *D. pter.*, *Dermatophagoides pteronyssinus*; PD₂₀FEV₁ metha, provocation dose of methacholine causing a 20% drop in FEV₁; eNO, exhaled nitric oxide; LAR, late asthmatic response; s.d., standard deviation.

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. After the challenge procedure, patients received two to four puffs of salbutamol (0.1 mg) until the FEV₁ value returned to at least 90% of the baseline value. Peak flow was measured for up to 10 h following the challenge, and a LAR was defined as a drop of $\geq 15\%$ PEF. In the prospective part, the patients measured the FEV₁ using the asthma monitor (AM-1; CareFusion), an LAR was defined as a drop of $\geq 15\%$ FEV₁. The patients received an action plan and rescue medication consisting of salbutamol and prednisolone.

Measurement of exhaled NO

Before the allergen challenge, measurements of exhalative NO were taken using the NIOX1 (Aerocrine, Solna, Sweden). NIOX1 measures eNO in exhaled air according to American Thoracic Society guidelines (15). This chemiluminescence gas analyser is sensitive to eNO at concentrations ranging from 1.5 to 200 ppb and demonstrates a deviation from the mean value of ± 2.5 ppb at NO < 50 ppb or $\pm 5\%$ of the measured value at > 50 ppb.

Statistical analysis

For statistical analysis, GraphPad Prism 5.01 (GraphPad Software, La Jolla, CA, USA), SPSS for Windows[®] version 11.0 (SPSS Inc., Chicago, IL, USA), BiAS. for Windows version 10 (epsilon-Verlag; Darmstadt, Germany) and Microsoft Excel were used. The parameters were tested for normal and log-normal distributions using the Kolmogorov–Smirnov test. The correlations between the predictors and the PD₂₀FEV₁ allergen were calculated using Spearman's rank correlation test. To define the sensitivity and specificity of a predictor to detect an EAR or an LAR, a receiver-operating characteristic (ROC) curve was plotted. Cut-off levels were optimized using the Youden index (sensitivity + specificity

– 1). The individual probability of the occurrence of an EAR in the retrospective group was calculated by logistic and log-logistic regression models, and the regression equations were validated in a prospective group. The accuracy was measured by area under the curve (AUC) analysis. Probability (p) values ≤ 0.05 were considered statistically significant.

Results

Retrospective group

Patients' characteristics

We screened 416 bronchial provocations and found 350 patients (mean age, 8.7 yr) who fulfilled the inclusion criteria (see Table 1). At the time of the provocations, 171 patients were on ICS, 31 were on LTRA, 7 had a therapy break of at least 14 days (ICS and LTRA), 138 were on beta-agonists alone, and the remaining 3 had incomplete information on their treatment history. The patients underwent allergen-specific bronchial challenge with *D. far* (n = 42) and with *D. pter* (n = 308). As an expression of an EAR, the patients had a maximum fall in FEV₁ of $25.6\% \pm 11.9$. In 290 of the 350 patients (82.8%) a significant EAR with a fall of $\geq 20\%$ in FEV₁ occurred. The mean PD₂₀FEV₁ was $73.1 \text{ SBU} \pm 73.5$. In 297 patients, the allergen-specific IgE, in 217 patients, the threshold in MCT and in 334 patients, the eNO had been measured. In those patients with a non-significant reaction in MCT (n = 9), the maximum dose of 2.9 mg methacholine was assumed for the calculations (13). In terms of the PD₂₀ allergen, the groups with available data did not significantly differ from the groups without data (allergen-specific IgE against *D. far*. p = 0.35, PD₂₀FEV₁ methacholine p = 0.17, eNO p = 0.06).

Test for normal distribution and correlations

The parameters were tested for normal distribution. The FVC was normally distributed; we achieved a pseudo-normal

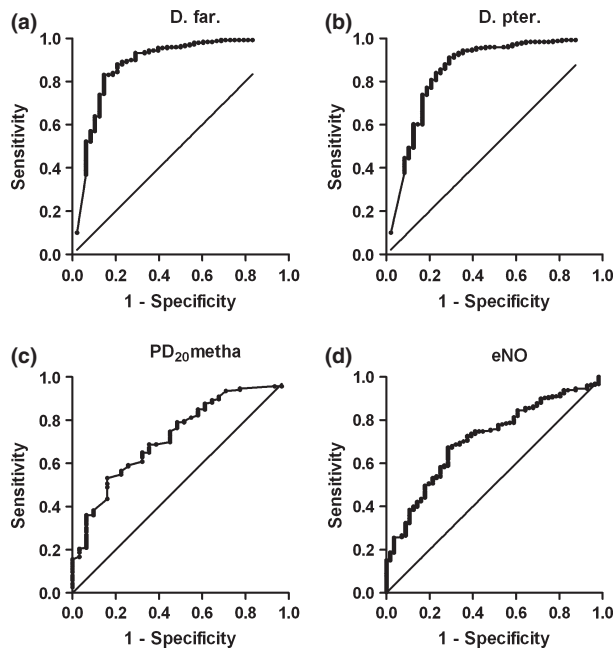


Figure 1 Receiver operating characteristic (ROC) curves to predict an early asthmatic response after bronchial challenge with house dust mites. (a) A specific IgE value of 19.6 kU/l against *D. far.* was the optimum cut-off, with a sensitivity of 83% and a specificity of 85%. Area under the curve (AUC) 0.88 ($p < 0.001$). (b) Specific IgE against *D. pter.*, cut-off 20.3 kU/l, sensitivity 84%, specificity 77%, AUC 0.85 ($p < 0.001$). (c) PD₂₀FEV₁ methacholine, cut-off 0.13 mg, sensitivity 53%, specificity 83%, AUC 0.73 ($p < 0.001$). (d) eNO, cut-off 20.1 ppb, sensitivity 66%, specificity 71%, AUC 0.71 ($p < 0.001$).

distribution for total IgE, PD₂₀FEV₁ methacholine, and eNO by log transformation (Table S1). We correlated the different parameters with the PD₂₀FEV₁ mite allergen in bronchial provocation. The allergen-specific IgE, the PD₂₀FEV₁ methacholine and the eNO showed a significant correlation (Table S2). The eNO significantly correlated with the allergen-specific IgE *D. far.* ($r = 0.37$, $p < 0.001$) and the PD₂₀FEV₁ methacholine ($r = 0.17$, $p = 0.01$).

ROC analysis to detect an EAR

We calculated receiver-operator characteristic (ROC) curves to evaluate the sensitivity and specificity of the allergen-specific IgE, the PD₂₀FEV₁ methacholine and the eNO to predict a positive reaction subsequent to the allergen challenges. The allergen-specific IgEs had the best accuracy (Fig. 1a–d, Table 2).

We stratified our patients by those that had been receiving ICS (ICS+) or had not (ICS–) and calculated the specific IgE against *D. far.*, the PD₂₀FEV₁ methacholine and the eNO thresholds accordingly. For the specific IgE against *D. far.*, the mean values and the AUCs were similar between both groups, whereas the optimal cut points were lower in the ICS+ group. For the PD₂₀FEV₁ methacholine, the mean and the optimal cut-off points were similar between both groups, whereas the

AUC was significant only in the ICS+ group. For the eNO, the mean was significantly lower in the ICS+ group, as the optimal cut-off points and AUCs were lower in the ICS+ group (Table 3).

Logistic regression models

The different parameters were calculated in a logistic regression model. The level of ¹⁰log specific IgE against *D. far.* predicted a positive reaction in the allergen challenge with an accuracy of 88% (equation: individual probability $p = 1/[1 + \exp\{-(-1.45 + 2.43 \cdot ^{10}\log D. far.)\}]^{-1}$, AUC = 0.88), while the accuracy of the ¹⁰log PD₂₀ methacholine and eNO was less. The combination of the ¹⁰log specific IgE against *D. far.* with the ¹⁰log PD₂₀ methacholine or the eNO, as well as the combination of all three parameters, did not improve the predictive value compared with the ¹⁰log specific IgE against *D. far.* alone (Table 4).

Prospective group

Patients' characteristics

A total of 75 patients (mean age, 9.0 yr) were challenged in 2011. At the time of the provocations, 53 (70.6%) patients were on ICS, five (6.6%) were on LTRA, and 17 (22.6%) had a therapy break of at least 14 days. All patients were challenged with *D. pter.* During an EAR, the maximum fall in FEV₁ was $27.8\% \pm 12.2$. In 69 of 75 patients (92%), a significant EAR with a fall of $\geq 20\%$ in FEV₁ occurred. The mean PD₂₀FEV₁ was $76.0 \text{ SBU} \pm 77.9$. In all patients, the specific IgE had been measured; in 71 patients, the PD₂₀FEV₁ methacholine; and in 62 patients, the eNO. Except for the LAR, all parameters did not significantly differ from those of the retrospective group (Table 1). The oxygen saturation before the provocation was $99.2\% \pm 0.97$ and dropped during the EAR to $96.7\% \pm 1.7$, $p < 0.001$. A PFT protocol to assess the LAR was available for 71 patients. Of these, 65 patients (91.5%) developed an LAR after a median of 8:10 hrs (range, 1:40–12:15 hours) with a mean maximal fall in FEV₁ of $38.7\% \pm 15.5$ (Table S3). A thirteen-year-old boy developed an asthma exacerbation with dyspnoea and increased use of salbutamol during the night. He presented the next day with a completely reversible airway obstruction and was placed on prednisone and salbutamol for the following 3 days.

Logistic regression models

The regression equations calculated in the retrospective group were tested in the prospective group. In comparison with the retrospective group, the ¹⁰log specific IgE against *D. far.* had similar AUCs as the ¹⁰log PD₂₀FEV₁ methacholine, whereas the results for the eNO were lower. The combination of ¹⁰log specific IgE against *D. far.* and ¹⁰log PD₂₀ methacholine revealed a significant AUC with excellent accuracy in the prediction of the individual probability of an EAR (equation: probability $p = 1/[1 + \exp\{-(-1.78 + 2.46 \cdot ^{10}\log D. far. - 1.25 \cdot ^{10}\log PD_{20} \text{ metha})\}]^{-1}$, AUC = 0.88) (Table 4).

Table 2 ROC analysis to detect an EAR

	Optimal cut-point	Sensitivity (95% CI)	Specificity (95% CI)	AUC	p
FVC (%pred)	94.3	0.62 (0.56–0.67)	0.58 (0.44–0.70)	0.58	0.06
FEV ₁ (%pred)	110.2	0.81 (0.76–0.85)	0.41 (0.28–0.53)	0.57	0.41
Total IgE (kU/l)	340.0	0.49 (0.42–0.55)	0.68 (0.53–0.80)	0.60	0.03
Spec. IgE <i>D. far.</i> (kU/l)	19.6	0.83 (0.78–0.87)	0.85 (0.72–0.94)	0.88	<0.001
Spec. IgE <i>D. pter.</i> (kU/l)	20.3	0.84 (0.79–0.88)	0.77 (0.63–0.88)	0.85	<0.001
PD ₂₀ FEV ₁ metha (mg)	0.13	0.53 (0.45–0.60)	0.83 (0.66–0.94)	0.73	<0.001
eNO (ppb)	20.1	0.66 (0.60–0.71)	0.71 (0.57–0.81)	0.71	<0.001

ROC, Receiver operating characteristic; EAR, early asthmatic response; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; *D. far.*, *Dermatophagoides farinae*; *D. pter.*, *Dermatophagoides pteronyssinus*; PD₂₀FEV₁ Metha, provocation dose of methacholine causing a 20% drop in FEV₁; eNO, exhaled nitric oxide; CI, confidence interval; AUC, area under curve.

Table 3 Predictors of an EAR in patients with ICS (+) and without ICS (–)

Predictor	Mean ± s.d.	Optimal cut-point	Sensitivity (95% CI)	Specificity (95% CI)	AUC	p
<i>IgE D. far.</i>						
ICS+ (n = 145)	56.2 kU/l ± 38.29*	15.6 kU/l	0.89 (0.83–0.94)	0.77 (0.55–0.92)	0.88	<0.001
ICS– (n = 127)	55.6 kU/l ± 38.42*	19.6 kU/l	0.84 (0.77–0.91)	0.90 (0.70–0.99)	0.87	<0.001
PD ₂₀ FEV ₁ methacholine						
ICS + (n = 93)	0.38 mg ± 0.66**	0.14 mg	0.65 (0.53–0.75)	0.94 (0.70–1.0)	0.82	<0.001
ICS– (n = 100)	0.41 mg ± 0.60**	0.09 mg	0.31 (0.21–0.41)	0.92 (0.62–1.0)	0.54	0.63
eNO						
ICS + (n = 165)	27.5 ppb ± 18.9***	20.1 ppb	0.64 (0.55–0.72)	0.66 (0.44–0.83)	0.66	0.01
ICS– (n = 139)	42.6 ppb ± 34.8***	27.6 ppb	0.65 (0.56–0.74)	0.79 (0.58–0.93)	0.74	<0.001

EAR, early asthmatic response; ICS, inhaled corticosteroid; *D. far.*, *Dermatophagoides farinae*; PD₂₀FEV₁ methacholine, provocation dose of methacholine causing a 20% drop in FEV₁; eNO, exhaled nitric oxide; CI, confidence interval; AUC, area under curve.

*p = 0.85.

**p = 0.07.

***p < 0.001.

Table 4 Logistic regression to predict an EAR. Predictor variables were calculated in the retrospective group and were tested in the prospective group

Predictor variables (regression coefficient)	Retrospective group (n = 350)		Prospective group (n = 75)	
	AUC (95% CI)	p	AUC (95% CI)	p
¹⁰ log IgE <i>D. far.</i> (kU/l) (2.43) intercept: –1.45	0.88 (0.82–0.94)	<0.001	0.81 (0.66–0.97)	0.009
¹⁰ log PD ₂₀ metha (kU/l) (–1.35) intercept: 0.933	0.72 (0.63–0.81)	<0.001	0.76 (0.55–0.96)	0.05
eNO (ppb) (0.04) intercept: 0.49	0.73 (0.66–0.79)	<0.001	0.55 (0.25–0.85)	0.67
¹⁰ log IgE <i>D. far.</i> (kU/l) (2.46)	0.87 (0.82–0.94)	<0.001	0.88 (0.79–0.96)	0.002
¹⁰ log PD ₂₀ metha (mg) (–1.25) intercept: –1.78				
¹⁰ log IgE <i>D. far.</i> (kU/l) (2.4) eNO (ppb) (0.034) intercept: –2.39	0.84 (0.79–0.89)	<0.001	0.72 (0.48–0.96)	0.06
¹⁰ log IgE <i>D. far.</i> (kU/l) (2.58)	0.87 (0.82–0.91)	<0.001	0.79 (0.64–0.94)	0.01
¹⁰ log PD ₂₀ metha (mg) (–0.94) e NO (ppb) (0.04) intercept: –2.99				

EAR, early asthmatic response; *D. far.*, *Dermatophagoides farinae*; PD₂₀metha, provocation dose of methacholine causing a 20% drop in FEV₁; eNO, exhaled nitric oxide; AUC, area under curve; CI, confidence interval.

Roc analysis to detect the LAR

To detect the LAR, the specific IgE against *D. far.* and *D. pter.*, the PD₂₀FEV₁ methacholine, the eNO, the maximal fall in FEV₁ during the EAR and the PD₂₀FEV₁ HDM were calculated. Only the allergen-specific IgE was a significant predictor (Table 5).

Discussion

We investigated the predictive value of the allergen-specific IgE, the BHR to methacholine and the eNO in a retrospective analysis of 350 paediatric patients challenged with HDM. While most studies have proven the validity of the SPT,

Table 5 ROC analysis to detect an LAR in the prospective group

	Optimal cut-point	Sensitivity (95% CI)	Specificity (95% CI)	AUC	p
FVC (%pred)	87.3	0.80 (0.68–0.89)	0.50 (0.12–0.88)	0.59	0.46
FEV ₁ (%pred)	103.1	0.64 (0.52–0.76)	0.50 (0.12–0.88)	0.50	0.97
Total IgE (kU/l)	592.0	0.69 (0.56–0.80)	0.66 (0.22–0.96)	0.57	0.57
Spec. IgE <i>D. far.</i> (kU/l)	12.3	0.93 (0.85–0.98)	0.66 (0.22–0.96)	0.84	<0.01
Spec. IgE <i>D. pter.</i> (kU/l)	23.9	0.78 (0.67–0.88)	1.0 (0.54–1.0)	0.92	<0.001
PD ₂₀ FEV ₁ metha (mg)	0.17	0.54 (0.4–0.67)	0.66 (0.22–0.96)	0.54	0.74
eNO (ppb)	26.7	0.51 (0.39–0.65)	1.0 (0.54–1.0)	0.63	0.29
MaxFEV ₁ EAR (%pred)	28.5	0.47 (0.35–0.60)	1.0 (0.54–1.0)	0.69	0.11
PD ₂₀ FEV ₁ mite (SBU)	58.9	0.60 (0.47–0.72)	0.83 (0.36–0.99)	0.70	0.10

ROC, Receiver operating characteristic; LAR, late asthmatic response; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; *D. far.*, *Dermatophagoides farinae*; *D. pter.*, *Dermatophagoides pteronyssinus*; PD₂₀FEV₁ metha, provocation dose of methacholine causing a 20% drop in FEV₁; eNO, exhaled nitric oxide; MaxFEV₁ EAR, maximal drop in FEV₁ during the early asthmatic response; PD₂₀FEV₁ mite, provocation dose of mite allergen causing a 20% drop in FEV₁; CI, confidence interval; AUC, area under curve.

allergen-specific IgE and MCT, this study, to the best of our knowledge, was the first to show that eNO is a significant predictor of a bronchial reaction in allergen challenges.

Very few studies exist that have examined the safety of bronchial allergen challenges in children. Warner et al. (16) challenged 69 children with HDM extracts. Among those patients with a significant EAR, 73% developed an LAR that was characteristically more prolonged and more severe than the EAR. In another investigation, a total of 137 bronchial provocation tests with allergen extracts were performed. Of these, 110 were positive, nine developed an LAR, and all patients with LAR had been provoked with HDMs. None of the patients were admitted to a hospital later (17).

In our prospective approach, 91% of the patients developed an LAR, which was more pronounced than the EAR. This was more than we expected based on the results from our retrospective analysis. In our prospective trial, we used an asthma monitor to detect the LAR, revealing that the measurement of the FEV₁ is more sensitive than measuring the PEF. To rule out systematic bias, we did not change our provocation procedure during the time period that was analysed. Another reason for the higher percentage of LARs might be that the patients were more efficiently pre-selected. Although the characteristics were similar between both groups, more patients from the prospective group received ICS, suggesting that more severe asthmatic patients were referred to the challenges. Additionally, one patient from the prospective group had to be treated at our walk-in clinic for asthma symptoms combined with an upper airway infection the next day, while no additional medical care was required for patients in the retrospective group. However, this observation reminds us that patients undergoing bronchial challenges should be pre-selected very carefully, and the stabilization of the asthma is recommended before provocations.

Due to ethical reasons patients inhaled salbutamol when they developed an EAR. Concerns exist that beta-agonists might influence the LAR. Intermediate-acting beta-agonists inhibit or reverse the EAR and reverse the LAR when it is not too severe; however, they do not prevent the LAR or any of the associated inflammatory events (18).

In former studies, the predictive value of the SPT had been predominantly investigated (7, 9–11, 19, 20). In children, a wheal size of ≥ 4.5 mm showed a sensitivity of 75% and a specificity of 86% to predict an EAR (AUC 0.85) (11). In adults, a positive SPT (wheal size 3 mm) failed to discriminate between challenge-positive and challenge-negative patients (3). In HDM allergies, the skin sensitivity did not significantly contribute to the prediction of an EAR (20). We used the SPT only to screen the patients and focused on the allergen-specific IgE.

IgE plays a critical role in both the EAR and the LAR, as the EAR results from IgE-mediated mast cell degranulation demonstrate (21). In a small group of 26 paediatric patients, an allergen-specific IgE of 5.1 kU/l (UniCAP) predicted a positive response with a sensitivity of 82% and a specificity of 85% (AUC 0.79) (11). In 64 adults with a cat allergy, an allergen-specific IgE of ≥ 13 kU_a/l (CAP) had a sensitivity of 48% and a specificity of 100% (AUC 0.85) (3). While the results of the ROC analyses were very similar to our results, optimal cut-off values were lower than in our group (median log specific IgE 1.3 [0.9–1.7] (11) vs. 1.9 [1.4–2.0] and 21.7 ± 28.9 13 kU/l (3) vs. 63.2 ± 35.4 kU/l, respectively). We used the Immulite test to determine specific IgE; it has been demonstrated in the literature that for samples with more than 10 kU/l of specific IgE, the Immulite results tend to be higher than those measured by ImmunoCAP (22). Thus, data obtained with different systems cannot be reliably correlated with each other (22).

It is known that the responsiveness to histamine or methacholine correlates with a positive response during bronchial allergen challenges. The correlation between the PC₂₀ histamine and the PD₂₀ allergen was $r = 0.43$, $p < 0.01$ (23), and it has been shown that methacholine challenge correlates with the PC₂₀ allergen ($r = 0.25$, $p = 0.0015$) (9) and with the PD₁₅ allergen ($r = 0.25$) (7). We found a correlation similar to the results of Haugaard et al. (23). To the best of our knowledge, a ROC analysis of the predictive value of the MCT has not yet been published. MCT is more useful in excluding a diagnosis of asthma due to its superior negative predictive power (24). In our analysis, MCT had a noteworthy specificity, as indicated

by the proportion of negatives that were correctly identified. The results were independent of corticosteroid treatment.

The PC₂₀ methacholine is frequently used in research provocations to predict the allergen PC₂₀ (9). Therefore, the PC₂₀ methacholine as an inclusion criteria would bias the outcome and turn out to be a significant predictor. In the present study, we used the methacholine challenge as an independent tool in asthma diagnostics and not to predict the airway responsiveness to mite allergen.

The predictive value of eNO in allergic responses has not yet been published. In children, only a few studies have investigated the eNO as a predictor of asthmatic exacerbations. In a group of 40 children, a raised eNO of ≥ 22 ppb was a significant predictor for those who failed during a steroid reduction (25). In another observational study, a cut-off point of 22.9 ppb eNO was the best predictor for asthmatic exacerbations (26). Interestingly, we showed that an eNO of ≥ 20.1 ppb predicted a significant reaction during bronchial challenges. ICS are well known to reduce the eNO (27), and, as expected, the group treated with ICS displayed significant lower eNO levels before challenge. Thus, it was surprising that the cut-off point to predict an EAR was not very different between both groups; the similar results suggest that ICS treatment influences the eNO level greater than the allergen-specific hyperreactivity. In healthy, non-atopic children aged 4–17 yr, the upper 95% limit in normal eNO values is 18.5 ppb (28). Therefore, an eNO of 20 ppb seems to be a plausible cut-off value that differentiates patients developing an EAR from those who would not.

The parameters specific IgE, PD₂₀ methacholine and eNO were not available in all patients, and this might bias the results. However, in terms of the PD₂₀ allergen, the groups with available data did not significantly differ from the groups without these data.

In subjects allergic to cats, specific IgE- values of ≥ 17 kU/l predicted in a logistic regression model the probability of a positive bronchial provocation with $\geq 93\%$ (3). We showed that an allergen-specific IgE of ≥ 20 kU/l predicted the individual probability of a positive response with 81 to 88%. In the prospective validation, the combination of allergen-specific IgE and PD₂₀FEV₁ methacholine revealed more robust results, with an 88% probability of a positive response.

The goal of logistic regression is to correctly predict the category of an outcome for individual cases, and a model is created that includes all predictor variables that are useful in foreseeing the response variable. Logistic regression can test the fit of the model after each coefficient is added or deleted, a process that is referred to as stepwise regression (29). Using this model, the inclusion of eNO did not improve the prediction in the retrospective or in the prospective group.

We found a weak, but significant, correlation between the eNO and the PD₂₀ methacholine and a moderate correlation between eNO and the allergen-specific IgE. In children with asthma, the eNO and the PC₂₀ methacholine significantly correlated ($r = -0.31$ and $r = -0.63$, respectively) (30,31), whereas in another publication, the correlation between eNO and PC₂₀ methacholine failed to be significant (32). The studies were heterogeneous regarding the inclusion criteria, the

treatment, the asthma severity and phenotype, and therefore, the results are difficult to compare.

In asthmatic children sensitized to HDM, serum levels of mite-specific IgE correlated with FeNO levels ($r = 0.33$ and $r = 0.74$, respectively) (33,34). These results are confirmed by our data.

In the prospective study, allergen-specific IgE was the only parameter that significantly predicted an LAR. Compared with the EAR, the cut-off values were higher with a similar accuracy. We observed a difference between specific IgE against *D. far.* and *D. pter.* Because we challenged all patients with *D. pter.*, this might favour the specific IgE against *D. pter.*

The cause and significance of the LAR is not as well understood. Eosinophils are important, and in response to IgE binding to the Fc[epsilon]RI receptor, eosinophil cytoplasmic granules and a number of cytokines are synthesized and released by degranulation. The low level of Fc[epsilon]RI expression on eosinophils means that these cells may not be the major mediators of LAR (35). In the literature, the correlation between the allergen-specific IgE determined by RAST and the LAR was $r = 0.25$ and $r = 0.33$, respectively (7, 19). In adults, a multiple regression analysis demonstrated that the LAR could be predicted by a model that incorporated the PD₁₅ methacholine, the specific IgE and the baseline values of FEV₁ (7). A logistic regression revealed that the methacholine PC₂₀ and the decrease in FEV₁ at 20 min during the EAR were significantly associated with an LAR, whereas the baseline spirometry failed to be significant (8). We showed that both the baseline pulmonary function and the PD₂₀ methacholine did not predict an LAR. In other studies, no relationship was found between non-specific bronchial reactivity to histamine or methacholine and an LAR (36,37).

Whenever the specificity was high, an eNO > 26.7 ppb and a maximal fall in FEV₁ $> 28.5\%$ during an EAR failed to predict an LAR, due to low sensitivity. In atopic asthmatic children, eNO levels correlated with eosinophil counts in the sputum or broncho-alveolar lavage fluid (27). In bronchial allergen challenges, an LAR was associated with elevated exhaled eNO concentrations; however, there was no significant difference in baseline eNO between dual responders, single responders or subjects who underwent control challenges (38). Conflicting results were demonstrated for correlations of eNO with asthma severity, PFTs and BHR (27). These data suggest that the predictive value of the eNO is associated with eosinophilic inflammation during an LAR, but other factors may contribute to the development of an LAR. Avila et al. (8) showed that a threshold value of 27% decline in FEV₁ 20 min after challenge represents the best cut-off value to predict an LAR and that the incidence of LAR increased if the decline was $>27\%$. This result is similar to our cut-off revealed by ROC analysis. However, other studies have demonstrated that there was no evidence of a correlation between the magnitude of an EAR and the magnitude of an LAR (36).

Conclusion

In bronchial allergen challenges with HDMs, allergen-specific IgE was the parameter that best predicted an EAR and an

LAR. In a logistic regression model that calculates the probability of an EAR, the inclusion of the PD₂₀FEV₁ methacholine variable increased the statistical significance. We showed that eNO is a significant predictor of an EAR. The predictive values of the allergen-specific IgE, PD₂₀FEV₁ methacholine and eNO were, to a large extent, independent of therapy with ICS.

Authors contributions

J Schulze contributed to the conception and design, the acquisition, analysis and interpretation of data and drafted the article. W Rheinmüller contributed to the acquisition,

analysis and interpretation of data and revised the article critically. E Hermann contributed to the analysis and interpretation of data and revised the article critically. M Rosewich and MA Rose contributed to the acquisition of data and revised the article critically. S Zielen contributed to the conception and design, the acquisition of data and revised the article critically. All authors approved the final version of the manuscript.

Conflict of interest statement

All authors have no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Kolmogorov-Smirnov-test for normal distribution.

Table S2. Spearman's rank correlation association with the PD₂₀FEV₁ mite.

Table S3. FEV₁ (% predicted, mean ± SD) during an EAR and an LAR in the prospective group.

Table e1		Kolmogorov-Smirnov-test for normal distribution	
		Normal distribution	Log-normal distribution
FVC	(%pred)	+	-
FEV ₁	(%pred)	-	-
PD ₂₀ FEV ₁ mite	(SBU)	-	-
Total IgE	(kU·L ⁻¹)	-	+
Spec. IgE D.far.	(kU·L ⁻¹)	-	-
Spec. IgE D.pter.	(kU·L ⁻¹)	-	-
PD ₂₀ FEV ₁ metha	(mg)	-	+
eNO	(ppb)	-	+

FVC, forced vital capacity; FEV₁, forced expiratory volume in one second; PD₂₀FEV₁ mite, provocation dose of mite allergen causing a 20 percent drop in FEV₁; D. far., Dermatophagoides farinae; D. pter., Dermatophagoides pteronyssinus; PD₂₀FEV₁ Metha, provocation dose of methacholine causing a 20 percent drop in FEV₁; eNO, exhaled nitric oxide.

Table e2		Spearman's rank correlation Association with the PD ₂₀ FEV ₁ mite	
		correlation coefficient (r)	p
FVC	(%pred)	0.06	0.29
FEV ₁	(%pred)	0.15	0.01
Total IgE	(kU·L ⁻¹)	- 0.08	0.19
Spec. IgE D.far.	(kU·L ⁻¹)	- 0.43	< 0.001
Spec. IgE D.pter.	(kU·L ⁻¹)	- 0.43	< 0.001
PD ₂₀ FEV ₁ metha	(mg)	0.43	< 0.001
eNO	(ppb)	- 0.29	< 0.001

PD₂₀FEV₁ mite, provocation dose of mite allergen causing a 20 percent drop in FEV₁; FVC, forced vital capacity; FEV₁, forced expiratory volume in one second; D. far., Dermatophagoides farinae; D. pter., Dermatophagoides pteronyssinus; PD₂₀FEV₁ Metha, provocation dose of methacholine causing a 20 percent drop in FEV₁; eNO, exhaled nitric oxide.

Table e3	FEV ₁ (% predicted, mean ± SD) during an EAR and an LAR in the prospective group			
	FEV ₁ baseline	FEV ₁ during EAR	FEV ₁ during LAR	Bronchodilator post LAR
LAR + (n = 65)	100.4 ± 12,4	71.4 ± 11,7	61.3 ± 15,5	91.0 ± 26,5
LAR - (n = 6)	99.7 ± 11,3	79.5 ± 11,1	96.5 ± 5,8	-

FEV₁, forced expiratory volume in one second; SD, standard deviation; EAR, early asthmatic response; LAR, late asthmatic response.