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REGULAR ARTICLE

Establishing IMMULITE® 2000 cut-off values for serum allergen-specific immunoglobulin and exploring their relationship to exhaled nitric oxide

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ABSTRACT

Aim: Paediatric cut-off values for serum allergen-specific IgE (sIgE) using the Siemens IMMULITE® 2000 system to diagnose allergic rhinoconjunctivitis have not been established. We aimed to determine cut-off levels for sIgE for 10 common inhalant allergens and to study the relationship between sIgE, total IgE and fractional exhaled nitric oxide (FE_{NO}).

Methods: We enrolled 243 schoolchildren, including 164 with allergic rhinoconjunctivitis. Parental interviews, skin prick tests, sIgE, total IgE, FE_{NO} measurements, spirometry and exercise tests were performed.

Results: Cut-off values with the best combined sensitivity and specificity were above the detection limit of the assay for seven of the ten allergens (0.23–1.1 kU/L). The overall accuracy of the IMMULITE® in detecting allergic rhinoconjunctivitis was good. sIgE was superior to total IgE and FE_{NO} in predicting allergic rhinoconjunctivitis to timothy, birch, mugwort, cat, dog and house dust mite. FE_{NO} was elevated in children with allergic rhinoconjunctivitis, irrespective of asthma.

Conclusion: Cut-off values for sIgE were dependent on the allergic phenotype and were above the IMMULITE® detection limit for seven of ten inhalant allergens. Consequently, using the detection limit for sIgE as the decision point would result in over-diagnosing allergic rhinoconjunctivitis. When measuring elevated FE_{NO} in children, allergic rhinoconjunctivitis should be suspected.

INTRODUCTION

The diagnosis of allergic diseases involves confirming sensitisation by detecting allergen-specific immunoglobulin E (sIgE). Such sIgE antibodies can be determined by skin prick testing or by a variety of *in vitro* immunoassays (1). In most studies, IgE mediated sensitisation is considered a dichotomous variable: in other words, there is either sensitisation or not. However, IgE quantification by sIgE has been shown to predict allergic rhinoconjunctivitis and asthma better than using sIgE as a dichotomous variable (2,3). Besides, underestimated cut-off points of sIgE may lead to over-diagnosing allergic rhinoconjunctivitis. Previous studies using different assays have demonstrated wide disparity among sIgE levels (4,5). The levels of sIgE are used to guide diagnosis and treatment decisions in clinical practice. However, little comparative data is available for sIgE testing using the Siemens IMMULITE® 2000 system and skin prick test results in children. The relationship between sIgE, total IgE and fractional exhaled nitric oxide (FE_{NO}) has not been fully elucidated.

FE_{NO} is commonly used as a non-invasive marker of eosinophilic airway inflammation. The FE_{NO} level depends on the allergic phenotype and the degree of sensitisation (6,7). FE_{NO} is increased in children with allergic rhinoconjunctivitis and the highest FE_{NO} values have been found in children with allergic asthma (7–10). However, increased

Abbreviations
FE_{NO}, Fractional exhaled nitric oxide; IgE, Immunoglobulin E; sIgE, Allergen-specific IgE.

Key notes

- Paediatric cut-off values for serum allergen-specific IgE (sIgE) using the Siemens IMMULITE® 2000 system to diagnose allergic rhinoconjunctivitis have not been established.
- Our study found that the cut-off values for sIgE depended on the allergic phenotype and using the detection limit for sIgE as the decision point would result in over-diagnosing allergic rhinoconjunctivitis.
- Allergic rhinoconjunctivitis should be suspected when measuring elevated fractional exhaled nitric oxide in children.

FE_{NO} has been observed in atopic individuals regardless of respiratory tract symptoms (9,11). It has been suggested that this might reflect subclinical airway inflammation (11,12).

The aim of this study was to determine cut-off levels of sIgE for the IMMULITE® 2000 system for ten common inhalant allergens using a positive skin prick test and related allergic rhinoconjunctivitis symptoms as the gold standard. Another aim was to study the relationship between sIgE, total IgE and FE_{NO} in children with allergic rhinoconjunctivitis.

METHODS

Study design

This study was a part of the 'Asthma and allergy among schoolchildren in Nordland' study, which included children aged from eight to 16 years. In 2008 (phase one), 4150 parents completed a questionnaire on their children's asthma, allergic rhinoconjunctivitis and eczema (13). In phase two of the study, 1144 pupils living near the study locations were invited to take part and 801 children participated. The parents completed a questionnaire and a structured interview regarding allergic rhinoconjunctivitis and asthma (14). Total IgE, sIgE, skin prick test, FE_{NO} measurements, spirometry and exercise treadmill testing were performed. Blood samples and FE_{NO} measurements were requested for all children. During the initial study period skin prick tests were requested for all children. Thereafter, skin prick tests were requested for children with asthma and/or allergy symptoms. This part of the study included 303 children with total IgE, sIgE, skin prick test and FE_{NO} measurements. Subjects with a reaction to the negative skin prick test control, food allergy and subjects with rhinoconjunctivitis symptoms who did not fulfil the allergic rhinoconjunctivitis definition were excluded. This analysis included 164 children with allergic rhinoconjunctivitis and 79 children without. The participants were examined during the school season from March 2009 to June 2010.

The study was approved by the Regional Committee for Medical and Health Research Ethics and was performed in accordance with the ethical standards of the 2000 Helsinki Declaration. Written informed consent was obtained from all children and their parents.

Methods

Blood samples were obtained using standard venepuncture using Vacutainer® tubes (Becton Dickinson, Plymouth, UK). Serum was collected and stored at −80°C until assayed. Total IgE and sIgE levels were analysed employing the IMMULITE® 2000 (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA) using 3gAllergy® kits. The detection range for sIgE was ≥0.10–100 kU/L. The following were tested: sIgE to timothy, birch and mugwort pollens; dog dander, cat and rabbit epithelial dander; house dust mite *Dermatophagoides pteronyssinus*; moulds *Alternaria tenius* and *Cladosporium herbarium* and German cockroach. Of a total of 2673 serum analyses, 23 measurements of sIgE or total IgE were missing due to a low sample volume.

The skin prick test was performed for the above listed allergens and egg white, milk, peanut and codfish with Soluprick® allergens (ALK Abello, Denmark), with histamine as positive and saline as negative controls. A skin prick test was considered positive in the presence of a wheal diameter ≥3 mm larger than the negative control (15).

FE_{NO} was measured online at mean exhalation flow 50 mL/sec by the single breath method using EcoMedics Exhalyzer® CLD 88sp with Denox 88 (Eco Medics, Duernten, Switzerland). FE_{NO} measurements, spirometry and the exercise tests were performed in accordance with published guidelines (16–18) and as previously described (14). The exercise test was considered positive with a fall of at least 10% in FEV_1 at three to 20 min post exercise.

Definitions

Allergic rhinoconjunctivitis symptoms: a history of watery rhinorrhea, blocked nose, sneezing, nasal itching accompanied by itchy watery eyes in absence of airway infection.

Allergic rhinoconjunctivitis: a positive skin prick test and a history of related allergic rhinoconjunctivitis symptoms as evaluated by a doctor.

Food allergy: a positive skin prick test and a history of related food allergy symptoms as evaluated by a doctor.

Asthma: At least two of the following three criteria fulfilled at any time in life: (1) recurrent dyspnoea, chest tightness and/or wheeze; (2) a doctor′s diagnosis of asthma; (3) Use of asthma medication including β-2 agonist, sodium chromoglycate, corticosteroids, leukotriene antagonists and/or aminophylline.

Current asthma: asthma as defined above plus symptoms and/or medication within the last year, and/or a positive exercise test (18).

Asthma in remission: asthma not defined as current asthma.

Statistical analysis

The distribution of FE_{NO} values was right skewed, thus analyses were executed with natural logarithm transformed data. The results were presented as back-transformed values, and expressed as geometric means with 95% confidence intervals. Inter-group comparisons were executed with an independent *t*-test for continuous variables and Pearson′s chi-square test for categorical variables. Receiver operating characteristic curves were made using MedCalc version 12.5.0 (MedCalc software, Ostend, Belgium). Spearman's rank correlation coefficient was used for correlations. Correlations were assessed with sIgE values ≤100 kU/L. Normally distributed values were presented as means and standard deviations or 95% confidence intervals. Categorical data were presented as percentages. All tests were two-sided, using a significance level of 0.05. Statistical analyses were performed using IBM SPSS Statistics version 21.0 (SPSS Inc. IBM, Chicago, IL, USA).

RESULTS

Demographic features of the study population

Of the 303 children enrolled, 223 had allergic rhinoconjunctivitis symptoms and 80 did not have allergic rhinoconjunctivitis symptoms. In the group with allergic rhinoconjunctivitis symptoms children with a reaction to the negative control (n = 5), food allergy (n = 23) and individuals who did not fulfil the allergic rhinoconjunctivitis definition (n = 31) were excluded. In the group without allergic rhinoconjunctivitis symptoms one child had food allergy and was excluded. Demographic data of the 164 children with allergic rhinoconjunctivitis and the 79 children without allergic rhinoconjunctivitis are presented in Table 1.

Diagnostic value of sIgE

Receiver operating characteristic curve analysis (Figure S1, Table 2) demonstrated that sIgE predicted allergic rhinoconjunctivitis to the tested pollen, animal and mite allergens. The area under the curves yielded values in the range 0.852–0.954, i.e. from moderate to excellent. The cut-off values of sIgE with the best combined sensitivity and specificity were found to be in the range 0.23–1.1 kU/L depending on the allergen. However, the sIgEs for *Alternaria tenius*, *Cladosporium herbarium* and German cockroach were not significant predictors of allergic rhinoconjunctivitis (data not presented). The positive and negative likelihood ratios were from moderate to high for the pollens, cat, rabbit and house dust mite allergens. However, the positive likelihood ratio for sIgE to dog was low. Cut-off values for a diagnostic test at 90% specificity and for a screening test at 90% sensitivity are presented in Table 3.

FE_{NO} levels and the correlations with IgE

FE_{NO} was significantly elevated in the allergic rhinoconjunctivitis groups in non-asthmatics, asthmatics in remission and current asthmatics (Fig. 1). In comparison, the FE_{NO} concentrations were similar in children without allergic rhinoconjunctivitis, irrespective of asthma. The highest level, 29.7 (23.3–37.7) parts per billion was found in children with allergic rhinoconjunctivitis and current asthma and was significantly elevated compared to non-asthmatics and asthmatics in remission (both $p < 0.001$).

In children with allergic rhinoconjunctivitis, FE_{NO} correlated moderately with total IgE (Spearman's rank correlation coefficient = 0.28, $p < 0.001$), sIgE to cat (Spearman's rank correlation coefficient = 0.38, $p = 0.002$) and dog (Spearman's rank correlation coefficient = 0.59, $p < 0.001$), (Figure S2). FE_{NO} did not correlate positively with sIgE to birch (Figure S2d), or the other tested allergens (data not presented).

Table 1 Demographic features of the study population

	With AR		Without AR		p-value
N (male)	164	(112)	79	(46)	
Age (years)*	12.7	(1.9)	12.3	(1.9)	0.094
Height (cm)*	157.4	(13.3)	154.3	(13.9)	0.101
Weight (kg)*	50.6	(14.4)	50.1	(17.4)	0.819
Current disease					
Asthma (%)	34.1		21.5		0.044
Eczema (%)	23.1		20.2		0.608
Urticaria (%)	7.3		5.1		0.507

AR = Allergic rhinoconjunctivitis defined by a positive skin prick test to ≥1/10 inhalant allergens and related AR symptoms.
*Mean (standard deviation).

Pairwise comparisons of receiver operating characteristic curves

Pairwise comparisons of receiver operating characteristic curves confirmed that sIgE was superior to FE_{NO} in predicting allergic rhinoconjunctivitis to the tested pollen, animal and house dust mite allergens (Table S1, Figure S1). Total IgE predicted allergic rhinoconjunctivitis to timothy, birch and rabbit, while FE_{NO} did not (Table S1, Figure S1). FE_{NO} and total IgE had equal power to predict allergic rhinoconjunctivitis in children sensitised to dog and *Dermatophagoides pteronyssinus*. In comparison, FE_{NO} and total IgE had both low power to predict allergic rhinoconjunctivitis in children sensitised to mugwort. Pairwise comparisons of receiver operating characteristic curves for *Alternaria tenius*, *Cladosporium herbarium* and German cockroach demonstrated as expected non-significant differences in the power of sIgE, total IgE and FE_{NO} to diagnose allergic rhinoconjunctivitis to these allergens.

DISCUSSION

This study showed that the sIgE to the tested pollen, animal and mite allergens analysed on the IMMULITE® was highly sensitive and superior to FE_{NO} in predicting allergic rhinoconjunctivitis. The cut-off levels for sIgE with the best combined sensitivity and specificity required to detect allergic rhinoconjunctivitis to these allergens were above the detection limit of the IMMULITE®. Therefore, labelling sIgE as a dichotomous variable, positive or negative based on the detection level of the immunoassay, would result in over-diagnosing allergic rhinoconjunctivitis. FE_{NO} was significantly elevated in individuals with allergic rhinoconjunctivitis regardless of asthma status, and the highest FE_{NO} values were found in children with current allergic asthma. Elevated FE_{NO} levels may therefore indicate allergic rhinoconjunctivitis in children.

Cut-off levels for the IMMULITE® to some common inhalant allergens have been reported for adults (19), but not in children. In this study of 243 schoolchildren we found that sIgE was a powerful predictor of allergic rhinoconjunctivitis to the tested pollen, animal and mite allergens. Different cut-off points were reported according to the purpose of the test. The sIgE cut-off value for a general optimal test was the value with the best combined sensitivity and specificity. In this case the cut-off levels were above the detection limit of the assay for seven of ten

Table 2 ROC curve statistics for specific IgE to inhalant allergens in children with allergic rhinoconjuntivitis

Allergen	N*/Positive†	AUC	95% CI	p-value	Cutoff value‡	Sensivitity	Specificity	LR+	LR−
Timothy	241/96	0.954	0.920–0.977	<0.001	1.1	94.8	84.1	6.0	0.06
Birch	241/73	0.905	0.861–0.939	<0.001	0.93	91.8	85.1	6.2	0.09
Mugwort	240/17	0.937	0.899–0.964	<0.001	0.59	82.4	94.2	14.1	0.19
Cat dander	240/89	0.924	0.882–0.954	<0.001	0.91	95.5	83.4	5.8	0.05
Dog dander	242/77	0.852	0.801–0.894	<0.001	0.27	83.1	78.2	3.8	0.22
Rabbit dander	242/23	0.856	0.805–0.897	<0.001	0.23	78.3	93.6	12.2	0.23
D. pteronyssinus	242/31	0.917	0.875–0.949	<0.001	1.00	87.1	97.2	30.6	0.13

AUC = Area under the curve; CI = Confidence interval; LR+ = Likelihood ratio positive; LR− = Likelihood ratio negative; *D. pteronyssinus* = *Dermatophagoides pteronyssinus.*

*Complete result sets of specific IgE, skin prick test (SPT) and allergic rhinoconjunctivitis (AR) symptoms.

†Positive SPT and related AR symptoms as evaluated by a doctor.

‡Specific IgE cutoff values (kU/L) with the best combined sensitivity and specificity.

Table 3 Cut-off values and diagnostic utility of allergen-specific IgE for identifying children with allergic rhinoconjunctivitis

Allergen	Purpose	Cut-off value*	Sensitivity (95% CI)	Specificity (95% CI)	LR+	LR−
Timothy	Diagnostic†	4.1	78.1 (68.5–85.9)	90.3 (84.3–94.6)	8.1	0.24
	Screening‡	1.7	90.6 (82.9–95.6)	87.6 (81.1–92.5)	7.3	0.11
Birch	Diagnostic	2.8	80.2 (69.1–88.6)	90.2 (84.6–94.3)	8.2	0.22
	Screening	1.0	90.4 (81.2–96.1)	85.1 (78.8–90.1)	6.1	0.11
Mugwort	Diagnostic	0.35	82.4 (56.6–96.2)	91.0 (86.5–94.4)	9.2	0.19
	Screening	0.16	94.1 (71.3–99.9)	82.1 (76.4–86.9)	5.3	0.07
Cat dander	Diagnostic	7.4	69.7 (59.0–79.0)	90.1 (84.1–94.3)	7.0	0.34
	Screening	1.3	91.0 (83.1–96.0)	85.4 (78.8–90.6)	6.3	0.11
Dog dander	Diagnostic	1.7	52.0 (40.3–63.5)	90.3 (84.7–94.4)	5.4	0.53
	Screening	0.1	88.3 (79.0–94.5)	67.3 (59.5–74.4)	2.7	0.17
Rabbit dander	Diagnostic	0.11	78.3 (56.3–93.5)	90.4 (85.7–94.0)	8.2	0.24
	Screening	0.1	78.3 (56.3–93.5)	88.6 (83.6–92.5)	6.9	0.25
D.pteronyssinus	Diagnostic	0.36	87.1 (70.2–96.4)	90.5 (85.7–94.1)	9.2	0.14
	Screening	0.1	87.1 (70.2–96.4)	82.9 (77.2–87.8)	5.1	0.16

CI = Confidence interval; LR+ = Likelihood ratio positive; LR− = Likelihood ratio negative; *D.pteronyssinus* = *Dermatophagoides pteronyssinus.*

*Specific IgE cutoff values (kU/L).

†Diagnostic test; specificity at 90% or the closest specificity identified with the best combined sensitivity.

‡Screening test; sensitivity at 90% or the closest sensitivity identified with the best combined specificity.

allergens. At these levels the sIgEs were good predictors of allergic rhinoconjunctivitis to pollens, cat and rabbit, and sIgE was a very good predictor of allergic rhinoconjunctivitis to house dust mite. However, sIgE to dog had a low positive likelihood ratio and was poor in detecting allergic rhinoconjunctivitis to dog.

For a diagnostic test 90% specificity was preferred to reduce false positive results. At these cut-off points, the sensitivity was moderate. Hence most individuals with allergic rhinoconjunctivitis to pollens, rabbit and house dust mite were diagnosed. However, this approach resulted in under-diagnosing allergic rhinoconjunctivitis to cat and dog. Using sIgE as a screening test a sensitivity of approximately 90% lead to lower sIgE cut-off values accompanied by a moderate specificity in most allergens. However, the low specificity for sIgE to dog indicated that this cut-off level cannot be used to rule out allergic rhinoconjunctivitis to dog. Consequently, the cut-off values used in the clinic should be chosen according to the purpose of the test. However, the cut-off values found in this study may be affected by the severity of allergic rhinoconjunctivitis disease in the school children.

The sIgEs for *Alternaria tenius, Cladosporium herbarium* and German cockroach were not significant predictors of allergic rhinoconjunctivitis to these allergens. This may partly be explained by the few children with allergic rhinoconjunctivitis to these allergens in this study. A low number of children (six of the 243 studied) had allergic rhinoconjunctivitis to German cockroach, while 9.5% had a positive sIgE. sIgE antibodies to German cockroach have been suggested to cross react with mite, shrimp and mosquito (20,21). We were surprised to find that 9.5% of this subarctic population was sensitised to German cockroach. However, this finding may also reflect the travelling habits of northern Norwegians to Mediterranean countries.

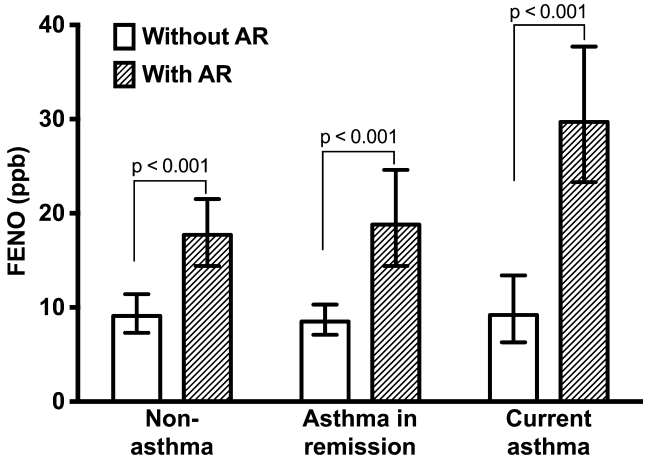

Figure 1 Comparison of fractional exhaled nitric oxide (FE_{NO}) levels in children without asthma (non-asthma, n = 110), asthma in remission (n = 60) and current asthma (n = 73) with (shaded bars) or without (white bars) allergic rhinoconjunctivitis. Allergic rhinoconjunctivitis was defined by a positive skin prick test and related allergic rhinoconjunctivitis symptoms. FE_{NO} was measured using the single breath technique and was expressed as parts per billion (ppb). Group comparisons were analysed by independent *t*-test with natural logarithm transformed data. Data are given as geometric means with 95% confidence intervals.

There exist no absolute sIgE antibody reference standards against which to judge true accuracy. However, ImmunoCAP® (Phadia) was the first established assay and has been accepted and validated as a quasi-standard (4,19,22). In a proficiency survey by Hamilton et al. carried out in 2010 excellent agreement was demonstrated for total IgE measurements between the most commonly used assays including the IMMULITE® (4). They reported a trend towards higher estimates of sIgE to common inhalation antigens for the IMMULITE®, compared to those of ImmunoCAP® at sIgE levels above 1 kU/L (4). In children, the IMMULITE® has been found to overestimate sIgE levels to cat, birch and *Dermatophagoides farinae* (5). However, better agreement between sIgE and skin prick test for the IMMULITE® compared to ImmunoCAP® has been reported (22). Allergen reagents produced by different manufactures vary in its protein composition and have been shown to detect unlike sIgE populations (4,23). Thus sIgE cut-off levels reported for one *in vitro* assay defining clinical allergy cannot be used with sIgE results from a different one.

FE_{NO} has been shown to be elevated in individuals with food allergy (24). In this study, children with allergy to major food allergens were excluded. Reports are conflicting as to whether eczema influence the FE_{NO} level (8,10,25). Nordvall et al. (9) found that FE_{NO} was not related to eczema after adjusting for wheeze and allergic rhinoconjunctivitis. In the present study the burden of eczema was equal in children with and without allergic rhinoconjunctivitis. FE_{NO} has been shown to correlate positively with age and height, but not gender (8,10). In the present study, children with and without allergic rhinoconjunctivitis were similar in respect to age and height, thus demographic features cannot explain differences in FE_{NO}. In line with other studies, we found the FE_{NO} level to be elevated in children with allergic rhinoconjunctivitis with the highest values in children with current allergic asthma (7–10). Identifying and treating patients with allergic rhinoconjunctivitis and contaminant asthma is essential as the risk of severe asthma exacerbations is reduced (26).

In children with allergic rhinoconjunctivitis, total IgE correlated significantly with FE_{NO}. High total IgE is a well-known predictive marker of FE_{NO} increase in children (7,27). However, unlike correlations with FE_{NO} have been demonstrated in different phenotypes of allergic rhinoconjunctivitis and allergic asthma (6,28). In children with allergic rhinoconjunctivitis, sIgE to cat and dog correlated significantly with FE_{NO}. This may partly be explained by allergen size. Sensitisation to small molecules is associated with bronchial hyperresponsiveness, whereas sensitisation to larger molecules such as pollen allergens is associated with allergic inflammation in the upper airways (12,29). Allergens inhaled to the lower respiratory tract may induce FE_{NO} production by increased expression of inducible nitric oxide synthase (30). sIgE to pollen allergens did not positively correlate with FE_{NO}, in line with other studies (6). This may depend on the level of exposure as the study was performed mainly out of the pollen season, and pollen exposure is time-limited in cold climates. We found sIgE to be superior to total IgE and FE_{NO} to predict allergic rhinoconjunctivitis in pollen, cat, dog and house dust mite-sensitised children. In children sensitised to timothy and birch, total IgE predicted allergic rhinoconjunctivitis better than FE_{NO}, suggesting that these allergens may trigger IgE production in the blood more than FE_{NO} production in the lower airways. The non-significant differences found in sIgE, total IgE and FE_{NO} to predict allergic rhinoconjunctivitis in *Alternaria tenius*, *Cladosporium herbarium* and German cockroach-sensitised children may be explained by the few with allergic rhinoconjunctivitis to these allergens.

The diagnostic accuracy of sIgE, total IgE and FE_{NO} to detect allergic rhinoconjunctivitis were examined, and cut-off values for sIgE were estimated. All examinations were performed by two investigators to ensure reproducibility, and a large number of children were included. Ideally the participants should have been randomly selected, and the study should have been blinded. A positive skin prick test and related allergic rhinoconjunctivitis symptoms as evaluated by a doctor served as a quasi-gold standard. The gold standard of type I hypersensitivity, namely a double-blinded provocation test, is difficult to perform in a reproducible manner in an outpatient setting.

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CONFLICT OF INTEREST

The 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:

Figure S1 Receiver operating characteristic curves for specific IgE, total IgE and fractional exhaled nitric oxide (FE_{NO}) to predict allergic rhinoconjunctivitis.

Figure S2 Scatter plots of the correlation between total IgE/ specific IgE and the natural logarithm fraction of exhaled nitric oxide ($LnFE_{NO}$) for (a) all children with allergic rhinoconjunctivitis and in children with allergic rhinoconjunctivitis to: (b) cat, (c) dog and (d) birch.

Table S1 Pairwise comparison of ROC curves for specific IgE, total IgE and fractional exhaled nitric oxide according to allergic rhinoconjunctivitis in children demonstrating differences in area under the curve