

ORIGINAL ARTICLE

Can Immunoglobulin E-measurement replace challenge tests in allergic rhinoconjunctivitis to grass pollen?

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Summary

Background There is minimal data available concerning the dose–response relationship between allergen exposure and clinical reactivity for outdoor aeroallergens, such as timothy grass pollen. Timothy pollen-specific IgE (sIgE) determinations might assist in predicting the clinical reactivity in patients with allergic rhinoconjunctivitis (ARC).

Methods Allergen-sIgE antibody levels of timothy grass pollen were correlated with individual threshold doses eliciting allergic reactions in skin prick test (SPT), conjunctival (CPT) and nasal (NPT) provocation tests in patients suffering from pollen-induced rhinoconjunctivitis and healthy controls.

Results One hundred and four patients with ARC (median age: 27 years; range: 18–64; females: 58%) and 36 controls (25 years (22–77); females: 70%) were included in the study. Ninety-six percent of the patients showed a positive reaction in the nasal and 57% showed a positive reaction in the conjunctival provocation. With regarding to titrated SPT, 98% of the patients showed a positive skin test reaction; correlating with the level of sIgE for timothy ($P < 0.001$). For both provocation protocols, there was no correlation between the provocation concentration at the reaction and the level of sIgE for timothy. The ratio of sIgE/total IgE correlated with the dilution level of SPT ($P < 0.002$) and CPT ($P < 0.01$), respectively.

Conclusion and Clinical Relevance A dose–response relationship between the levels of sIgE and clinical outcome of timothy allergen exposure could not be established. Although IgE-determination remains an important key element in allergy diagnosis, provocation tests are procedures of choice if the clinical relevance of an allergen has to be confirmed.

Keywords conjunctival provocation, dose–response relationship, nasal provocation, pollinosis, skin prick test

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Introduction

In addition to patient history, further diagnostic tests are crucial in the diagnosis of allergic disease. In some cases the history does not correspond to the test results, which should be a prerequisite to establish a relationship between allergen exposure and the elicitation of clinical symptoms [1]. In this situation, provocation tests are considered as the gold standard in assessing the causality between the triggering allergens and the development of signs and symptoms of an allergic disease [2].

For >40 years *in vitro* allergy diagnosis has been mainly based on the measurement of specific-IgE (sIgE)

antibodies in the blood and can be regarded as a major contributor to the clinical management of allergic patients [3, 4], giving additional information and complementing skin and provocation tests. It has been demonstrated that serum levels of sIgE may predict the probability of an allergic response following food allergen provocation [5, 6]. Because provocation tests are time consuming, labor intensive and potentially risky for the patient, there is also a medical need to investigate the predictive value of sIgE levels for other common allergens and allergic diseases, such as allergic rhinoconjunctivitis (ARC) and asthma, where grass pollens represent important triggering factors [7, 8].

Even though the elicitation of an allergic disease seems to follow a dose–response characteristic, the exact amount of allergen necessary to trigger symptoms of allergic reaction in a population of hayfever patients is not clearly established [1, 9–11]. Based on the working hypothesis that sIgE levels to grass pollen allergen may help to predict the probability of symptoms in grass pollen allergic individuals, it was the aim of this study to investigate the dose–response relationship between allergen exposure, the degree of sensitization and the intensity of clinical symptoms elicited by grass pollen in patients with ARC and healthy controls. Serum sIgE and total IgE (tIgE)-levels were compared with the outcome of titrated skin tests as well as nasal and conjunctival provocation tests (CPT) in patients who reported symptoms during the grass pollen season. Because the majority of pollinosis patients are polysensitized and often express other allergic diseases, we also took the total serum IgE concentration into account and investigated sIgE levels in relation to the levels of tIgE, as demonstrated previously for food allergy in children [12]. The ratio of allergen-sIgE to tIgE, also termed specific activity, was shown to influence effector cell activation in the allergic cascade and to be an important parameter for translating sensitization into clinical manifested allergic symptoms [13].

Methods

Study design

In this monocentric prospective four visit study, adult patients with ARC and allergy to timothy grass pollen as well as healthy individuals without allergy serving as controls were recruited during the grass pollen season. For inclusion, the serum level of sIgE-timothy had to be at least 0.35 kU_A/L. Individuals recruited as controls had to have no history of pollinosis (to timothy grass pollen) and the degree of sIgE-timothy had to be below 0.35 kU_A/L. For both groups, pregnancy, diseases such as HIV or hepatitis B/C infection, severe bronchial asthma or history of mastocytosis, as well as current or previous immunotherapy and treatment with beta-blockers and ongoing treatment with systemic medication influencing skin and challenge test were exclusion criteria. All participants signed an informed consent before participating in the study. The protocol for this study was approved by the local ethics committee before the initiation of the study.

Patients and controls

Patients and controls were included in the study during two consecutive grass pollen seasons (2006 and 2007). Each patient visited the clinic on four occasions. The first visit took place during the grass pollen season in Germany

from May to July (run-in visit). The other three visits were scheduled outside the grass pollen season: the second and third visits were both performed in October, while the fourth and last visit took place in December. The interval between visits 1 and 2 ranged from 3 to 5 months, while the interval between visits 3 and 4 ranged from 1 to 2 months.

At visit 1, demographic data, medication at inclusion, symptom observations, history of atopic/non-atopic disease and heredity of atopy were recorded. A thorough physical examination was performed in all individuals and sIgE-timothy was determined. At baseline total serum IgE and allergen sIgE antibodies to common atopy-relevant allergens were determined as well.

At visits 2 and 3, sIgE-timothy was determined, a skin prick test (SPT) was carried out as a stepwise titrated test and nasal provocation test (NPT) (visit 2) and CPT (visit 3), respectively, were performed. Standardized questionnaires regarding the occurrence of allergy-related symptoms during the current grass pollen season were used in order to obtain symptom data. Symptom intensity, between 0 and 100, was scored by the patients on a visual analogue scale. The symptoms were sneezing, runny nose, congestion, itchy nose, itchy-watery eyes, wheezing, chronic dry cough and headache (data not shown).

At the last visit, a further blood sample was collected and the levels of sIgE-timothy and tIgE, as well as a repeated stepwise-titrated SPT, were determined.

Skin prick tests

Skin testing was performed using a lancet with a 1 mm tip (Hollister-Stier Laboratories, Spokane, WA, USA) and allergenic extracts from Allergopharma (Reinbek, Germany). Histamine phosphate (10 mg/mL in 50% glycerosaline) and glycerosaline (50%) were used as positive and negative controls, respectively. Weal and flare diameters were assessed after 15 min by a clear plastic calliper disk scaled in tenths of millimetres. A diameter of the weal ≥ 3 mm was considered as positive. For timothy pollen allergens SPT was performed as a stepwise titrated test, with dilutions from 1 : 10 000 to 1 : 1. For each step the allergen extract dilution was reduced 10-fold.

Conjunctival provocation test

In the CPT, timothy pollen allergen extract was applied into the conjunctival sac of the eye. The test was performed in serial dilutions starting with 1 : 100 of the undiluted SPT concentration. The following concentration steps were used: 1 : 10, 1 : 1, 1 : 0.1 and 1 : 0.01. First, 0.1% saline was applied as a negative control; thereafter for each step a 20 μ L drop of the test solution was applied into the conjunctival sac. The concentration was increased at

10-min intervals, switching from one eye to the other until a clinical reaction appeared.

Conjunctival signs and symptoms (itching, burning/foreign body sensation, tearing, redness, chemosis, eyelid swelling) were assessed using a score of 0 (none) to 3 (severe) for each eye before allergen challenge and 5 and 10 min after challenge.

Nasal provocation test

The NPT was performed with dilutions of timothy grass pollen allergen extracts (Provo-Spray, HAL Allergie, Düsseldorf, Germany) starting with 1 : 10.000 of the stock solution (10 000 AU/mL) using the RhinoScreen-device for anterior rhinomanometry (Jaeger, Höchberg, Germany). The concentration steps used were 1 : 1000, 1 : 100, 1 : 10 and 1 : 1. First, the side of the nose with a higher air flow was identified. Further testing was performed on this side of the nose starting with application of physiologic saline as a control in order to exclude individuals with hyperreactivity of the nasal mucosa. A reduction of nasal flow by > 20% at a pressure of 150 Pa or above indicated non-specific hyperreactivity and excluded patients from further testing. Additionally, clinical symptoms following provocation testing were evaluated by a score (0–6). Nasal challenge was regarded positive (according to the guidelines) if a flow reduction of > 40% was measured or a flow reduction of > 20% in addition to a symptom score > 2 or a symptom score > 3 alone occurred after rhinomanometry [14].

Laboratory tests and Statistics

Serum samples were analyzed for IgE antibodies using the ImmunoCAP® System (Phadia AB, Uppsala, Sweden).

All statistical analyses were performed using the SAS® statistical software system version 9.1. All statistical tests were two-sided and a significance level < 0.05 was regarded as statistically significant. The results of the provocation test and the titrated SPT were analyzed, when applicable, both as an ordered categorical variable with six classes and as a dichotomized variable. Subjects with a positive reaction to the provocation test were referred to as responders and subjects who did not have a positive reaction to the provocation test were referred to as non-responders. Depending on the lowest concentration of the timothy allergen extract to which a positive reaction was obtained, there were five responder classes in the study. The Jonckheere–Terpstra test was used to examine whether there was a significant difference between the classes [15], and the Wilcoxon (Mann–Whitney) rank-sum test was used for a pair-wise comparison of the classes.

In the case of significant relationships, a dose–response curve was fitted to the relationship between dose of the timothy allergen extract and median levels of sIgE antibody to timothy pollen as well as the IgE ratio between

sIgE antibody to timothy pollen and total IgE (sIgE/tIgE ratio) using the following equation:

$$Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{1 + 10^{\log \text{EC50} - X}}$$

where X is the logarithm of the dose, Y is the response (starts at Bottom and goes to Top with a sigmoid shape) and $\log \text{EC50}$ is the the logarithm of the EC50 (effective concentration, 50%).

Results

Patients

In total, 140 subjects (104 ARC patients; 36 controls) were included (Table 1).

On average, each patient with ARC was sensitized to 5.5 of 13 allergens analyzed (SD 3.3, min = 1; max = 10). Corresponding figures for the controls were 0.5 allergens (SD 0.9, min = 0; max = 5). Aside from timothy pollen, the most common allergens to which the patients with ARC were sensitized were birch, hazel, house dust mite (HDM) and mugwort. For the patients, median levels of sIgE to timothy pollen were 10.72, 11.73 and 9.88 kU_A/L at visit 1 (May/June), visit 2 (October) and visit 4 (December), respectively. Test values for tIgE and sIgE to timothy pollen are presented at both run-in and at out-of-season. As demonstrated for both tests, there were small changes between these time-points. Furthermore, as shown in Fig. 1, the distribution of the patients' ratio of sIgE to timothy pollen and tIgE were quite similar at the run-in and at out-of-season visits.

The number of patients and the minimum dose concentrations of the timothy allergen extract that resulted in a positive reaction is shown in Table 2. For each dose, the number of patients with ARC and their distribution are given. P -values and correlation coefficients for the threshold dose as compared with sIgE-timothy levels and the sIgE/tIgE ratio for the timothy allergen extract are shown in Table 3.

Skin prick test

For SPT, there was a dose–response relationship to sIgE-timothy levels ($R = -0.350$; $P < 0.001$). Also for the relative IgE and the dose causing a response relationships was established ($R = -0.311$, $P < 0.008$). Figures 2a and b demonstrates the dose–response curves for the two significant relationships along with median values and $\pm 95\%$ confidence intervals. In Table 4 calculated coefficients for the fitted functions and goodness of fit are shown.

Six of the 104 patients showed false-positive reactions in SPT (at all test sites) due to urticarial dermatographism. These patients were excluded from the analysis of the SPT test results, but were kept in the overall study because this was no exclusion criterion for study participation.

Table 1. Demographic and background characteristics of patients with allergic rhinoconjunctivitis and controls

	Patients		Controls	
<i>Sex (n, %)</i>				
Male	44 (42.3)		11 (30.5)	
Female	60 (57.7)		25 (69.5)	
	Mean (SD)	Median (range)	Mean (SD)	Median (range)
Age (years)	30.1 (9.8)	27 (18–64)	28.7 (10.4)	25 (22–77)
Total clinical symptom score	243.5 (115.5)	256 (0–580)	28.2 (40.4)	9 (0–159)
Serum total IgE (IU/mL)*	237 (296)	118 (0–1664)	68 (127)	28 (0–658)
Serum total IgE†	251 (352)	115 (11–1750)	–	–
<i>Serum-specific IgE (kU_A/L)</i>				
Birch pollen	18.0 (32.2)	3.0 (0–181)	1.3 (7.4)	0 (0–44.4)
House dust mite	5.85 (14.7)	0.3 (0–97.7)	0.42 (0.9)	0 (0.0–3.1)
Hazel	11.3 (19.0)	1.8 (0–105)	0.5 (2.7)	0 (0–16.1)
Mugwort pollen	0.7 (1.2)	0.3 (0–6.2)	0.1 (0.2)	0 (0–0.8)
Cat epithelium	1.7 (5.8)	0.1 (0–45)	0.1 (0.2)	0 (0–1.2)
Dog dander	1.7 (8.3)	0.26 (0–82)	0.0 (0.0)	0.02 (0–0.2)
Cow milk	0.1 (0.1)	0 (0–0.9)	0.0 (0.0)	0 (0–0.2)
Hen's egg	0.1 (0.1)	0 (0–1.4)	0.1 (0.1)	0 (0–0.4)
Hazelnut	4.6 (10.7)	0.4 (0–60.1)	0.0 (0.1)	0 (0–0.5)
Celery	0.7 (1.2)	0.2 (0–7.5)	0.0 (0.1)	0 (0–0.4)
<i>Alternaria alternata</i>	0.3 (1.5)	0.1 (0.1–13.3)	0.0 (0.0)	0 (0–0.2)
Timothy pollen*	20.5 (25.8)	10.7 (0.4–127)	0.0 (0.0)	0 (0–0.23)
Timothy pollen†	20.8 (26.7)	10.9 (0.3–157)	0.0 (0.0)	0 (0–0.47)
Common ragweed	0.9 (2.1)	0.3 (0–17.3)	0.1 (0.0)	0.1 (0–0.3)

*During grass pollen season (run-in value).

†Outside grass pollen season.

Provocations

The correlation between sIgE-timothy and the dose causing a reaction was weak for both the NPT and CPT ($R = -0.036$, -0.146 ; $P = 0.867$, 0.128), respectively. Among patients with ARC almost all reacted in SPT and NPT (3% negative in SPT; 7% negative in NPT), whereas in CPT 44% remained negative. There was no correlation found between relative IgE and the dose causing a reaction for the NPT ($R = -0.143$; $P < 0.281$) nor for the CPT ($R = -0.134$; $P < 0.255$). Concerning the outcome of the SPT (positive/negative and weal size) no correlation to the results of the nasal and conjunctival provocations was detected (NPT: $R = -0.095$; $P < 0.429$; CPT: $R = -0.005$; $P < 0.994$).

Performance characteristics of:

Specific immunoglobulin E-timothy. sIgE to timothy grass accurately predicted positive reactions in 97% for SPT, 93% for NPT and 56% for CPT at 0.35 kU_A/L as the cut-off point. The accuracy of sIgE to timothy grass for predicting a negative outcome for the provocations was 94–100% high in all provocations. Performance characteristics of sIgE to timothy grass pollen are summarized in Table 5.

Relative immunoglobulin E antibody concentration. The optimal cut-off of 0.36% for the sIgE/tIgE ratio as compared with the results of the SPT gave the following characteristics; positive predictive value (PPV): 96%, sensitivity: 96% and specificity: 91%. Corresponding figures for NPT were; optimal cut-off: 1.44%, PPV: 94%, sensitivity: 95% and specificity: 91%. For CPT the figures were; optimal cut-off: 2.2%, PPV: 63%, sensitivity: 92% and specificity: 65%.

Discussion

Challenge tests are needed in the diagnosis of IgE-mediated allergic diseases to prove the relevance of a given allergic sensitization, if the history is not suggestive. This holds true in spite of the fact that these tests are time consuming, expensive to perform and connected with the risk of discomfort or even anaphylaxis for the patient in the case of a positive reaction [16]. *In vitro* tests that are able to predict the outcome of an allergen provocation test, thus allowing determination of the relevance of a given sensitization for the elicitation of clinical symptoms, would be very useful.

This study demonstrated that sIgE and its ratio with tIgE was significantly correlated to the outcome of SPT. It also

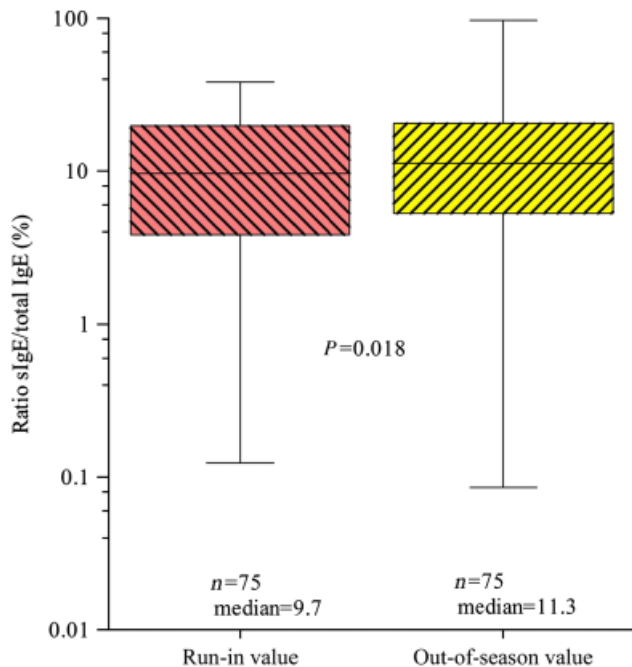


Fig. 1. Ratio between specific IgE (sIgE) to timothy pollen and total IgE in 75 patients with allergic rhinoconjunctivitis and allergy to timothy grass pollen. Comparison of data observed during the pollen season (May until July) and after the pollen season (October until December). Boxes include median value and the interquartile range (25–75% observation). Whiskers extend to the most extreme data point.

Table 2. Minimum dose of timothy allergen extract resulting in a positive reaction in patients* with allergic rhinoconjunctivitis

Dose concentration (AU/mL)	SPT n (%)	NPT n (%)	CPT n (%)
0.01	–	–	1 (1.0)
0.1	–	–	6 (5.98)
1	31 (31.6)	14 (18.2)	5 (4.9)
10	18 (18.4)	14 (18.2)	20 (19.6)
100	41 (41.9)	13 (16.9)	25 (24.5)
1000	4 (4.1)	23 (29.9)	–
10 000	1 (1.0)	8 (10.4)	–
No reaction	3 (3.1)	5 (6.5)	45 (44.1)
Total (n)	98	77	102

Number of patients and percentage in SPT, NPT and CPT provocations are given. The dose relates to the lowest concentration to which a positive reaction was obtained.

*In the control group, three participants reacted to any of the provocations, one on nasal provocation (at 10 000 AU/mL) and two on skin prick testing (at 100 and 10 000 AU/mL, respectively). SPT, skin prick test; NPT, nasal provocation test; CPT, conjunctival provocation test.

showed that no significant relationships were detected between the sIgE-timothy concentration and the allergen dose causing a reaction in NPT or CPT. The use of the sIgE/tIgE ratio was demonstrated to be more appropriate

than that of sIgE only in regard to the correlation with SPT. The approach of using the sIgE/tIgE ratio was demonstrated earlier in the evaluation of the outcome of food provocations in allergic children, because the relevance of sIgE may differ depending on the level of tIgE in the individual patient [12, 17]. A recent publication has also pointed out this as an important parameter, termed specific activity, for the relationship between sensitization and clinical symptoms [13].

The results of the titrated SPT correlations with sIgE clearly reflect experiences from the clinical setting, in which a correlation between the IgE-measurements and the skin test is commonly observed, and is in line with earlier studies also reporting strong correlations between these two test methods [18, 19]. Data analysis revealed no relationship between SPT and challenge results, which means that a positive SPT reaction does not necessarily predicts a clinical reaction and therefore is not indicative for the diagnosis of an allergic disease alone.

Because the dose–response results were negative for the CPT and NPT, we also analyzed the performance characteristics of sIgE-timothy at the cut-off point of 0.35 kU_A/L with the challenge outcomes dichotomized into positive (any reaction irrespective of concentration) or negative (no reaction). Owing to substantial skewness in parts of the data, log transformation did not give satisfying symmetric data. Therefore, we chose to use more robust non-parametric statistical methods. As shown for NPT and SPT, both sensitivity and specificity were high, indicating that sIgE-timothy tests perform well in subjects with or without allergy. Furthermore, the high PPV indicated a high likelihood of disease in those with elevated sIgE-timothy levels (SPT: 97%; NPT: 93%).

All patients included in the study were selected based on clinical symptoms during the grass pollen season and reported nasal complaints in all cases (e.g. sneezing, runny nose congestion). The NPT was carried out with an up-dosing scheme aimed at provoking clinical symptoms, which was achieved in 93.5% of the patients, underlining correct patient selection and provocation protocol.

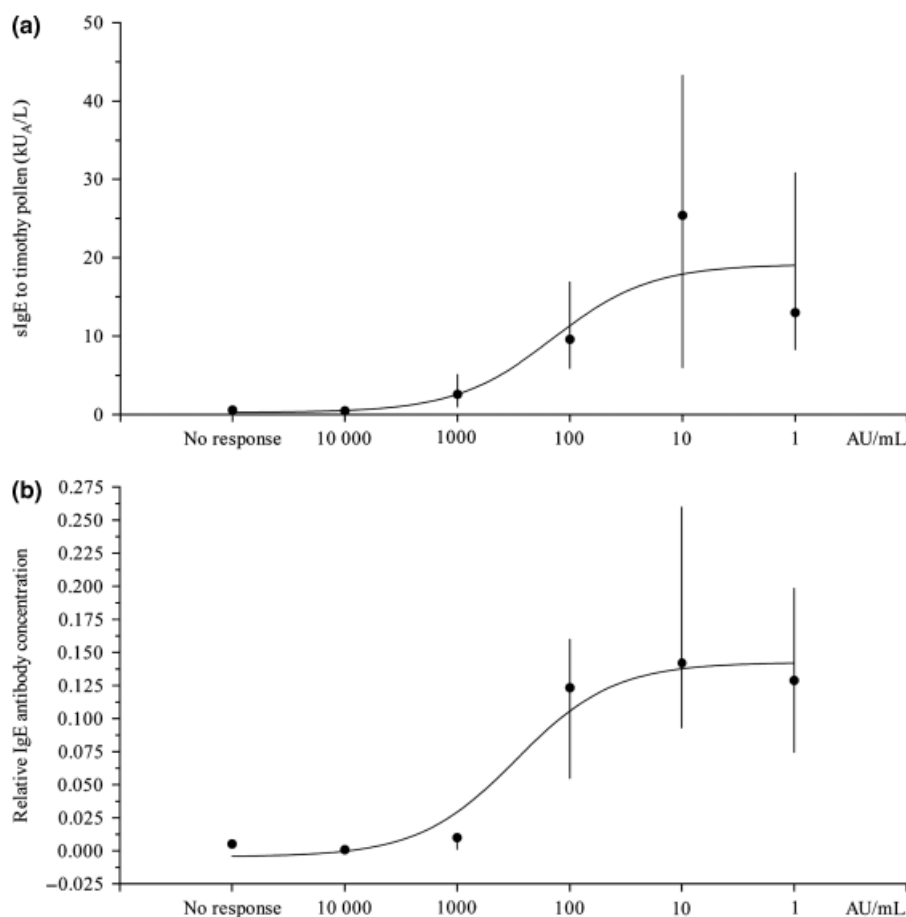
However, for CPT the specificity was quite low indicating a higher risk of false-positive results. Also, the use of sIgE evaluated against CPT has to be discussed critically with regard to the rather low predictive value of this combination. With concern to the high number of individuals with a negative CPT it has to be taken into account that all of the patients included in the study stated nasal complaints when describing their clinical symptoms during the grass pollen season while only less than half of the patients (mean 47.4%) reported the occurrence of 'itchy-watery eyes' as conjunctival symptoms during this time. So, conjunctival symptoms caused by natural exposure during the grass pollen season occurred to a much lower extent than nasal symptoms. This fact is reflected by the outcome of the CPT (negative in 44% of patients).

Table 3. Levels of specific IgE antibody to timothy pollen and the ratio between levels of specific anti-timothy IgE to total IgE in comparison with the dose of the lowest concentration of the timothy allergen extract to which a positive reaction was obtained in SPT, NPT and CPT

Dose vs.	SPT		Nasal		Conjunctival	
	<i>P</i> -value [†]	<i>R</i> [‡]	<i>P</i> -value	<i>R</i>	<i>P</i> -value	<i>R</i>
Specific IgE	<0.001***	−0.350***	0.867 ^{ns}	−0.036 ^{ns}	0.128 ^{ns}	−0.146 ^{ns}
sIgE/tIgE ratio	0.008**	−0.311**	0.281 ^{ns}	−0.143 ^{ns}	0.255 ^{ns}	−0.134 ^{ns}

[†]Jonckheere–Terpstra test.[‡]Spearman's rank-correlation coefficients.***P* ≤ 0.01,****P* ≤ 0.001.

SPT, skin prick test; NPT, nasal provocation test; CPT, conjunctival provocation test; ns, not significant.

**Fig. 2.** (a) Dose–response curve relating the degree in titrated skin prick test to the level of specific IgE (sIgE) to timothy pollen. (b) Dose–response curve relating the degree in titrated skin prick test to the ratio of sIgE to timothy pollen and total IgE.

Concerning the extracts for the different test procedures for SPT and NPT, commercially available extracts especially designed for this purpose were used. For CPT, the SPT extract was used at different concentrations because no CPT extract can be commercially obtained in Germany. Because the SPT extract contains glycerol as an additive while the NPT extract is glycerol-free it was necessary to use different extracts for these test procedures.

With regard to other studies investigating the relationship between IgE-measurements and allergic symptoms, the following results have been published previously: In a study focusing on inhalant allergens from HDM, cat and dog a correlation between sIgE-levels and the presence of a doctor-diagnosed allergic disease associated with clinical symptoms in 894 patients with ARC, asthma, atopic eczema and urticaria [20, 21] could be revealed. The

drawback of this study was the wide range of entities included in the investigation and the fact that the analysis was based on the physician's diagnosis, only without actual provocation tests proving the relevance of an allergen for eliciting symptoms.

Another investigation showed the probability of wheeze and reduced lung function to be increased when sIgE-levels to HDM, cat and dog were elevated [22]. Data concerning the importance of the sensitization pattern was generated in a study focusing on the relationship between bronchial hyperresponsiveness (BHR) and allergic sensitization within the European Community Respiratory Health Survey in the Netherlands: In this study multiple sensitizations to both outdoor and indoor allergens had a relatively strong association with BHR in young adults, while this did not hold true for monosensitization to grass or birch independent of the sIgE-level [23].

Early evidence for the impact of sIgE-levels to common aeroallergens and the clinical expression of allergy was already provided in the mid 1990s where cut-off values for seasonal and perennial aeroallergens were suggested [24]. However, these cut-off levels were calculated based on patient history, SPT and sIgE-data alone and can therefore not be considered as reliable as the conclusions drawn from studies applying provocation procedures to assess the clinical relevance of a given allergen.

Previous studies on food allergy [5, 6, 16, 25, 26] associated the levels of sIgE to foods with the probability of developing a clinical reaction upon allergen exposure with the help of double-blind placebo-controlled food challenges (DBPCFC) for several allergens. The higher the sIgE antibody concentration the higher the probability for a clinical reaction [20]. Only one of these studies [26]

focused on the allergen doses eliciting positive challenge reactions with concomitant calculations to predict dose-response relationships. In that study, and in accordance with our results, the dose causing a reaction during challenge was not predictable by sIgE. As reported, we were unable to demonstrate such a relationship in our study, even though sIgE-timothy at the cut-off of 0.35 kU_A/L clearly differentiated between a positive or negative outcome in both the SPT and NPT challenges with 90–95% predicted probability of clinical reactivity.

The missing correlation between sIgE and the outcome of some provocation tests obtained in our study could be due to several reasons: (I) In the above-mentioned studies on food allergy, DBPCFCs were performed whereas, in our study, provocation tests on eye and nose represent a substantially different methodology, which might explain the different findings. (II) Of several allergens investigated in food allergy the probability of a clinical reaction was only established for some (e.g. peanut, hen's egg), whereas this was not possible for others. In our study only timothy grass pollen allergen was investigated, which is the first aeroallergen to be evaluated in this context with the help of provocation tests of nose and eye – which to our knowledge have not been compared previously within one study. (III) Furthermore, the results could be due to differences in end-organ reactivity between nose and eye, explaining the different findings for NPT and CPT in our study but also the discrepancy with the outcome of the DBPCFC in the studies on food allergy. The importance of a non-specific end-organ hyperreactivity determining the outcome of NPT was also pointed out in the update of the ARIA-guidelines from 2008 [7]. (IV) Also, the study collective is of great importance for the outcome of investigations relating IgE-values to clinical parameters. Probability curves and decision points valid for patient groups seen in highly specialized centers may not apply to the general population, from which our patients and healthy individuals were recruited. Therefore the results cannot be readily transferred from one setting to the other and have to be obtained for each group and allergen separately. (V) Finally, it has to be taken into account that aeroallergen-induced rhinitis in most individuals involves sensitization to a broad spectrum of grasses, trees and other aeroallergens which might substantially decrease the predictive value regarding clinical symptoms that can be expected from the sIgE determination against timothy

Table 4. Coefficients for the dose-response function calculated from estimates of specific IgE to timothy pollen and IgE ratio in five different classes of responders plus one non-responder class

Measure	Model parameters			
	Bottom	Top	LogGEC50	¹⁾ R ²
sIgE vs. SPT	0.2684	19.16–0	3.849	0.7960
IgE ratio vs. SPT	–0.004569	0.1424	3.523	0.9599

¹⁾ R² = Goodness of fit.

Number of patients in each class are given in Table 2.

sIgE, specific IgE; SPT, skin prick test.

Table 5. Performance characteristics of sIgE for predicting skin prick test, nasal and conjunctival provocation outcomes to timothy grass pollen

Characteristics of sIgE	Number of individuals (ARC; controls)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)
SPT	137 (101; 36)	97	94	98	92
NPT	113 (80; 33)	93	97	99	84
CPT	138 (102; 36)	56	100	100	44

SPT, skin prick test; NPT, nasal provocation test; CPT, conjunctival provocation test; PPV, positive predictive value; NPV, negative predictive value; ARC, rhinoconjunctivitis; sIgE, specific IgE.

alone. In order to assess this question all patients mono-sensitized to timothy were identified ($n = 17$; 16.4%), but this group proved too small for further subgroup analysis. Because clinical symptoms during the grass pollen season were an inclusion criterion for all patients in our study, the clinical relevance of grass pollen allergens as triggers of symptoms can be expected. However, for evaluating the relationship between allergen exposure and the outcome of provocation tests as performed in this study, multiple aeroallergen sensitization might be an important confounder explaining the lack of correlation between quantitative IgE data and the magnitude of provocation tests. Because we could not perform separate data analyses for mono- and polysensitized patients, the conclusion drawn from our study may only be valid for this heterogeneous group of polysensitized patients and not for highly selected patients monosensitized to timothy only. Nevertheless, the group of patients included in the study were recruited from the general population and therefore represents the 'real live' situation where polysensitization frequently occurs and sIgE to timothy alone is of limited value in predicting the probability of clinical symptoms.

Our findings indicate the need for considering the total amount of circulating IgE when evaluating specific IgE-

measurements even though they cannot predict the outcome of provocation tests. In general, the use of provocation tests should be restricted to certain clinical situations, when decisions regarding allergen-specific immunotherapy or avoidance strategies cannot be based on patient history and routine allergy tests alone. Because challenge methods are associated with the above-mentioned drawbacks they should not be performed routinely in the standard situation, when patient history and IgE-associated sensitization are in concordance and sufficient to allow the diagnosis of an allergic disease.

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