

Molecular allergy diagnosis: A potential tool for the assessment of severity of grass pollen-induced rhinitis in children

To the Editor

Clinical anamnesis, in vivo provocation testing, and in vitro determination of allergen-specific IgE comprise the traditional three pillars of diagnosis of IgE-associated allergy. In this setting, the in vivo challenge tests, in particular skin testing, are used to link IgE sensitization to certain allergens to the development of allergic symptoms.¹ At present, only allergen extract-based tests are available for in vivo diagnosis. But since allergen extracts have been classified as medical products by the European Directive 2001/83/EC, increased regulatory demands have prompted manufacturers to reduce the number of available extracts for economic reasons over the past few years and it can be expected that allergen extract-based provocation tests may disappear from the market in the future.¹ Moreover, diagnostic allergen extracts were shown to provide only limited clinical information due to quality matters and the presence of cross-reactive allergens as well as clinically non-relevant IgE-reactive carbohydrates.¹ As these problems are an inherent part of the production process and its starting material, they cannot be resolved by improving allergen extract-based technology.¹ For in vitro allergy diagnosis, it seems that these problems can be overcome by the introduction of molecular allergology, measuring IgE reactivity to recombinant allergens with defined purity and biological activity. It allows diagnosing genuine versus cross-reactive sensitization with high specificity and thus identifying patients for specific immunotherapy.^{2,3} Furthermore, an emerging field of research deals with the establishment of serological parameters based on sensitization patterns which are associated with phenotypes of allergy. It was shown that in food allergic patients, the sensitization to food allergens with high stability, like the major peanut allergen Ara h 2, is associated with a higher risk of severe systemic reactions, while sensitization to food allergens which are less stable to heat and digestion is linked to local reactions.² In a recent study, even IgE titers specific for certain "high-risk" food allergens could be correlated with the severity of symptoms during oral food challenges.⁴ Regarding pollen allergy, the sensitization to distinct pollen allergens was found to be predictive for disease progression in children,⁵ but much less is known about the possible usefulness of molecular allergy diagnosis for the severity of allergic symptoms. A recent study identified IgE levels to the major grass pollen allergens Phl p 1 and Phl p 5 as biomarkers for allergic rhinitis persistence.⁶ Our study aimed to establish serological parameters based on the sIgE measurement to single grass pollen allergens in grass pollen allergic children which could be associated with the

severity of allergic rhinitis. Sequential patients who fulfilled the inclusion criteria, that is, diagnosis of grass pollen allergy due to a positive SPT and/or sIgE to timothy grass pollen extract and allergic rhinitis during the pollen season, were invited at the Allergy Department, 2nd Pediatric Clinic University of Athens, Greece, to participate. The response rate was >80%. Written informed consent was obtained from parents, and the study was approved by the local ethics committee in Greece. Anonymized samples were analyzed in Austria with permission of the ethics committee of the Medical University of Vienna. Fifty-nine children with grass pollen allergy and 9 negative controls without grass pollen allergy were included. According to ARIA guidelines, allergic rhinitis can be classified as mild or moderate/severe depending on the severity of the symptoms and their impact on social life, school, and work.⁷ Following these criteria, patients were divided into two groups with mild ($n = 14$) and moderate ($n = 45$) rhinitis. Demographic, clinical, and serological characteristics of children within the two groups and of the negative controls are summarized in Table 1. We measured sIgE levels to single timothy grass pollen allergens for all children using an allergen microarray containing 176 allergens, which was developed previously within the MeDALL project.⁸ Results ≥ 0.3 ISU were considered as positive, statistics are presented as scatter plots with medians, and the Mann-Whitney U test was applied for statistical analysis, using GraphPad Prism Software 5.0 (GraphPad Software). Specific IgE levels to the clinically relevant grass pollen allergens Phl p 1, Phl p 2, Phl p 5, and Phl p 6 were significantly higher in the group with moderate rhinitis compared to children having mild rhinitis (Figure 1A). The difference between the two groups was even more apparent ($P = .009$) when we compared the sum of IgE levels to Phl p 1, Phl p 2, Phl p 5, and Phl p 6 in both groups (Figure 1B). These results point out the high ability of these allergens to induce allergic symptoms in patients.⁹ Phl p 4-specific IgE levels were also included in our analysis, as 64% of patients from the mild rhinitis group and 78% from the moderate rhinitis group had a positive result to Phl p 4. Though, we could not find a significant difference of Phl p 4-specific IgE levels between the two groups, confirming the minor relevance of this allergen for the elicitation of allergic symptoms.⁹ IgE levels to other grass pollen allergens were not compared due to the low number of patients with a positive IgE result (Phl p 7:1 patient; Phl p 11:5 patients; Phl p 12:5 patients). We also compared the number of allergens (Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7, Phl p 11, and Phl p 12) which were recognized by IgE antibodies from grass pollen allergic children with mild and moderate rhinitis. As shown in Figure 1C, children suffering from moderate rhinitis showed IgE reactivity to a significantly higher

TABLE 1 Demographic, clinical, and serological characteristics of grass pollen allergic children and controls without grass pollen allergy

	Mild rhinitis (n = 14)	Moderate rhinitis (n = 45)	Negative controls (n = 9)
Sex	11 (79%) male 3 (21%) female	38 (84%) male 7 (16%) female	6 male 3 female
Age (y)	12 (10-14.25)	11 (9-13)	13 (9.5-14)
Positive family history	11 (79%)	32 (71%)	3 (33%)
Conjunctivitis	8 (57%)	30 (67%)	2 (22%)
Asthma	9 (64%)	23 (51%)	3 (33%)
Atopic dermatitis	7 (50%)	21 (47%)	6 (67%)
Food allergy	5 (36%)	8 (18%)	0
SPT timothy grass (wheal diameter, mm)	4 (2.88-4.63)	4.5 (3-6.5)	0
Sensitizations to other pollens	Amb: 1 (7%), Art: 2 (14%), Che: 1 (7%), Ced: 3 (21%), Cup: 8 (57%), Ole: 10 (71%), Par: 2 (14%), Pla: 5 (36%), Pol: 1 (7%)	Amb: 3 (7%), Art: 7 (16%), Che: 2 (4%), Ced: 13 (29%), Cup: 22 (49%), Ole: 19 (42%), Par: 10 (22%), Pla: 12 (27%), Plan: 3 (7%), Pro: 8 (18%)	Ole: 3 (33%), Par: 1 (11%)
Total IgE (kU/L)	180 (99.5-656)	472.5 (226.5-814.5)	331 (142.3-662.5)

Note: Values are given as n (%) or median (interquartile range).

Abbreviations: Amb, Ambrosia; Art, Artemisia; Ced, Cedar; Che, Chenopodium; Cup, Cupressus; Ole, Olea; Par, Parietaria; Pla, Platane; Plan, Plantago; Pol, Polcalcin; Pro, Profilin.

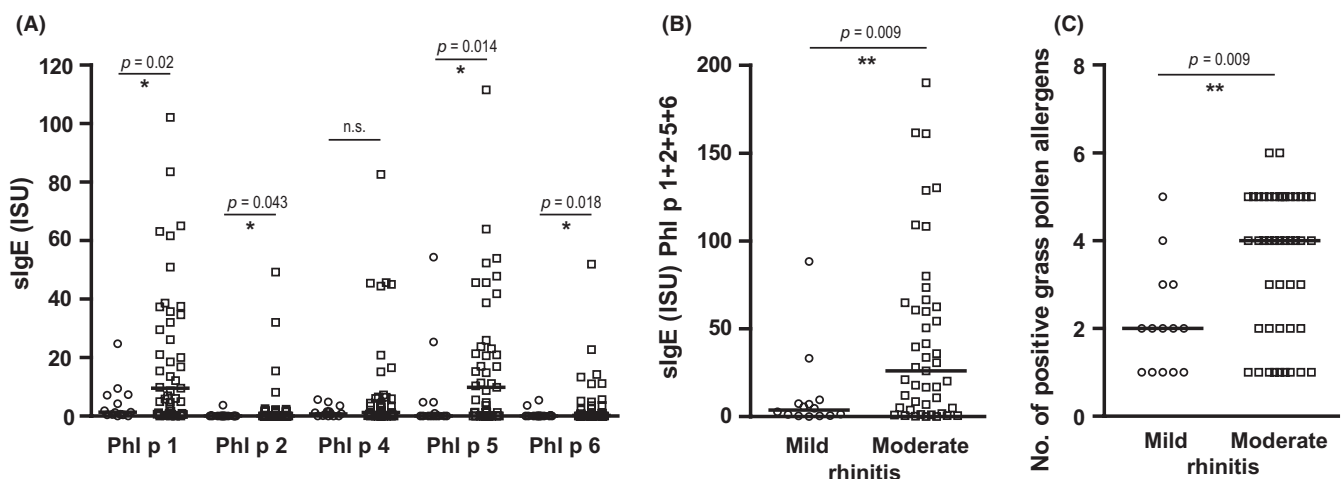


FIGURE 1 Grass pollen allergen-specific IgE in grass pollen allergic children with mild and moderate rhinitis. A, sIgE levels (y-axis) of grass pollen allergic children with mild (open circles) and moderate (open squares) rhinitis are shown for Phl p 1, Phl p 2, Phl p 4, Phl p 5, and Phl p 6 (x-axis). B, The sum of major grass pollen allergen-specific IgE levels (sIgE [ISU] Phl p 1 + 2 + 5 + 6; y-axis) and C, the number of grass pollen allergens recognized by IgE (y-axis) of children with mild vs moderate rhinitis (x-axis) are displayed. Horizontal lines represent the medians, and asterisks indicate statistically significant differences between the two groups (* $P < .05$, ** $P < .01$, n.s. not significant)

($P = .009$) number of grass pollen allergens (median: 4) than the group of children with mild rhinitis (median: 2).

As we were interested, whether diagnostic tests based on timothy grass pollen extract were also able to distinguish between the two groups of patients, we performed ImmunoCAP™ measurements (Thermo Fisher; g6 ImmunoCAP) and skin prick testing (timothy grass pollen extract, Stallergenes) in all grass pollen allergic children. ImmunoCAP results are expressed in kUA/L; values >0.35 were

classified as positive. SPT was performed on the forearms using histamine hydrochloride as positive control and physiological saline as negative control. Results were read after 20 minutes, and mean wheal diameters >3 mm were considered positive. While significantly higher IgE titers to timothy grass pollen extract were detected in children with moderate rhinitis compared to mild rhinitis ($P = .002$), there was no significant difference in skin reactions to timothy grass pollen extract between the two groups (Figure 2A,B). A low sensitivity or specificity of

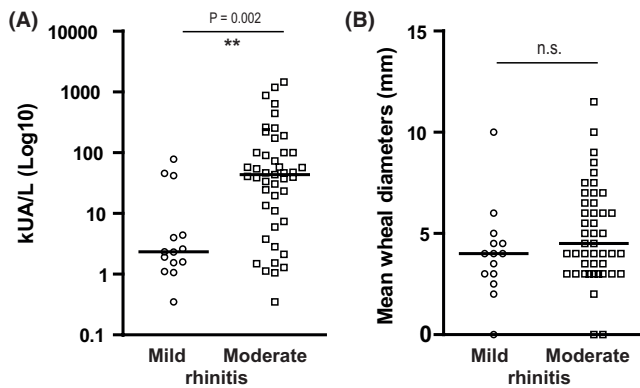


FIGURE 2 IgE titers and skin reactions to timothy grass pollen extract in grass pollen allergic children with mild and moderate rhinitis. A, sIgE levels to timothy grass pollen extract were measured by ImmunoCAP (kUA/L, y-axis) in grass pollen allergic children with mild (open circles) and moderate (open squares) rhinitis (x-axis). B, Skin prick test results in response to timothy grass pollen extract are depicted as mean wheal diameters (mm, y-axis) for grass pollen allergic children with mild (open circles) and moderate (open squares) rhinitis (x-axis). Medians are shown for each group, as well as statistically significant differences between the two groups (* $P < .05$, ** $P < .01$, n.s., not significant)

the skin prick test solution, related to the extract composition, might be an explanation for this result.¹ In this context, it should be mentioned that also in vitro IgE measurement based on allergen extracts greatly depends on the quality of the used extract and the respective allergen source. Good concordance was found between recombinant allergens and allergen extracts for grass pollen but not for house dust mite.^{10,11} No IgE or positive skin reaction to timothy grass pollen allergens was detected in the control group (data not shown).

Taken together, our study is the first one suggesting the usefulness of measuring sIgE levels to recombinant major grass pollen allergens as serological parameters for the assessment of rhinitis severity in grass pollen allergic children. We are aware that the relatively low number of patients, who were investigated, as well as the lack of severe rhinitis patients, and an imbalanced gender distribution are limitations of our study. Additional studies in larger groups of patients will be necessary to translate these findings into clinical practice. We believe that with regard to the limited availability of diagnostic allergen extracts in the future, the use of serological parameters like allergen-specific IgE levels associated with phenotypes of allergic symptoms will become an integral part of allergy diagnosis.

CONFLICT OF INTEREST

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AUTHOR CONTRIBUTION

N.D, VG, KP, and BL contributed to acquisition of data. NGP, VR, and BL made the conception and design. ND, VG, MFT, RV, NGP, and B.L carried out the analysis and interpretation. BL and RV wrote the

original draft. ND, VG, MFT, RV, NGP, and B.L reviewed and edited the manuscript.

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Children with wheat allergy usually tolerate oats

To the Editor:

Wheat is among the five most common food allergies in children with a prevalence of 0.3-0.5% worldwide.¹ In Finland, Japan, and Germany, wheat is the third most common food allergy in young children.² In about 70%, wheat allergy resolves by the age of 6 years. Sensitization to gliadin predicts persistent wheat allergy and a slower course of recovery.³ The gluten-containing cereals wheat, rye, and barley are taxonomically closely related, and their storage proteins display extensive sequence homology and IgE cross-reactivity. Oats belong to the same grass family but are more distantly related to wheat. Oats do not contain allergens cross-reacting with wheat omega-5-gliadin, an allergen associated with severe wheat allergy.⁴ Oats are tolerated by most patients with celiac disease.¹

Oats have been reported to cause non-IgE-mediated reactions and are a well-known elicitor of FPIES.⁵ On the contrary, reports on severe IgE-mediated oats allergy are scarce with only a few published case reports.^{6,7} The use of topical emollient creams containing oats seems to be a risk factor for percutaneous sensitization to oats in patients with atopic dermatitis and may cause even severe reactions to ingested oats. Oral food challenges to oats were, however, negative in 85% of oat-sensitized children with atopic dermatitis.⁸

Children with wheat allergy are frequently sensitized to oats, but they seem to tolerate ingested oats in their diet. Studies on clinical oats allergy in children with wheat allergy are lacking. Here, we demonstrate that children with challenge-proven wheat allergy and sensitization to oats tolerate ingested oats.

The Helsinki University Hospital of Children and Adolescents Ethics Committee approved the study, and each participant as well as his/her guardian gave written informed consent. SPSS Statistics version 22.0 (IBM Corp) was used in the analysis of the data.

The study included 15 wheat-allergic children, aged 9-16 years (median age of 12.1 years), from the Department of Allergology, Helsinki University Central Hospital, Finland. The baseline characteristics of the study children are shown in Table 1. Of the 15 children, 10 (67%) were male, 11 (70%) had asthma, and 10 (67%) had

atopic eczema. Nine (60%) had another food allergy—typically hen's egg or nut allergy (Table 1).

All 15 children had persistent wheat allergy and were evaluated for forthcoming wheat oral immunotherapy. All were sensitized to wheat, gliadin, and oats (specific IgE $\geq$ 0.35 kU/L and/or skin prick test $\geq$ 3 mm) (Table 1). All had an adrenaline autoinjector as an emergency rescue medication.

Serum IgE levels to oats, wheat, gliadin, and omega-5-gliadin were measured by ImmunoCAP (Thermo Fisher). Specific IgE levels to oats were lower than the levels to wheat in all 15 children ($P < .05$, t test, of independent samples). The median IgE level to oats was 6.6 kU/L, wheat 90.2 kU/L, and gliadin 15.2 kU/L. The median ratio of specific IgE to total IgE to oats was 0.015 (range: 0.003-0.032), to wheat 0.201 (range: 0.050-0.688), and to gliadin 0.028 (range: 0.002-0.097). Ten children were sensitized to omega-5-gliadin with a median of 0.40 kU/L. Skin prick tests (SPTs) to wheat and oats (1:10 w/v 0.9% NaCl), and gliadin (1 mg/mL, Sigma) were performed as previously described in 14 of 15 children.³ The median SPT reactions to oats, wheat, and gliadin were 7 mm, 10 mm, and 6 mm, respectively. One child had a negative oat SPT (Table 1).

The oral food challenges were performed according to the PRACTALL consensus criteria.⁹ Reaction severity was assessed using a dose-adjusted severity scoring of patients with a positive oral food challenge.¹⁰ Thirteen patients underwent an open oral wheat challenge. Two patients were not challenged because of a recent severe reaction treated with adrenaline within the past 3 months. Wheat challenges were performed with wheat bread in increasing doses (10 mg, 90 mg, 500 mg, and 1000 mg) given at 30-60-minute intervals with a cumulative protein dose of 1600 mg. All 13 wheat challenges were positive. The median cumulative dose that elicited symptoms was 370 mg (range: 10-1600 mg). Four children (31%) needed intramuscular adrenaline. All were treated with antihistamines. Moderate or severe reactions occurred in 11 of 13 children (85%) (Table 2).

All 15 patients underwent an open oral oats challenge. The median time between the oats and wheat challenges was 15 days