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Misleading Allergens in the Diagnosis of Latex Allergy: Profilin and Cross-Reactive Carbohydrate Determinants

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Abstract
Background: It has been suggested that latex-specific IgE analysis may lead to false-positive results, especially in patients with pollen allergy. In the present study, the reasons underlying clinically irrelevant latex-specific IgE positivity were investigated. ***Methods:*** Thirty patients with latex allergy (group 1), 89 patients sensitised to aeroallergens (group 2a), and 98 healthy individuals without allergy (group 2b) were enrolled. Participants from all 3 groups were subjected to skin prick tests with aeroallergens including latex, latex-specific IgE analysis (ImmunoCAP), and nasal provocation test with latex. All cases demonstrating positive latex-specific IgE also underwent specific IgE tests (ImmunoCAP) with latex profilin, birch pollen profilin, peach lipid transfer protein, and pineapple bromelain as cross-reactive carbohydrate determinants. ***Results:*** Comparison of the atopic and healthy control groups showed that the rate of positive latex-specific IgE was significantly higher in group 2a. Latex profilin-, birch pollen profilin-, and bromelain-specific IgE were remarkably higher in group 2a. ***Conclusion:*** False positivity to latex-specific IgE in ImmunoCAP analysis may be observed in approximately 19% of patients with pollen allergy. Profilins and bromelain are the main contributors to clinically irrelevant positive latex-specific IgE.

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Introduction

Definitive diagnosis of latex allergy is important due to the risk of anaphylaxis and the challenge of providing a latex-free environment in health-care facilities. Skin prick tests (SPTs) and in vitro tests are commonly used in the diagnosis. Specific IgE analysis and SPT using non-recombinant natural latex allergens may yield positive results in asymptomatic patients. In these patients, provocation tests such as glove use test are used to establish a

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diagnosis of latex allergy. We previously showed for the first time by means of the nasal provocation test (NPT) that SPT and latex-specific IgE may yield false-positive results in asymptomatic patients [1]. In a subsequent study we showed that latex-specific IgE analysis performed by Phadia ImmunoCAP (Pharmacia, Uppsala, Sweden) would also have a high false-positive rate, especially in patients presenting allergic sensitisation to more than one pollen. Furthermore, we underscored that clinically irrelevant positive latex-specific IgE may be attributed primarily to cross-reactive allergen profilins and cross-reactive carbohydrate determinants (CCD) and, to a lesser extent, lipid transfer proteins (LTP) [2]. In the present study, we investigated the influence of profilins, CCD, and LTP on clinically-irrelevant positive specific IgE to latex in larger patient and control groups.

Subjects and Methods

Subjects

The patient group (group 1) consisted of 30 cases with latex allergy (rhinitis, asthma, contact urticaria) confirmed by history and SPT. The atopic control group (group 2a) consisted of 100 patients who presented with allergy to at least one aeroallergen (pollens, house dust mites, moulds, animal dander) and negative SPT to latex. One hundred individuals with no history of allergic disorder participated in the study as a healthy control group (group 2b). Group 2a was selected randomly from patients referred to our outpatient clinic and group 2b was randomly selected from a healthy population.

Study Design

All participants in the 3 groups were subjected to SPT with aeroallergens including latex, latex-specific IgE analysis, and NPT with latex. All patients demonstrating positive latex-specific IgE were then subjected to specific IgE tests with latex profilin, birch pollen profilin, peach LTP, and pineapple bromelain CCD. Informed consent was obtained from all patients and the study was approved by the Ege University Ethics Committee (approval No. 2.100.2012.0136).

Skin Prick Test

SPTs were performed with a standardised prick test needle (Stallerpoint). SPTs were carried out with latex and commercially available common aeroallergens (grass, weed, tree pollen, house dust mites, moulds, animal dander; ALK-Abello, Madrid, Spain). Latex SPT material contained 500 μg/ml latex protein. Physiological saline was used as a negative control, and histamine (10 mg/mL) was used as a positive control. SPT results were assessed after 20 min. The presence of an induration with a diameter at least 3 mm greater than that of the negative control with associated erythema was considered positive.

Specific IgE Analysis

Phadia ImmunoCAP (Pharmacia) kits were used for specific IgE analysis. k82 (latex), k221 (rHev b8 profilin, latex), t216 (rBet v2 profilin, birch), f420 (rPru p3 LTP, peach), and k202 (nAna c2 bromelain, CCD pineapple) were used for measuring specific IgE. For latex-specific IgE analysis, values higher than 0.35 kU/L were considered positive. The results were graded on a 6-point scale as recommended by the manufacturer.

Nasal Provocation Test

NPT was applied to groups 1, 2a, and 2b to determine rhinitis symptoms associated with latex protein. The test was conducted according to NPT protocols using the active anterior rhinomanometry technique. Latex-specific sublingual immunotherapy vial containing 500 μg/mL latex protein, 0.5 mL of glycerol, 3 mg of phenol, and 9 mg of sodium chloride (ALK-Abello) was used as the allergen source. The diluent portion of this vial containing no latex protein was used as a placebo. The diluent was produced at Ege University Pharmacology Laboratories. The allergen was diluted 10, 100, 1,000, and 10,000 times, corresponding to 50, 5, 0.5, and 0.05 μg/mL latex protein, respectively, and the placebo was diluted 10 times with physiological saline prior to the NPT. The incremental doses of allergen were applied to all groups using nasal applicators delivering 0.1 mL per application. Nasal and eye symptoms were recorded during the15-min observation, and changes in the nasal flow rate were measured by rhinomanometry (Jager Rhinoscreen, Hochberg, Germany). The first step of the NPT was placebo application. Individuals who had no symptoms and did not show more than 20% decrease in nasal flow rate in reaction to the placebo were then subjected to allergen challenge. In all patients and healthy controls, the NPT was discontinued when a positive result or the maximum allergen concentration (50 μg/mL latex protein) was reached. Symptom scores during the NPT were recorded as previously reported [1]. The test was considered positive if the symptom score reached 5, or reached 4 with a decrease in the nasal flow rate of 40% of the baseline value [1].

Respiratory Function Test

A respiratory function test was administered before and after NPT. FEV_1 (forced expiratory volume in 1 s percentage) was measured 5 min after the test.

Statistical Analysis

Mann-Whitney U tests were used for comparison of the groups. Categorical variables were compared by the Fisher exact χ^2 test. Statistical analysis was performed using the Statistical Package for the Social Sciences, version 18.0 for Windows. Data were expressed as median, mean ± standard deviation, and percentage. A p value less than 0.05 was accepted as statistically significant.

Results

Subjects

All patients in group 1 completed the study. Eleven of the 100 patients in group 2a and 2 of the 100 patients in group 2b were excluded because they did not provide written informed consent. Group 1 comprised 4 male (13.3%) and 26 female patients (86.7%) ranging in age from 23 to 50 years (median 33.5 years). Twenty-five of

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Table 1. The distribution of positive latex SPT, NPT, and k82 ImmunoCAP results in the study groups

Group	*n*	Latex SPT, *n* (%)	NPT, *n* (%)	k82, *n* (%)	k82 ImmunoCAP classes, *n* (%)					
					I	II	III	IV	V	VI
1	30	30 (100)	30 (100)	27 (90)	1 (3.7)	8 (29.6)	6 (22.2)	9 (33.3)	2 (7.4)	1 (3.7)
2a	89	0	0	17 (19.1)	10 (11.2)	4 (4.5)	2 (2.2)	1 (1.1)	0	0
2b	98	0	0	2 (2)	1 (1)	1 (1)	0	0	0	0

SPT, skin prick test; NPT, nasal provocation test.

the 30 patients were health-care workers. All patients in group 1 presented respiratory reactions (rhinitis, asthma) upon latex exposure. In group 2a, 48 patients (53.9%) were male and 41 (46.1%) were female, with ages ranging from 17 to 66 years (median 30 years). All patients in this group had respiratory allergy symptoms (rhinitis, asthma) with aeroallergen exposure. Of the 98 healthy subjects in group 2b, 47 (48%) were male and 51 (52%) were female, with ages ranging from 21 to 55 years (median 31 years).

There was no significant difference between the groups in terms of age, whereas group 1 had a significantly larger proportion of females in comparison to the other groups ($p < 0.0001$), as 21 of the 30 patients in this group were female health workers.

Skin Prick Test

All 30 patients in group 1 had positive SPT to latex. Eighteen (60%) were monosensitised to latex. Five (16.7%) were sensitised to grass pollens, 9 (30%) to weed pollens, and 6 (20%) to tree pollens. One patient (3.3%) presented combined pollinosis (grass + weed + tree pollinosis). Eight patients (26.7%) presented sensitisation to house dust mites and 4 (13.3%) to other aeroallergens (animal dander or moulds).

All patients in group 2a ($n = 89$) were sensitised to one or more pollens: grass pollens in 72 patients (80.9%), weed pollens in 58 (65.2%), tree pollens in 52 (58.4%), and combined pollens in 32 (34.8%) patients. Twenty patients (22.5%) were sensitised to house dust mites and 14 (15.7%) to other aeroallergens (animal dander or moulds).

As a result, the frequency of pollen, grass, weed, and combined pollinosis was significantly higher in group 2a when compared with the patient group ($p < 0.0001$, $p = 0.001$, $p = 0.001$, $p = 0.0001$, respectively), while no difference was observed between the groups in terms of mite and other aeroallergen sensitisations.

Specific IgE Analysis

Latex-specific IgE was positive in 27 (90%) of the 30 patients in group 1, and their latex-specific IgE test results ranged from class I to class VI. Latex-specific IgE was also positive in 17 (19.1%) of the 89 patients in group 2a, and positivity ranged from class I to class IV. Two (2%) of the 98 subjects in group 2b were positive for latex-specific IgE, with results of class I or class II (Table 1). The percentage of latex-specific IgE positivity was significantly higher in group 1 when compared with either of the control groups ($p < 0.0001$, Fisher exact test). When the control groups were compared with each other, group 2a had a significantly higher percentage of patients with positive latex-specific IgE ($p = 0.001$, Fisher exact test).

In group 1, both latex profilin- and birch pollen-specific IgE were positive in a single patient (1/27 [3.7%]) and were class I and class II, respectively (Fig. 1). Of the 17 patients in group 2a who were positive for latex-specific IgE, 7 (41.2%) were also positive for latex profilin-specific IgE (2 class II, 5 class III) and 8 (47.1%) were positive for birch pollen-specific IgE (1 class I, 4 class II, 3 class III). All 7 of the patients in group 2a with latex profilin-specific IgE positivity were also positive for birch pollen-specific IgE. The percentage of positive latex profilin- and birch pollen profilin-specific IgE were found to be significantly higher in group 2a when compared with group 1 ($p = 0.002$ and $p = 0.001$, respectively). Peach LTP-specific IgE was positive in 1 (3.7%) of 27 patients in group 1 and 2 (11.8%) of 17 patients in group 2a. Pineapple bromelain (CCD)-specific IgE was positive in 2 of 27 patients (7.4%; 1 class I, 1 class II) in group 1 and 12 of 17 patients (70.6%; 4 class I, 5 class II, 3 class III) in group 2a. The positivity rate of bromelain (CCD)-specific IgE was remarkably higher in the atopic control group ($p < 0.0001$) (Fig. 1). None of the healthy subjects tested positive for specific IgE to any of the recombinant allergens (profilin, LTP, or CCD).

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Table 2. SPT and specific IgE results of patients in group 1 with positive latex-specific IgE (n = 27)

Patient No.	Age, years	Gender	SPT without pollen	SPT with pollen	Latex-specific IgE (k82)	Latex profilin-specific IgE (rHev b8, k221)	Profilin-specific IgE (rBet v2, t216)	Bromelain-specific IgE (nAna c2, k202)	LTP-specific IgE (rPru p3, rf420)
1	40	M	+	–	4+	–	–	–	–
2	29	M	–	–	5+	–	–	–	–
3	26	F	–	+	3+	–	–	–	–
4	36	F	–	–	2+	–	–	–	–
5	38	F	–	–	2+	–	–	–	–
6	43	F	+	+	4+	–	–	–	–
7	28	F	–	+	4+	–	–	–	–
8	35	F	–	+	2+	–	–	–	–
9	31	F	+	–	5+	–	–	–	–
10	23	M	–	–	4+	–	–	–	–
11	30	F	–	–	4+	2+	1+	–	–
12	29	F	+	+	4+	–	–	–	–
13	50	F	+	–	3+	–	–	–	–
14	32	F	–	–	2+	–	–	–	–
15	39	M	–	–	3+	–	–	–	–
16	32	F	–	+	2+	–	–	–	–
17	33	F	+	–	2+	–	–	2+	–
18	38	F	–	–	4+	–	–	–	–
19	34	F	–	–	2+	–	–	–	–
20	36	F	–	+	3+	–	–	–	–
21	30	F	–	–	3+	–	–	–	–
22	43	F	–	–	6+	–	–	1+	2+
23	36	F	+	+	3+	–	–	–	–
24	35	F	–	+	2+	–	–	–	–
25	25	F	+	–	4+	–	–	–	–
26	25	F	–	+	1+	–	–	–	–
27	28	F	–	+	4+	–	–	–	–

SPT, skin prick test; LTP, lipid transfer protein.

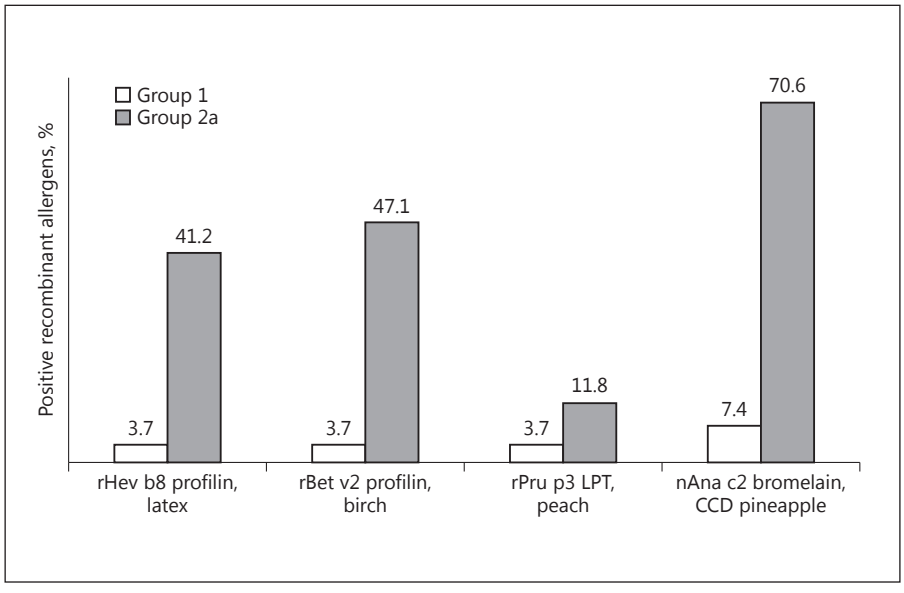

Fig. 1. The percentages of positive recombinant allergens in latex-specific IgE-positive patients. Latex profilin-specific IgE was more common in the atopic control group (group 2a), despite negative skin prick test with latex (p = 0.002), as were birch pollen profilin-specific IgE and bromelain-specific IgE (p = 0.001 and p < 0.0001, respectively). LTP, lipid transfer protein; CCD, cross-reactive carbohydrate determinant.

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Table 3. SPT and specific IgE results of patients in group 2a with positive latex-specific IgE (n = 17)

Patient No.	Age, years	Gender	SPT without pollen	SPT with pollen	Latex-specific IgE (k82)	Latex profilin-specific IgE (rHev b 8, k221)	Profilin-specific IgE (rBet v2, t216)	Bromelain-specific IgE (nAna c 2, k202)	LTP-specific IgE (rPru p3, f420)
1	23	M	+	+	1+	–	–	1+	–
2	31	F	–	+	2+	3+	3+	2+	–
3	35	M	+	+	3+	–	–	3+	–
4	32	F	–	+	1+	2+	2+	–	–
5	28	F	–	+	1+	–	–	3+	–
6	32	M	–	+	1+	2+	2+	–	–
7	17	M	+	+	1+	–	–	2+	–
8	32	F	–	+	1+	–	1+	1+	4+
9	21	F	–	+	1+	–	–	–	–
10	30	M	+	+	1+	–	–	2+	–
11	37	M	+	+	3+	3+	3+	–	–
12	53	M	–	+	4+	–	–	3+	–
13	24	F	+	+	2+	–	–	2+	3+
14	49	M	–	+	1+	3+	2+	–	–
15	19	M	–	+	2+	3+	3+	2+	–
16	50	F	–	+	1+	3+	2+	1+	–
17	36	F	–	+	2+	–	–	1+	–

The mean latex-specific IgE level was significantly higher in group 1 ($p < 0.0001$) compared to group 2a. Since there was only 1 patient with IgE to latex profilin, birch pollen profilin, and LTP, and only 2 patients with IgE to bromelain, we could not perform statistical analysis comparing means between the groups.

Nasal Provocation Test

NPT was performed in all patients who participated in the study. NPT was positive in all patients in group 1 and negative in both control groups. No allergic reaction (urticaria, asthma, or anaphylaxis) was observed during NPT.

Discussion

In the present study, which was conducted with larger patient and control groups in comparison to our previous study [2], we showed once again that latex-specific IgE analysis using ImmunoCAP can yield a high false-positive rate (19.1%) among atopic patients with pollen sensitisation. When the atopic control and latex allergic groups were evaluated in terms of allergen sensitisation profile, the atopic control group exhibited more grass, weed, and tree pollen sensitisations and combined pollinosis, while no significant difference was observed regarding mite and other aeroallergen sensitisations. These findings confirm that pollen allergy is responsible for the clinically irrelevant latex-specific IgE positivity.

The proportion of patients positive for specific IgE to latex profilin (Table 1), birch pollen profilin, and pineapple bromelain (CCD) was remarkably higher in the atopic control group when compared to the latex-allergic group. It was an intriguing finding that latex profilin-specific IgE was positive in only 1 patient in the latex-allergic group while it was positive in 7 patients in the atopic control group, and all 8 of these patients were also positive for birch pollen profilin-specific IgE (Tables 2, 3). The aforementioned patient in the latex allergy group did not have pollen or food allergy, yet all 7 of the atopic control patients were sensitised to pollen (Tables 2, 3). These findings show that latex profilin (rHev b8) sensitisation develops primarily on the basis of latex allergy, but also develops secondarily through pollen profilin found as a panallergen in pollen, latex, and plant-based foods. The latex allergen (k82) used in the latex-specific IgE analysis was responsible for the high prevalence of false-positive specific IgE (19.1%) in the atopic patients sensitised to pollens since it contains sufficient amounts of profilin [3].

Like profilins, LTP is one of the important allergens in certain grass, weed, and tree pollens, and in latex (Hev b12). In the present study, LTP-specific IgE was positive in 1 patient in the latex-allergic group who did not have pollen allergy, while it was positive in 2 control patients with pollen allergy. LTP does not seem to be responsible

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for clinically irrelevant latex-specific IgE observed in pollen allergy without latex allergy. Additionally, it is unknown whether k82 contains Hev b12.

It is known that bromelain may lead to clinically irrelevant specific IgE positivity. In our study, bromelain-specific IgE positivity was highest (70.6%) in the atopic control group, and all of those patients were sensitised to pollen. However, only 2 patients (7.4%) in the latex-allergic group tested positive for bromelain-specific IgE. The fact that the prevalence of pollen sensitisation was high in the atopic control group suggests that the increased positive bromelain-specific IgE in this group was caused mostly by pollens.

In a study with similar patient and control groups conducted by Ebo et al. [4], specific IgE to latex, latex profilin (Hev b8), bromelain (CCD), and recombinant latex allergens (rHev b1, 3, 5, 6.01, 6.02, 9, 11) was assessed using ImmunoCAP, and basophil activation test with latex was performed. The study included 26 healthy subjects without allergy history, 22 latex-allergic patients with positive SPT and serum latex-specific IgE, and 20 latex-sensitive patients with no latex allergy history but positive latex-specific IgE and negative SPT to latex. Nineteen of the 20 latex-sensitive patients had positive specific IgE to a major grass allergen. More than half of the latex-allergic patients were positive for specific IgE to rHev b5, 6.01, and 6.02, whereas these ratios were reported as less than 10% in the latex-sensitive group. Positivity for rHev b8-specific IgE was reported in 5% of the latex-allergic patients versus 70% of the latex-sensitive group. Bromelain-specific IgE was positive in 13.6% of the latex-allergic group and 40% of the latex-sensitised group. The basophil activation test was negative in all cases in the latex-sensitive and control groups, though it was positive in more than 70% of the latex-allergic group [4].

In the study by Ebo et al. [4] and in the present study, rHev b8- and bromelain-specific IgE percentages were comparable in the latex-allergic groups (5 vs. 3.7% and 13.6 vs. 7.4%, respectively). In both studies, rHev b8- and bromelain-specific IgE were significantly higher in the control groups (atopic and latex-sensitive) in comparison to latex-allergic groups. However, in our atopic control group, the prevalence of rHev b8-specific IgE was lower (41.2 vs. 70%), while bromelain-specific IgE was higher (70.6 vs. 40%). In both studies, NPT was negative in the control groups. Our results demonstrated that all latex-allergic patients showed positive NPT.

Garnier et al. [5] analysed specific IgE against recombinant latex allergens (rHev b1–3, 5, 6.01, 6.02, 8, 9, 11) and bromelain using ImmunoCAP in 130 patients with positive latex-specific IgE, 97 with latex allergy history, and 33 with no history of latex allergy. Twenty-nine of the 33 patients with no history of latex allergy were allergic to pollen and 20 presented with a food allergy. In the latex-allergic group, the highest prevalence was observed for rHev b6.01 specific IgE (78%), followed by rHev b6.02 (71%), rHev b2 (68%), and rHev b5 (61%). Forty-three patients in this group were found to be allergic to latex in the absence of pollen allergy and only 1 of them (2.3%) had specific IgE against rHev b8. In contrast, 26 (78%) of the 33 patients without latex allergy were monosensitised. The other 7 patients exhibited negative IgE response to all recombinant latex allergens.

The prevalence of positive rHev b8-specific IgE among latex-allergic patients in our study was comparable to that reported by Garnier et al. [5] (2.3 vs. 3.7%). However, compared to their results, we determined a lower prevalence of rHev b8-specific IgE (41.2 vs. 78%) and a higher prevalence of bromelain-specific IgE (70.6 vs. 21.2%) in the atopic control group.

The two aforementioned studies and our study were carried out on the basis of similar patient and control groups and also employed the same method (ImmunoCAP) for specific IgE analysis. The most striking common feature among the latex-specific IgE-positive control groups was the high prevalence of pollen allergy. In all three studies, the prevalence of positive rHev b8- and bromelain-specific IgE response was higher in the pollen-allergic control groups compared to the latex-allergic groups. Furthermore, although the rHev b8- and bromelain-specific IgE rates were similar in the latex-allergic groups in all three studies, these rates differed in the latex-specific IgE-positive control groups. The differences observed in the control groups may be attributable to the high prevalence of pollen sensitisation or diversity of the pollen allergens. Detailed information on aeroallergen sensitisations was not provided in the other studies; however, the high prevalence of polysensitisation to pollens in the atopic control group was noteworthy in our study. Polysensitisation is prevalent in our region. rHev b8 was shown not to be a major allergen in the two studies discussed above. In the present study, we used the highly specific and sensitive NPT to demonstrate that specific IgE to latex and rHev b8 detected by ImmunoCAP was proved to be clinically irrelevant in pollen-allergic cases without latex allergy history. In our study, 16 out of 17 patients with positive latex-specific IgE in the atopic control group also showed positive results in at least one of the latex profilin-, birch profilin-, or bromelain-specific IgE tests, and latex profilin-specific IgE was positive in all

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7 patients with birch pollen profilin-specific IgE in this group (Table 3). These findings not only reveal that latex and pollen profilins are highly cross-reactive, but also elucidates the underlying reason for the higher prevalence of false-positive latex-specific IgE in pollen-allergic cases. Furthermore, unlike other studies, the present study demonstrated a 19.1% prevalence of false-positive latex-specific IgE in pollen-allergic cases (ImmunoCAP), and our results indicated that LTP is not responsible for clinically irrelevant latex-specific IgE in patients with pollen allergy.

In conclusion, false-positive latex-specific IgE in ImmunoCAP analysis may be observed in approximately 19% of patients with pollen allergy. Profilin and bromelain are primarily responsible for clinically irrelevant positive latex-specific IgE, whereas LTP does not seem to play a role. SPT remains valuable in the diagnosis of latex allergy due to its high sensitivity. Given the difficulty of implementing NPT in routine clinical practice, it may be beneficial to analyse specific IgE against rHev b5 and 6.

Disclosure Statement

None of the authors have financial or personal relationships which could result in a conflict of interest with regard to the presented article. No financial support was provided for this study.

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