

Serum immunoglobulin E reactivity to cross-reacting panallergen components in north-eastern Poland patients pollen sensitized

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ABSTRACT

Background: The presence of immunoglobulin E (IgE), which cross-reacts with allergen components, such as profilins, polcalcins, and cross-reacting carbohydrate determinants (CCD), creates a problem when selecting patients for allergen immunotherapy by using conventional methods. The aim of this study was to evaluate the prevalence of sensitization to profilins, polcalcins, and CCDs in patients with seasonal pollen allergic rhinitis.

Methods: The study was performed on a group of 112 patients with seasonal pollen allergic rhinitis, ages 14 to 55 years, with sensitization to at least one seasonal allergen (IgE > 0.7 kUA/L). The presence of IgE sensitization to recombinant (r) Bet v 2, rPhl p 12, rBet v 4, rPhl p 7, and CCDs, in addition to rBet v 1, rPhl p 1, rPhl p 5, was evaluated by using a multi-parameter immunoblot.

Results: Among the studied patients, 64.3, 80.4, and 41.1% were sensitized to birch, timothy grass, and mugwort pollen, respectively. Sensitization to profilins rBet v 2/Phl p 12 was demonstrated in 28.6%, to polcalcins Bet v 4/Phl p 7 in 8.9%, and to CCDs in 25%. In 29.3%, serum IgE reactivity to any of the cross-reactive components could be demonstrated. Serum IgE reactivity to rBet v 2 was always accompanied by IgE reactivity to rPhl p 12, and IgE reactivity to rBet v 4 was always accompanied by IgE reactivity to rPhl p 7. Among the patients with pollinosis co-sensitized to at least two allergen sources according to extract-based diagnosis, possible false-positive results due to sensitization to cross-reactive components were detected in 17.9%.

Conclusion: Evaluation of sensitization to cross-reacting components may be useful in evaluation of patients with pollen allergy who are being assessed for allergen immunotherapy to optimize the constitution of their immunotherapy vaccines.

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Demonstration of immunoglobulin E (IgE) specific to a clinically relevant allergen by skin testing or *in vitro* testing remains a key step in allergy diagnosis.¹ Allergen extracts currently used for diagnostics and therapy of allergic diseases are standardized chemically, immunologically, and biologically.^{2–4} However, extracts obtained from the same allergenic sources by individual

manufacturers may contain different amounts of distinct allergens and may differ in extract potency.^{5–7} This is particularly true for certain allergens whose concentration and composition are not routinely assessed in the allergenic extract.^{5,6} Many of these minor allergens, such as profilins or polcalcins, are characterized by high structural similarities with those derived from distantly related species and may cross-react on determination by a specific IgE (sIgE) assay.^{8–10} Due to their high cross-reactivity, these allergens are frequently called panallergens.¹⁰ Moreover, the posttranslational modification of individual allergens by glycosylation may create novel highly cross-reactive epitopes, called cross-reactive carbohydrate determinants (CCD).^{11,12}

Concentrations of individual panallergens are different in various allergen extracts, being dependent not only on the manufacturer but also on the allergen source.¹³ Sensitization to individual panallergens is different in different geographic regions. Panallergen cross-reactivity can result in a wider range of allergen sIgE responses to distinct allergen sources. This can produce positive results not due to the assessed allergens but instead due to cross-reacting panallergens,

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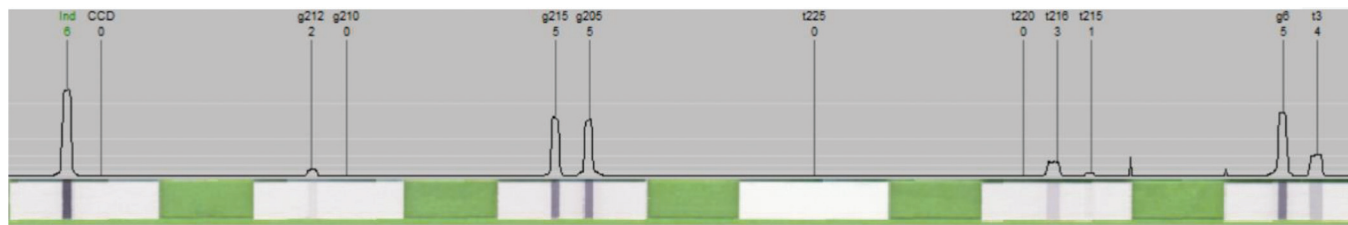


Figure 1. A representative result of immunoglobulin E (IgE) binding to individual allergen components in a patient with positive skin-prick test results to grass and birch pollen extracts possibly related to sensitization to profilins (Phl p 12 and Bet v 2): t3-birch pollen extract, t6-timothy pollen extract, t215- recombinant (r) Bet v 1, t216 – rBet v 2, t220 – r Bet v 4, t225 – rBet v 6, g205 – rPhl p 1, g215 – rPhl p 5, g210 – rPhl p 7, and g212 – rPhl p 12. CCD = Cross-reactive carbohydrate determinant; Ind = internal standard.

which complicates both diagnosis and treatment.¹⁰ Thus, sensitization to individual panallergens may adversely impact the diagnosis of pollen allergy and selection of allergens for immunotherapy by giving the appearance of specific allergen sensitization, whereas, in fact, there is IgE reactivity to a nonspecific panallergen.^{14–16} This study assessed the frequency of sensitization to cross-reacting allergens, including birch and timothy pollen profilins (Bet v 2 and Phl p 12), polcalcins (Bet v 4 and Phl p 7), and CCDs in a cohort of patients with seasonal allergic rhinitis who were sensitized to pollens who had been qualified for allergen immunotherapy based on routine extract-based diagnostic methods.

METHODS

A total of 112 patients with patients, ages 14–55 years, with moderate-to-severe seasonal allergic rhinitis referred to the allergy clinic of the Medical University of Bialystok due to at least 2 years of rhinitis and/or conjunctivitis symptoms related to exposure to pollens were included in this study. All the patients had positive skin-prick test results for at least one pollen allergen extract and elevated serum concentration of allergen sIgE (≥ 0.7 kU/L). A patient was considered to be sensitized to an individual allergen source when a positive skin-prick test result and sIgE level ≥ 0.7 kU/L to a given allergen extract were demonstrated. Patients who had been treated with allergen immunotherapy were excluded from the study. All the subjects signed an informed consent. The study was approved by the local ethics committee.

Skin-Prick Testing

All the persons were skin tested by using prick methodology with a screening panel of aeroallergens (Allergopharma, Reinbek, Germany), including the following allergen extracts: (1) *Dermatophagoides pteronyssinus*, (2) *Dermatophagoides farinae*, (3) birch pollen, (4) alder pollen, (5) hazel tree pollen, (6) grass mix (*Holcus lanatus*, *Dactylus glomerata*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *Festuca pratensis*), (7) mugwort

pollen, (8) cat allergen, (9) dog allergen, (10) *Alternaria tenuis*, and (11) *Cladosporium herbarum*. After 15 minutes, the longest wheal size was measured. Positive skin-prick test results were defined as wheal diameter larger than that of histamine control with a reaction to histamine of at least 3 mm.

Immune Assays

Concentrations of serum total IgE and allergen sIgE (sIgE) with extracts of birch pollen (T3), timothy grass pollen (G6), mugwort pollen (W6), *Alternaria* (M6), *D. pteronyssinus* (D1), *D. farinae* (D2), cat (E1), and dog (E5) were evaluated by using UniCap (Thermo Fisher, Uppsala, Sweden). The concentration of serum sIgE to individual allergen components was evaluated by using a multiparameter immunoblot Euroline DPA-Dx Pollen 1 DP 3210-1601-1-E (Euroimmun AG, Luebeck, Germany) according to the manufacturer's instructions. The following allergens were included: t215- recombinant (r) Bet v 1, t216 – rBet v 2, t220 – rBet v 4, t225 – rBet v 6, g205 – rPhl p 1, g215 – rPhl p 5, g210 – rPhl p 7, g212 – rPhl p 12, CCDs, timothy grass pollen (G6), and birch pollen (T3) (Fig. 1).

Statistics

Data were analyzed by using Statistica software (StatSoft, Tibco Software Inc., Palo Alto, CA). Values of $p < 0.05$ were considered to be significant. The χ^2 test was used to compare discrete parameters. IgE concentrations were logarithmically transformed for analysis. Regression analysis was used to determine the associations between sensitization to individual allergenic sources or allergen components.

RESULTS

Among the studied patients, 72 (64.3%) were sensitized to birch, 90 (80.4%) to timothy grass, and 47 (41.1%) to mugwort pollen. In addition, sensitization to *D. pteronyssinus* and/or *D. farinae* occurred in 42 patients (37.5%), to cat in 10 (8.9%), to dog in 8 (7.1%),

Table 1 Patient characteristics

Characteristic	Patients			
	All (n = 112)	Pro+ (n = 32)	Pol+ (n = 10)	CCD+ (n = 28)
Age range, y	14–55	15–55	15–52	17–55
Males, n (%)	66 (58.9)	20 (62.5)	6 (60)	19 (67.9)
T3 > 0.7 kUA/L	72 (64.3)	24 (75)	8 (80)	22 (78.6)
G6 > 0.7 kUA/L	90 (80.4)	31 (96.9)*	10 (100)*	26 (92.9)*
W6 > 0.7 kUA/L	47 (41.1)	31 (96.9)*	10 (100)*	25 (89.3)*
D1 > 0.7 kUA/L	42 (37.5)	10 (31.3)	3 (30)	7 (25)
E1 > 0.7 kUA/L	10 (8.9)	2 (6.3)	1 (10)	3 (10.7)
E5 > 0.7 kUA/L	8 (7.1)	2 (6.3)	1 (10)	3 (10.7)
M2 > 0.7 kUA/L	2 (1.8)	0	1 (10)	1 (3.6)
M6 > 0.6 kUA/L	14 (12.5)	3 (9.4)	1 (10)	2 (7.1)
T3 > 0.7 kUA/L and G6 > 0.7 kUA/L and W6 > 0.7 kUA/L	34 (30.4)	22 (68.8)*	8 (80)*	17 (60.7)*
T3 > 0.7 kUA/L and G6 > 0.7 kUA/L	25 (22.3)	1 (3.1)	0	3 (10.7)
T3 > 0.7 kUA/L and W6 > 0.7 kUA/L	5 (4.5)	1 (3.1)	0	2 (7.1)
G6 > 0.7 kUA/L and W6 > 0.7 kUA/L	9 (8)	8 (25)*	2 (20)*	6 (21.4)*
Phl p 1 and Phl p 5 > 0.7 kUA/L	66 (50)	24 (75)*	8 (80)*	20 (71.4)
Bet v 2 and Phl p 1 and Phl p 5 > 0.7 kUA/L	42 (37.5)	18 (56.3)*	6 (60)	14 (50)

pro+ = Sensitized to profilin; pol+ = sensitized to polcalcin; CCD+ = sensitized to cross-reactive carbohydrate determinant; T3 = birch pollen extract; G6 = timothy grass pollen extract; W6 = mugwort pollen extract; D1 = *Dermatophagoides pteronyssinus* extract; E1 = cat dander extract; E5 = dog dander extract; M2 = *Cladosporium herbarum* extract; M6 = *Alternaria alternata*.

*Significantly different.

Table 2 Frequency of IgE reactivity to individual cross-reactive allergens

	Bet v 2/Phl p 12, %	Bet v 4/ Phl p 7, %	CCD, %
0.35 ≥ IgE > 0.0 kUA/L	67.8	84.8	62.5
0.7 ≥ IgE > 0.35 kUA/L	5.4	6.3	12.5
3.5 ≥ IgE > 0.7 kUA/L	7.2	1.8	10.7
17.5 ≥ IgE > 3.5 kUA/L	6.3	0.9	7.1
50 ≥ IgE > 17.5 kUA/L	12.5	1.8	5.4
100 ≥ IgE > 50 kUA/L	2.8	4.4	1.8
IgE > 100 kUA/L	0	0	0
IgE > 0.7 kUA/L	26.8	8.9	25
IgE > 0.7 kUA/L		29.3	

CCD = Cross-reacting carbohydrate determinant; IgE = immunoglobulin E.

to *Alternaria* in 14 (12.5%), and to *Cladosporium* in 2 (1.8%) (Table 1). Most of the studied patients (63.4%) were sensitized to at least two pollen allergen sources as determined by skin-prick testing and UniCap tests. Sensitization to all three pollens was detected in 34 patients (30.4%), whereas sensitization to two pollen sources was detected in 39 patients (33%), including 25 patients (22.3%) sensitized to birch and timothy grass pollens, 9 patients (8%) sensitized to mugwort and timothy grass pollens, and 5 (4.5%) sensitized to mugwort

and birch pollens. Representative results of the immunoblots are presented in Fig. 1. Sensitization to rBet v 2/Phl p 12 was demonstrated in 26.8%, to Bet v 4/Phl p 7 in 8.9%, and to CCDs in 25% of the patients (Table 2). In 29.3% of the patients, serum IgE reactivity > 0.7 kUA/L to any one of the cross-reactive components could be demonstrated (Table 2).

Patients sensitized to profilins (pro+) were characterized by a different pattern of sensitization to individual pollens than patients not sensitized to profilins (pro–)

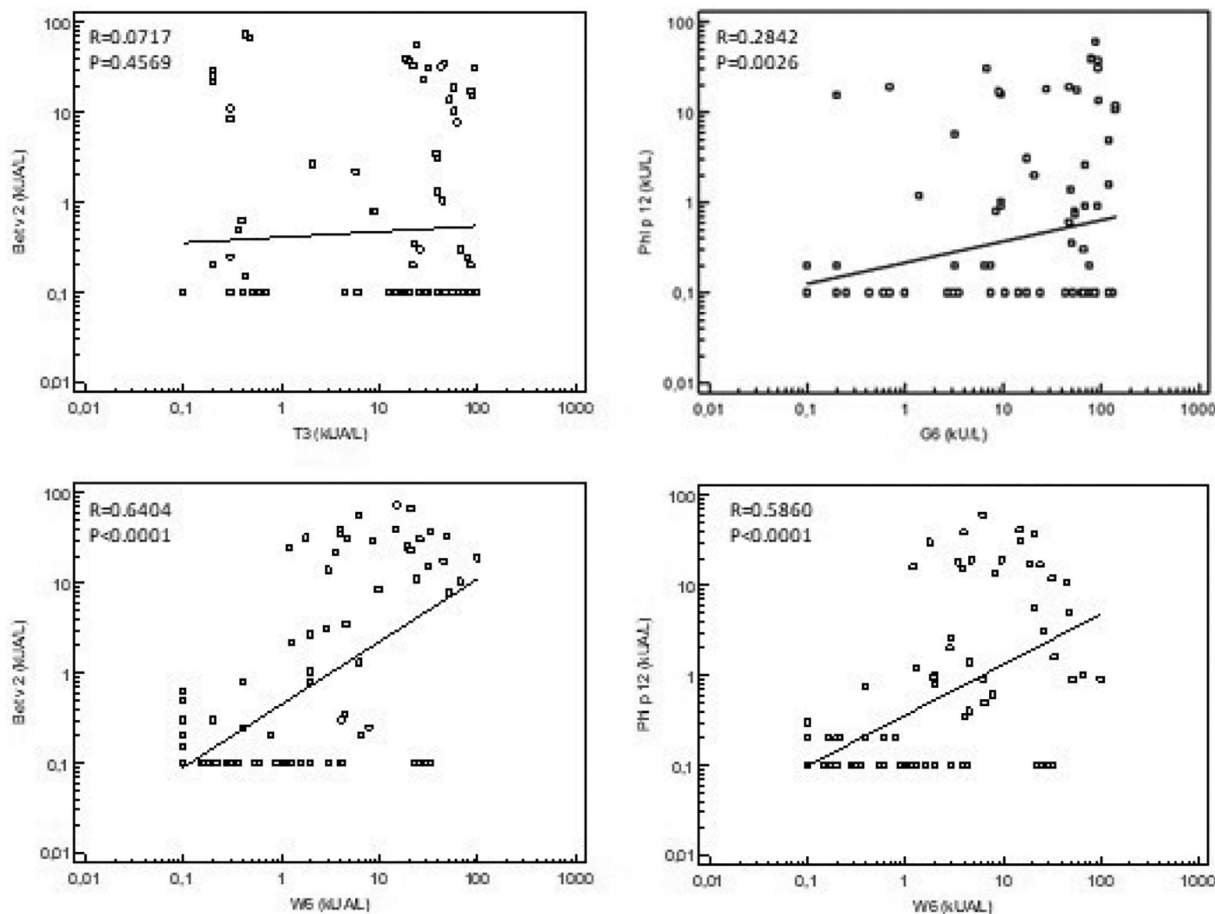


Figure 2. Correlation of serum immunoglobulin E (IgE) concentration to pollen extracts birch (T3), timothy grass (G6), mugwort (W6) with that to Bet v2 or Phl p 12.

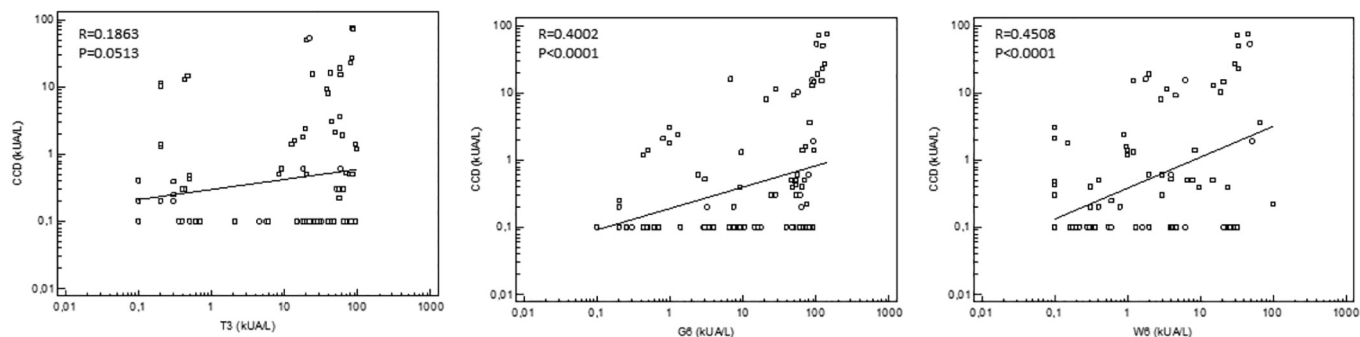


Figure 3. Correlation of serum immunoglobulin E (IgE) concentration to pollen extracts birch (T3), timothy grass (G6), mugwort (W6) with that to cross-reactive carbohydrate determinant (CCD).

(Fig. 2). Sensitization to mugwort was more frequently seen in patients who were pro+ (96.9%) than in patients who were pro- (20%) ($p < 0.0001$). Similarly, the frequency of sensitization to timothy grass pollen was more frequent in patients who were pro+ than in those who were pro- (96.9 versus 73.8%; $p = 0.004$).

However, sensitization to birch pollen was not significantly different in patients who were pro+ versus those who were pro- (75 versus 60%; $p = 0.19$). No patient was sensitized to a single pollen among the pro+ group, whereas, in the pro- group, monosensitization to birch, timothy grass, or mugwort pollen was

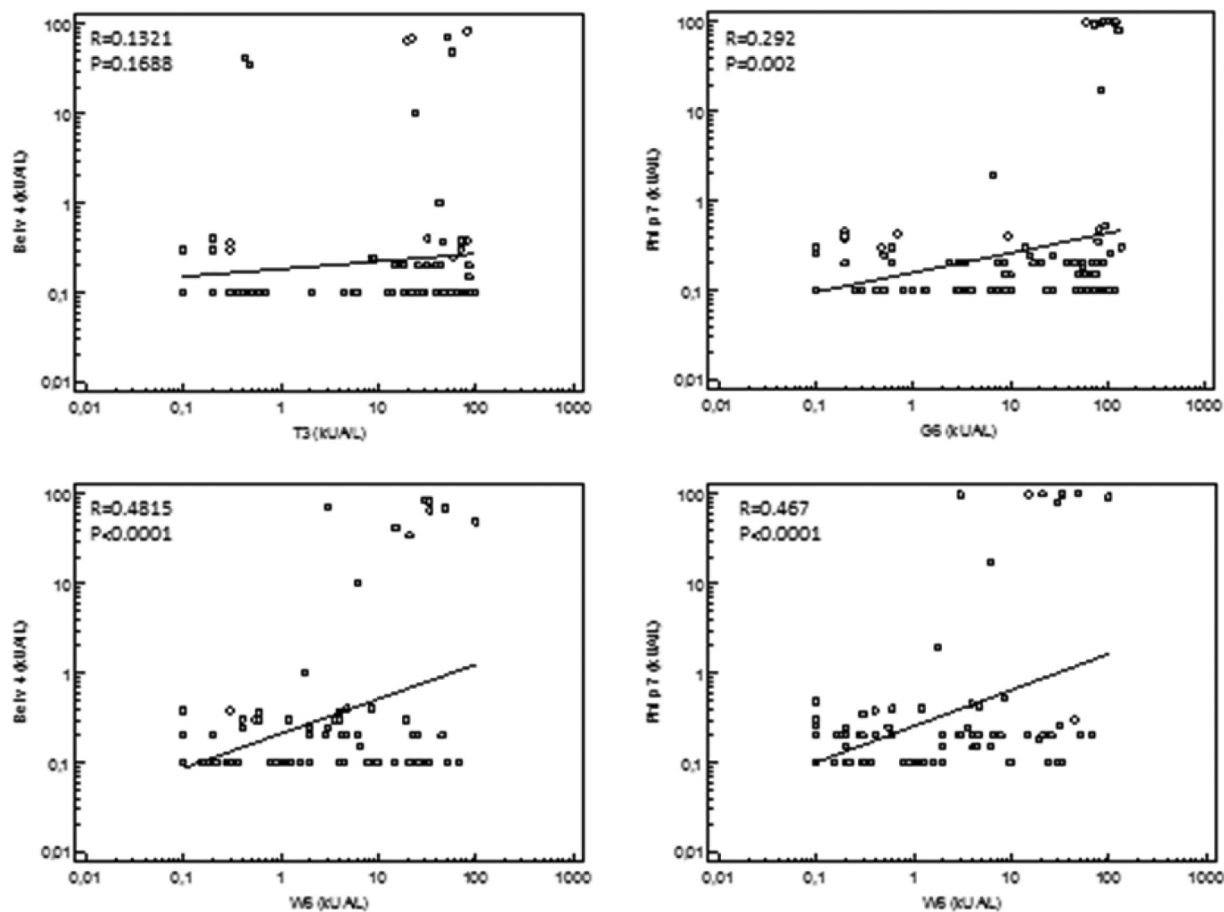


Figure 4. Correlation of serum immunoglobulin E (IgE) concentration to pollen extracts birch (T3), timothy grass (G6), mugwort (W6) with that to polcalcins Bet v 4 and Phl p 7.

Table 3 Frequency of co-sensitization to panallergens

	Profilin	CCD	Polcalcin	Both	Any
Profilin+, n (%) (n = 32)		16 (50)	8 (25)	6 (18.8)	18 (56.3)
CCD+, n (%) (n = 28)	16 (57.1)		8 (28.6)	6 (21.4)	18 (64.3)
Polcalcin+, n (%) (n = 10)	8 (80)	8 (80)		6 (60)	10 (100)

+ = Sensitized; CCD = cross-reacting carbohydrate determinant.

demonstrated in 37 patients (46.3%). Sensitization to all three pollens was more frequent in patients who were pro+ (68.8%) than in patients who were pro- (12.5%; $p < 0.0001$).

Among the patients sensitized to cross-reactive carbohydrate determinants (CCD+), the frequency of sensitization to mugwort pollen (89.3%) was significantly greater than in patients who were CCD- (26.2%; $p < 0.0001$) (Fig. 3). However, the frequency of sensitization to birch pollen did not differ significantly with CCD+ versus CCD- (71.4 versus 61.9%; $p = 0.5$), whereas sensitization frequency to timothy grass

pollen reached borderline significance related to CCD+ versus CCD- status (92.9 versus 76.2%; $p = 0.059$). All the patients sensitized to polcalcins (pol+) were characterized by significant serum IgE reactivity to timothy grass and mugwort pollens (100%), with 80% of these patients also sensitized to birch pollen (Fig 4).

In patients who were pro+, co-sensitization to CCD was seen in 16 patients (50%) and to polcalcins in 8 patients (25%), significantly greater than in patients who were pro- (15% [$p = 0.0002$] and 2.5% [$p = 0.0006$], respectively) (Table 3). Among patients who were CCD+, co-sensitization to profilins was

Table 4 Regression analysis of serum IgE concentration to individual allergen sources and individual panallergens

	Profilin		CCD		Polcalcins	
	R^2	p	R^2	p	R^2	p
Birch	0.005	0.46	0.035	0.051	0.017	0.177
Timothy grass	0.08	0.003	0.161	<0.001	0.076	0.004
Mugwort	0.425	<0.001	0.245	<0.001	0.236	0.001
<i>Dermatophagoides pteronyssinus</i>	0.002	0.608	0.001	0.95	0.01	0.295
Cat	0.004	0.54	0.01	0.288	0.013	0.244
Dog	0.0001	0.94	0.036	0.051	0.035	0.06
<i>Cladosporium</i>	0.0008	0.77	0.03	0.058	0.03	0.07
<i>Alternaria</i>	0.002	0.628	0.001	0.95	0.03	0.07

Significant association is highlighted in bold.

detected in 57.1% of the patients and to polcalcins in 8 (28.6%), significantly greater than in patients who were CCD- (19% [$p=0.0007$] and 2.4% [$p=0.0003$], respectively). Among patients who were pol+, co-sensitization to both profilins and CCD was seen in 80% of patients, being significantly greater than in patients who were not sensitized pol- (23.5 [$p<0.0001$] and 2.0% [$p<0.0001$], respectively).

When sIgE responses to individual major allergens of birch and grass pollens were analyzed, patients who were pro+ were characterized by more frequent sIgE responses to all three allergens, Bet v 1, Phl p 1, and Phl p 5, than patients who were pro- (56.3 versus 30%; $p=0.017$) (Table 1). Similarly, in patients who were pro+ more frequently demonstrated sIgE responses to both Phl p 1 and Phl p 5 was seen than in patients who were pro- (75 versus 52.5%; $p=0.034$) (Table 1). No significant difference in frequency of sIgE responses to either the three major allergens, Bet v 1, Phl p 1, and Phl p 5, or the two major timothy allergens could be demonstrated in patients who were pol+ versus pol- or CCD+ versus CCD- (Table 1).

The intensity of sIgE reactivity to individual panallergens varied. A sIgE level of >17.5 kUA/L was detected in 57.1% of those sensitized to profilins, 69.7% of those sensitized to polcalcins but only 28.8% of those sensitized to CCD (Table 3). Serum sIgE reactivity to rBet v 2 was always accompanied by serum sIgE reactivity to rPhl p 12, and serum sIgE reactivity to rBet v 4 was always accompanied by reactivity to rPhl p 7 (Online Supplemental Fig. S1). Regression analysis indicated that mugwort and timothy grass pollen IgE concentrations are predictors of sensitization to profilins ($R^2 = 0.425$, $p<0.001$; and $R^2 = 0.08$, $p=0.003$, respectively), CCD ($R^2 = 0.245$, $p<0.001$; and $R^2 = 0.161$, $p<0.001$, respectively) and polcalcins ($R^2 = 0.236$, $p<0.001$; and $R^2 = 0.076$, $p=0.004$, respectively). No association between sensitization to birch pollen, *D. pteronyssinus*,

cat, dog, *Cladosporium*, or *Alternaria*, and sIgE reactivity to the panallergens could be demonstrated (Table 4). Multiple regression analysis confirmed the results, which indicate that sensitization to mugwort pollen is the best predictor of sensitization to panallergens among patients with seasonal allergic rhinitis.

Analysis of serum concentration of IgE to individual allergen components allowed for detection of possible false-positive results of extract-based diagnosis in 20 patients (17.9%) (Online Supplemental Table S1). In 12 of 72 patients (16.7%) sensitized to birch pollen according to the extract-based testing, no sensitization to a major birch pollen allergen Bet v 1 could be demonstrated. All these patients were also co-sensitized to grass and mugwort pollen, and had elevated IgE concentration to profilins Bet v 2/Phl p 12 (Online Supplemental Table S1). In addition, in these patients, concomitant sensitization to polcalcins Bet v 4/Phl p 7 and CCDs could be demonstrated in two patients (18.2%) and four patients (36.4%), respectively. In 8 of 90 patients (8.9%) sensitized to grass pollen according to extract-based testing, no sensitization to either timothy pollen major allergen (Phl p 1, Phl p 5) could be demonstrated (Online Supplemental Table S1). In these patients, sensitization to profilins Bet v 2/Phl p 12, polcalcins Bet v 4/Phl p 7, and CCDs was demonstrated in three patients (37.5%), zero patients (0%), and four patients (50%), respectively.

In addition, in these patients concomitant sensitization to profilins Bet v 2/Phl p 12 and CCDs was demonstrated in one patient (12.5%). All these patients were also co-sensitized to birch pollen but co-sensitization to mugwort pollen was only seen in those sensitized to profilins Bet v 2/Phl p 12. In summary, among patients with pollinosis co-sensitized to at least two allergen sources according to extract-based diagnosis possible false-positive results due to sensitization to cross-reactive components were detected in 17.9%. In these patients,

sensitization to cross-reactive components was responsible for positive responses to extract-based test results.

DISCUSSION

In the current study, an analysis was presented of the multiparameter allergen sIgE immunoblot results, which focused on cross-reacting molecules related to pollen allergy. Patients selected for this study represented a population of candidates for allergen immunotherapy due to seasonal allergic rhinitis caused by plant pollens. The frequency of sensitization to individual pollens is similar to that described in other populations, with grass pollen sensitization being the most prevalent, followed by tree and weed pollens.^{17,18} Moreover, the majority of patients with allergy were sensitized to at least two allergen sources, which is consistent with observations made by other investigators.^{17,18} It has already been demonstrated that, in patients with seasonal allergic rhinitis, sensitization to panallergens can be frequently demonstrated.¹⁹

In the current study, the focus of the assessment of cross-sensitization was the common cross-reacting allergens: profilins, which are present in all eukaryotes; polcalcins, which are present in all pollens; and CCDs, which are present on glycoproteins in plants and invertebrates.¹⁰ Sensitization to such allergens has been reported in 10 to 30% of patients in various populations of patients with seasonal pollen allergy.^{15,17,19} However, in certain populations, >30% of adults with pollen allergy are sensitized to these proteins.^{20,21} This concurs with the results of this study, wherein the presence of sensitization to cross-reacting allergens occurred in approximately 30% of patients with seasonal allergic rhinitis.

Several factors have been reported to affect the rate of sensitization to cross-reacting allergens,²²⁻²⁴ including patient's age, geographic region, poly- versus monosensitization, sensitization to particular allergen sources, and the presence or absence of food allergy.²²⁻²⁴ Our study evaluated a population of older adolescents and younger adults. In these study patients, a higher frequency of sensitization to panallergens was seen than has been reported in younger adolescents and children.²² A longitudinal study of molecular spreading of sensitization to individual allergens in patients with timothy grass allergy demonstrated that the IgE response to profilins and polcalcins appear late, usually after sensitization to major allergens, such as Phl p 1 and Phl p 5, is well developed.²⁴

Moreover, sensitization to a greater number of allergens, including polcalcins and profilins, was demonstrated in those with a longer duration of allergic rhinitis.²⁴ Our study demonstrated an association of sensitization to all three cross-reacting allergens with sensitization to mugwort and grass pollen. However, only sensitization to profilins was detected more

frequently in those with a more-expanded IgE response to timothy grass major allergens. Interestingly, there was no association demonstrated between sensitization to birch pollen and cross-reacting allergens. Other studies performed in Europe and the United States support these observations.^{21,23} In central Europe, the reported frequency of sensitization to profilins among patients with birch allergy was <15%.²⁵ This is clearly less than the sensitization to profilins seen in patients with allergic rhinitis due to grass or mugwort pollen allergy.^{21,23} These observations support the findings in this study, which link panallergen sensitization, predominantly with grass and mugwort pollen allergy.

Problems in making a clear demonstration of the culprit panallergen is caused by the fact that sensitization to profilins, polcalcins, or CCDs rarely leads to clinical symptoms.¹⁰ However, recent reports demonstrate that, in specific populations, panallergen sensitization may produce reactions, sometimes even systemic reactions.²⁶ Moreover, differences in content and stability of cross-reacting allergens in individual allergen sources may determine the likelihood and type of allergic reaction in patients sensitized to those panallergen proteins.^{9,13} Therefore, in some populations, a diagnosis of sensitization to panallergens should result in appropriate avoidance measures to prevent allergic reactions produced by pollens or foods that contain these cross-reacting proteins.

In clinical practice, the presence of sensitization to cross-reacting allergens can significantly affect the diagnostic process. It may produce positive results to commonly used allergen extracts in the absence of sensitization to major allergens.^{17,21} This, in turn, can jeopardize the decision-making about the choice of allergen extracts for immunotherapy. In fact, it has already been demonstrated that, in some populations, evaluation of sensitization to cross-reacting allergens can affect the decision-making process while selecting appropriate immunotherapy vaccines in patients with pollen allergy.¹⁷ In some populations, in patients with polysensitized pollen allergy, the level of concordance between the skin-prick testing and IgE concentration determination was low, especially in patients sensitized to profilins and polcalcins.²⁷ The association between sensitization to cross-reacting allergens and polysensitization has also been demonstrated in other populations in which most sensitization to cross-reacting allergens was found among patients who are polysensitized.^{21,28}

This is also consistent with the current study, which indicates that sensitization to profilins, polcalcins, and CCDs was seen exclusively in patients with polysensitized seasonal allergic rhinitis, which reinforces a possible role of co-sensitization to cross-reactive allergens in diagnostic assessment of patients who are polysensitized. Moreover, cross-reacting allergen-dependent "false-positive" IgE test results to other allergen sources

have been reported.²⁹ In the case of latex sIgE in ImmunoCAP analysis, false-positive results may be observed in approximately 19% of patients with pollen allergy.²⁹ Profilins and CCDs were shown to be the main contributors to clinically irrelevant positive latex sIgE results.²⁹

Similarly, in our study, possible false-positive skin-prick test results with birch and grass pollen extracts were mainly associated with elevated serum IgE to profilins and CCDs. In almost 18% of patients with pollinosis, the application of component-resolved diagnostics affected the selection of allergen vaccine composition for immunotherapy. In each of these patients, the extract-based allergy diagnosis to one of the pollen sources could not be supported by the presence of IgE against major allergen components. This, in turn, argues against inclusion of those allergenic extracts in the preparation of vaccines for allergen immunotherapy.

Unfortunately, in this study, we included only a number of allergen components of birch and timothy grass pollens. Inclusion of a broader number of molecules, including major mugwort allergens would help in the detection of more false-positive results of extract-based diagnostic methods in a population of patients with pollinosis. Nonetheless, even application of a limited number of allergen components in evaluation of patients with polysensitized pollinosis can result in substantial improvements of the diagnostic process. Our observations were consistent with other studies, which indicates that component-resolved diagnosis helps in the selection of patients who are truly sensitized to a given allergen source.³⁰

In north-eastern Poland, birch, grass, and mugwort pollen seasons follow each other, but they typically do not overlap (Online Supplemental Fig. S2). It, therefore, seems highly unlikely that, in an individual patient who develops allergy to a given allergen source, concomitant co-exposure to panallergens derived from other allergen sources cooperates in the sensitization process. The observed associations of sensitization to an individual panallergen with that to an individual allergen source suggests that it is truly exposure to a given allergen source that participates in a mounting IgE response to cross-reactive allergens. Sensitization to panallergens does not seem to be a simple derivative of intensity of pollen exposure.

In north-eastern Poland, birch pollen counts reach much higher values than that of grass pollen or mugwort pollen (Online Supplemental Fig. S2). It is possible that the amount of panallergens and/or their availability in a given pollen may be responsible for the sensitization process. In fact, differences in the amount of some panallergens in birch and grass pollens have already been demonstrated.¹³ It is possible that sequential exposure to cross-reacting allergens present in tree, grass, and weed pollens are necessary

for development of sensitization to panallergens. The latter hypothesis is more likely because all patients sensitized to panallergens were also sensitized to at least two allergen sources in this study, and, in this region of Poland, mugwort pollen counts are usually the lowest when compared with grass or birch pollen levels. The mechanisms that lead to sensitization to panallergens requires further investigation.

Our study was restricted to only some of the cross-reactive allergens. Others, such as lipid transfer proteins (LTP) may play a role in producing false-positive results due to the presence of cross-reacting IgE.^{31–35} Moreover, LTPs, which are more stable than profilins, may be responsible for triggering anaphylaxis in patients who are pollen sensitized after ingestion of foods that contain cross-reactive LTPs.³⁴ Sensitization to LTPs is particularly frequent in Mediterranean countries where foods, *e.g.*, peaches, are the primary sensitizers.^{31–33} In the areas where birch is a dominate pollen allergen, sensitization to LTP is much lower.³¹

Analysis of amino-acid identity and similarity indicates that IgE cross-reactivity between individual LTPs is less likely than that among profilins.⁸ In fact, in 27% of Italian patients who were LTP sensitized, IgE reactivity to a single LTP was demonstrated, whereas IgE binding to all seven studied LTPs was found in only 15% of the patients.³³ Serum IgE reactivity to a mugwort pollen LTP Art v 3 was detected in approximately 50% of the patients with a commercial mugwort extract in some patients only able to inhibit 50% of IgE binding to other LTPs, *e.g.*, Pru p 3.³³ An increasing body of evidence indicates that LTPs are important allergens that trigger both respiratory and gastrointestinal symptoms.³³ Due to their low amino-acid sequence identity, several LTPs should be included in a component-resolved diagnosis.^{8,33}

CONCLUSION

The current study demonstrated that sensitization to cross-reacting allergens is frequent in patients with seasonal allergic rhinitis who, on routine clinical evaluation, would qualify for allergen immunotherapy. Assessment of sensitization to individual allergen components may help in the selection appropriate composition of allergen immunotherapy vaccines to avoid inclusion of allergen in the immunotherapy, which are only positive on a skin test or allergen sIgE test due to the presence of sIgE directed toward a panallergen. This study supported the use of component resolved allergy diagnostics for assessment of appropriate allergens to include in allergen vaccines for patients being considered for allergen immunotherapy.

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