

Birch pollen-associated peanut allergies in children

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Abstract Panallergens show structural similarities, and they are responsible for many cross-reactions between pollen and plant food sources. The aim of the present study was to investigate IgE reactivity to peanut allergen components in children with birch pollen allergy. Patients experienced symptoms of allergic asthma, allergic rhinitis, and urticaria, and they underwent a complete diagnostic evaluation, including skin prick test (SPT), specific IgE (sIgE) to birch pollen allergen (t3), peanut allergen (f13). In addition, measurement of sIgE to the major birch allergen components, *Betula verrucosa* (Bet v1, Bet v2), and to peanut allergen components, *Arachis hypogaea* (genuine components: Ara h1, Ara h2, Ara h3, and cross-reactive Ara h8) was performed, by using a microarray technique (component resolved diagnosis, CRD). SPT to birch extract was positive in all children, and SPT to peanut extract was positive in 51 % of them. sIgE to both allergens was increased in 39 % of children, 55 % of them had increased sIgE (t3), and one child had increased sIgE (f13). CRD results confirmed that some children were sensitized to

Bet v1 only, and some children to genuine Ara h only. Bet v1/Ara h8 cross-reactivity was found in 16 % of children. Results of the present study reveal that SPT, sIgE, and CRD may detect sensitization and co-sensitization with birch and peanut allergens/allergen components, and CRD may help to differentiate sensitization to genuine peanut components from sensitization to peanut cross-reactive component in birch-sensitive children. Diagnostic approach has to be individualized for each patient.

Keywords Allergy · Birch · IgE · Component resolved diagnosis · Microarray · Peanut

1 Introduction

Pollen allergy is a common disease caused by hypersensitivity to pollen allergens. In Europe, early springtime allergies are commonly caused by pollen from the family *Betulaceae*, that is, *Alnus* (alder), *Betula* (birch) and *Corylus* (hazel) (D'Amato and Spieksma 1992). In Croatia, birch was found to disseminate the highest pollen load from February to May (Peternel et al. 2007). During pollen season, the onset of symptoms in sensitized patients depends on the allergenic pollen grain counts.

As panallergens show structural similarities, they are responsible for many IgE-mediated cross-reactions between pollen and plant food sources (Hauser et al. 2010). Birch-sensitive patients may react to fruits

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(e.g., nuts, cherry, apple, peach), due to cross-reactivity among birch and related plant proteins (cluster proteins). IgE-antibodies specific for the major birch allergen Bet v1 are known to cross-react with homologous proteins in these foods (Bohle 2007). Bet v1 is homologous to the group of pathogenesis-related proteins (PR-10 proteins) (Mogensen et al. 2002).

Profilins, the most abundant vegetal panallergens, rarely cause clinical reactions in sensitized persons, and they are often milder, urticaria with or without angioedema (Valenta et al. 1992). In birch, the representative of profilin is Bet v2, which is recognized by 10–40 % of allergic patients (Móvérare et al. 2002).

Allergy to common food allergens in childhood (e.g., cow's milk, egg, soybean, and wheat proteins) appears to outgrow it up to 10 years of life (Wood 2003). In contrast to these food allergies, peanut allergy is usually lifelong. Only 20 % of individuals may outgrow peanut allergy (Hourihane et al. 1998). Peanuts (*Arachis hypogaea*) are a major cause of severe IgE-mediated allergic reactions to food allergens in children. Peanuts belong to the plant family Fabaceae. Eleven peanut allergens, that is, Ara h1 to Ara h11, have been identified until now (Nicolau and Custovic 2011). Peanut major allergens, that is, Ara h1 (vicilin), Ara h2 (conglutin), and Ara h3 (glycinin), are the most potent allergens (Palmer and Burks 2006). These allergens are risk markers for severe allergic reaction (Asarnoj et al. 2010). Ara h8 belongs to PR-10 proteins, and it is cross-reactive allergen to the homologous Bet v1 allergen (Mittag et al. 2004).

Diagnosis of peanut allergy relies on a careful history and diagnostic in vivo and in vitro tests, that is, skin prick test (SPT), serum-specific IgE measurement, and oral food challenges (OFC) as the gold standard for food allergy diagnosis. Both SPT and specific IgE tests to the whole allergen extracts have low specificity and low positive predictive value, since some sensitized individuals may be tolerant to peanut ingestion (Wainstein et al. 2007; Nicolaou and Custovic 2011). Development of microarray technology in allergy diagnosis, that is, component resolved diagnosis (CRD) offered significant progress as promising tool for simultaneous measurement of specific IgE against multiple purified natural or recombinant allergen components. Using CRD, disease-eliciting molecules can be identified, and detailed analysis of a

sensitization profil can be made (Nicolau and Custovic 2011). In addition, CRD make it possible to study cross-reactions between allergenic components with similar structures present in food and plant pollen allergenic molecules.

The aim of the present study was to investigate IgE reactivity to peanut allergen components in children with birch pollen sensitization, by using a microarray technique.

2 Materials and methods

2.1 Patients

The study included 31 patients from outpatient pediatric allergy clinic, aged 1–18 years ($\bar{x} \pm SD = 9 \pm 6$ years), referred for allergy work-up during the months of October and November, 2011. Diagnosis of birch allergy was made based on clinical symptoms in birch season (March–April) and positive birch sensitization by SPT and/or sIgE.

The mothers answered a standardized questionnaire about clinical manifestations of their children. Patients experienced symptoms of allergic asthma (wheezing, coughing, especially at night, shortness of breath, chest tightness), allergic rhinitis (rhinorrhea, post-nasal drip, nasal congestion, itching, sneezing), and urticaria (red, raised, itchy hives). Selected children underwent a complete diagnostic evaluation: asthma was diagnosed based on GINA guidelines (GINA 2009) and PRACTALL consensus report (Bacharier et al. 2008), allergic rhinitis was diagnosed according to the Clinical Practice Guidelines (Bousquet et al. 2003), and urticaria on EAACI/GA(2)LEN/EDF/WAO guidelines (Zuberbier et al. 2009). The mothers could not identify the cause of urticaria. All patients had positive skin prick test to *Betula verrucosa* allergen, increased serum total IgE (Dodig et al. 2006). Children with peanut-sIgE ≥ 15 kUA/L and/or SPT ≥ 8 mm and positive one or more of Ara h1, Ara h2, and Ara h3 were considered allergic (Nicolau et al. 2010). Standard work-up included history, physical examination, skin prick test (SPT), determination of total (tIgE), and specific IgE (sIgE). In addition, allergen component analysis (i.e., CRD) was performed. At the time of testing, children were without antihistamine therapy, while inhaled corticosteroids (ICS) in asthma patients were continued.

Diagnostic work-up was performed in line with Declaration on Human Rights from Helsinki 1975 and Seoul amendments 2008.

2.2 Methods

2.2.1 Skin prick test

Sensitization was tested by SPT with allergen extract from pollen and fruit/nuts (Alyostal[®] Stallergenes, France) according to the European Academy of Allergy and Clinical Immunology (EAACI) guidelines (Clark and Ewan 2003). The tests have been considered positive if mean wheal diameter was ≥ 3 mm compared with negative control.

2.2.2 Specific IgE

According to aeroallergens and food positive SPT results, the concentration of sIgEs to birch (t3) and peanut (f13) allergens was determined by standardized UniCAP fluoroimmunoassay method (Phadia AB, Uppsala, Sweden) on a selective UniCAP 100 auto-analyzer (Phadia, Uppsala, Sweden), using reagents from the same manufacturer (Paganelli et al. 1998). The confidence interval for sIgE is from 0.35 to 100 kIU_A/L.

2.2.3 Allergen component analysis

Sensitization profiles of birch major allergens Bet v1 and Bet v2, genuine peanut allergens (Ara h1, Ara h2, Ara h3), as well as cross-reactive allergens in peanut (Ara h8), have been analyzed by CRD, that is, by microarray technique ImmunoCAP ISAC[®] method (Phadia, Uppsala, Sweden). Allergen components were immobilized on a solid substrate in a microarray format. Binding of sIgE to allergen components was detected by the addition of a fluorescence-labeled anti-IgE antibody. The procedure was followed by image acquisition using an appropriate microarray scanner. The results were analyzed with proprietary software (Microarray Image Analysis Software, MIA). IgE internal calibration was made against an in-house reference serum, which has been standardized against ImmunoCAP. Subsequently, CRD results expressed as ISAC Standardized Units (ISU) are indirectly linked to the WHO 2nd IRP 75/502 IgE. Each allergen component is bound to the solid phase in triplicate, to ensure

reproducibility of the test. The within run coefficient of variation (CV) was estimated to 15 %, and the total CV was estimated to 25 %. The concentrations of allergen components-IgE were expressed as arbitrary units, that is, ISAC Standardized Units, ISU. The confidence interval is from 0.3 to 100 ISU. Interpretation of the results: <0.3 ISU = undetectable or very low; ≥ 0.3 to <1 ISU = low; ≥ 1 to <15 ISU = moderate to high; ≥ 15 ISU = very high.

2.3 Statistics

Data were stored and prepared for statistical analysis by use of the Microsoft Office Excel 2000 software (Microsoft, USA). On data processing, MedCalc (Medisoftware Mariakerke, Belgium) was used. The variables with normal distribution (age) were described by arithmetic mean and standard deviation ($\bar{x} \pm SD$), and those not showing normal distribution were presented by median (M) and interquartile range (IQR). Concordance between SPT, sIgE, and CRD was tested using interrater agreement. Agreement was quantified by kappa (κ) coefficient of agreement with κ values from 0.81 to 1.00 indicating very good concordance; 0.61–0.80, good concordance; 0.41–0.60, moderate concordance; 0.21–0.40, fair concordance; and, 0.20, poor concordance. The values $p < 0.05$ were considered statistically significant. (Marusteri and Bacarea 2010).

3 Results

Table 1 summarizes the results of the main findings of studied subjects. Clinical diagnoses included asthma and/or rhinitis and/or urticaria (14/31 = 45 %) and isolated asthma (4/31 = 13 %), rhinitis (9/31 = 29 %), and urticaria (4/31 = 13 %), respectively. SPT to birch extract was positive in all children, and in 51 % of them (16/31), SPT was positive to both, birch and peanut extracts. In two patients (No 15, 28), mean wheal diameters for birch allergen extract were 8 mm, in others it ranged 3–5 mm. For peanut allergen extract, in one patient only, mean wheal diameter was 8 mm (No 15), while in other patients it ranged 3–5 mm.

Specific IgEs to both allergens were positive in 39 % of children (12/31). Seventeen children (17/31 = 55 %) had positive sIgE to birch allergen t3 only,

Table 1 Results of skin prick test (SPT), specific IgE (sIgE), and component resolved diagnostics (CRD) in children

	Age	Symptoms	SPT positive	t3 (kIU _A /L)	f13 (kIU _A /L)	Bet v1 (ISU)	Bet v2 (ISU)	Ara h1 (ISU)	Ara h2 (ISU)	Ara h3 (ISU)	Ara h8 (ISU)
1	6	A, U	Birch, peanut	7.34	11.70	3.3	<0.3	<0.3	15.0	<0.3	<0.3
2	5	A, U	Birch, peanut	7.70	66.90	<0.3	3.1	5.8	9.2	<0.3	<0.3
3	18	A	Birch, peanut	2.10	3.63	<0.3	6.4	<0.3	<0.3	<0.3	<0.3
4	1	R	Birch, peanut	0.70	0.80	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
5	1	U	Birch, peanut	<0.35	<0.35	6.2	<0.3	<0.3	<0.3	<0.3	<0.3
6	17	R, U	Birch, peanut	4.50	0.40	9.4	<0.3	<0.3	<0.3	<0.3	<0.3
7	17	R, A	Birch, peanut	15.00	2.35	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
8	5	R, A	Birch, peanut	6.80	<0.35	11.0	<0.3	25.0	6.6	28.0	<0.3
9	18	U	Birch, peanut	0.70	<0.35	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
10	13	R, A	Birch, peanut	1.50	3.40	4.3	<0.3	<0.3	<0.3	<0.3	3.0
11	7	U	Birch, peanut	0.35	100.0	<0.3	0.4	1.0	5.9	3.2	<0.3
12	13	R, U	Birch, peanut	3.14	1.80	43.0	<0.3	<0.3	<0.3	<0.3	6.1
13	7	R	Birch, peanut	17.90	<0.35	5.8	<0.3	<0.3	<0.3	<0.3	0.8
14	8	A	Birch, peanut	<0.35	16.70	6.2	<0.3	4.5	1.8	10.0	<0.3
15	4	R	Birch, peanut	91.30	31.20	46.0	<0.3	<0.3	<0.3	<0.3	24.0
16	5	R, U	Birch, peanut	0.54	<0.35	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
17	10	A	Birch	0.50	0.42	1.1	<0.3	<0.3	<0.3	<0.3	<0.3
18	14	R, A	Birch	1.19	<0.35	26.0	<0.3	<0.3	<0.3	<0.3	<0.3
19	14	R, U	Birch	0.73	<0.35	1.2	<0.3	<0.3	<0.3	<0.3	0.6
20	7	A	Birch	0.41	<0.35	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
21	2	R	Birch	2.86	<0.35	2.9	<0.3	<0.3	<0.3	<0.3	<0.3
22	3	R	Birch	0.60	<0.35	1.1	<0.3	<0.3	<0.3	<0.3	<0.3
23	1	R, U	Birch	1.60	<0.35	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
24	8	R	Birch	0.80	<0.35	3.1	<0.3	<0.3	<0.3	<0.3	<0.3
25	18	R, A	Birch	10.10	<0.35	6.5	<0.3	<0.3	<0.3	<0.3	<0.3
26	3	R, U	Birch	0.90	<0.35	3.4	0.4	0.5	<0.3	<0.3	4.2
27	12	R	Birch	16.82	<0.35	28.0	21.0	<0.3	<0.3	<0.3	<0.3
28	4	R, A, U	Birch	10.62	0.82	10.0	2.4	<0.3	<0.3	<0.3	<0.3
29	18	R	Birch	0.50	<0.35	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
30	18	U	Birch	2.30	<0.35	<0.3	<0.3	0.6	1.0	<0.3	<0.3
31	6	R	Birch	<0.35	<0.35	4.3	<0.3	<0.3	<0.3	<0.3	<0.3

Interpretation of symptoms: A asthma, R rhinitis, U urticaria

SPT skin prick test, sIgE specific IgE concentration, CRD component resolved diagnosis, sIgE: t3 birch allergen, f13 peanut allergen, CRD: Bet v1 birch allergen component; Ara h1, Ara h2, Ara h3, Ara h8 peanut allergen components

one child (No 14) had positive sIgE to peanut allergen f13, and in one child (No 5) sIgEs to these allergens were not confirmed. The median level of sIgE to peanut allergen (f13) [M(IQR)] = [0 (0–2.21) kIU_A/L] was statistically lower ($p = 0.005$) than to birch allergen (t3) [M(IQR)] = [1.6(0.56–7.61) kIU_A/L].

CRD results (Table 1) confirmed that eleven children (11/31 = 36 %) had positive Bet v1 (No 5, 6, 17, 18, 21, 22, 24, 25, 27, 28, 31), six children

(6/31 = 19 %) had positive Bet v2 (No 2, 3, 11, 26, 27, 28), three of them (3/31 = 10 %) had positive both Bet v1 and Bet v2 (No 26, 27, 28). Three children (3/31 = 10 %) had positive Bet v1/genuine Ara h (No 1, 8, 14), two children (2/31 = 6 %) had positive genuine Bet v2/Ara h (No 2, 11), one child (1/31 = 1 %) had positive genuine Ara h (No 30), one child (1/31 = 1 %) had positive Bet v1, Bet v2/Ara h1/Ara h8 (No 26) five children (5/31 = 16 %) had

Bet v1/Ara h8 positive results (No 10, 12, 13, 15, 19), and seven children (7/31 = 23 %) (No 4, 7, 9, 16, 20, 23, 29) had negative CRD results.

Concentration of sIgE to peanut allergen ≥ 15 kIU_A/L (Table 1) was found in 4 children (No 2, 11, 14, 15). In three of them, sensitivity to genuine peanut allergen components was positive, and in one child (No 15), cross-reactive Ara h8 was confirmed.

In one patient (No 14), sensitivity to birch was confirmed by SPT and CRD, although sIgE was <0.35 kIU_A/L. In addition, one patient (No 8) with sIgE to

peanut allergen f13 < 0.35 kIU_A/L, concentration of genuine peanut allergen components was moderate to high (Ara h2) and very high (Ara h1, Ara h3), respectively. Peanut cross-reactivity was confirmed in 6 children (No 10, 12, 13, 15, 19, 26). In these children, sIgE to Bet v1 was ≥ 1.2 ISU.

According to the results of SPT, sIgE, and CRD, diagnosis of hypersensitivity is summarized in Table 2. Completely concordant diagnosis (either birch sensitivity or birch/peanut-co-sensitivity confirmed by all three tests) was achieved in 13 cases

Table 2 Diagnosis of hypersensitivity according to the results of skin prick test (SPT), specific IgE (sIgE), and component resolved diagnostics (CRD) in children

	Hypersensitivity according to SPT	Hypersensitivity to sIgE	Hypersensitivity according to CRD
1	Birch, peanut	Birch, peanut	Genuine birch and peanut
2	Birch, peanut	Birch, peanut	Genuine birch and peanut
3	Birch, peanut	Birch, peanut	Genuine birch
4	Birch, peanut	Birch, peanut	Negative
5	Birch, peanut	Negative	Genuine birch
6	Birch, peanut	Birch, peanut	Genuine birch
7	Birch, peanut	Birch, peanut	Negative
8	Birch, peanut	Birch	Genuine birch and peanut
9	Birch, peanut	Birch, peanut	Negative
10	Birch, peanut	Birch, peanut	Genuine birch, cross-reactive peanut
11	Birch, peanut	Birch, peanut	Genuine birch and peanut
12	Birch, peanut	Birch, peanut	Genuine birch, cross-reactive peanut
13	Birch, peanut	Birch	Genuine birch, cross-reactive peanut
14	Birch, peanut	Peanut	Genuine birch and peanut
15	Birch, peanut	Birch, peanut	Genuine birch, cross-reactive peanut
16	Birch, peanut	Birch	Negative
17	Birch	Birch	Genuine birch
18	Birch	Birch	Genuine birch
19	Birch	Birch	Genuine birch, cross-reactive peanut
20	Birch	Birch	Negative
21	Birch	Birch	Genuine birch
22	Birch	Birch	Genuine birch
23	Birch	Birch	Negative
24	Birch	Birch	Genuine birch
25	Birch	Negative	Genuine birch
26	Birch	Birch	Genuine birch and peanut, cross-reactive peanut
27	Birch	Birch	Genuine birch
28	Birch	Birch, peanut	Genuine birch
29	Birch	Birch	Genuine birch
30	Birch	Birch	Genuine peanut
31	Birch	Birch	Genuine birch

(42 %). In other 58 %, discordant results were obtained. In addition, cross-reactive peanut allergen component (Ara h8) was observed in six (19 %) children—some of them were both birch and peanut positive, and some of them were positive only to birch allergen in SPT and sIgE tests.

Table 3 illustrates the relationship between in vivo and in vitro tests for birch and peanut allergens. Moderate concordance ($\kappa = 0.551$) has been confirmed only between SPT and sIgE for peanut allergens, and poor concordance ($\kappa = 0.175$; $\kappa = 0.151$) or even negative κ has been found between other tests. For the birch allergens, $\kappa = 0$ was obtained between SPT and sIgE results and between SPT and CRD results.

4 Discussion

Results of the present study reveal that CRD modified the conventional diagnosis in children with birch allergy, since it can identify genuine and cross-reactive allergen components. In our group of birch allergic children, 51 % was also peanut sensitized, according to SPT, or 39 % according to sIgE. High percentage of cross-reactivity between birch and peanut allergens is well known (Mittag et al. 2006).

Table 3 Concordance between results of skin prick test (SPT), specific IgE (sIgE), and component resolved diagnosis (CRD) for birch and peanut allergens or allergen components

Test		Birch	Peanut
SPT/sIgE	kappa	0.0	0.551
Positive/positive	N (%)	28 (90)	11 (36)
Negative/negative	N (%)	0 (0)	13 (42)
Positive/negative	N (%)	3 (10)	5 (16)
Negative/positive	N (%)	0 (0)	2 (6)
SPT/CRD	kappa	0.0	0.175
Positive/positive	N (%)	24 (47)	5 (16)
Negative/negative	N (%)	0 (0)	13 (42)
Positive/negative	N (%)	7 (23)	11 (36)
Negative/positive	N (%)	0 (0)	2 (6)
sIgE/CRD	kappa	−0.157	0.151
Positive/positive	N (%)	21 (68)	4 (13)
Negative/negative	N (%)	0 (0)	15 (48)
Positive/negative	N (%)	7 (22)	9 (29)
Negative/positive	N (%)	3 (10)	3 (10)

CRD confirmed that 42 % of children were sensitized to genuine peanut allergen components Ara h 1, 2 and 3, responsible for clinical reactions to peanut, especially severe (Nicolaou and Custovic 2011), while 19 % to cross-reactive component Ara h8, responsible for mild symptoms. It means that peanut allergen extract-depending tests (i.e., SPT and sIgE) may produce either false-positive or false-negative results. Completely concordant diagnosis (confirmed with all three tests) was achieved in 42 % of children, and in others discordant results were obtained.

We analyzed two major birch proteins included in the microarray test, Bet v1 as a member of widely distributed defense plant proteins PR-10, and Bet v2 as one of the plant panallergens, profilins. PR-10 proteins behave as major allergens in plant food and birch pollen allergy in patients from northern and central Europe (Midoro-Horiuti et al. 2001). Bet v1 sensitivity was confirmed in 36 % of children and Bet v2 sensitivity in 19 % of children. These results are consistent with the results of Vieths et al. (2002), who demonstrated that minor allergenic components, including profilins, may sensitize approximately 10–20 % of patients. Sekerková and Poláčková (2011) revealed that specificity of birch-induced IgE synthesis is age dependent. However, it is not clear whether some sIgEs will be increased depending on age or whether their synthesis will be decreased. As this study was performed out of birch pollen season, exposure to birch pollen grains had no influence on the concentration of sIgE against birch allergen components. It must be emphasized that diagnosis of birch allergy was made based on clinical symptoms in birch season and positive sensitization by SPT and/or sIgEs. We may assume that the concentration of sIgE to birch pollen allergen and allergen components would be higher during the pollen season, as it was already shown for sIgE to ragweed (Špehar et al. 2010).

Conventional methods, that is, SPT and determination of sIgE-antibodies, use crude birch and peanut extracts, and these extracts contain a mixture of both allergic and non-allergic proteins that may cross-react with other allergens. Results of SPT and sIgE determination may confirm sensitivity to the mix complex, but cannot distinguish sensitivity among genuine and cross-reactive allergen proteins. Poor concordance of specific birch IgE and sIgE to birch allergen components could be explained by sensitization to minor birch allergens (Bet v3, Bet v4, Bet v5, Bet v7),

recognized by 10–30 % of birch allergic patients (Karamloo et al. 1999), or Bet v1 cross-reactive allergens from other Betulaceae/Fagales family trees (alder, hazel, oak) (Valenta et al. 1991).

Hence, SPT and sIgE determination may be used as screening methods for peanut allergy (Rancè et al. 2001). Overall, a negative peanut SPT has a negative predictive value of more than 95 % (Sampson and Ho 1997). SPT results are more sensitive to inhalant allergens than to food allergens in children (Cantani and Micera 2003). In the present study, only two children who had genuine peanut positive CRD results were negative in SPT test (false-negative SPT). For identification of peanut allergy in children, cutoff values of both SPT and sIgE have to be determined. According to the investigation of Nolan et al. (2007), wheal diameter of ≥ 7 mm predicted an allergic outcome with sensitivity 83 %, specificity 97 %, and positive predictive value 93 %. Results of Johanssen et al. (2011) have shown that wheal diameter < 7 mm and the concentration of peanut-specific IgE < 2 kIU_A/L may identify children with tolerance to peanut. Rancè et al. co-authors have shown that in cases when SPT results were ≥ 16 mm, and sIgE were ≥ 57 kIU_A/L, peanut OFC can be avoided. Determination of food-specific IgE concentration may identify patients (> 95 %) who are likely to experience allergy (Rancè et al. 2001). For peanut, sIgE > 15 kIU_A/L could predict clinical symptoms (Sampson and Ho 1997). On the basis of SPT and sIgE in our study, peanut allergy was confirmed in one patient, since he had mean wheal diameter ≥ 7 mm and concentration of peanut-specific IgE was 31.2 kIU_A/L. In addition, positive reactions to peanut showed comparable patterns in SPT and sIgE results, and moderate concordance between these two tests was confirmed. Concentration of sIgE to peanut allergen ≥ 15 kIU_A/L (Table 1) was found in 4 children. In three of them, sensitivity to Ara h1–3 components was positive, and in one child cross-reactive Ara h8 was confirmed.

As the increased concentration of sIgE to Ara h 1, 2, or 3 is associated with severe peanut reactions (Moverare et al. 2011), measurement of sIgE to these major peanut allergen components is more useful in predicting clinical peanut allergy than currently used SPT or sIgE on whole extract (Nicolau et al. 2010), but clinical manifestation could be dependent on the geographical region (Vereda 2011). Sensitization to Ara h8 is associated with mild symptoms (Asarnoj

et al. 2010). Therefore, CRD may be used to distinguish peanut-sensitized children at risk of severe symptoms from the children with mild or no symptoms to peanut allergens (if they are sensitized to pollen allergens and their homologue allergens).

Immunochemical methods (immunoblotting, RAST inhibition) (Sung et al. 2012; Mittag et al. 2004) and microarray methods (Moverare et al. 2011; Nicolaou and Custovic 2011) allow the identification of the protein that is giving the rise to allergic reaction in certain patients. Although the use of CRD enables more detailed analysis of a sensitization profile (Nicolaou and Custovic 2011), it may not replace controlled OFC (Ott et al. 2008) as the gold standard for the diagnosis of peanut allergy. Studies directly comparing SPT and sIgE with allergen component analysis are still scarce.

As expected, moderate concordance ($\kappa = 0.551$) has been found between SPT and sIgE for peanut allergens, as both tests use crude allergen extracts. Poor concordance between peanut SPT/IgE and CRD can be due to low levels of allergens in the extract to bind IgE, as shown by Mittag et al. (2004) or lower sensitivity of SPT.

We are aware of limitations of the study, such as small number of children, lack of comparison with oral peanut challenge, and incomplete panels of sIgE to allergen components.

In conclusion, this preliminary study demonstrated that allergen component diagnosis helps in differentiating sensitization to major peanut components from sensitization to peanut cross-reactive component in birch-sensitive children. Positive sIgE to genuine peanut allergen components may indicate an increased risk for systemic and more severe reactions to peanut. Poor concordance reveals that these results do not have general acceptability and that the CRD enables individualized approach for each patient.

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