

Peanut Allergy Diagnosis in the Context of Grass Pollen Sensitization for 125 Patients: Roles of Peanut and Cross-Reactive Carbohydrate Determinants Specific IgE

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Key Words

Peanut allergy • Specific IgE • Cross-reactive carbohydrate determinants • Immulite • ImmunoCap

Abstract

Background: In vitro testing for food allergy may yield clinically irrelevant results due to cross-reactive carbohydrate determinants (CCD) specific immunoglobulin E (sIgE) induced by pollen exposure. The performances of 2 in vitro methods were evaluated for peanut sIgE measurement in patients allergic to grass pollen with or without subsequent allergy to peanuts. The correlation between clinically irrelevant peanut sIgE and the presence of CCD sIgE was investigated. **Methods:** In vitro measurement of peanut sIgE was performed using the Pharmacia ImmunoCapTM system Radio Immuno Assay (RIA) and the Immulite[®] 2000 3gAllergyTM system. Discrepancies between in vitro results and peanut allergy diagnosis were evaluated by measurement of CCD sIgE using bromelain and ascorbic acid oxydase (AAO). **Results:** The sensitivity was 100% with both systems for the diagnosis of allergy to peanut (58 patients), nevertheless the specificity obtained with Immulite (73%) was better than that obtained using ImmunoCap (46%) in patients who were not allergic to

peanuts, but who had a grass pollen allergy (n = 41). In 22 out of 41 patients who presented clinically irrelevant peanut sIgE results using ImmunoCap, CCD sIgE was detected in 72% of the cases by bromelain and in 86% by AAO. In 11 patients out of 41 who presented irrelevant peanut sIgE results using Immulite, CCD sIgE was detected in 81% of the cases by bromelain and in 100% by AAO. **Conclusion:** The Immulite 2000 system had better specificity than the ImmunoCap system for accurate diagnosis of peanut allergy in patients allergic to grass pollen. CCD sIgE was identified in most of the false-positive peanut sIgE results.

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Introduction

Peanut allergy is a relatively common food allergy and it is reported to be one of the most fatal. The diagnosis of peanut allergy is usually made on the basis of clinical history and examination, with confirmation by investigative procedures such as skin prick tests (SPT), measurement of serum-specific immunoglobulin E (sIgE) and challenge procedures.

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The widespread presence of asparagine-linked glycoproteins with core 1,3-fucose and 1,2-xylose modifications in plants and invertebrates is the basis for the high incidence of cross-reactivity that has been reported for carbohydrate IgE antibodies (also known as cross-reactive carbohydrate determinant, CCD, sIgE) [1], which leads to extensive cross-reactions between vegetable foods, pollens and insect venoms. It is now well accepted that pollen exposure [2, 3] and, to a lesser extent, hymenoptera stings [4] are the primary inducers of CCD sIgE antibodies (Ab). The biological and clinical relevance of these CCD sIgE is still a matter of debate [5–12]. However, in vitro diagnostic tests show that these Ab are the basis of many irrelevant results for food allergy [6, 13–14], and the frequency of these results may be method dependent.

This study had 2 aims. First, to compare the risk of clinically irrelevant serology to peanuts between the Pharmacia ImmunoCapTM system Radio Immuno Assay (RIA; Phadia, Uppsala, Sweden) and the Immulite[®] 2000 3gAllergyTM System (Siemens Medical Solutions Diagnostics, Tarrytown, N.Y., USA) in a group of control patients not allergic to peanuts who were further divided into subgroups of those with symptoms related to at least grass pollen and those without sensitization to any pollen. Secondly, to investigate the correlation between clinically irrelevant peanut sIgE results and the presence of CCD sIgE.

Methods

Patients

A total of 125 subjects were recruited from patients referred to the Department of Internal Medicine, Clinical Immunology and Allergology, Nancy University Hospital, France. Four groups were investigated, and patients were selected according to their clinical history and sensitization to peanuts and/or pollens. Group Ia consisted of 29 peanut allergy patients without symptoms related to pollen and with negative SPT to grass pollen. Group Ib consisted of 29 peanut allergy patients with positive SPT to grass pollen. Groups Ia and Ib had convincing clinical allergy to peanuts with positive SPT results to peanuts and either a positive double-blind placebo-controlled food challenge (DBPCFC) or a positive labial challenge to peanuts. Groups IIa and IIb had no history of peanut allergy and a negative SPT to peanuts. Group IIa consisted of 26 patients not sensitized to any pollen (15 nonatopic subjects and 11 patients allergic to house dust mites with rhinitis or asthma). Group IIb consisted of 41 patients with hay fever and positive SPT results to grass pollen. Control subjects ate peanuts regularly without any symptoms.

This study did not require any institutional review board approval. In France allergologists are alone to decide how many prick-in-prick tests are needed to ensure a correct diagnosis for their patients according to environmental circumstances, food

habits or atopic disease. Because of the high prevalence of peanut sensitization (8.7–22.7%) in the French atopic population [15] allergologists routinely look for peanut sensitization with the informed consent of the patient.

Group IIa and IIb patients who confirmed that they ate peanuts on multiple occasions without any adverse effect gave their informed consent to add a prick-in-prick-test to peanuts to the current prick-tests to inhalants, and to use the remainder of the blood sample to perform sIgE to peanut.

Patients allergic to peanuts were routinely tested by the following procedure, according to the consensus guidelines [16]: a prick-in-prick test to roasted peanuts, sIgE and DBPCFC were carried out with their informed consent. Patients corresponding to the selection criteria were successively included in the different groups, according to their respective previously defined allergic profile, in order to reach a minimum of 25–30 patients per group for statistical analysis.

Skin Prick Tests

SPT were performed on the volar side of the forearm using a positive control (9% codeine phosphate), a negative control (50% glycerol and 50% buffered saline) and a peanut extract (Allerbio, Varennes en Argennes, France). Sensitization to grass pollen was determined by a positive SPT to a mixture of grass pollens (*Anthoxanthum odoratum*, *Poa pratensis*, *Arrhenatherum odoratum*, *Cynodon dactylon*, *Festuca elatior* and *Agrostis vulgaris*; Allerbio). Skin reactions were recorded after 15 min. The test was considered positive if the mean wheal diameter was >75% of the positive control wheal or ≥ 3 mm, the negative control being negative.

Food Challenges

DBPCFC or, in a few cases, labial challenges were performed, as previously described [17]. Briefly, for DBPCFC, roasted peanut extract doses were increased every 15–20 min until symptoms were observed (doses ranged from 4 to 7,000 mg). DBPCFC was performed in 46 out of the 58 patients diagnosed with peanut allergy. DBPCFC was considered positive in the cases showing allergic objective symptoms, which have been previously detailed [17], or in the cases showing abdominal pain lasting >30 min and only observed after peanut ingestion, according to the position paper from the European Academy of Allergology and Clinical Immunology [16]. Labial challenges were performed in 6 of the remaining 12 patients. Challenges were not performed in 6 patients due to the high risk of a severe anaphylactic reaction, a contraindication for DBPCFC. Food challenges were not carried out in control patients with no history of allergic reaction.

Allergy Diagnosis

The gold standard was a consensus allergy diagnosis derived from clinical history, skin testing and food challenges. A diagnosis of peanut allergy was defined by a convincing clinical history, a positive SPT result and, as appropriate, a positive DBPCFC or labial challenge. The absence of peanut allergy was documented by a negative clinical history and a negative SPT result. Hay fever patients were diagnosed by convincing clinical history during the grass pollen season and positive SPT results.

Laboratory Studies

Peanut sIgE was measured using the ImmunoCap system RIA and the Immulite 2000 3gAllergy system, following the

Table 1. A summary of skin tests, food challenges and peanut sIgE measurements performed in this study

Patient group	Atopic	Peanut SPT, mm		DBPCFC threshold of reactivity, mg		ImmunoCap peanut sIgE kU/l		Immulite peanut sIgE kU/l	
		median	range	median	range	median	range	median	range
Ia (n = 29)	22	8	5–16	100	4.4–7,000	92	1.4 to >100	>100	1.1 to >100
Ib (n = 29)	29	10	0.5–17	265	4.4–7,000	49	3.3 to >100	>100	3.1 to >100
IIa (n = 26)	11	0	0–0	n.d.	n.d.	<0.35	<0.35–0.35	<0.10	<0.10–0.91
IIb (n = 41)	41	0	0–1.5	n.d.	n.d.	0.68	<0.35–53	0.11	<0.10–14

Ia = Peanut-allergic patients without symptoms related to pollen; Ib = peanut-allergic patients with grass pollen sensitization; IIa = control patients not sensitized to any pollen; IIb = control patients with hay fever; n.d. = not determined.

manufacturers' instructions. Values of sIgE >0.35 kU/l were considered positive. Both bromelain (MUXF³ glycan type detected by ImmunoCap) and ascorbic acid oxidase (AAO; MMXF³ glycan type detected by Immulite) were used as CCD IgE markers [13, 18].

Statistical Analysis

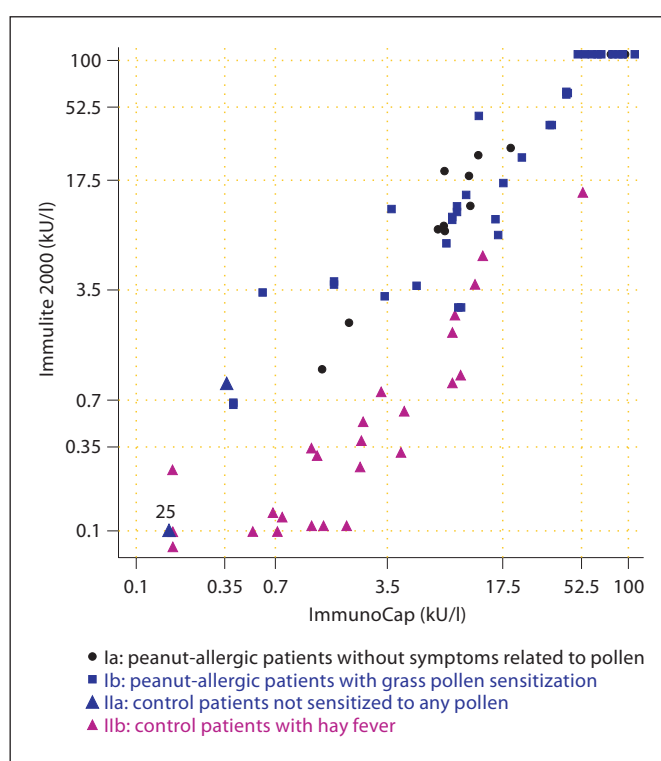
The sensitivity, specificity, positive predictive value (PPV) and likelihood ratio were calculated on the basis of the consensus diagnosis. Confidence intervals for proportions were calculated using Newcombe or Wilson methods [19]. For the confidence intervals for both systems, values that were >100 kU/l were assigned the value of 101 kU/l, and those <0.35 kU/l on ImmunoCap were assigned the value 0.35 kU/l, while those <0.1 kU/l on Immulite were assigned the value 0.1 kU/l.

Results

The general characteristics of the 4 patient groups are given in table 1 and figure 1.

Peanut-Allergic Groups (Ia and Ib).

Detailed results for peanut and CCD sIgE are reported in tables 2 and 3. For group Ia, the median peanut sIgE values for ImmunoCap were 92 kU/l (range 1.4 to >100 kU/l) and for Immulite they were >100 kU/l (range 1.1 to >100 kU/l). For group Ib, the median peanut sIgE values for ImmunoCap were 49 kU/l (range 3.3 to >100 kU/l) and for Immulite they were >100 kU/l (range 3.1 to >100 kU/l). Values measured by either method were not significantly different between groups Ia and Ib. In both groups sensitivity was 100% (95% CI 88–100%) for both ImmunoCap and Immulite. CCD sIgE in group Ia was detected at >0.35 kU/l in 5 out of 26 patients (19%) by ImmunoCap bromelain and in 8 of 26 (31%) by Immulite AAO. CCD sIgE in group Ib was detected at >0.35 kU/l

**Fig. 1.** Peanut-specific IgE. Immulite 2000 versus ImmunoCap in the 4 different groups.

in 7 of 29 patients (24%) by ImmunoCap bromelain and in 8 of 29 (28%) by Immulite AAO.

Control Groups (IIa and IIb)

Group IIa

Among the 26 patients in group IIa, only 1 patient (4%) had clinically irrelevant peanut sIgE by both the ImmunoCap and Immulite AAO.

Table 2. Peanut and CCD sIgE results for group Ia

Patient No.	ImmunoCap peanut, kU/l	Immulite peanut, kU/l	ImmunoCap bromelain, kU/l	Immulite AAO, kU/l
1	1.4	1.1	<0.35	<0.10
2	2.0	2.1	<0.35	<0.10
3	6.9	8.4	n.d.	n.d.
4	7.6	19.6	<0.35	<0.10
5	7.7	8.3	0.97	0.54
6	7.7	9.0	<0.35	1.4
7	10.6	18.4	n.d.	n.d.
8	10.9	11.8	n.d.	n.d.
9	12.2	24.7	<0.35	<0.10
10	19.2	27.5	<0.35	2.6
11	65.7	>100	<0.35	<0.10
12	77.9	>100	1.2	0.54
13	82.0	>100	<0.35	<0.10
14	83.4	>100	<0.35	<0.10
15	92.4	>100	<0.35	<0.10
16	94.5	>100	<0.35	<0.10
17	98.2	>100	<0.35	<0.10
18	>100	>100	<0.35	<0.10
19	>100	>100	1.4	3.5
20	>100	>100	0.40	3.3
21	>100	>100	<0.35	0.14
22	>100	>100	<0.35	<0.10
23	>100	>100	<0.35	<0.10
24	>100	>100	<0.35	0.25
25	>100	>100	<0.35	<0.10
26	>100	>100	0.50	1.4
27	>100	>100	<0.35	<0.10
28	>100	>100	<0.35	3.5
29	>100	>100	<0.35	<0.10

Ia = Peanut-allergic patients without symptoms related to pollen; n.d. = not determined

Table 3. Peanut and CCD sIgE results for group Ib

Patient No.	ImmunoCap peanut, kU/l	Immulite peanut, kU/l	ImmunoCap bromelain, kU/l	Immulite AAO, kU/l
1	3.3	3.1	<0.35	<0.10
2	3.7	11.4	<0.35	0.17
3	5.2	3.7	0.78	<0.10
4	7.9	6.8	0.81	1.5
5	8.6	9.9	<0.35	0.12
6	9.0	11.9	<0.35	<0.10
7	9.0	10.6	<0.35	<0.10
8	10.3	14.0	<0.35	<0.10
9	15.7	9.8	1.2	1.1
10	16.1	7.8	6.5	5.9
11	17.5	16.7	<0.35	0.24
12	22.3	23.9	3.8	7.6
13	33.6	39.4	<0.35	<0.10
14	41.9	62.7	<0.35	<0.10
15	48.8	>100	<0.35	<0.10
16	50.9	>100	4.8	6.6
17	55.0	>100	<0.35	<0.10
18	59.5	>100	<0.35	<0.10
19	66.4	>100	<0.35	0.14
20	67.5	>100	<0.35	0.45
21	67.9	>100	2.6	0.91
22	80.4	>100	<0.35	0.15
23	87.9	>100	<0.35	<0.10
24	92.8	>100	<0.35	<0.10
25	>100	>100	<0.35	<0.10
26	>100	>100	<0.35	<0.10
27	>100	>100	<0.35	0.39
28	>100	>100	<0.35	<0.10
29	>100	>100	<0.35	<0.10

Ib = Peanut-allergic patients with grass pollen sensitization.

noCap (0.36 kU/l) and Immulite (0.91 kU/l) techniques for a specificity of 96% (95% CI 81–99%). We performed tests for bromelain and AAO sIgE, all results were negative, except for the patient with the positive peanut sIgE (AAO 0.92 kU/l).

Group IIb (Pollinic Patients)

Results for peanut and CCD sIgE in group IIb are reported in table 4. The values of peanut sIgE for ImmunoCap ranged from <0.35 to 53 kU/l, and those for Immulite ranged from <0.1 to 14 kU/l. At the 0.35 kU/l cutoff, 22 patients were positive by ImmunoCap and 11 by Immulite for a specificity of 46% (95% CI 32–61%) and 73% (95% CI 58–84%) respectively, showing a statistically significant difference ($p < 0.02$). CCD sIgE was detected by at least 1 marker in 21 of 41 (51%) patient samples. Among the clin-

ically irrelevant peanut results for ImmunoCap, CCD sIgE was detected at levels >0.35 kU/l in 16 of 22 (72%, 95% CI 52–87%) by bromelain and in 19 of 22 (86%, 95% CI 67–95%) by AAO (table 4). Among the clinically irrelevant peanut results for Immulite, CCD sIgE was detected at levels >0.35 kU/l in 9 of 11 (81%, 95% CI 52–95%) by bromelain and in 11 of 11 (100%, 95% CI 74–100%) by AAO (table 4). In true negative peanut sIgE results with ImmunoCap assays, CCD sIgE was observed only in 2 of 19 patients (10%), and in true negative peanut sIgE results with Immulite, CCD sIgE was observed in 10 of 31 (30%).

All Groups

While both in vitro tests had a clinical sensitivity of 100% for peanut (95% CI, 94–100%), the Immulite had a significantly ($p < 0.02$) better specificity (82%, 95% CI

71–89% vs. 66%, 95% CI 54–76%), better PPV (not statistically significant, 82%, 95% CI 72–90% vs. 71%, 95% CI 61–80%) and a more positive likelihood ratio (5.6 vs. 2.9) than the ImmunoCap system. The percentage of CCD sIgE among grass pollen sensitized patients (Ib and IIb) was 41% versus 17% in nonpollinic patients (Ia and IIa).

Discussion

In this study, ImmunoCap and Immulite techniques both demonstrated 100% sensitivity for detecting sIgE in patients with true peanut allergy (groups Ia and Ib), and the range of peanut sIgE values was similar for both techniques.

Our results confirmed the poor clinical specificity of peanut sIgE values (46% for the ImmunoCap system vs. 73% for Immulite) in patients allergic to grass pollen (group IIb) as observed by Van der Veen et al. [6] and Mari [3]. The better specificity of Immulite is not due to a better sensitivity of the ImmunoCap technique.

With the ImmunoCap method the upper limit value for clinically irrelevant peanut sIgE was 53 kU/l. This level can be compared to the 100% PPV level (57 kU/l) observed for peanut allergy and reported by Rance et al. [20] to reduce the need for DBPCFC. This ImmunoCap upper limit value is higher than the first and traditional cutoff proposed by Sampson and Ho [21] (15 kU/l at a PPV of 95%), which is used by many physicians to reduce DBPCFC. This suggests that solely relying on an absolute peanut sIgE cutoff value may lead to falsely classifying some patients as peanut allergic, if the pollinic sensitization status is not taken into account. Furthermore, our data indicate that the upper limit value is method dependent. At a 100% PPV the sIgE value for Immulite was 14 kU/l. Using these upper limits for a 100% PPV for peanut sIgE, DBPCFC had to be performed on patients suspected of allergy to peanuts in 26% of cases when tested by Immulite and in 45% when tested by ImmunoCap.

Our study showed a level of sensitization to CCD in pollinic patients (from 28% for peanut allergy patients to 51% for patients not allergic to peanuts) within the range reported in the literature (20–65%), which was dependent on the allergen and patient's number of pollen sensitizations [3, 22]. The low level of CCD sensitization (4%) in nonpollinic patients (group IIa) agrees also with results reported in the literature [3, 22]. The single positive peanut sIgE result in control group IIa was for a patient with severe atopic dermatitis, negative SPT to pollen, no symp-

Table 4. Peanut and CCD sIgE results for group IIb

Patient No.	ImmunoCap peanut, kU/l	Immulite peanut, kU/l	ImmunoCap bromelain, kU/l	Immulite AAO, kU/l
1	<0.35	<0.10	<0.35	1.6
2	<0.35	<0.10	<0.35	<0.10
3	<0.35	<0.10	<0.35	<0.10
4	<0.35	<0.10	<0.35	<0.10
5	<0.35	0.25	2.6	<0.10
6	<0.35	<0.10	<0.35	<0.10
7	<0.35	0.24	<0.35	<0.10
8	<0.35	<0.10	<0.35	<0.10
9	<0.35	<0.10	<0.35	<0.10
10	<0.35	<0.10	<0.35	<0.10
11	<0.35	<0.10	<0.35	<0.10
12	<0.35	<0.10	<0.35	<0.10
13	<0.35	<0.10	<0.35	<0.10
14	<0.35	<0.10	<0.35	<0.10
15	<0.35	<0.10	<0.35	<0.10
16	<0.35	<0.10	<0.35	<0.10
17	<0.35	<0.10	<0.35	<0.10
18	<0.35	<0.10	<0.35	<0.10
19	<0.35	<0.10	<0.35	<0.10
20	0.52	0.10	1.2	2.0
21	0.68	0.13	<0.35	0.88
22	0.73	0.10	<0.35	<0.10
23	0.78	0.12	0.97	2.1
24	1.2	0.34	0.39	0.54
25	1.2	0.11	0.84	1.23
26	1.3	0.30	<0.35	0.23
27	1.4	0.11	1.6	0.41
28	1.9	0.11	1.2	1.0
29	2.3	0.26	<0.35	0.20
30	2.4	0.38	<0.35	0.74
31	2.4	0.50	1.6	7.7
32	3.2	0.76	<0.35	5.0
33	4.1	0.32	2.2	2.4
34	4.3	0.59	6.3	11.5
35	8.5	1.8	0.42	2.2
36	8.5	0.89	10.3	17.3
37	8.8	2.4	2.8	6.2
38	9.4	0.99	10.1	35.8
39	11.5	3.8	5.1	8.9
40	13.0	5.7	12.6	11.7
41	53.3	14.0	54.5	>100

IIb = Control patients with hay fever.

toms of hay fever, a total IgE level above 14,000 kU/l and positive SPT to mites and cockroach. However, this patient tested positive for timothy grass pollen (ImmunoCap 0.5 kU/l, Immulite 1.0 kU/l) and for CCD (AAO 0.92 kU/l).

The results obtained in this study show a higher percentage of CCD sIgE Ab in pollinic patients who were not

allergic to peanuts with irrelevant peanut sIgE (72–100%) when compared to pollinic patients allergic to peanuts (24–28%). Moreover, the level of the CCD sIgE was also higher, up to 54.5 kU/l (bromelain) or >100 kU/l (AAO) versus 6.5 kU/l (bromelain) or 7.6 kU/l (AAO). Until very recently the concept of low binding affinity of anti-CCD IgE was not confirmed [23], our study could suggest that CCD sIgE level and percentage are effectively lower in peanut allergy patients when compared to patients who did not have peanut allergy.

While CCD testing can be a useful tool, there is a lack of complete agreement between CCD and clinically irrelevant peanut sIgE results, since 14% (3 cases) of irrelevant peanut sIgE did not correspond with the presence of CCD sIgE. Profilin and Bet-v-1-like proteins have also been shown to be sources of clinically irrelevant in vitro results for allergens such as apple and latex in the case of pollinosis [16]. However, in these instances, SPT are generally also positive [24, 25], which was not the case in our study. In our series, 2 determinations out of the 3 for recombinant Phl p 12 were carried out, 1 being positive (data not shown).

At present, the availability of commercial reagents for the detection of specific seric IgE to nonglycosylated re-

combinant proteins rAra h 1, rAra h 2 and rAra h 3 will allow for a better discrimination between clinically irrelevant and relevant seric sIgE to peanuts [26].

Conclusions

Our study underscores the high occurrence of clinically irrelevant results for peanut sIgE in patients sensitized to grass pollen who have no symptoms related to peanuts. The comparison between 2 techniques for the determination of peanut sIgE leads to the same clinical sensitivity and indicates a better specificity for the Immulite 2000 as compared to the ImmunoCap system. The detection of CCD sIgE should be done systematically to improve the in vitro diagnosis of peanut allergy.

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