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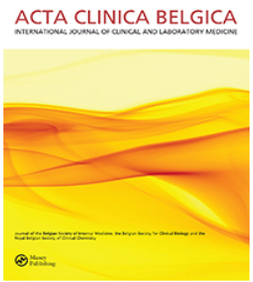

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Sensitization profiles to peanut allergens in Belgium; cracking the code in infants, children and adults

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Objectives: Peanut allergy shows distinct clinical patterns that can be predicted by component resolved diagnosis. However, data about peanut sensitization profiles in populations with a broad age stratification are scarce.

Methods: Sera of 89 peanut allergic patients (age 1–70 years), 21 infants (<1 year) with atopic dermatitis (AD) sensitized to peanut, 24 age matched peanut-tolerant individuals with positive specific IgE (sIgE) to peanut and 15 healthy individuals were tested for sIgE reactivity to rAra h 1, rAra h 2, rAra h 3, rAra h 8, rAra h 9 and rBet v 1 (FEIA ImmunoCAP, Thermo Fisher Scientific).

Results: In infants with AD, Ara h 1, Ara h 2 and Ara h 3 enabled to explain 14/21 (67%) of peanut sensitizations. No sensitization to Ara h 8 or Bet v 1 was observed. Patients with generalized reactions were more frequently sensitized to Ara h 1, Ara h 2 and Ara h 3 compared to patients with an oral allergy syndrome (OAS) and peanut-tolerant patients. Sensitization to Ara h 8 was significantly more observed in patients with an OAS. Ara h 2 showed to be the best marker to distinguish patients with generalized reactions from patients with an OAS and/or peanut sensitized patients but tolerating the legume.

Conclusion: Sensitization to Ara h 1, Ara h 2 and Ara h 3 can have an early onset and is predominantly associated with a more severe outcome. Ara h 2 is the best marker of a generalized peanut allergy.

Keywords: Ara h 2, Component resolved diagnosis, Food allergy, Peanut allergy

Introduction

Peanut (*Arachis hypogaea*) constitutes an increasing cause of food allergy in children and adults.[1,2] The clinical presentation of peanut allergy is highly variable and can vary from localized allergic reactions such as an oral allergy syndrome (OAS) to severe generalized reactions, which can be life threatening.[3,4] Given the severity and impact of peanut allergy, correct diagnosis is mandatory.

In the last decade, different studies have shown that additional quantification of specific IgE (sIgE) antibodies to the different allergenic components of peanut by component resolved diagnosis (CRD) is more reliable to predict clinical outcomes than only conventional assays measuring sIgE to whole peanut extracts.[5–11]

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Overall, Ara h 2, a member of the prolamin superfamily, has been described as the most important component for discrimination between peanut allergic patients with generalized symptoms and peanut-tolerant individuals.[5,6,10–15] Other clinically important peanut components, also predominantly associated with generalized allergic reactions, are Ara h 1 and Ara h 3, both members of the cupin superfamily and Ara h 6, another member of the prolamin superfamily that is displaying a high sequence identity with Ara h 2.[10,12,16]

In southern Europe, Ara h 9, the non-specific lipid transfer protein (ns-LTP) of peanut, has been described as a relevant peanut allergen and sensitization to this allergen is oftentimes observed to be associated with severe allergic reactions.[17] In contrast, in northern Europe, patients frequently present with a mild OAS resulting from a cross-reactivity between Bet v 1 the major allergen component from birch (*Betula verrucosa*) pollen and its homologue Ara h 8 from peanut, both proteins belonging to the pathogenesis related (PR10) protein group.[11,17]

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Besides geographic differences, it has been observed that food allergy can also exhibit distinct age-dependent sensitization profiles.[11,18] However, for the time being, the large majority of studies assessing the peanut sensitization profile has been performed in paediatric populations whereas data in adults remain scarce.[19] Therefore, in this study we aimed (1) at evaluating the sensitization patterns to five peanut components in a population ranging from infants (<1 year of age), over preschool and school children to adults with different clinical presentations, and (2) to assess the predictive value of sIgE quantification against the various peanut components.

Methods

Subjects

A total of 150 individuals were enrolled via the outpatients' clinics of Allergology and Paediatrics of the Antwerp University Hospital.

The study group consisted of infants less than 1 year old with atopic dermatitis (AD), all with an sIgE to whole peanut extract ≥0.35 kUa/L (FEIA ImmunoCAP, Thermo Fisher Scientific, Uppsala, Sweden) were selected. Second, patients with a definite history of immediate allergic symptoms due to consumption of raw and/or processed peanut and an sIgE to whole peanut extract ≥0.35 kUa/L were included. These patients were stratified into six groups according to the extent of the clinical reaction (OAS or generalized reaction) and age (<7 years, 7–18 years, >18 years) as detailed elsewhere.[18] Third, peanut-tolerant individuals with an sIgE to whole peanut extract ≥0.35 kUa/L were included and finally non-allergic age-matched individuals were enrolled. At the time of inclusion, all peanut-tolerant individuals indicated to consume peanut regularly without allergic symptoms.

The diagnosis of a generalized peanut allergy was based upon a compelling history of an adverse reaction upon roasted or processed peanut. Allergic symptoms could involve skin or subcutaneous tissues (e.g. generalized erythema, urticaria, or angioedema) and/or the respiratory tract (e.g. dyspnoea, stridor, wheezing, chest/throat tightness or cyanosis) and/or the gastrointestinal system (e.g. nausea, vomiting or abdominal pain) and/or the cardiovascular system (dizziness, diaphoresis, hypotension, confusion, loss of consciousness).[20] The diagnosis of an OAS to peanut relied upon a compelling history of repetitive pruritus and/or angioedema of the lips, tongue and/or palate on consumption of peanut.

Challenges were conducted neither in patients with a compelling history of generalized reactions due to the severity of the reported symptoms and the potential risk of eliciting serious reactions nor in patients with an OAS, since challenges were deemed unnecessary as the clinical history in such cases was highly reliable and symptoms were easily recognized and described by the patient.

The local ethics committee approved this study (B300201316182). Patients, healthy controls or their representatives approved an informed consent in accordance with the Declaration of Helsinki.

Total and specific IgE

Total IgE and sIgE to the whole peanut extract, the recombinant (r) peanut components rAra h 1, rAra h 2, rAra h 3, rAra h 8 and rAra h 9 and recombinant birch pollen major allergen rBet v 1 were quantified by the FEIA ImmunoCAP technique (Thermo Fisher Scientific) according to the manufacturer's instructions.

Calculation of predictive values

We calculated predictive values of different peanut components and a combination thereof to evaluate their ability to discriminate patients with generalized allergic reactions from patients with OAS and individuals tolerant to peanut but demonstrating a positive sIgE to this legume.

Statistical analysis

Data are expressed as median and range. IBM SPSS 20 software was used for data analysis. Evaluation of the diagnostic performance of the different peanut components in discriminating peanut allergic patients from healthy control individuals was performed by using receiver operating characteristic (ROC) curve analysis. Non-parametric tests and χ^2 analysis were used where appropriate. A $P < 0.05$ was regarded as statistically significant.

Results

Patient characteristics

Table 1 summarizes the demographics of patients and healthy control inividuals. In total, 68 patients exhibited a generalized reaction to peanut ingestion, whereas 21 patients reported an OAS to peanut. We were unable to enrol sufficient number of children younger than 7 years old with an OAS for robust analysis.

ROC analysis

ROC analyses between patients with generalized reactions upon peanut consumption and healthy control individuals generated a cut-off value of 0.10 kUa/L for all components. As a consequence, 0.10 kUa/L was applied as a decision threshold for further analysis of the sensitization profiles and for calculation of predictive values.

Sensitization to Ara h 1

As displayed in Fig. 1, sensitization towards Ara h 1 was mainly observed in patients with generalized reactions as compared to OAS patients (χ^2 analysis, $P < 0.05$). None of the patients with generalized reactions were monosensitized to Ara h 1. Sensitization to Ara h 1 can have an early onset as it was observed in 12/21 (57%) infants with AD. Three out of 24 peanut-tolerant individuals (13%) with a sensitization to whole peanut extract, showed sIgE reactivity to Ara h 1.

Table 1 Demographics and characteristics

		No. (female)	Age (years) median (range)	Total IgE (kU/L) median (range)
Peanut allergic patients with generalized reactions (sIgE peanut extract ≥0.35 kUa/L)	Preschool	32 (10)	3.9 (0.7–6.7)	202 (10–13450)
	School	25 (11)	11.3 (7.1–17.9)	441 (20–28500)
	Adults	11 (4)	21.5 (18.0–38.4)	642 (58–6500)
	Total	**68 (25)**		
Peanut allergic patients with localized reactions (OAS) (sIgE peanut extract ≥0.35 kUa/L)	Preschool	1 (1)	*	*
	School	7 (3)	13.4 (6.3–16.0)	422 (211–833)
	Adults	14 (12)	42.3 (20.1–69.5)	133 (20–1702)
	Total	**22 (16)**		
Infants with atopic dermatitis (sIgE peanut extract ≥0.35 kUa/L)	≤1 year	**21 (7)**	0.6 (0.5–1.0)	159 (9–4149)
Peanut-tolerant individuals (sIgE peanut extract ≥0.35 kUa/L)	Preschool	4 (2)	5.7 (4.9–6.3)	606 (270–4292)
	School	8 (5)	10.8 (8.2–14.3)	746 (531–2206)
	Adults	12 (8)	35.4 (18.1–56.2)	311 (76–5800)
	Total	**24 (15)**		
Healthy control individuals (sIgE peanut extract <0.35 kUa/L)	Preschool	5 (4)	6.4 (2.8–6.5)	28 (16–278)
	School	5 (2)	10.0 (8.2–15.8)	182 (8–290)
	Adults	5 (5)	32.4 (22.0–44.3)	7 (2–73)
	Total	**15 (11)**		
	Total	**150 (74)**		

*No sufficient number of preschool children with an Oral Allergy Syndrome (OAS) were included for robust analysis. Bold values demonstrate the total of each clinical group and provide an overview of the total study population.

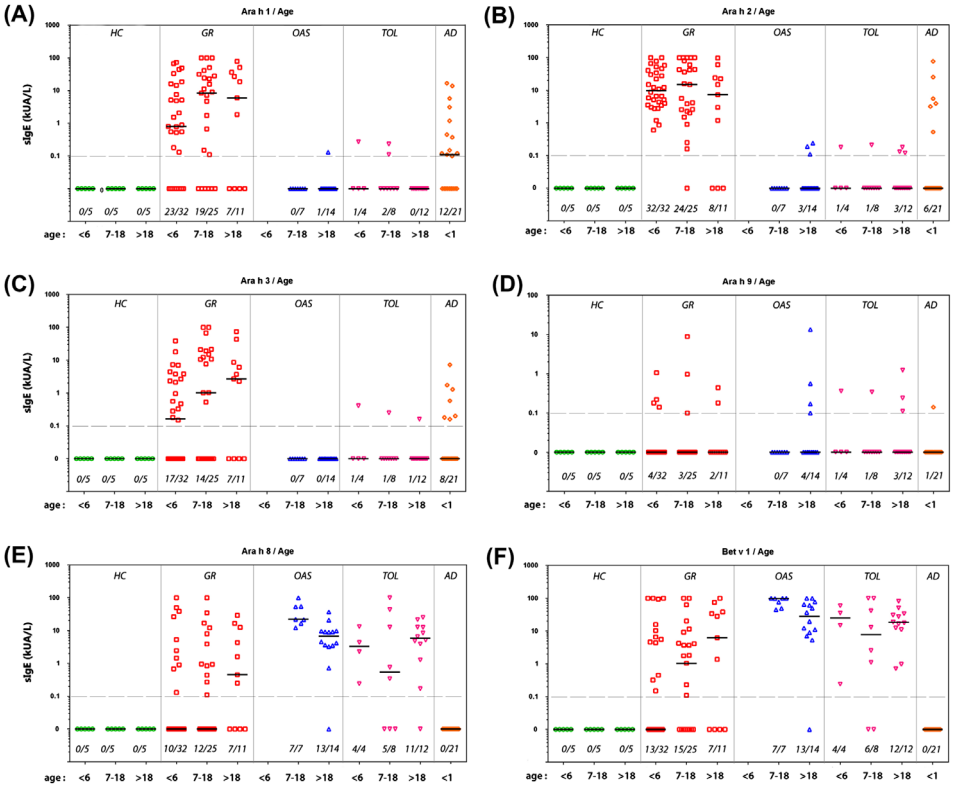

Figure 1 Sensitization to five different peanut components in healthy control individuals (HC), peanut allergic patients with generalized reactions (GR), patients with an oral allergy syndrome (OAS), infants sensitized to peanut (<1 year of age) with atopic dermatitis (AD) and peanut-tolerant individuals with positive sIgE to peanut (TOL). Number of positive sIgE results on the total number of patients in the corresponding group is written below the age groups.

Sensitization to Ara h 2

As demonstrated in Fig. 1, similarly to Ara h 1, sensitization to Ara h 2 was significantly more observed in patients with generalized allergic reactions compared to patients with an isolated OAS (χ^2 analysis, $P < 0.05$). Monosensitization to Ara h 2 was seen in only 10/68 (7%) patients with generalized reactions. With respect to age, sensitization to Ara h 2 was significantly more frequent in preschool and school children than in adults with generalized reactions (χ^2 analysis, $P < 0.05$). As a matter of fact all 32 preschool children, 24/25 (96%) schoolchildren and 8/11 (73%) adults with generalized reactions showed sIgE reactivity to Ara h 2.

Table 2 Performance characteristics of specific IgE to Ara h 1, Ara h 2 and Ara h 3

To differentiate	Age (years)	rAra h 1		rAra h 2		rAra h 3		rAra h 1 and/or rAra h 2 and/or rAra h 3	
		PPV (%) (95%CI)	NPV(%) (95%CI)	PPV (%) (95%CI)	NPV (%) (95%CI)	PPV (%) (95%CI)	NPV (%) (95%CI)	PPV (%) (95%CI)	NPV (%) (95%CI)
Patients with generalized reactions vs. OAS and peanut-tolerant patients	0–6	96 (79–100)	25 (5–57)	97 (84–100)	100 (29 100)	94 (73–100)	17 (4–41)	97 (84–100)	100 (29–100)
	7–18	90 (70–99)	68 (43–87)	96 (80–100)	93 (68–100)	93 (68–100)	56 (35–76)	92 (75–99)	93 (66–100)
	>18	88 (47–100)	86 (68–96)	57 (29–82)	87 (66–97)	88 (47–100)	86 (68–96)	60 (32–84)	91 (71–100)
	Overall	83 (61–95)	46 (35–56)	89 (79–95)	90 (77–97)	93 (80–98)	58 (46–70)	88 (78–94)	92(79–98)

Note: OAS: Oral Allergy Syndrome, PPV: Positive Predictive Value, NPV: Negative Predictive Value.

Six of the 21 infants with AD (29%) were sensitized to Ara h 2. Five out of 24 peanut-tolerant individuals with a positive sIgE to peanut (21%) showed IgE reactivity to rAra h 2.

Sensitization to Ara h 3

Sensitization to Ara h 3 was also predominantly observed in patients with generalized reactions compared to patients with an OAS (χ^2 analysis, $P < 0.05$). None of the patients with generalized reactions showed a monosensitization to Ara h 3.

Sensitization to the legumin Ara h 3 was observed in 8/21 (38%) infants with AD (Fig. 1). Furthermore, three patients in the group of peanut tolerant but sensitized individuals (13%) showed sIgE antibodies to Ara h 3.

Sensitization to Bet v 1 and its peanut homologue Ara h 8

Unlike sensitization to Ara h 1, Ara h 2 and Ara h 3, sensitization to the Bet v 1 homologue, Ara h 8, was significantly more observed in patients with an OAS vs. patients with generalized reactions upon peanut (χ^2 analysis, $P < 0.05$). However, it should be noticed that only three patients with generalized reactions were monosensitized to the Bet v 1 homologue, the remainder patients demonstrated a cosensitization to Ara h 1 and/or Ara h 2 and/or Ara h 3 and/or Ara h 9.

Sensitization to Ara h 8 is significantly correlated with sensitization to Bet v 1 (Pearson correlation coefficient = 0.81). In the group of peanut-tolerant patients, 20/24 (83%) showed a positive sIgE to Ara h 8 and 22/24 (92%) were sensitized to Bet v 1. In the group of infants with AD and demonstrating a positive sIgE to crude peanut, no sensitization to Bet v 1 and Ara h 8 was demonstrable.

Sensitization to Ara h 9

Sensitization to Ara h 9 was found in 9 out of the 68 patients (13%) with generalized reaction and in 4/21 (19%) of patients with an OAS. Neither in the group of patients with generalized reactions nor in the group of patients with an OAS there was a monosensitization to the ns-LTP of peanut demonstrable. In the group of peanut-tolerant individuals 5/24 (21%) were sensitized to Ara h 9. One infant with AD was sensitized to the ns-LTP of peanut.

Sensitization to Ara h 1 and/or Ara h 2 and/or Ara h 3

When evaluating sIgE to Ara h 1 and/or Ara h 2 and/or Ara h 3, a positive result was found in all 46 preschool children, in 24/25 (96%) school children and in 9/11 (82%) of adults with generalized reactions. Furthermore, at least 1 out of 3 storage proteins were recognized in 14/21 infants with AD.

In contrast, only 3/21 of patients with an OAS (14%) ($P < 0.05$) and 6/24 peanut-tolerant patients with a positive sIgE to peanut (25%) ($P < 0.05$) were sensitized to Ara h 1 and/or Ara h 2 and/or Ara h 3.

Predictive values of Ara h1 and/or Ara h 2 and/or Ara h 3

Because sensitization to Ara h 1, Ara h 2 and Ara h 3 was significantly more seen in patients with generalized reactions, we wondered whether these components or a combination thereof could be predictive for a more severe clinical outcome. Therefore, positive and negative predictive values (PPV and NPV) were calculated for these components between patients with generalized allergic reactions vs. patients with an OAS and peanut-tolerant patients with a positive sIgE to peanut.

As demonstrated in Table 2, applying the ROC-generated decision thresholds of 0.10 kUa/L we found highest overall PPV and NPV for Ara h 2. As a matter of fact, the NPV of Ara h 2 was found to be absolute in preschool children. Furthermore, combining the sIgE results of Ara h 1 and/or Ara h 2 and/or Ara h 3 did not significantly increase the PPV and/or NPV of the CRD technique.

Correlation between different peanut components

Fig. 2 shows a hierarchic cluster analysis of the sensitization to different peanut components. As expected, sensitization to the major birch pollen allergen Bet v 1, and sensitization to the Bet v 1 homologue Ara h 8 is highly correlated. In the group of seed storage proteins there is a high correlation between sensitization to Ara h 1 and sensitization to Ara h 2 (Pearson correlation coefficient = 0.83), furthermore sensitization to Ara h 3 is correlated to sensitization to Ara h 1.

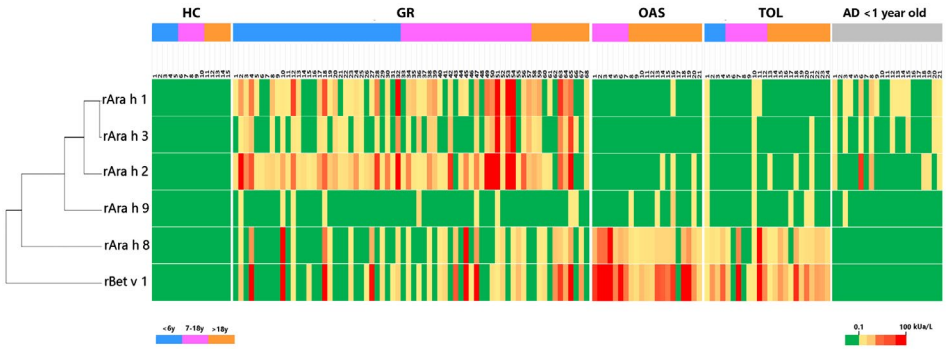

Figure 2 Graphical representation and hierarchical cluster analysis of all individual sIgE results in healthy control individuals, patients allergic to peanut with generalized reactions or OAS, peanut-tolerant individuals with positive sIgE to peanut and infants with AD sensitized to peanut.

Discussion

To our knowledge, this is the first study that investigates peanut sensitization profiles using five components in an age-differentiated population composed of infants, preschool and school children and adults. It reveals several particularities. First it confirms that patients with peanut allergy can display various sensitization profiles with distinct clinical outcomes. Sensitization to the 2S albumin, Ara h 2 and to members of the bicupin family, such as the vicilin Ara h 1 and legumin Ara h 3, is predominantly, but certainly not uniquely, associated with more generalized reactions. Whereas a sensitization to the Bet v 1 homologue Ara h 8, is generally associated with an OAS.[5–15]

Second, this study reveals that these sensitization profiles also exhibit significant age variations. Sensitization to Ara h 1, Ara h 2 and Ara h 3, unlike sensitization to the Bet v 1 homologue Ara h 8, can already start in infants without an overt peanut exposure as two-thirds of our group of infants with AD < 1 year of age with a positive sIgE to peanut extract was sensitized to Ara h 1 and/or Ara h 2 and/or Ara h 3. The exact mechanisms and routes of exposure for these early sensitizations remain elusive but probably relate to cutaneous exposure to peanut allergen present in for example house dust or in peanut-derived cosmetic products[21,22] and/or sensitization due to peanut exposure during pregnancy or breastfeeding.[23,24] In contrast, the observation that sensitization to the Bet v 1 homologue, Ara h 8 is absent in infants is likely to be explained by the fact that sensitization to the major birch pollen allergen generally starts at later age in childhood.[25] In any way, longitudinal follow-up studies seem mandatory in order to ascertain the clinical significance and prognosis of sensitization against Ara h 1, Ara h 2 and Ara h 3 in infants without overt peanut allergy. These studies should focus on the fact whether infants with AD demonstrating such a sensitization pattern are at particular risk to develop generalized peanut allergies or do outgrow their sensitization during childhood. In addition, longitudinal studies are also mandatory to explain the observation that adults seem less sensitized to Ara h 2 as compared to children. From our cross-sectional data, it cannot be concluded whether this lower prevalence of Ara h 1 and Ara h 2 sensitization is due to a genuine outgrow[26,27] or merely reflects a loss of medical follow-up, i.e. adults with established peanut allergy not attending the clinics anymore.

Third, it is well known that peanut allergy can constitute a severe condition with potentially fatal outcome.[4] On the other hand, overdiagnosis of peanut allergy with the set-up of unnecessary dietary prevention measures should be avoided. Therefore, correct identification of patients with more severe allergic reactions upon peanut is of paramount importance.

The most significant finding of our study is that Ara h 2 is the best marker to distinguish patients with generalized reactions from patients with an OAS and/or peanut sensitized patients but tolerating the legume. As a matter of fact, the negative predictive value of Ara h 2 was absolute in preschool children, meaning that the absence of sIgE reactivity to Ara h 2 precludes a more severe generalized reaction in the youngest peanut allergic children. Furthermore, this study shows that addition of sIgE measurement to Ara h 1 and Ara h 3, does not significantly improve the capacity of the CRD technique to identify patients with generalized reactions compared to sIgE measurement to Ara h 2 alone.

In contrast, monosensitization to the Bet v 1 homologue Ara h 8 in our cohort was predominantly associated with an OAS. Alternatively, it should be kept in mind that in a region where birch trees are endemic that sIgE antibodies to Bet v 1 homologues were confirmed to be the most prevalent cause of clinically irrelevant sIgE results and not to contribute to discriminate between birch pollen allergic individuals with or without peanut allergy.[28]

A sensitization rate of ≥10% to Ara h 9 was observed in the group of peanut allergic individuals with generalized reactions as well as in the group of patients with an OAS upon peanut, although sensitization to the ns-LTP of peanut was also observed in the group of peanut-tolerant patients. To be conclusive about clinical correlation, more studies are needed.

A potential criticism on our study could be the absence of double-blind placebo controlled challenges (DBPCFC) with peanut. However, from the literature it appears that

even DBPCFC's are not absolutely predictive for the clinical outcome, as both false negative and false positive food challenges have been described in approximately 5 and 13% of cases.[29–31] Second, almost half of our population are preschool children aged less than 7 years in whom DBPCFC remains particularly dangerous. Finally, in patients with a compelling history of OAS upon peanut, challenges were deemed unnecessary as the clinical history in such cases is highly reliable and symptoms are easily recognized and described by the patients.

Another potential criticism could be that skin tests have not been systematically carried out in this study, therefore no robust and reliable statistical analysis in order to evaluate the diagnostic value of skin prick tests could be done. However, it is the authors' experience that in children, not sensitized to pollen, skin prick tests can add to the diagnosis, particularly in cases where quantification of sIgE yields borderline positive results. In contrast, in adolescent and adults, skin testing frequently yields clinically irrelevant responses mainly because of the extensive cross-reactivity with birch pollen components such as the major birch pollen allergen Bet v 1 and profilin (Bet v 2). To our knowledge, no purified or recombinant peanut components are available for skin testing.

In conclusion, this study emphasizes the importance of a broad age stratification when assessing sensitization profiles in food allergy. First it reveals that, unlike Ara h 8, sensitization to Ara h 1, 2 and 3 can occur in infants without overt peanut allergy. Second, it confirms that sensitization to Ara h 1,2 and 3 is predominantly associated with a more severe outcome, whereas sensitization to Ara h 8 is generally associated with an OAS. Finally, and most importantly, our study indicates that Ara h 2 is the best marker to identify peanut allergic patients in risk of generalized reactions.

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