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Ana o 3-specific IgE is a good predictor for clinically relevant cashew allergy in children

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Abstract

Introduction

Component resolved diagnostics using specific IgE to 2 S albumins has shown to be a valuable new option in diagnostic procedure. Ana o 3 is a 2 S albumin from cashew. Aim of this study was to investigate the role of Ana o 3 specific serum IgE in the diagnosis of cashew allergy and to identify cut-off levels to replace oral food challenges. Moreover, the value of additional determination of total IgE has been investigated.

Methods

In a multicentre study we analysed specific IgE to cashew extract and Ana o 3 as well as total IgE in children with suspected cashew allergy using the ImunoCAP-FEIA and a standardized diagnostic procedure including oral challenges where indicated.

Results

61 patients were included in the study. 42 were allergic to cashew, 19 were tolerant. In receiver operating curves Ana o 3 discriminates between allergic and tolerant children better than cashew-specific IgE with an area under the curve of 0.94 versus 0.78. The ratio of Ana o 3-specific IgE to total IgE did not further improve the diagnostic. Probability curves for Ana o 3-specific IgE has been calculated and a 95% probability could be estimated at 2.0 kU/l.

Conclusion

Specific IgE to Ana o 3 is a valuable tool for the diagnosis of cashew allergy. Considering its positive predictive value it might allow to make a considerable number of oral challenges superfluous.

Key words

Ana o 3, cashew, children, component resolved diagnostics, food allergy, allergen specific IgE

Introduction

Cashew seed (*Anacardium occidentale*) is a food allergen with increasing importance (1). In Germany, cashew is the second most common seed allergen eliciting anaphylactic reactions in children and adolescents (2). In a multicentre European study on anaphylaxis including children and adults cashew seed was the sixth most common single food allergen (3) with large local differences in different countries. The first allergic reactions occur often already at preschool age (4). Moreover the worldwide production and consumption of cashew is rising (1,5). Cashew seeds are used as ingredient in different processed food products such as pesto, pastries, sweets and confectionary and in Asian cuisines. About 60% of the cashew products are consumed as snacks. Increased exposure might lead to increased sensitisation rates. This was hypothesized because of a 20-fold increase of cashew imports in Sweden and a parallel increase in cashew allergy (1). Children with Asian background in the United Kingdom had a higher rate of suspected cashew allergy as a result of a higher consumption due to different dietary habits (6).

Cashew is often termed as nut but botanically it is a seed. It belongs to the Anacardiaceae family along with pistachio and mango. A high degree of clinical relevant cross-reactivity is known to pistachio (7,8), but not to mango (5). Data on cross-reactivity to other seeds or nuts are scarce.

The necessary strict diet for allergic patients with its impact on quality of life (9) makes a correct diagnosis mandatory. Gold standard of the food allergy diagnosis is the oral food challenge, but it is costly, time-consuming and not without risk. Component resolved diagnostics is a new tool that supports the diagnostic procedure. It had been shown in peanut and hazelnut allergy that specific IgE to Ara h 2 and Cor a 14, both 2 S albumins from peanut and hazelnut, respectively, improves the diagnostic procedure in children (10).

Ana o 3 is the 2 S albumin of cashew (11). Recently it was shown in a Greek single center study, that sensitization to Ana o 3 is highly predictive for cashew allergy in children (7). The aim of our study was to investigate the potential of Ana o 3-specific IgE in the diagnostic work-up of cashew allergy and to determine cut-off levels for the prediction of clinically apparent allergy in a multicentre study in Germany.

Methods

Study population

Infants and children referred to specialised outpatient clinics in 7 German children's hospitals in Bonn, Berlin, Aachen, Cologne, Aue, Hannover and Osnabrück for evaluation of suspected cashew allergy or because of acute anaphylactic reactions were eligible for the study. Diagnosis of cashew allergy was based on a positive food challenge or a clear-cut history of a recent anaphylactic reaction to cashew combined with sensitisation to cashew extract. The study was approved by each local ethical committee and the parents gave written informed consent.

Oral food challenges

Oral food challenges were performed for clinical reasons with ground cashew. Increasing amounts of 0.02 g, 0.05 g, 0.18 g, 0.5 g, 1.8 g, 5 g, 18 g of cashew were given orally every 30 min. The challenges were scored positive if objective clinical reactions were noted such as urticaria, angioedema, vomiting, diarrhoea, wheezing, stridor, rhinoconjunctivitis or drop in blood pressure (12,13). In case of clinical tolerance the patient received a subsequent cumulative dose of cashew with a total amount of 25 g another day (14).

IgE measurements

Venous blood samples were collected prior to oral provocation tests and stored at -20°C. Cashew- and Ana o 3-specific IgE were determined by fluorescence enzyme immunoassay using ImmunoCAP 100 System (ThermoFisher, Uppsala, Sweden). Specific IgE titers were quantified in protein units designated as kilounits of antibody per liter (kUA/l) with a lower detection limit of 0.1 kUA/l and an upper detection limit of 100 kUA/l.

Statistical analysis

The highest and the lowest measured IgE levels had the values 0.1 and 100 kUA/l. All IgE levels were log-transformed (base 10) before logistic regression analysis and graphical representation. Separately for specific IgE to cashew and Ana o 3, stepwise logistic regression analysis was used to derive a multifactorial model for the prediction of positive challenge results. Based on the resulted single component models, univariate receiver operating characteristics (ROC) were calculated and compared with Delongs test for correlated ROC-curves. All statistical computations were performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA) and R (Version 3.1.3).

Results

61 patients were included in the study, the clinical characteristics are described in **Table 1**. 42 children were allergic to cashew based on a positive open food challenge (n=14) or a convincing history in combination with cashew-specific IgE ≥ 0.1 kUA/l (n=28). 19 patients were clinically tolerant following cashew ingestion. Three of these had a regular consumption without clinical symptoms and 16 had a negative oral cashew challenge.

50 patients with cashew sensitization had concomitant other food allergies as described in detail in **Table 1**. Specific IgE to cashew was higher in cashew-allergic children (0.3 – 100 kUA/l, median 3.19 kUA/l) compared to cashew tolerant children (0.1 – 17.8 kUA/l, median 1.0 kUA/l) but showed a remarkable overlap (**Figure 1A**). When analysing specific IgE to Ana o 3 the overlap was much smaller (allergic 0.15 – 100 kUA/l, median 2.2 kUA/l; tolerant 0.1 – 15.6 kUA/l, median 0.1 kUA/l) except one patient with tolerance during food challenge but high specific IgE both to cashew extract and Ana o 3 (**Figure 1A**). The patient with the highest level in the tolerant group was birch pollen allergic and had total IgE of 3,092 kU/l. He had multiple sensitizations to other nuts and seeds including storage proteins (walnut, hazelnut, peanut, sesame, pumpkin seed) without any clinical relevant allergy in food challenge tests.

In receiver operating characteristic curve analysis the ability of Ana o 3 to discriminate between allergic and tolerant patients turned out to be significantly superior to whole cashew extract with an AUC of 0.944 for Ana o 3 compared to 0.776 for cashew extract ($p < 0.005$, **Figure 2**).

Sensitivity of Ana o 3 was 0.93 and specificity 0.9 at IgE-level of 0.3 kUA/l. Continuous estimation of diagnostic accuracy for Ana o 3 is shown in **Table 2**.

Calculating decision points (10), a 90% probability for a positive cashew challenge was estimated for Ana o 3-specific IgE at 1.3 kUA/l, a 95% probability at 2.0 kUA/l, for cashew extract specific IgE these probability were 10.9 kUA/l and 27.0 kUA/l (**Figure 3 A and B**). As not all patients were challenged the same calculations were done only with challenged children (30 patients, 16 allergic, 14 tolerant). In this calculation the 90% probability for Ana o 3-specific IgE for a positive challenge was 2.7 kUA/l, 95% 4.8 kUA/l (data not shown). For cashew-specific IgE neither a 95% probability nor a 90 % probability for a positive challenge could be calculated (data not shown).

In order to investigate the hypothesis that the ratio of specific IgE to total IgE to either cashew or Ana o 3 might influence the test results the ratio was calculated additionally in 50 children with available total IgE values (**Figure 1B**). Using the ratio of Ana o 3- or cashew-specific IgE to

total IgE did not improve AUC indicating no superior diagnostic value for the ratio (**Figure 2**). Moreover, it was not possible to calculate a 95% probability for the ratio of cashew-specific IgE to total IgE (**Figure 3 C**). Calculating decision points for the ratio of Ana o 3-specific IgE to total IgE are shown in **Figure 3D**.

Discussion

In our multicentre study conducted in various children's hospitals in Germany we evaluated the value of component resolved diagnostics for children with cashew allergy. Ana o 3, the 2 S albumin of cashew, proved to have an excellent diagnostic accuracy. Only one patient was clinically tolerant in a standardized food challenge including a cumulative dose on the next day despite high levels of Ana o 3-specific IgE. Although this one patient changed the whole calculation basis and the results to a large extent, he reflects the clinical reality. This was already shown for Ara h 2 as marker for peanut allergy before (10, 15). In larger cohorts there are single patients that develop tolerance mechanisms despite high levels of sensitization. Even if component specific IgE, such as Ana o 3 or Ara h 2, have an excellent diagnostic value in general, in single patients it does not prove clinical relevance of sensitization.

Nevertheless in situations with limited access to hospitals conducting a standardized food challenge, a sensitization to risk markers such as Ana o 3 might help to establish a firm diagnosis for proper counselling of the patients. In those preselected due to a reasonable clinical suspicion for cashew allergy and a sensitisation to Ana o 3, a clinical relevant allergy is very likely. Remarkably every allergic patient in our cohort was sensitised to Ana o 3, which means on the other hand, that negative specific IgE to Ana o 3 might be a good predictor of a negative challenge outcome. Using a cut-off level of 2.0 kUA/l (95% positive predictive value for a positive challenge outcome), 25 of the 42 allergic patients (60%) would have correctly been identified as food allergic without oral food challenge, 1/19 tolerant patients (5%) would have been incorrectly labelled as allergic.

2 S albumins seem to be the most important and potent allergens in seeds and nuts of different kind. This is well known and thoroughly documented for the peanut allergen Ara h 2 and the hazelnut allergen Cor a 14 (10). Recent studies were able to indicate a helpful role of Jug r 1 of walnut (16) and Gly m 8 of soy (17,18) in the diagnostic process. The high degree of stability of 2 S albumins to heat and digestion and the high content of these proteins in these seeds and nuts might be the main reason for their superior role as main allergens in many different foods

(19). In peanuts for instance Ara h 2 constitutes 5.9 – 9.3% of the total protein fraction, which is 24 – 29% of the allergen (20).

Nevertheless the diagnostic accuracy seems to be even better for the cashew 2 S albumin Ana o 3 differentiating between allergic and tolerant paediatric patients compared to other 2 S albumins. The calculated AUC for Gly m 8 was 0.75 (18), for walnut 0.79 (16), for hazelnut 0.89 (10) and for Ara h 2 0.92 (10). For Ana o 3 our study indicates an AUC of 0.94 and in the Greek population an AUC of 0.97 (7).

Only one study on the value of component resolved diagnostics in food allergy calculated the probability for a positive food challenge (10) in relation to specific IgE against seed storage proteins. For Ara h 2 a 95% probability for a positive challenge was calculated at a specific IgE of 42.2 kUA/l, whereas for Cor a 14 a 95% probability could not been calculated and the cut-off for a 90% probability was 47.8 kUA/l. In our study the 95% probability for a clinical relevant cashew allergy was 2.0 kUA/l.

Using the ratio of specific IgE to total IgE did not improve diagnostic accuracy as a diagnostic test to distinguish between allergic and tolerant children as shown by comparable AUC. Two other studies came to the same results (21,22) for milk, egg, hazelnut and peanut. In a recent publication by our group (21) only extremely high total IgE-levels decreased the probability for a positive challenge at given specific IgE levels.

The weakness of the current study is that not all children underwent oral food challenge as the gold standard in food allergy diagnostics. However, the determination of clinical relevant cashew allergy was obtained very carefully focusing on clear objective reactions to cashew in the patients' history. Moreover, the performed sub-analysis using only the children with challenge proven food allergy showed similar results.

Few data exist on the pattern of concomitant food allergies in seed allergic patients. In our patients 45% were only allergic to cashew and had no further concomitant food allergy. Sensitization patterns in cashew allergic patients did not show any significant difference compared to tolerant patients.

Concerning other allergic diseases, tolerant patients were significantly more often suffering from allergic rhinitis (58% vs. 18%), mostly due to birch pollen allergy (47% vs. 21%). To date Bet v 1-homologs in cashew are not yet described but it is tempting to hypothesize that some sensitizations to cashew in tolerant patients are based on cross-reactive PR 10 proteins.

In conclusion we were able to demonstrate in a multicentre study that the detection of Ana o 3-specific IgE offers a clear improvement in the diagnostic procedure in children with suspected cashew allergy. The additional measurement of total IgE in order to determine the ratio did not further improve the diagnostic procedure.

Figures and tables

Figure 1:

- A) Immunoglobulin E against whole cashew and Ana o 3 by challenge outcome.
- B) Ratio of specific IgE against cashew and Ana o 3 to total IgE by challenge outcome.

Figure 2:

Receiver operating characteristic (ROC) curves predicting positive challenge outcome.

Figure 3:

Probability for a positive cashew challenge by cashew-specific IgE (A), Ana o 3-specific IgE (B), ratio of cashew-specific IgE to total IgE (C) and Ana o 3-specific IgE to total IgE (D). Individual IgE-levels or ratios sorted by challenge outcome above figure (plus sign)

Table 1: Patient characteristics

		Tolerant (n=19)	Allergic (n=42)	Significance
Sex, male		12 (63%)	24 (57%)	ns
Age (median, quartiles)		80 (42, 158)	46 (26, 75)	ns
Oral food challenge		16 (84%)	14 (43%)	
Comorbidities	Atopic dermatitis	7 (42%)	22/40 (55%)	ns
	Allergic rhinitis	11 (58%)	7/40 (18%)	0.02
	Asthma	5 (26%)	5/40 (13%)	ns
	Other food allergy	12 (63%)	23 (55%)	ns
Sensitisation	Birch	9 (47%)	9 (21%)	ns
	Grass	8 (42%)	6 (14%)	ns
	Indoor-allergens	5 (26%)	11 (26%)	ns
Concomitant food allergy	Peanut	7 (37%)	7 (17%)	ns
	Nut	4 (21%)	7 (17%)	ns
	Egg	1 (5%)	8 (19%)	ns
	Milk	0	4 (10%)	ns
	Other	2 (11%)	5 (12%)	ns

Table 2: Diagnostic sensitivity and specificity of Ana o 3-specific IgE concentrations

IgE cut-off	Specificity	Sensitivity
0.4	0.94	0.91
0.3	0.9	0.93
0.23	0.89	0.95
0.2	0.83	0.98
0.15	0.74	1
Ratio Ana o 3-specific IgE/total IgE		
0.0006%	0.53	0.91
0.001%	0.71	0.88
0.002%	0.93	0.85
0.005%	1	0.75

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