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Continuing Education for Nutrition Professionals

Association Between IgE and Food Allergies

A novel combination of an IgE mediated adult onset food allergy and a suspected mast cell activation syndrome presenting as anaphylaxis

Abstract

Background: Adult onset food allergy is a rare, but increasingly recognized phenomenon. Mast cell activation syndromes present an ongoing diagnostic and classification challenge. The combination of the two has been rarely described in the literature.

Case presentation: We present a case of a new onset, IgE mediated food allergy in combination with a mast cell activation syndrome in an elderly patient not known to have a history of atopy. He presented to a hospital with a first presentation of anaphylaxis manifesting profound hypotension following consumption of a stew consisting of fish and shellfish. He had a persistently elevated serum tryptase and demonstrated evidence of high titre serum specific IgE to shellfish. He responded well to histaminergic blockade.

Conclusions: Given that mast cell activation syndromes pose an increased risk for recurrent, severe anaphylaxis and that secondary causes of mast cell activation syndromes are more prevalent with aging, this case highlights the importance of considering this entity when evaluating an elderly patient with a first presentation of anaphylaxis.

Keywords: Adult onset food allergy, Mast cell activation syndrome, Anaphylaxis

Background

Food allergy is reported to affect approximately 2 % of the adult population, with the majority having had onset during childhood [1, 2]. There is an increasing recognition of adult onset food allergy, however these typically are associated with pollen-plant association, and are more often food-dependent, exercise-induced, anaphylaxis (FDEIAn) [3, 4]. Profound cardiovascular derangements during anaphylaxis suggest the possibility of an underlying mast cell activation syndrome and should prompt further investigation [5, 6]. The association between IgE mediated Hymenoptera anaphylaxis with hypotension and limited cutaneous findings and underlying mast cell activation disorders has been well established, and guideline recommendations include assessment of a serum tryptase, including in patients with negative skin prick testing, positive serum specific IgE who have had severe anaphylaxis [5].

According to recent consensus statements, mast cell activation syndromes are divided into three subtypes: Primary mast cell activation syndromes (MCAS), Secondary MCAS, and idiopathic MCAS. A mast cell activation disorder requires clinical symptomatology that is in keeping with the disorder, a transient, measurable increase in either serum tryptase or other markers of mast cell mediators and a response to agents that interfere with mast cell mediators [7, 8]. Recent attempts have been made to standardize an approach to suspected mast cell disorders, in order to appropriately classify individuals with evidence of mast cell activation that did not meet diagnostic criteria for systemic mastocytosis [7, 8].

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The combination of adult onset food allergy with an underlying mast cell disorder has not been described previously in the literature, and this case report demonstrates the investigation of an elderly gentleman who presented with first onset anaphylaxis due to food ingestion with evidence of a suspected underlying mast cell activation syndrome.

Case presentation

A 75-year-old male presented to the local emergency department exhibiting symptoms consistent with anaphylaxis. When found by his son in law he was flushed and unresponsive. On arrival of emergency medical personnel he was found to be hypotensive with blood pressure values of 80/60 mm Hg, hypothermic at 34.7 °C orally, and hypoxemic with a sPO_2 of 88 % by pulse oximetry. He received a 500 cc IV normal saline bolus with EMS. In the emergency department he remained hypotensive with a blood pressure of 71/44 mm Hg. He was treated with 3000 cc of IV crystalloid, epinephrine 1:1000 0.3 mg IM once, diphenhydramine 25 mg IV with a second 50 mg IV dose, ranitidine 150 mg IV once, and methylprednisolone 250 mg IV once with improvement. He suffered a type 2 MI, which was felt to be related to anaphylaxis-associated hypotension after assessment by Cardiology. A serum tryptase value was not obtained by the emergency room physician at the time of his presentation.

He had no previous history of anaphylaxis, atopy, lymphoproliferative disorder or other neoplasm. His daily medications included aspirin 81 mg daily, and this, in addition to his other regular medications was continued post reaction. He did not take additional doses of aspirin, over the counter or herbal products on the day of reaction. He had not been started on any new medications. There was no family history of atopy. The Allergy and Clinical Immunology Service was contacted by the emergency physician, at which time the food intake history was unclear, and given his profound hypotension at presentation, a concern of a MCAS was raised. This is consistent with suggestions to investigate for MCAS in patient's presenting with anaphylaxis with profound cardiovascular derangement and lacking documented urticaria, even if likely attributed to an IgE mediated reaction [5]. He was therefore discharged on cetirizine 10 mg orally daily, prednisone 50 mg orally for 5 days, diphenhydramine 25–50 mg orally q6 h as needed, and an epinephrine auto-injector. He was subsequently assessed in the Adult Allergy and Clinical Immunology outpatient clinic.

Between the anaphylactic episode and his appointment at the Allergy Clinic [approximate time 1 month] he consumed Atlantic cod without reaction. A food history obtained at his first visit revealed his initial event had developed following ingestion of a mixed fish and shellfish stew, which he had consumed without reaction on a regular basis. Following the meal he was alone in his room, until being found unresponsive by his son-in-law 3 h later. The patient could not recall the timeline of onset of symptoms, as his recall of the entire event was limited. Due to the concern of a potential underlying MCAS and the severity of his initial reaction it was felt safest to continue H1 receptor antagonist therapy, and due to the interference of anti-histamines on skin prick testing this was deferred for use of ImmunoCAP® [serum specific IgE (Phadia, Sweden)] for shellfish, finned fish and Hymenoptera venom. He was given an epinephrine auto-injector to be used in the event of subsequent anaphylactic reaction. At follow up his serum specific IgE was high positive for Shrimp at 13.7 kUA/L, and Crab at 7.3 kUA/L, moderately positive for Lobster at 2.9 kUA/L and Clam at 0.9 kUA/L, while testing negative with values <0.35 kUA/L for Salmon, Walleye Pike and Whitefish [9].

While both skin prick testing and serum specific IgE demonstrate the existence of clinical sensitization, they do not provide evidence for clinical allergy. Serum specific IgE has been reported to be in agreement with skin prick testing from between 50 and 90 % of the time, with average values between 70 and 75 %, and similar values in agreement between supervised challenges [10]. The interpretation of skin prick tests and serum specific IgE requires evaluation of historical features, physical examination and, at times, supervised challenge [10]. Although a supervised oral food challenge would have provided definitive evidence of an IgE mediated food allergy rather than sensitization, this was not undertaken due to the severity of his presentation with development of a myocardial infarction, his baseline limited myocardial reserve, and multiple comorbidities. It is acknowledged this limits the definitive confirmation of IgE mediated food allergy, but was made on a risk–benefit decision in the best interests of the patient.

A serum tryptase level drawn during his first appointment at the Allergy Clinic was 15 ng/ml [with a normal range from 1 to 11.4 ng/ml], an elevation not diagnostic of systemic mastocytosis, but suggestive of a mast cell activation disorder [11]. A creatinine obtained demonstrated a value of 92, corresponding to an eGFR of >60 ml/min, ruling out reduced renal clearance as a cause of accumulation of serum tryptase [12].

To further evaluate for a potential mast cell disorder, a 24-h urine methylhistamine level was obtained, demonstrating a value of 103 µg/g Cr (normal range 30–200 µg/g Cr). A serum protein electrophoresis demonstrated no evidence of an M protein. He was seen by the Adult Hematology/Oncology Service at Cancer Care

Manitoba to definitively exclude a diagnosis of systemic mastocytosis, for which a bone marrow biopsy and c-KIT testing were completed. His c-KIT mutation testing was negative. A bone marrow biopsy demonstrated normal trilineage hematopoiesis with normal differentiation and maturation without definitive morphological evidence of mastocytosis or lymphoma, specifically revealing no large lymphoid aggregates, abnormal plasma cells, or spindle cells suggestive of mastocytosis. Accompanying flow cytometry demonstrated revealed a sample composed of 23 % lymphocytes, of which 84 % were T cells, 8 % NK cells, and there was a CD4/8 ratio of 0.9. Remaining cells were polyclonal B cells without evidence of lymphoma, plasma cell neoplasm, or mastocytosis. Tryptase was consistently elevated at 17 ng/ml on repeat testing.

Given his elevated tryptase, he was maintained indefinitely on cetirizine, and continued to avoid both fish and shellfish, but did require emergency department monitoring following administration of his epinephrine auto injector in January 2015 following ingestion of a perogy, of which the precise constituents were unknown, and development of a diffuse urticarial rash. He was treated with a 3 day course of 50 mg of oral prednisone.

He fulfills the proposed diagnostic criteria for diagnosis of a suspected MCAS based on guidelines published by Valent and colleagues, however we acknowledge the challenge of establishing the diagnosis in the context of a documented IgE mediated food allergy, and he may be best classified as a Secondary MCAS [IgE-dependent disease related] [7, 8, 13].

Conclusions

The combination of food allergy with a mast cell activation syndrome with onset in the elderly population represents a novel combination that is not well described. It is unclear whether the patient in this case had an underlying mast cell disorder that was previously quiescent and was detected only due to the new development of an IgE mediated food allergy, or if sub-clinical sensitization to shellfish was able to manifest clinically with acquisition of a mast cell disorder. Theoretically the patient could have developed proliferation of a non-clonal mast cell population due to a subclinical entity such as malignancy, infection, subclinical thrombosis, an underlying autoimmune condition, increased stimulatory cytokines, or increased vasoactive peptides; as all are postulated triggers of mast cell activation [14].

The increasing prevalence of food allergy worldwide, and the heightened propensity for life threatening anaphylactic reactions in those with underlying mast cell reactivity highlights the importance of an educated, stepwise, evidence-based diagnostic approach to older adults presenting with anaphylaxis. The combination of IgE mediated allergy presenting with anaphylaxis associated with hypotension and underlying mast cell activation disorders is well established in Hymenoptera IgE hypersensitivity, and guideline recommendations to assess for serum tryptase exist in this situation [6]. According to a recent case series the absence of urticaria or angioedema with severe anaphylaxis and associated hypotension in response to Hymenoptera stings provides the strongest indicator of an underlying mast cell disorder [15]. This provides precedence for the combination of IgE mediated anaphylaxis and an underlying mast cell activation disorder, and the authors believe this case report highlights the importance of a high level of suspicion for mast cell disorders in patient's presenting with anaphylaxis with profound cardiovascular manifestations and limited cutaneous manifestations, as has been previously described [5, 16].

A case such as this emphasizes the importance of an approach with thorough investigation of all potential allergen exposures, including those to which a patient has been exposed on multiple occasions without systemic or local reaction. This case suggests the need for further elucidation of patient characteristics that predispose to an underlying mast cell activation syndrome despite identification of an IgE mediated trigger, to better facilitate identification of patients who would benefit from additional intervention with mast cell stabilizing therapy.

It could be theorized that new sensitization to previously tolerated food antigens with resultant anaphylaxis could be associated with underlying mast cell activation disorders in adults without a history of atopy, particularly when presenting with profound hypotension. Further research is needed into the incidence of adult onset IgE mediated food allergies, anaphylaxis, and the rate at which it is associated with underlying mast cell activation, particularly given the association with MCAS and a heightened risk for a more severe anaphylactic reaction [5].

Retrospective analysis on the agreement between skin prick test and serum food specific IgE antibody results in adults with suspected food allergy

Abstract

Background: Food allergy is a common clinical problem in adults. Given logistical barriers to conducting food challenges, the use of skin prick test (SPT) and specific IgE (sIgE) are important in establishing the diagnosis. The purpose of this study is to investigate the agreement of SPT and sIgE results in adults presenting to an allergy clinic with suspected food allergy.

Methods: Retrospective analysis of medical records at the University of Alberta Allergy Clinic between September 2013 and May 2015 was performed. Demographic, medical history as well as SPT and specific IgE results were recorded. Agreement of SPT and sIgE for individual food allergens was analyzed by Kappa statistics.

Results: Data from 260 patients was collected. The population was predominantly female, often having other atopic diseases. Very few food challenges were performed; IgE mediated food allergy was diagnosed in a minority (29.6 %) of cases. Kappa values which reached statistical significance were moderate for peanut $\kappa = 0.535$ ($p = 0.0002$, CI 0.364–0.707), walnut $\kappa = 0.408$ ($p = 0.001$ CI 0.159–0.657), pecan $\kappa = 0.530$ ($p = 0.001$ CI 0.211–0.848), and lobster $\kappa = 0.543$ ($p = 0.004$ CI 0.197–0.889), substantial for pistachio $\kappa = 0.657$ ($p = 0.023$ CI 0.224–1.000), codfish $\kappa = 0.770$ ($p = 0.0002$ CI 0.558–0.983), shrimp $\kappa = 0.627$ ($p = 0.0006$ CI 0.383–0.871) and egg white $\kappa = 0.625$ ($p = 0.002$ CI 0.293–0.957), almost perfect for cashew $\kappa = 0.894$ ($p = 0.0008$ CI 0.693–1.000) and salmon $\kappa = 0.874$ ($p = 0.004$ CI 0.705–1.000).

Conclusions: The agreement between SPT and sIgE results on adults being evaluated for food allergy is at least moderate or better for peanut, walnut, pecan, pistachio, cashew, lobster, shrimp, codfish, salmon and egg white. This should be reassuring for patients who have contraindications or restricted access to either test as the results for the above allergens will likely agree. These findings may suggest that these tests could possibly be interchangeable in adults being evaluated for suspected food allergy and will aid primary care physicians in the triage of patients requiring allergist care.

Keywords: Adult, Food allergy, Skin prick test, Specific IgE, Agreement

Background

Food allergy is an increasingly common diagnosis in adults. Recent prevalence estimates indicate that food allergies affect nearly 5 % of adults [1]. Oral food challenge (OFC) remains the gold standard diagnostic test for food allergy. In clinical practice, however, there are often logistical barriers to performing food challenge in outpatient settings. Lack of human resources and time are the most often listed impediments reported by allergists in an American survey [2]. As well, the possibility of inducing a systemic reaction likely weighs heavily on clinicians.

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Patients may resist food challenge due to fear of a reaction or are unable to make time for a prolonged visit. Due to these factors, clinicians are often relying upon skin prick test (SPT) or measurement of the antigen specific Immunoglobulin E (sIgE) to adjunct history and physical exam to make the diagnosis. The accuracy of SPT can be confounded by patients' medication and the preparation of the allergen used in the SPT. Both SPT and sIgE can reflect cross reactivity with other allergens or asymptomatic sensitization [1]. The 2010 National Institute of Allergy and Infectious Diseases Sponsored Expert Panel Report recommends either skin prick test (SPT) or serum sIgE level as adjunctive objective testing [3]. This recommendation likely reflects the paucity of literature on the level of agreement between these two tests, despite their good performance characteristics. In Schoos et. al's [4] examination of a birth cohort of children, agreement of SPT and sIgE to common food allergens was initially poor to moderate and deteriorated to slight agreement with age. In contrast, Asha'ari et al. [5] showed a positive correlation between SPT and sIgE in an adult population unselected for common food allergens. As these studies on the agreement of SPT and sIgE seem to have contradictory findings, further study is needed to clarify this question. The aim of this study is to investigate the level of agreement between SPT and serum sIgE results in a selected adult population referred to an academic allergy clinic for investigation of suspected food allergy. Because these two tests are independent methods of testing for sensitization, we hypothesized their results will show high levels of agreement but that the level of agreement would vary between individual food allergens.

Methods

We performed a retrospective study/chart review of the SPT and sIgE results of adult patients seen at the University of Alberta Allergy Clinic in Edmonton (Alberta) for suspected food allergy from September 2013 to May 2015. This is an outpatient clinic affiliated with a tertiary care academic hospital. This clinic has approximately 100 new patient visits a month. Ethics approval was obtained from the University of Alberta Research Ethics Board. Electronic medical records of visits to the clinic in the selected time period were reviewed. Minimum referral age was at least 16 years with referral base of either primary care or specialist physicians. Data retrieval was performed by one author (LL).

Demographic information including age, gender were collected for all patients. Medical history variables noted were known previous diagnosis of food allergy and offending allergen, allergic rhinitis, isolated angioedema, spontaneous urticaria, atopic dermatitis, medication allergy, venom allergy, and pollen food syndrome. Details of their presenting complaint including the nature of their reaction, whether the history of the reaction was compatible with IgE mediated food allergy, and the specific food trigger of concern were extracted from the chart. The nature of the reaction was classified as cutaneous with or without angioedema, gastrointestinal, respiratory, anaphylaxis, other or unclear. The reaction was classified as other if the symptoms were not cutaneous, gastrointestinal, and respiratory. An unclear reaction was noted if the patient could not recall the details of the reaction. The history of the reaction was classified as suggestive of IgE mediated food allergy if there was a clear immediate temporal relationship between ingestion of the food and symptoms, reaction was reproducible on subsequent exposure and responded to epinephrine or antihistamines. If not all elements were noted on the chart, record made by the attending allergist of the presence of a suggestive history was also accepted. The diagnosis of IgE mediated food allergy made by the attending allergist was based on clinical impression which included a combination of factors including the history, SPT, sIgE, oral food challenge (where available) as recommended in current guidelines. The diagnosis entered by the allergist on the electronic medical record or in their consultation letter to the referring physician was used in the data abstraction. Where available, the results of skin prick test, sIgE, complete blood count, total serum IgE and other immunoglobulin quantification were also recorded.

Skin prick testing performed at the clinic used DUOTIP-TEST® (Lincoln Diagnostics Inc) and commercial extracts purchased from Omega Laboratories Ltd (Montreal, Canada). Histamine and saline were used as positive and negative controls respectively. SPT was performed by trained clinic staff under the supervision of the attending allergist. A wheal size >3 mm was considered a positive SPT result [6]. Wheal size 3 mm or smaller were considered negative.

Specific IgE testing was either ordered by the allergy clinic at the time of the visit or already available having been ordered by the referring physician. The local laboratory uses Phadia 250 Immunocap serum assay. Specific IgE titre >0.35kU/L was considered a positive result [7]. The use of 0.35kU/L or greater as the level for a positive sIgE test was chosen to give uniformity to the data analysis and was based upon the Immunocap assay's antibody detection threshold. This detection threshold was used for every food allergen tested. For adults, there is currently no evidence to support another positive threshold value for sIgE assays.

The agreement between SPT and sIgE for individual food allergens was analyzed by using Kappa statistics. Kappa values <0 indicate poor agreement; 0 to 0.2: slight agreement; 0.21 to 0.40: fair agreement; 0.41 to 0.6:

moderate agreement; 0.61 to 0.80 substantial agreement; and 0.81 to 1.00: almost perfect agreement [8]. Only patients who underwent both SPT and sIgE for a particular food allergen were included in the kappa analysis for that allergen. All data were analyzed using IBM SPSS Statistics for Macintosh (version 23.0).

Results

A total of 260 patients were referred to the University of Alberta Adult Allergy Clinic during the study period for evaluation of a possible food allergy. These patients were typically referred after experiencing symptoms attributed to food ingestion.

Demographic information and medical history of the patient population are summarized in Table 1. The patient population was predominantly female (70.4 %), with a mean age of 38.8 years. With the exception of two patients, one 16 and one 17 years of age, the population was comprised of adults. The majority of referrals came from primary care physicians (88.8 %) and the rest from specialists, mostly respirologists. A notable portion of patients had a history of other allergic diseases, with the most common being allergic rhinitis, followed by asthma and atopic dermatitis.

Table 2 summarizes the nature of the food reaction as well as the rate of diagnosis of food allergy in the study population. The majority of patients reported their primary symptoms as cutaneous either with or without angioedema. Gastrointestinal symptoms, unknown reaction, and anaphylaxis followed in frequency. The nature of the reaction was recorded as unknown if the patient could not recall the specifics in the history or if the chart was incomplete. The medical records revealed that the attending allergist determined the history alone was suggestive of an IgE mediated food allergy in a minority (37.3 %) of the patients and either not suggestive of food allergy or not clear enough to discern in the majority of patients (62.7 %). The patients who underwent food challenges generally had testing results that were discordant from their clinical history. A total of nine oral challenges were performed; almost all (7/9) had history suggestive of food allergy. Of these patients with suggestive histories, 5/7 had negative SPT and sIgE to the allergen of concern. The diagnosis of an IgE-mediated food allergy was made by the attending allergist in 77 (29.6 %) of patients and refuted in 126 (48.5 %). There was a sizeable minority of patients (n = 57; 21.9 %) in which the diagnosis remained unclear. Reasons for the ambiguity of diagnosis included active patients who still required follow up testing or incomplete chart.

Table 3 details SPT and sIgE results for most common individual food allergens tested. The most frequently tested foods were peanuts and treenuts, fish, shellfish, milk, soy, sesame, egg and wheat. The majority of patients were tested to more than one allergen. Positive SPT and sIgE results were in the minority for all foods recorded. In particular, shellfish has the lowest rate of positive SPT and sIgE tests. Not all patients had both SPT and sIgE tests for a particular allergen. However, the kappa

Table 1 Demographic and clinical characteristics of patient population (N = 260)

Characteristic	N (%) or Mean ± SD
Sex	
Female	183 (70.4 %)
Male	77 (29.6 %)
Age (years)	38.8 (13.7)
Referral source	
Primary Care	231 (88.8 %)
Respirologist	7 (2.7 %)
Allergist	2 (0.8 %)
Other	10 (3.8 %)
Unknown	10 (3.8 %)
Diagnosis of atopy	
Allergic rhinitis	120 (46.2 %)
Asthma	90 (34.6 %)
Atopic dermatitis	61 (23.5 %)
Venom allergy	5 (1.9 %)
Drug allergy	42 (16.2 %)
Urticaria	60 (23.1 %)
Eczema	61 (23.5 %)
Pollen food syndrome	35 (13.5 %)

Table 2 Food reaction characteristics and food allergy diagnosis rate in patient population

Characteristic	N (%)
Nature of reaction	
Cutaneous/angioedema	120 (46.2 %)
Gastrointestinal	47 (18.1 %)
Unknown	38 (14.6 %)
Anaphylaxis	29 (11.2 %)
Other/non specific	14.2 (5.4 %)
Respiratory	9 (3.5 %)
None	3 (1.2 %)
History suggestive of food allergy	
Yes	97 (37.3 %)
No/unclear	163 (62.7 %)
Positive	2 (22.2 %)
Negative	7 (77.8 %)
Diagnosis of food allergy	
Yes	77 (29.6 %)
No	126 (48.5 %)
Unclear	57 (21.9 %)

Table 3 SPT and specific IgE results for Patients Included in Kappa Analysis

Food	Positive sIgE positive SPT	Negative sIgE negative SPT	Positive sIgE negative SPT	Negative sIgE positive SPT	Total
Peanut	26	41	16	4	87
Walnut	8	33	11	2	54
Hazelnut	11	19	28	2	60
Almond	3	35	21	1	60
Pistachio	6	4	1	1	12
Cashew	10	8	0	1	19
Pecan	5	26	5	1	37
Salmon	12	19	2	0	33
Codfish	12	21	2	2	37
Shrimp	9	32	6	1	48
Lobster	4	30	4	1	39
Crab	1	19	9	1	30
Clam	0	16	1	1	18
Milk	2	16	8	3	29
Soy	0	6	2	3	11
Sesame	6	2	2	0	10
Egg white	6	14	2	2	24
Wheat	1	16	8	0	25

analysis for each allergen only included patients who had both SPT and sIgE to that allergen. Table 3 reflects the test results of patients who had both tests and were subsequently included in the kappa analysis.

Figure 1 illustrates the kappa agreements coefficients for individual foods. SPT and sIgE agreement for cashew and salmon were near perfect. Agreement was substantial for pistachio, shrimp, and egg white, moderate for peanut, walnut, pecan, lobster, sesame, slight for hazelnut, almond, crab, milk, wheat, poor for soy and clam. There were not enough data points to perform kappa analysis on tuna, halibut, codfish, oyster, scallop, mussel and whole egg.

The kappa analyses reached significance ($p < 0.05$) to reject the null hypothesis for peanut, walnut, pistachio, cashew, pecan, salmon, shrimp, lobster and egg white. All of these kappa values showed at moderate or better agreement between the SPT and sIgE.

Discussion

Our study shows that in adult patients being evaluated for a possible food allergy, agreement between the SPT and sIgE of common food allergens is at least moderate or better. Of the analyses that reached statistical significance, SPT and sIgE results showed near perfect agreement for cashew and salmon; substantial agreement for pistachio, codfish, shimp and egg white; moderate agreement for peanut, walnut, pecan, lobster. The value of objective testing in the diagnosis of IgE mediated food allergy is belied by the fact that the history of the reaction was frequently unclear and therefore not helpful. In this population, there appears to be substantial barriers to performing oral food challenges as very few were done. Our data did not supply any particular reason for the low number of oral challenges performed at our site. In general, both patients and physicians contribute to the reluctance to perform oral challenge. While clinicians often have financial or logistical barriers, patients generally contend with fear of experiencing a reaction or the significant time a challenge requires.

In our analysis, a significant p value ($p < 0.05$) rejects the null hypothesis that the kappa analysis result was due to chance. Peanut, walnut, pistachio, cashew, pecan, salmon, shrimp, lobster and egg white analyses reached a significant p value. Hazelnut, almond, crab, clam, milk, soy, wheat, sesame did not reach a significant p value probably because of small sample sizes and therefore their kappa analyses cannot be interpreted.

There is a paucity of literature examining the concordance of SPT and sIgE in adult populations. Asha'ari et al. showed that when tested for a predetermined panel of food allergens including peanut, egg, flour and chicken without clinical correlation, the agreement of SPT and sIgE was between fair to good [5]. This is comparable to our study in that there were no instances of poor agreement. Our data demonstrated a relatively stronger agreement between the SPT and sIgE to common food allergens. This may be due to the fact that the study population was tested for foods to which there was a history of symptoms after ingestion. Given the targeted approach to selecting food allergens to test for, our study population was less likely to produce discordant SPT and sIgE results due to asymptomatic sensitization.

The major strength of this study is that it is reflective of the routine clinical practice. Our study population was specific to patients in whom food allergy was already suspected because a reaction had occurred: highly representative of daily practice. The food allergens they were tested for at our allergy clinic reflected their clinical history or concern, making the results clinically relevant.

There are several limitations to our study. Inevitably, there was some heterogeneity to the data because this was a retrospective analysis; we could only abstract the data available on the medical records. We could also not obtain oral food challenges to validate the diagnosis of food allergy. We did not aim to correlate the agreement or the individual test results with an OFC validated diagnosis and it is not possible to do so from our data. Additionally, not every patient underwent both SPT and sIgE testing to the same panel of allergens because the

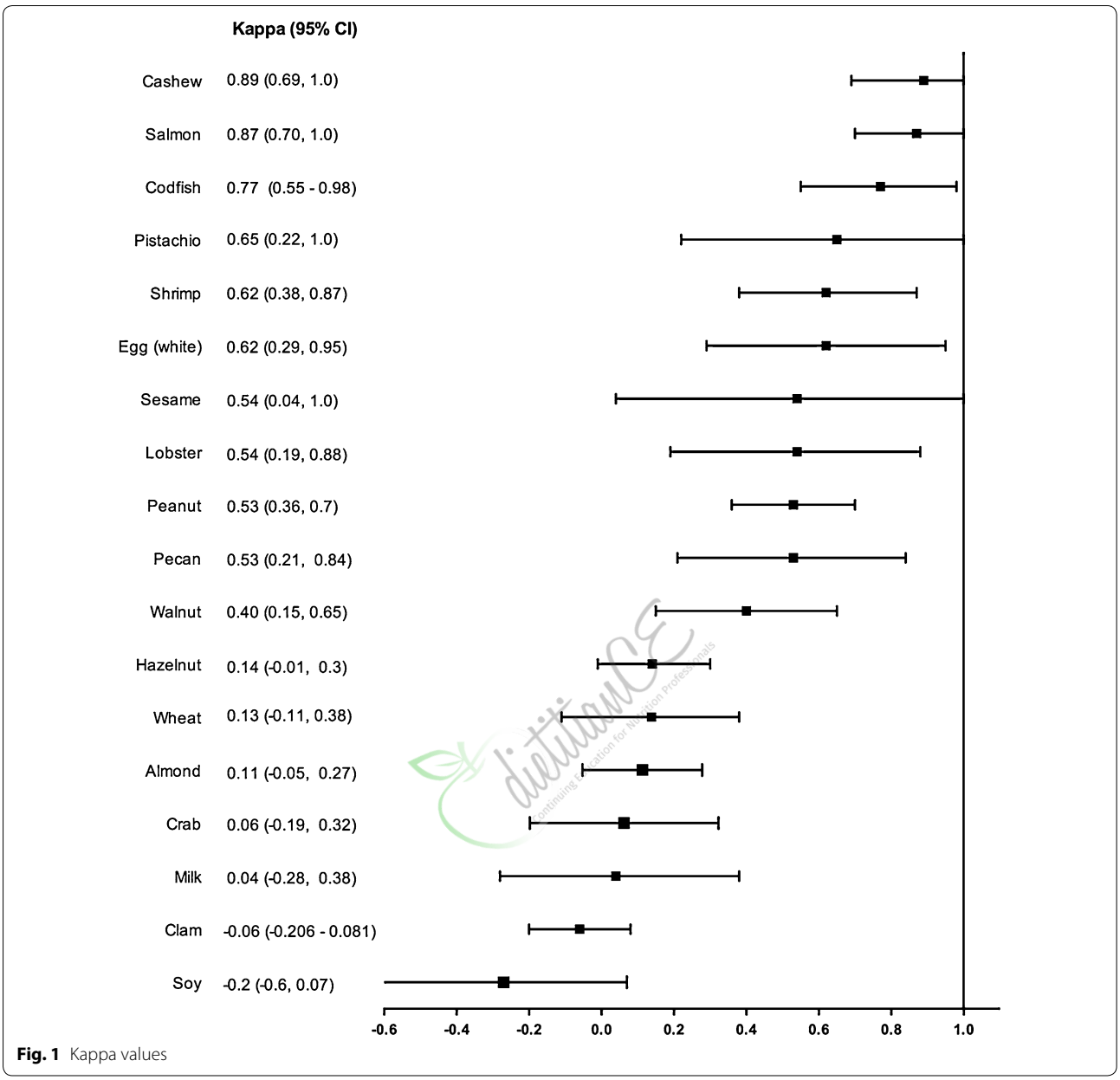

Fig. 1 Kappa values

referring physician had frequently ordered a broad panel of sIgE titres and at our allergy clinic a smaller selection of foods were skin prick tested. This reduced the number of patients who could be included in the kappa analysis and subsequent statistical analysis of several food allergens was not possible due to the limited sample size.

The literature in pediatric food allergy has established the utility of SPT and sIgE in predicting the result of oral food challenges and therefore their use in diagnosing food allergy. SPT wheal size and sIgE titre cutoffs that predict oral food challenge response have been characterized in children. This is so for peanut, fish, egg and milk while wheat and soy remain a challenge [9–12]. This advancement has likely led to greater roles of SPT and sIgE in diagnosing food allergy in children. In adults however, the utility and validity of sIgE is less well studied despite its frequent use. So far, there are no validated SPT or sIgE values that can predict a reaction on oral challenge test in adults as there are in children. It is further unclear whether the predictive values from the pediatric population can carry over to the adult population. The objective of our study was not to identify such cut offs, but the identification of these threshold values should be the direction of future research. The strength of the

agreement between SPT and sIgE for common food allergens demonstrated by our study represents the first step of characterizing the utility of these tests in adults with suspected food allergy.

The data from this study may serve to reassure clinicians when both testing modalities are not available concurrently that the results will likely agree and that these two tests are possibly interchangeable. It may be useful for non-allergists who evaluate patients with complaints suggestive of IgE mediated food allergy to obtain sIgE to the foods of concern. Therefore when a patients presents with a history strongly suggestive of IgE mediated food allergy and a positive sIgE to the food of concern, referring physicians will be in a more confident position to triage the patient to specialist care or to counsel the patient appropriately while waiting for a specialist's evaluation.

Our study also found a substantial minority of patients in whom a diagnosis of food allergy is still unclear after a thorough history and SPT or sIgE testing while the number of food challenges performed remained low. This finding should encourage allergists to use the oral challenge where appropriate and the provincial health services to remove any logistical or incentive barriers that discourage their use.

Conclusion

In adults presenting with a concern of food allergy, the history alone cannot provide enough information to suggest the presence or absence of IgE mediated food allergy the majority of the time. Skin prick test and sIgE agree at least moderately well or better for peanut, walnut, pistachio, cashew, pecan, salmon, shrimp, lobster and egg white. These results may provide reassurance to clinicians when only one testing modality is available that the SPT and sIgE have a reliable degree of agreement.

Reproducibility of serum IgE, Ara h2 skin prick testing and fraction of exhaled nitric oxide for predicting clinical peanut allergy in children

Abstract

Background: Ara h2 sIgE serum levels improve the diagnostic accuracy for predicting peanut allergy, but the use of Ara h2 purified protein as a skin prick test (SPT), has not been substantially evaluated. The fraction of exhaled nitric oxide (FeNO) shows promise as a novel biomarker of peanut allergy. Reproducibility of these measures has not been determined. The aim was to assess the accuracy and reproducibility (over a time-period of at least 12 months) of SPT to Ara h2 in comparison with four predictors of clinical peanut allergy (Peanut SPT, Ara h2 specific Immunoglobulin E (sIgE), Peanut sIgE and FeNO).

Methods: Twenty-seven children were recruited in a follow-up of a prospective cohort of fifty-six children at least 12 months after an open-labelled peanut food challenge. Their repeat assessment involved a questionnaire, SPT to peanut and Ara h2 purified protein, FeNO and sIgE to peanut and Ara h2 measurements.

Results: Ara h2 SPT was no worse in accuracy when compared with peanut SPT, FeNO, Ara h2 sIgE and peanut sIgE (AUC 0.908 compared with 0.887, 0.889, 0.935 and 0.804 respectively) for predicting allergic reaction at previous food challenge. SPT for peanut and Ara h2 demonstrated limited reproducibility (ICC = 0.51 and 0.44); while FeNO demonstrated good reproducibility (ICC = 0.73) and sIgE for peanut and Ara h2 were highly reproducible (ICC = 0.81 and 0.85).

Conclusions: In this population, Ara h2 SPT was no worse in accuracy when compared with current testing for the evaluation of clinical peanut allergy, but had—like peanut SPT—poor reproducibility. FeNO, peanut sIgE and Ara h2 sIgE were consistently reproducible despite an interval of at least 12 months between the repeated measurements.

Keywords: Peanut, Allergy, Anaphylaxis, Predict, Reproducibility, Ara h2, Skin prick test, Fraction exhaled nitric oxide, Peanut sIgE, Ara h2 sIgE

Background

Peanut allergy can be a life threatening event and accounts for approximately two-thirds of all fatal food-induced anaphylaxis [1]. In a recent Australian population based study [2], the prevalence of peanut sensitisation [by skin prick testing (SPT)] in infants was 6.4 %, with a prevalence of clinical allergy (confirmed by oral food challenge) of 2.9 %. Furthermore, clinical peanut allergy resolves in up to 20 % of children [3] but the processes involved in resolution are not fully understood [4].

Current testing to confirm sensitisation to peanut includes SPT to peanut protein and specific immunoglobulin E (sIgE) antibodies to peanut [5]. The gold standard for diagnosing clinical allergy is a double-blind placebo-controlled oral food challenge [6]. However, there is the associated risk of severe allergic reaction (including anaphylaxis), financial cost to health care (to provide beds and supervision), and finally time involved

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for patients, their families and health care professionals. To alleviate this, in clinical practice it is routine to conduct open-labelled food challenges [2, 7] and to exclude children at extremely low or high risk for clinical allergy from food challenge employing (a set of) non-invasive biomarkers. For instance, the resulting size of SPT wheal to whole peanut antigen or levels of sIgE antibodies to peanut is thought to correlate with an increasing likelihood of reaction [8, 9], but not with increasing severity of the reaction at food challenge [6]. While these tests confirm an allergy based on a significant clinical history, they do not suggest how severe the reaction will be on subsequent exposures. Monitoring these values over time may assist with identifying patients who are likely to outgrow their allergies (decreasing size of SPT wheal). However, as SPT is an operator driven test this type of deduction may at times be erroneous, placing children at risk of allergic reaction at a food challenge.

Prospectively measured levels of serum sIgE against the peanut component Ara h2 have been investigated and when used in combination with peanut SPT, found to improve the diagnostic accuracy and reduce the need for oral peanut challenge [5, 7]. Two studies published in 2007 [10, 11] have investigated the use of Ara h2 purified protein as a SPT reagent, but there has been no further published data reporting on its use.

Fraction of exhaled nitric oxide (FeNO) is a non-invasive marker that has been shown to correlate with allergic airways inflammation and IgE sensitisation [12]. Additionally, there appears to be enhanced prediction of peanut allergy prior to food challenge when combining measurement of FeNO with current testing (peanut SPT and Ara h2 sIgE) [7].

The primary aim of this study was to assess the accuracy of purified Ara h2 protein SPT in comparison with four predictors of peanut allergy (peanut SPT, peanut sIgE, Ara h2 sIgE and FeNO). The secondary aim was to assess the reproducibility (over a time-period of at least 12 months) of Ara h2 SPT, in comparison with the same four predictors of peanut allergy, by following-up a population of children studied previously [7].

Methods

Study population

Twenty-seven children were able to be recruited in follow-up from a cohort of fifty-six children enrolled in an earlier prospective study [7] that had involved children scheduled for open-labelled peanut food challenge by their paediatric allergist at a tertiary referral paediatric allergy centre in Newcastle, Australia. Their food challenge in the initial study had been scheduled to (1) confirm a peanut allergy diagnosis, (2) assess for the possibility of acquired tolerance, (3) test for clinical reactivity in children who had not consumed peanut but were sensitised, or (4) had significant parental concern and anxiety [7]. Participants were excluded from the earlier study if their SPT to whole peanut extract was ≥10 mm [7].

The original cohort (of fifty-six children) included thirty-two participants with a history of IgE mediated reactions, including anaphylaxis, not within the previous 12 months. Three participants who underwent food challenge in the original study had equivocal challenge results and were excluded from the data analysis.

Ethics and consent

The Hunter New England Health Human Research Ethics Committee approved both studies. Informed written consent was obtained from all parents or guardians prior to entry into the study, and from children as appropriate for their age. Participants from the earlier study were invited by telephone call from the Allergy Clinical Nurse Consultant to participate in this follow-up study.

Original cohort assessment

Prior to the food challenge, the original cohort (of fifty-six children) was assessed in a pre-challenge clinic. This clinic included assessing personal atopy and family history of atopy by way of a modified version of a previously validated parental questionnaire [13, 14]. Allergic rhinitis was assessed using paediatric validated allergic rhinitis and its impact on asthma (ARIA) criteria [15], where classification is according to symptom duration (intermittent or persistent) and severity (mild or moderate/severe). Each was then scored 1—intermittent mild, 2—intermittent moderate/severe, 3—persistent mild or 4—persistent moderate/severe. Eczema was assessed based on any previous medical diagnosis, and current "active treatment" including any current management other than emollients. Visible eczema was scored using the validated SCORing Atopic Dermatitis (SCORAD) system [16].

Patients in the original cohort then underwent SPT which was performed on the volar surface of the patient's forearm using standard whole peanut extract reagent, 1:10 w/v (Stallergenes, Antony, France). A positive result was ≥3 mm determined by averaging maximal perpendicular wheal diameters fifteen minutes after applying the lancet. Positive control was with histamine base, 6 mg/mL (Stallergenes, Antony, France) and with a wheal ≥3 mm indicating a valid test [17]. Negative control was glycerol saline. Later in the study, a small number were able to be skin prick tested with purified protein Ara h2 (100 μg/mL in glycerol saline solution). The Ara h2 protein was sourced commercially from Protein Labs, San Diego, California, where it was purified from peanut extract.

Serum was collected and analysed using ImmunoCAP 250 system (Phadia, AB, Uppsala, Sweden) for peanut sIgE and Ara h2-specific IgE.

FeNO was measured according to the American Thoracic Society and European Respiratory Society (ATS/ERS) guidelines [18]. Online single-breath analysis (ECOMEDICS, Duernten, Switzerland) was used with the requirement of an expiratory flow rate of 50 mL/s for a minimum of 2-s during at least a 4-s expiration time. A flow limiter maintaining constant minimum exhalation pressure of 5 cm H_2O prevented nasal nitric oxide (NO) measurement. Measurements were repeated until criteria were met (2 results within 5 % or 3 within 10 %) and the mean was recorded.

The open-labelled food challenge to peanut was conducted according to Australasian Society of Clinical Immunology and Allergy (ASCIA) food challenge protocol [19]. A medical officer, blinded to the results from the pre-challenge clinic, supervised all challenges. Challenges were declared successful if there was no reaction during the food challenge and throughout the following week with regular ingestion of peanut. Challenges were declared unsuccessful if they had (1) anaphylaxis [which was defined according to ASCIA guidelines [20] or (2) clinical allergy, not anaphylaxis (CANA) when they demonstrated an IgE mediated reaction consistent with published pre-defined objective criteria [21].

Follow-up cohort assessment

The follow-up study assessment was conducted over a six-month period and ranged from fifteen to thirty-two months after the individual participant's original assessment. The assessment was conducted in the paediatric outpatients department of a tertiary children's hospital and involved two stages—(1) undertaking the previously validated questionnaire [13, 14] (assessing the current degree of atopic disease and their family history of atopy) and —(2) SPT to whole peanut extract and purified Ara h2 protein, measuring FeNO, and blood collection to measure sIgE to peanut and Ara h2 (as described above).

Statistical methods

STATA 13.1 and GraphPad Prism 6.0 were used for statistical evaluation and graphical presentation. Participant clinical features are presented as medians with minimum and maximum values for continuous variables (due to non-normal distribution), and frequency and percentages for categorical variables. Differences in participant clinical features between groups (defined by the results of their open food challenge in the original study) were tested using Mann–Whitney two-tailed test for continuous variables and Fisher's exact test for categorical variables.

Receiver Operator Characteristic (ROC) curves were produced in STATA 13.1 and used to assess the ability of each measure in predicting an allergic reaction to peanuts. The area under the curve (AUC) is a summary measure of the sensitivity and specificity of the measure for all possible cut points.

Reproducibility was assessed using repeatability coefficient (C_R) (calculated using Bland–Altman test in GraphPad Prism) and the Intra-class Correlation Coefficient (ICC) was calculated using a one-way random effects model in STATA 13.1.

Results

Participant clinical features

The median time between the original and follow-up measurements was 2.2 years. The clinical features are outlined in Table 1. There were no significant differences when comparing the follow-up cohort with those who did not return for follow-up. (Additional file 1: Table S1).

Table 1 Participant clinical features of the follow-up cohort

		Follow-up (n = 26)
Age (years)	Median (min, max)	9.4 (4.1, 17.8)
Sex (%)	Males	18 (69)
Parental smokers (%)	Total	3 (12)
Previous adrenaline required (%)	Total	6 (23)
Other food allergy (%)	Total	11 (42)
Allergic rhinitis (%)	Total	17 (65)
AR severity for those with AR—max = 4[a]	Median (min, max)	4 (1, 4)
Eczema ever (%)	Total	22 (85)
Eczema active treatment (%)	Total	12 (46)
SCORAD for those with visible eczema	Median (min, max)	10.9 (3.0, 28.9)
Asthma ever (%)	Total	17 (65)
Current preventer (%)	Total	12 (46)
Current reliever (%)	Total	15 (58)
Anaphylaxis in challenge (%)	Total	5 (19)
CANA in challenge (%)	Total	9 (35)
No allergy in challenge (%)	Total	12 (46)
Ara h2 SPT (mm)	Median (min, max)	3.8 (0.0, 9.0)
Peanut SPT (mm)	Median (min, max)	6.3 (0.0, 13.0)
Ara h2 sIgE (kU/L)	Median (min, max)	0.66 (0.00, 22.10)
Peanut sIgE (kU/L)	Median (min, max)	0.99 (0.01, 35.60)
FeNO (p.p.b)[b]	Median (min, max)	24.3 (2.7, 119.2)

One patient had an equivocal result at challenge and was excluded from the analysis

AR allergic rhinitis; *SCORAD* SCORing Atopic Dermatitis; *CANA* clinical allergy not anaphylaxis; *SPT* skin prick test; *sIgE* serum-specific IgE; *FeNO* fraction of exhaled nitric oxide

[a] For determination of rhinitis severity, see "Methods" section

[b] Only 22 individuals in the follow-up cohort were able to perform FeNO

Clinical features of follow up cohort

In the follow-up cohort there were statistically significant differences between the successful [no clinical allergy (CA) at food challenge] and unsuccessful (CA at food challenge) groups in regards to age, male sex ratio, previous adrenaline usage, and current use of a preventer for asthma (Table 2). The patients without CA were younger (*P* value 0.015, Table 2). There were more males in the group of children without CA (*P* value 0.036, Table 2). Previous adrenaline usage in the CA group was higher (*P* value 0.017, Table 2). There were more participants currently using a preventer for asthma in the group of children without CA (*P* value 0.045, Table 2). As expected, children with CA did not have exposure to peanuts subsequent to the challenge test (<0.0001, Table 2). Interestingly, two children who were described as tolerant (no CA at food challenge) in the original study have subsequently developed symptoms of food allergy after eating peanut subsequent to the original study and as such now avoid eating peanut. All other clinical features across the two groups did not reach statistical significance for difference.

Data availability

Data for the peanut SPT were available for all twenty-seven individuals at the two time points. Only twelve individuals from the original group had data available for Ara h2 SPT, while twenty-seven individuals from the follow-up group had data available for Ara h2 SPT. Data for FeNO were available for twenty individuals at the two time points. Seven children had data missing from one or both time points due to being unable to perform single breath measurement of FeNO. Data were also available for peanut sIgE and Ara h2 sIgE for all twenty-seven individuals at the two time points. Due to an equivocal result in their challenge in the original study, one individual was excluded from the analysis of the follow-up cohort.

Accuracy of Ara h2 SPT at predicting clinical outcome

There was a statistically significant difference between groups for Ara h2 SPT wheal size (*P* value 0.0001, Table 2). This compared with peanut SPT (*P* value 0.0004, Table 2), Ara h2 sIgE (*P* value <0.0001, Table 2), peanut sIgE (*P* value 0.0073, Table 2) and FeNO (*P* value 0.0018, Table 2).

Table 2 Follow-up cohort – divided by clinical allergy or not at food challenge in original study

		No CA n = 12	CA n = 14	*P* value
Age (years)	Median (min, max)	6.8 (4.1, 15.9)	13.6 (4.5, 17.8)	*0.015*
Sex (%)	Males	11 (92)	7 (50)	*0.036*
Parental smokers (%)	Total	1 (8)	2 (14)	1.000
Previous adrenaline required (%)	Total	0 (0)	6 (43)	*0.017*
Other food allergy (%)	Total	6 (50)	5 (36)	0.692
AR (%)	Total	9 (75)	8 (57)	0.429
AR severity for those with AR—max = 4[a]	Median (min, max)	4 (1, 4)	3 (1, 4)	0.698
Eczema ever (%)	Total	12 (100)	10 (71)	0.478
Eczema active treatment (%)	Total	7 (58)	5 (36)	0.431
SCORAD for those with visible eczema	Median (min, max)	19.2 (3.0, 28.9)	7.4 (3.4, 24.4)	0.460
Asthma ever (%)	Total	9 (75)	8 (57)	0.429
Current preventer (%)	Total	8 (67)	3 (21)	*0.045*
Current reliever (%)	Total	8 (67)	7 (50)	0.453
Further exposure to peanut since challenge (%)	Total	12 (100 %)	0 (0 %)	*<0.0001*
Still eating peanuts at time of follow-up (%)[b]	Total	10 (83 %)	0 (0 %)	*<0.0001*
Ara h2 SPT (mm) (min, max)	Median (min, max)	2.3 (0.0, 5.0)	6.5 (2.0, 9.0)	*0.0001*
Peanut SPT (mm) (min, max)	Median (min, max)	4.0 (0.0, 8.5)	8.0 (5.0, 13.0)	*0.0004*
Ara h2 sIgE (kU/L)	Median (min, max)	0.08 (0.00, 4.79)	2.21 (0.41, 22.1)	*<0.0001*
Peanut sIgE (kU/L)	Median (min, max)	0.31 (0.01, 35.60)	2.84 (0.32, 23.3)	*0.0073*
FeNO (p.p.b)[c]	Median (min, max)	9.6 (2.7, 40.0)	42.1 (15.2, 119.2)	*0.0018*

One patient had equivocal result at food challenge and was therefore excluded from the analysis

Italics indicate statistical significance $P < 0.05$

AR allergic rhinitis; *No CA* no clinical allergy; *CA* clinical allergy; *SCORAD* SCORing Atopic Dermatitis; *CANA* clinical allergy not anaphylaxis; *SPT* skin prick test; *sIgE* serum-specific IgE; *FeNO* fraction of exhaled nitric oxide

[a] For determination of rhinitis severity, see "Methods" section

[b] Following the successful challenge, two children subsequently developed symptoms at home after eating peanut and now avoid eating peanut

[c] Only 9 individuals in the No CA group were able to perform FeNO, while 12 individuals in the CA group were able to perform FeNO

Clinical features of follow-up cohort stratified for severity of clinical allergy

In the follow-up cohort, when the CA group was divided into subgroups of children with anaphylaxis or clinical allergy not anaphylaxis (CANA) based upon the result of their food challenge, there were significant differences between the groups in regards to age and previous adrenaline usage (Table 3). Median age was 16.0 years in the anaphylaxis group compared to 10.9 years in the CANA group (*P* value 0.029, Table 3). Previous adrenaline usage in the anaphylaxis group was higher than the CANA group (*P* value 0.003, Table 3). All other clinical features across the two groups did not reach statistical significance for difference.

Accuracy of Ara h2 SPT at predicting severity of reaction at challenge

When stratifying the clinical allergy group by severity of reaction at challenge (CANA or anaphylaxis), Ara h2 SPT did not show ability to differentiate between the groups (*P* value 0.541, Table 3). This compared with peanut SPT, Ara h2 sIgE, peanut sIgE and FeNO (*P* values between 0.227 to 0.968).

Accuracy of Ara h2 SPT at predicting clinical outcome—Allergy

The AUC for Ara h2 SPT predicting allergy was 0.908, which compared with the AUC for peanut SPT, Ara h2 sIgE, peanut sIgE, and FeNO for predicting allergy (0.887, 0.935, 0.804 and 0.889 respectively, Fig. 1).

Accuracy of Ara h2 SPT at predicting clinical outcome—Anaphylaxis

The AUC for Ara h2 SPT predicting anaphylaxis was 0.738. This compared with the AUC for peanut SPT, Ara h2 sIgE, peanut sIgE and FeNO for predicting anaphylaxis (0.638, 0.857, 0.791 and 0.763 respectively. Figure 2).

Reproducibility

Reproducibility for Ara h2 SPT was limited [ICC = 0.44 and C_R value −2.0 (Table 4)]. Peanut SPT reproducibility was also limited [ICC = 0.51 and C_R value 0.85 (Table 4)]. Reproducibility was higher for FeNO [ICC = 0.73 and C_R value −8.2 (Table 4)]. Finally, reproducibility was highest for Ara h2 sIgE [ICC = 0.85 and C_R value 0.03 (Table 4)] and Peanut sIgE [ICC = 0.81 and C_R value −0.10 (Table 4)].

Table 3 Follow-up cohort – divided by severity of clinical allergy at food challenge in original study

		CANA (n = 9)	Anaphylaxis n = 5	*P* value
Age (years)	Median (min, max)	10.9 (4.5, 17.8)	16.0 (14.0, 17.4)	*0.029*
Sex (%)	Males	3 (33)	4 (80)	0.266
Parental smokers (%)	Total	2 (22)	0 (0)	0.506
Previous adrenaline required (%)	Total	1 (11)	5 (100)	*0.003*
Other food allergy (%)	Total	4 (44)	1 (20)	0.580
AR (%)	Total	5 (56)	3 (30)	1.000
AR severity for those with AR—max = 4[a]	Median (min, max)	4 (2, 4)	3 (1, 4)	0.750
Eczema ever (%)	Total	7 (78)	3 (60)	0.580
Eczema active treatment (%)	Total	4 (44)	1 (20)	0.580
SCORAD for those with visible eczema	Median (min, max)	7.4 (3.4, 24.4)	0.0 (0.0, 0.0)	N/A
Asthma ever (%)	Total	5 (56)	3 (60)	1.000
Current preventer (%)	Total	2 (22)	1 (20)	1.000
Current reliever (%)	Total	5 (56)	2 (40)	1.000
Ara h2 SPT (mm) (min, max)	Median (min, max)	7.0 (2.0, 8.0)	5.0 (3.5, 9.0)	0.541
Peanut SPT (mm) (min, max)	Median (min, max)	8.0 (5.5, 12.5)	6.0 (5.0, 13.0)	0.968
Ara h2 sIgE (kU/L)	Median (min, max)	1.15 (0.41, 14.50)	5.02 (0.80, 22.10)	0.227
Peanut sIgE (kU/L)	Median (min, max)	1.49 (0.32, 23.30)	3.88 (1.01, 21.80)	0.240
FeNO (p.p.b)[b]	Median (min, max)	28.3 (15.2, 119.2)	55.1 (15.4, 79.5)	0.631

Italics indicate statistical significance $P < 0.05$

AR allergic rhinitis; *SCORAD* SCORing Atopic Dermatitis; *CANA* clinical allergy not anaphylaxis; *SPT* skin prick test; *sIgE* serum-specific IgE; *FeNO* fraction of exhaled nitric oxide

[a] For determination of rhinitis severity, see "Methods" section

[b] Only 7 individuals in the CANA group were able to perform FeNO, while all 5 individuals in the anaphylaxis group were able to perform FeNO

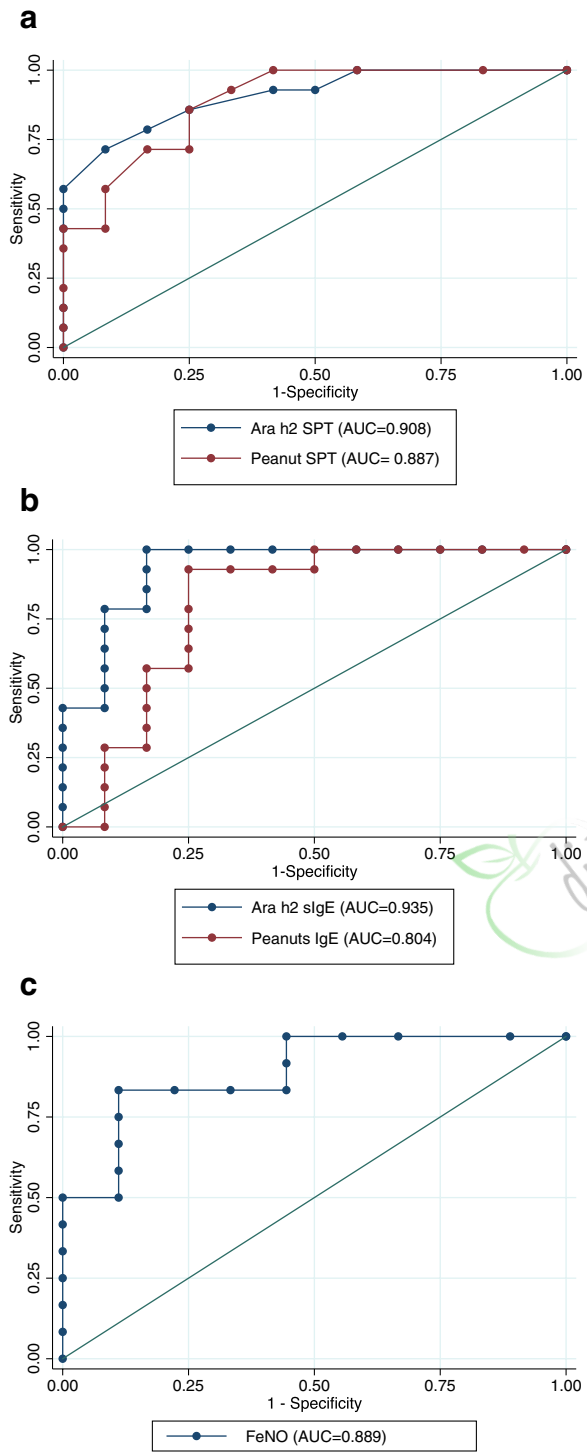

Fig. 1 ROC curves for predicting allergy. **a** Ara h2 SPT and Peanut SPT for predicting allergy, **b** Ara h2 sIgE and Peanut sIgE for predicting allergy, **c** FeNO predicting allergy

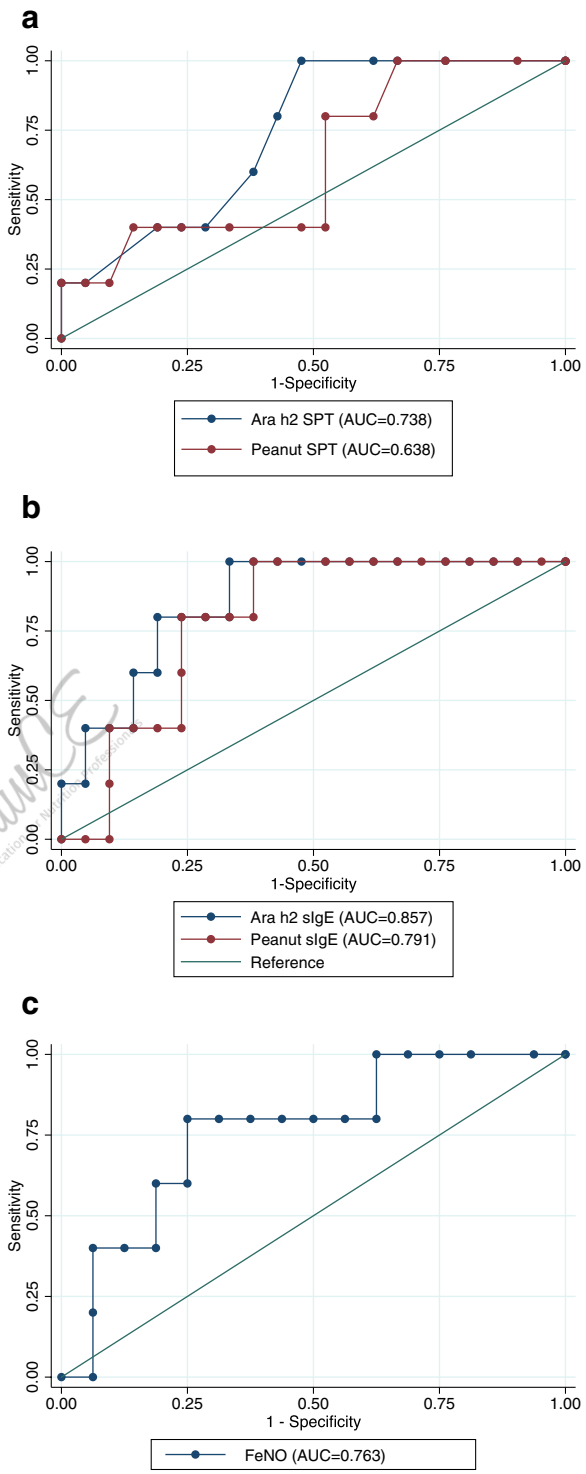

Fig. 2 ROC curves for predicting anaphylaxis. **a** Ara h2 SPT and Peanut SPT for predicting anaphylaxis, **b** Ara h2 sIgE and Peanut sIgE for predicting anaphylaxis, **c** FeNO predicting anaphylaxis

Table 4 Reproducibility of tests

Variables	Ara h2 SPT (n = 12)	Peanut SPT (n = 26)	FeNO (n = 19)	Ara h2 sIgE (n = 26)	Peanut sIgE (n = 26)
Subgroup assessment (median & min, max)	6.5 (0.0, 10.0)	6.3 (0.0, 9.0)	32.5 (4.6, 170.5)	0.35 (0.00, 17.90)	0.90 (0.01, 31.60)
Repeat assessment (median & min, max)	3.5 (0.0, 7.5)	6.3 (0.0, 13.0)	26.6 (5.2, 119.2)	0.66 (0.00, 22.10)	0.99 (0.01, 35.60)
P value	0.039	0.170	0.332	0.974	0.259
C_R value (95 % limits of agreement)	−2.0 (−8.0, 3.9)	0.85 (−4.9, 6.6)	−8.2 (−59, 42)	0.03 (−6.1, 6.2)	−0.10 (−10.9, 10.7)
ICC value (95 % CI)	0.44 (0.00, 0.90)	0.51 (0.23, 0.80)	0.73 (0.51, 0.94)	0.85 (0.75, 0.96)	0.81 (0.68, 0.95)

Discussion

This study demonstrates that Ara h2 SPT had similar accuracy to Peanut SPT, FeNO, Peanut sIgE, and Ara h2 sIgE at predicting allergic reaction at food challenge. While the accuracy of Ara h2 SPT was also similar with Peanut SPT, FeNO, Peanut sIgE and Ara h2 sIgE at predicting severity of reaction at food challenge, the AUC for that question is too low to be clinically useful. Larger population numbers would need to be studied to determine appropriate thresholds of Ara h2 SPT for diagnosis of peanut allergy or anaphylaxis.

To further clarify the utility of FeNO at predicting clinical allergy, we re-calculated the area under the ROC curve after excluding patients with a history of asthma but it remained unchanged (0.90). We acknowledge that this analysis is based on small numbers and future studies are required to confirm our observations.

Overall the accuracy of all tests for predicting allergic reaction at food challenge was higher than that previously reported [5, 7]. It is possible that a selection bias contributed to this result because only 48 % of the original cohort participated in the follow-up visit despite our very best recruitment efforts. Everyone who participated in the follow up study lived within 50 km of the research centre, while the original study included participants from up to 250 km away from the research centre providing some clue as why recruitment may have been less successful. It could be hypothesised that those with persisting allergy were more likely to return, as they are likely to have ongoing contact with the clinical team (some of whom were involved in the research) or having persisting allergy may make them more likely to contribute with the desire of improving diagnosis and management of peanut allergy. Thus in clinical practice all tests can be expected to have a lower predictive value than found in this study due to a regression to the mean phenomenon [22].

This study has demonstrated relatively poor reproducibility for both Ara h2 SPT and peanut SPT. This possibly relates to different operators performing the SPT in each cohort as would commonly happen in clinical practice. The ASCIA SPT manual highlights the likelihood of operator dependant technique significantly affecting SPT results [17]. Another possible cause for this poor reproducibility may be due to changes in SPT size related to further exposure to peanut, for instance by inclusion into regular diet or by accidental exposures. However, we did not observe such great variability in sIgE. Recent research on the natural history of SPT would suggest that those with persisting clinical allergy would have increasing SPT wheal size, while those with resolved clinical allergy would have decreased [23].

Despite the poor reproducibility of both Ara h2 SPT and peanut SPT, this study has demonstrated high levels of reproducibility for FeNO, and for Ara h2 sIgE and peanut sIgE serum levels. The excellent reproducibility of the sIgE results is likely contributed to by the lack of potential variation in operator technique affecting the serum sIgE result. A significant limitation remains the small population and that it may not be representative of the true value in the total population.

Two results of interest relate to the participants who appear to have lost tolerance to peanut after a successful challenge in the original study. While one participant's peanut and Ara h2 SPT and sIgE results have decreased compared to the original challenge, the other participant's peanut and Ara h2 SPT and sIgE results have increased. These seemingly contradictory results do not provide insight into why these two participants have developed symptoms of allergy after successful challenge (and therefore presumed tolerance) in the original study. While it is known that peanut sIgE tends to increase in children with persisting peanut allergy with repeated exposure to peanut [24], there is no published data of the natural history of peanut sIgE or Ara h2 sIgE results in children previously sensitised to peanut whose clinical allergy has resolved.

An obvious weakness of this study is the time that has passed since the food challenge with no repeat conducted on the second visit. Therefore we cannot be certain if each individual is still allergic based upon that original challenge. Those with unsuccessful challenges reported no further accidental exposures since the challenge, and all but two children who had successful challenges were continuing to eat peanut in their diet regularly. This

unfortunately does not help clarify their current allergy status. However, with no further clinical indication for food challenge arising in that period, we did not believe that a repeat food challenge at that time would have been ethically sound in this cohort of children.

Another limitation is the use of open challenges in the original study. While double-blind placebo-controlled food challenges are the gold standard for diagnosing food allergy [6], in clinical practice, open-labelled food challenges are routinely used [2, 7]. In the original study [7], to help minimise the chance of false positive results, outcomes were designated based upon pre-defined objective criteria [5, 25, 26], as participants were recruited sequentially from a list of children referred clinically for open-labelled food challenge at a tertiary referral paediatric allergy centre to alleviate the lack of a placebo-controlled challenge [7].

Conclusion

In summary, Ara h2 SPT was no worse in accuracy when compared with current testing for the evaluation of peanut allergy in this population of children. SPT with purified Ara h2 protein and peanut protein in this study demonstrate poor reproducibility and further studies could help determine inter-and intra-operator variability. FeNO demonstrated high accuracy and good reproducibility. Finally, peanut and Ara h2 sIgE collected in this study demonstrate high accuracy and excellent reproducibility over time, reaffirming the utility of these markers in assessing peanut allergy.

Abbreviations

ARIA: allergic rhinitis and its impact on asthma; ASCIA: Australasian Society of Clinical Immunology and Allergy; ATS/ERS: American Thoracic Society and European Respiratory Society; AUC: area under the curve; CA: clinical allergy; CANA: clinical allergy, not anaphylaxis; C_R: coefficient of repeatability; FeNO: fraction of exhaled nitric oxide; ICC: intra-class correlation coefficient; NO: nitric oxide; ROC: receiver operator characteristic; SCORAD: SCORing Atopic Dermatitis; sIgE: specific immunoglobulin E; SPT: skin prick test.

Peanut sensitization pattern in Norwegian children and adults with specific IgE to peanut show age related differences

Abstract

Background: Peanuts contain potent food allergens and the prevalence of allergy is reported to increase, especially in children. Since peanut sensitization may differ between different geographical regions, we wanted to investigate the sensitization pattern to the individual peanut allergens in a Norwegian population.

Methods: Cases reported to the Norwegian Food Allergy Register with sera positive to peanut extract were analyzed for specific IgE (sIgE) to the recombinant peanut allergens Ara h 1, Ara h 2, Ara h 3, Ara h 8 and Ara h 9 and to birch pollen extract. Serum samples negative to the above allergens were analyzed for sIgE to Ara h 6, and sIgE to Pru p 3 in peach were analyzed in sera positive to the cross-reactive allergen Ara h 9.

Results: Highest frequency of sIgE to Ara h 2, often co-sensitized to Ara h 1 and 3, were found in the small children up to 6 years of age. From the age of 6 years, sensitization to Ara h 8 was predominant. The sIgE levels to the storage proteins Ara h 1, 2 and 3 were strongly correlated, as was the sIgE levels to Ara h 8 and birch pollen extract. A low sensitization rate of sIgE to Ara h 9 in young adults was observed, which sIgE levels were very strongly correlated to Pru p 3.

Conclusion: The sensitization to peanut allergens in a Norwegian population shows a clear age dependent pattern. The results add to the previously published research on the sensitization patterns of peanut sensitized patients in different geographical areas.

Keywords: Peanut sensitization pattern, sIgE, Age related differences, Peanut allergens, Ara h 2, Ara h 8

Background

Peanut allergy represents a worldwide problem, it is often severe, potentially fatal and often persistent throughout life [1, 2]. The estimated prevalence of peanut allergy is between 0.5 and 2.0 % and appears to be increasing especially in children [3–5]. An accurate diagnosis of peanut allergy is essential since it may represent a significant burden on both quality of life and socio-economy [6]. Medically supervised oral food challenges (double-blind placebo-controlled food challenge, DBPCFC) are considered the gold standard for diagnosis but are resource-intensive and may be associated with risk of severe allergic reaction or anaphylaxis. In the last decades, however, several of the peanut allergens have been characterized, and analysis of specific IgE (sIgE) on a molecular basis has been evaluated as a diagnostic tool for peanut allergy [7–10].

The major peanut allergens Ara h 1, 2 and 3 belong to the seed storage proteins of the vicilin, conglutin and glycinin families, respectively, and are considered to be responsible for the original sensitization to peanut in susceptible individuals. The seed storage proteins are stable and associated with increased risk of severe reactions or anaphylaxis. The storage protein Ara h 6, a conglutin, has sequence identities to Ara h 2 and is also reported to be associated with clinical reactivity to peanut. [11]. The relationship between allergy to pollen and vegetables, nuts, peanuts and fruits is caused

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by cross-reacting epitopes due to homology between proteins and often give rise to milder symptoms such as the oral allergy syndrome. The peanut protein Ara h 8 is homologous to the birch pollen protein Bet v 1, and contributes to a substantial cross-reactivity between peanut and birch pollen [12]. Cross-reactivity between profilin in grass pollen and peanut may also occur [13]. The peanut allergen Ara h 9 is an enzyme-stable non-specific lipid transfer protein (LTP) with cross-reactive epitopes to other LTPs such as Pru p 3 in peach and Cor a 8 in hazelnut [14]. The protein Ara h 9 is reported to be an allergen of importance in the Mediterranean area that may cause systemic reactions in addition to oral allergy syndrome [15].

Recent studies have shown that peanut allergy in USA, Australia and different parts of Europe have different clinical and immunological patterns, due to differences in pollen exposures and differences in dietary traditions [13, 14, 16]. Since Norway is a birch endemic country and birch pollen gives rise to cross-reactions to peanut, we wanted to investigate the sensitization pattern to the individual peanut allergens in cases reported to the Norwegian National Reporting System and Register of Severe Allergic Reactions to Food (the Norwegian Food Allergy Register). The cases are submitted with serum samples routinely analyzed for a standard panel of allergen extracts [17]. All patients sensitized to peanut extract were analyzed for sIgE to the recombinant peanut allergens, in relation to age, gender, onset of reaction, symptoms and number of co-sensitizations to other foods and to birch pollen.

Methods

Patients

The Norwegian Food Allergy Register was established at the Norwegian Institute of Public Health in 2000 in collaboration with the Norwegian Food Safety Authority and the National Veterinary Institute [17]. Cases are reported on a voluntary basis by first-line doctors and submitted together with a serum sample. The reports contain patients' information such as a short case history including gender, age, the suspected or incriminating food, and onset of reaction, known allergies, symptoms and the medication given. A written consent form is signed by all patients. A total of 1250 sera submitted to the Food Allery Register, routinely analyzed for sIgE to a panel of food allergens including peanut, birch- and timothy pollen were screened for sIgE to peanut extract. Two hundred and fourteen sera had sIgE antibodies to peanut extract above the cut off value 0.35 kU/l and were included in the study. The 214 patients were equally distributed between genders, 101 females and 113 males, and comprised ages from <1 to 80 years.

Serological analysis

The patient sera were analyzed for sIgE using ImmunoCap® (Phadia AB, Uppsala, Sweden). Due to limited volume of serum available for some of the patients, specific IgE antibodies to the three storage proteins Ara h 1, Ara h 2 and Ara h 3 were analyzed in 192 patient sera. IgE reactivity to Ara h 8, Ara h 9 and to birch pollen extract were analyzed in all 214 sera. Sera with sIgE to Ara h 9 were analyzed for sIgE to the peach allergen Pru p 3 known to result in cross-reactions to the lipid transfer protein. Sera with sIgE antibody levels >0.35 kU/l were considered positive. Since ImmunoCap with Ara h 6 is not commercially available, sera negative to all the above peanut allergens were analyzed for sIgE to Ara h 6 by ImmunoSorbent Allergen bioChip assay, ISAC (Thermo Fisher Scientific, Oslo, Norway), reported in standard units (ISU). ISU >0.3 were considered positive.

Statistics

Pearson correlation was used to establish the strength of the relationship between sIgE antibody levels. Syntax of recoded combinations of sera with sIgE to the major allergens Ara 1, 2 and 3 made it possible to obtain the frequency of all combinations. Frequency analysis and plots of the collected data were made using the statistical programs IBM SPSS Statistics 22 and SigmaPlot 12.3.

Results

Gender, onset of reaction, symptoms and treatment in relation to age groups

Frequency analysis of the ages of the 214 patients sensitized to peanut showed four age groups (Fig. 1). As seen from the figure, the frequency of sensitization peaked

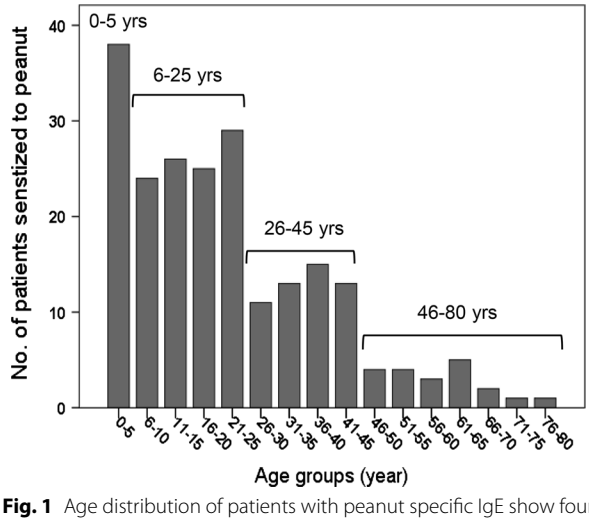

Fig. 1 Age distribution of patients with peanut specific IgE show four age groups; 0–5 years, 6–25 years, 26–45 years and 46–80 years of age

at ages 0–5 years, the second group comprised the ages from 6 to 25 years, the third group from 26 to 45 years and the lowest frequency of sensitization was seen for the ages 46 to 80 years. The gender distribution shifted from 76:24 % males:females in the small children's group to 23:77 % males:females in the oldest group (Table 1). The onset of reaction reported to occur within 1 h after intake of the suspected food was highest in the youngest group, 97 %, and decreased by age to 69 % in the oldest group (Table 1). The organ systems reported to be most often affected were symptoms in the skin (urticaria, and angioedema, sudden itching of eyes and nose), most often in combination with gastrointestinal tract (oral pruritus, lip swelling, abdominal pain, diarrhea, and vomiting) and/or respiratory symptoms (rhinorrhea, wheezing, chest tightness, cough, stridor, dyspnoe, and respiratory arrest). Patients reported to have symptoms affecting more than one organ system, which is related to high risk of severe reactions, were highest in the youngest age group, 76.7 %, declining with age to 54.5 % in the oldest age group (Table 1). The four age groups also differed in that severe skin symptoms were more often reported and loss of consciousness less reported in the youngest children. Cardiac arrest was not reported for the youngest and the oldest age groups. The therapeutic treatment employed was reported to be antihistamines alone (23.5 %), in combination with steroids (24.1 %) or most commonly in a combination of both epinephrine and steroids (32.1 %). Epinephrine alone and steroids alone was used in 14.2 and 6.2 % of the reported cases, respectively.

Specific IgE levels to the peanut allergens

Seventy-four (38.5 %) of the 192 patient sera analyzed had sIgE to the three seed storage proteins Ara h 1, 2, and 3 in different combinations and sIgE co-sensitized to all three proteins was seen in 36 (48.7 %) patient sera. The proteins Ara h 1 and Ara h 2 were co-sensitized in 51 (68.9 %) of the patients, Ara h 2 and Ara h 3 in 39 (52.7 %) patients and Ara h 1 and Ara h 3 were co-sensitized in 38 (51.4 %) patients. Seventeen patients were mono-sensitized to Ara h2, Ara h1 and Ara h3 in frequencies of 9 (12.2 %), 6 (8.1 %) and 2 (2.7 %), respectively. The IgE levels to all three recombinant allergens were strongly correlated ($r = 0.65–0.71$, $p < 0.01$).

One hundred and eight (50.5 %) of the 214 patients sensitized to peanut extract had sIgE to the birch pollen homologue Ara h 8 and were co-sensitized to the birch pollen extract in all but two patients. Their sIgE levels were strongly correlated ($r = 0.61$, $p < 0.01$). Thirty-seven (34 %) of the patients with sIgE to Ara h 8 were co-sensitized to the three peanut storage proteins in different combinations and showed no correlation with respect to sIgE levels.

Twenty-four (11 %) of the 214 patients, showed to be sensitized to the lipid transfer protein Ara h 9 with co-sensitization to Pru p 3 with similar sIgE levels. The sIgE levels to the two allergens were very strongly correlated ($r = 0.99$, $p < 0.01$). Thirteen (54.2 %) of these patients showed co-sensitization in different combinations to Ara h 1, 2, 3 and 8.

Thirty-five (16.3 %) patient sera had no sIgE to any of the above peanut allergens and were analyzed for sIgE to the Ara h 2 homologue Ara h 6. Four (11.4 %) patients had sIgE to Ara h 6 with ISU values characterized as low (0.3 ISU), moderate to high (4.0 ISU and 7.9 ISU) and very high (20.0 ISU).

Thirty-one patient sera were negative to all six peanut allergens and had low levels of sIgE to peanut extract. These sera were all from patients between the ages 0–10 years and showed to have sIgE to birch- and/or timothy pollen and/or to seeds and nuts indicating cross-reaction to peanut due to primary sensitization to pollen or to seeds or nuts. Sensitization to peanut due to cross-reactivity between 2S albumins in nuts like walnut, and sesame seeds has been reported [18].

Table 1 Gender ratio, onset of reaction within 1 h and symptoms affected in more than one organ system reported in the 214 patients in relation to the four age groups

Age group	Reported date			ImmunoCap® analysis		
Years	Gender ratio %	Onset of reaction %	Symptoms %	Ara h 2 %	Ara h 8 %	Birch pollen %
	Male:female	<1 h	>1 organ affected	sIgE >2.0 kU/l[b]	sIgE kU/l	sIgE kU/l
0–5 (N[a] = 43)	76: 24	97	76.7	51.2	23.3	41.9
6–25 (N = 96)	49: 47	80	72.0	31.3	56.3	83.3
26–45 (N = 53)	26.5: 73.5	90	58.5	15.0	54.7	79.2
46–80 (N = 22)	23: 77	69	54.5	0.0	68.2	86.4

The patients sera analyzed for sIgE to Ara h 2 with levels >2.0 kU/l, sIgE to the birch pollen homologue Ara h 8 and birch pollen extract is also shown

[a] Number of patient sera

[b] Sera with sIgE to Ara h 2/6 >2.0 kU/l (marker for clinical allergy)

Age related IgE profiles to peanut allergens

Specific IgE sensitization to the individual peanut allergens differed between the four age groups. Sensitization to the major storage proteins was highest in the youngest age groups and lowest in the oldest age group. The decrease according to age was especially marked with respect to Ara h 2 (Fig. 2). The youngest children (0–5 years) were most frequently sensitized to the seed storage protein Ara h 2 (58.0 %), but were frequently co-sensitized to Ara h 1 (44 %) and Ara h 3 (27 %) in different combinations. The four sera with sIgE to Ara h 6 were all from patients in the small children's group. Sensitization to the lipid transfer protein Ara h 9 was seen in all age groups (only one positive in the children's group) but most frequently among the young adults (26–45 years) (Fig. 2). The birch pollen homologue Ara h 8 increased markedly in frequency from 23.3 % in the youngest children to 56.3 % at the age of 6 years and was found to be 68.2 % in oldest age group. Similarly, sensitization to birch pollen showed a marked increase in frequency of sensitization from 41.9 % in the youngest children to 83.3 % at the age of 6 and showed similar high frequencies in the two older age groups (86.4 %) (Fig. 2). A level of sIgE to Ara h 2 > 2.0 kU/l, considered to be diagnostic for clinical peanut allergy [19], was found in 51.2 % of the sera from patients in the youngest age group. Sera with levels above this value decreased with age to 31.3 % in the second age group, 15 % in the third age group to none in the oldest age group (Table 1).

Co-sensitizations, to 1, 2 or 3 other food allergens or to more than 3 food allergens were equally common in all age groups. The most common sensitizations to allergens other than peanut were to other legumes, celery, wheat, seeds and tree nuts.

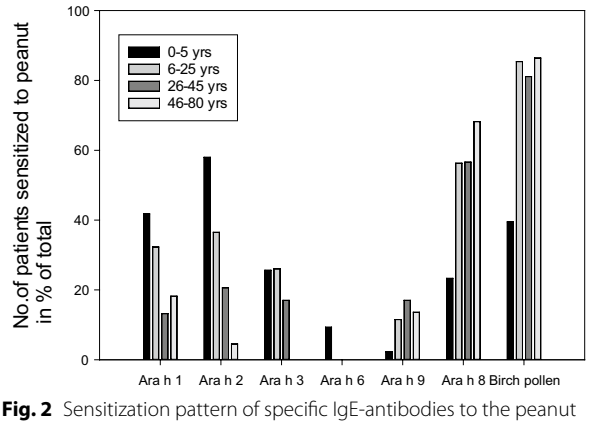

Fig. 2 Sensitization pattern of specific IgE-antibodies to the peanut allergens and birch pollen extract in the four age groups showing highest frequency of sensitization to Ara h 2 in the youngest age groups, and highest frequency of sensitization to Ara h8 from the age of 6 years and in the older age groups

Discussion

The pattern of sensitization to the six individual peanut allergens Ara h 1, 2, 3, 6, 8 and 9 was evaluated in patients reported to the Norwegian Food Allergy Register. Frequency analysis of the ages of the 214 patients sensitized to peanut showed four age groups; 0–5, 6–25, 26–45 and 46–80 years (Fig. 1). The sensitization pattern showed highest frequency of sIgE to the major peanut allergens Ara h 2/6, 1, and 3 in the youngest age group (58.5 %) and lowest frequency in the oldest age group (4.5 %), as opposed to the birch pollen homologue Ara h 8 which showed the highest frequency of sensitization in the oldest age group (68.2 %) and lowest frequency of sensitization in the youngest age group (23.3 %) (Fig. 2; Table 1).

Similar changes in gender distribution by age, as presently observed, from 76:24 % male:female in the youngest age group to the opposite ratio in the oldest group, has been reported for both asthma and allergy and is explained by hormonal changes, genetic susceptibility and differences in environmental exposure. The early onset of sensitization to the major peanut allergens and the early onset from intake of food to elicitation of symptoms in the children's group, together with high incident of more than one organ system affected, indicate that the reactions were severe in these patients. Although the medical treatment of allergic reactions will vary between the individual doctors, all patients were treated with antihistamines, epinephrine and steroids or a combination of the three, indicating that the reactions were considered to be severe. Severity, however, based on the reports of symptoms, and the medication given is difficult to measure since it will depend on at which time course of reaction the patients were treated. An early onset of effective treatment will always be aimed at to avoid the most severe reactions which may explain why loss of consciousness and cardiac arrest was seldom reported.

The results showed the highest frequency of sensitization to Ara h 2, less to Ara h 1 and to a much less extent to Ara h 3 in the youngest children. Ara h 2 and Ara h 6 have been found to account for the majority of the effector activity in crude peanut extract [20], with equal diagnostic value [21] and hence, to be more potent than Ara h 1 and Ara h 3 [22]. However, co-sensitization to all 3 allergens has been shown to be correlated to severity of symptoms [23]. In the present study, sIgE to Ara h 6 was detected in four sera from the youngest children. Sensitization to Ara h 6 without concomitant sensitization to Ara h 2 was also reported in a Swedish study to be responsible for severe reactions [24]. In two of the present cases, sIgE levels to Ara h 6 were high (20.0 and 7.9 ISU) and the patients were reported to react with acute anaphylactic reaction after intake of one peanut. In the other two cases, however, with low sIgE levels to Ara h 6 (ISU 0.3 and 4.0),

high levels of sIgE to cashew nut was detected. This may indicate primary sensitization to cashew nut with cross-reactivity to Ara h 6 due to sequence identity between storage proteins. The early onset of severe peanut allergy in children found in the present study is in line with findings in other population studies in children [7, 25, 26]. Further, in the studies comparing immunological differences among patients of different ages and in different geographical regions, early onset of sIgE to the three allergens Ara h 1, 2 and 3 were also reported, often presented with severe symptoms [13, 14, 16]. One may speculate if the high sensitization rate to the major peanut allergens in the youngest children is due to dietary changes with an increase in the overall use of peanuts in foods and as snacks over the last decades and/or as Ballmer-Weber et al. [16] speculates, an increase due to an intestinal permeability in genetically predisposed children. A recent study [27], however, showed that delayed oral exposure to peanut was associated with a greater frequency of clinical peanut allergy and hence may be responsible for the increased prevalence in this age group.

A study from Italy [28] reported no differences in sensitization among ages up to 16 years for the major peanut allergens, but reported increased levels of sIgE to Ara h 8 according to age, as found in the present study. The high correlation of sIgE levels to birch pollen extract and Ara h 8 may suggest primary pollen sensitization with following cross-reaction to Ara h 8, and possibly to other labile PR-10 proteins homologous to Bet v 1. The increase, however, in sensitization to birch pollen and Ara h 8 observed at the early age of 6 years, may, in part, be due to a milder climate and thereby longer pollen season [29]. Even if cross-reactions in general are considered to give milder reactions than sensitization to the major, stable allergens, the symptoms may have been experienced as severe and treated and reported as such. Thirty-seven (34 %) of the patients, however, with sIgE to Ara h 8 were co-sensitized to the three peanut storage proteins in different combinations, and may in these cases have been responsible for the severe reactions reported. Reactions caused by cross-sensitizations or co-sensitization to other food allergens than peanut cannot be ruled out.

All sera with sIgE to the lipid transfer protein Ara h 9, also had sIgE to Pru p 3 with similar sIgE levels and half of these patients were co-sensitized in different combinations to Ara h 1, 2, 3 and 8. All sera were in addition co-sensitized to other foods and often to hazelnut. The severe symptoms reported may, therefore, have been caused by sensitization to the major peanut allergens or by cross-reactions to LTP in hazelnut rather than to Ara h 9. The diagnostic value of Ara h 9 is said to be poor [19] and the clinical relevance of sensitization to Ara h 9 is difficult to interpret.

Various thresholds for sIgE to Ara h 2 have been suggested to predict clinically relevant peanut allergy but regional differences in addition to large individual variations make extrapolations between studies difficult [16]. The use of recombinant allergens, therefore, may be useful to distinguish patients with high risk of severe symptoms from those with less severe symptoms but cannot still replace oral challenges in determining thresholds and severity. Although the cases reported in the present study were submitted by first-line doctors who considered the reactions as being severe, the weaknesses of the study are that the results are based on cases with reported symptoms and serological analysis not verified by oral challenges. Hence, the overall information given including the severity of symptoms may have been biased by the reporting habits of the doctors. Further, the volume of the serum sample submitted, were in some cases small which limited the number of analysis. Still, the results from the submitted reports and the present analyses of peanut allergens in sensitized subjects, contribute to the information on peanut sensitization patterns in different populations.

Conclusion

Component based analysis of peanut in patient sera from cases reported to the Norwegian Food Allergy Register sensitized to peanut, demonstrate a clear age dependent pattern. The early onset of sensitization to the main allergens Ara 1, 2 and 3 found in the children below the age of 6 years, showed highest frequency of sIgE to Ara h 2, indicating the importance of using Ara h 2 in diagnosing small children sensitized to peanut. The early debut of pollen sensitization, may be caused by warmer climate and longer pollen season and suggest a majority of primary sensitization to birch pollen from the age of 6 years, with following cross-sensitization to the birch pollen homologue Ara h 8 in peanut.

Comparison of ImmunoCAP and Immulite serum specific IgE assays for the assessment of egg allergy

Abstract

Egg specific IgE levels are frequently used in combination with skin-prick tests to guide clinical decisions and to monitor egg allergy evolution in children. We compared both Immulite and ImmunoCAP egg specific IgE assays in egg allergic children, and found a linear correlation between both assays with a mean Immulite:ImmunoCAP ratio of 3. This is relevant information for clinicans wishing to estimate values from one assay to the other, as most literature has been published using the ImmunoCAP system.

Keywords: Egg allergy, Food allergy, Immulite, ImmunoCAP, UniCAP, Specific IgE assays

Findings

Egg allergy represents one of the most common food allergies encountered in pediatric practice, with an estimated prevalence of 0.5–2 % in infants and young children [1]. Many egg allergic children are able to tolerate baked eggs [2], which can greatly improve quality of life. Egg specific IgE levels are frequently used in combination with skin-prick tests (SPT) to guide clinical decisions and to monitor egg allergy evolution in children. Most reports on egg allergy have been using the ImmunoCAP (Phadia AB, Uppsala, Sweden) assay [3–5], which is a problem for the fraction of clinicians who do not have access to it, as their lab works with Immulite (Siemens Healthcare Diagnostics, Tarrytown, New York) for technical or administrative reasons. In this context, clinical literature is challenging to interpret and implement in practice. Some would recommend simply not using this ImmunoCAP literature, but this would mean depriving patients from useful information to guide management and therapy. Interestingly, it has been suggested that although egg-specific IgE results from either assays cannot be substituted [6], they may be adapted so that the results may still be used to guide management [7, 8].

The objective of this study was to directly compare Immulite and ImmunoCAP egg white-specific IgE assays and to determine whether their measurements can be applied equivalently and/or adapted to guide clinical management of egg allergic children.

Briefly, 37 egg allergic patients between 2 and 13 years of age were enrolled at Sainte-Justine University Hospital Center (Montreal, Canada) from July 2013 to January 2014. Patients with egg allergy had either a positive OFC or a history of at least one sign or symptom of allergy (ocular, respiratory, gastrointestinal, or cardiovascular) occurring within 1 h of egg ingestion and persistent sensitization at time of evaluation confirmed by a positive egg white skin prick test (3 mm greater than control), and either ImmunoCAP specific IgE levels $\geq$0.35 kU/L or Immulite specific IgE levels $\geq$0.1 kU/L. The project was approved by the ethics committee of Sainte-Justine University Hospital Center.

Patients' serum was aliquoted into two separate samples and sent on dry ice for analysis at the laboratories of the University of Montreal Hospital Center (CHUM) and Sainte-Justine University Hospital Center, each using a different specific IgE assay system:

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ImmunoCAP Phadia 250 and Siemens DPC Immulite 2000.

Descriptive analysis consisted of medians and range. Immulite and ImmunoCAP values were compared using Pearson's correlation (GraphPad Prism 6, San Diego, CA).

The median age of patients was 6.5 years (range, 2–13) and the median age at first reaction to eggs was 12 months (range, 4–96). The age of worst reaction was a median of 5.5 years before testing. Eighteen patients (49 %) had a history of anaphylactic reactions to eggs and 10 (27 %) tolerated baked eggs, while most of the remainder had never ingested baked egg before. Median egg white skin prick test diameter at time of specific IgE measurement was 10 mm (range, 3–25 mm).

In the whole cohort, Immulite median egg white-specific IgE levels was 24.80 [range, 0.72–100] kU/L compared to 6.45 [range, 0.33–100] kU/L for ImmunoCAP. In the subgroup tolerating baked eggs (n = 10), median egg white-specific IgE levels was 5.2 [range, 1.13–28.1] kU/L using Immulite and 3.17 [range, 0.38–8.93] kU/L using ImmunoCAP. When examining the subgroup of patients with anaphylactic reactions to eggs (n = 18), the median egg white-specific IgE levels using Immulite was 17.4 [range, 0.715–100] kU/L compared to 5.90 [range, 0.33–100] kU/L for ImmunoCAP.

Thus, as previously suggested, Immulite and ImmunoCAP egg-specific IgE values could not be substituted [6]. However, values were highly correlated (Pearson correlation factor of 0.864; Fig. 1) with egg white-specific IgE levels measured by Immulite a mean of 3.02 (± 0.44) times higher than when measured by ImmunoCAP(n = 29, ImmunoCAP values ≤30 kU/L). This correlation ratio was lost with ImmunoCAP values higher than 30 kU/L, which likely reflects the fact that values higher than 100 kU/L for Immulite exceeded the top point of the calibration curve. One can expect that diluting the samples with an appropriate serum diluent and reanalyzing the samples would have preserved this linear correlation [8]. Interestingly, the ImmunoCAP:Immulite ratio was of 1.64 in the subgroup tolerating baked eggs (n = 10). The significance of this lower ratio is difficult to interpret due to the small n in this sub-group. A possibility is that individual allergen components (i.e., ovomucoid vs ovalbumin) may be measured differently in each assay, which would become apparent when comparing subgroups with different sensitivity profiles (baked good tolerant vs allergic).

These observations are in line with previous studies. Wang et al. found an Immulite:ImmunoCAP ratio of 3.7 for egg-white specific IgE in 50 atopic patients [6]. Although the correlation coefficient was not included, qualitatively it appeared to be very high. Another study

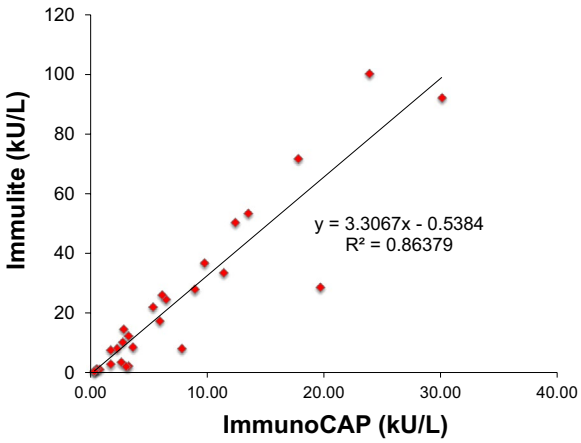

Fig. 1 Correlation plot of egg white specific IgE levels as measured with ImmunoCAP® and Immulite®

from South Korea evaluated atopic patients 1–75 years of age and found a very similar Pearson's correlation coefficient of 0.845 for egg white-specific IgE when comparing both assays [7]. Hamilton et al. [8] also found a mean Immulite:ImmunoCAP ratio of 4.85 and a high coefficient of determination of 0.95 in children aged 1–16 with a history of egg allergy (no skin prick tests or challenge), which is comparable to our results.

In conclusion, because of variability between Immulite and ImmunoCAP specific IgE assays, it is preferable to use a single assay to monitor the evolution of egg allergy and to assess the development of tolerance. This said, a linear correlation does exist between both assays, as has been observed in four independent cohorts including ours. Therefore in the absence of access to ImmunoCAP, a factor of 3–5 could be applied to egg-specific IgE published thresholds to guide clinical decisions. Although imperfect, this approach remains in our opinion preferable to withholding useful clinical information from patients and clinicians.

Abbreviations

SPT: skin-prick test; OFC: oral food challenge.

Role of specific IgE to β-lactoglobulin in the gastrointestinal phenotype of cow's milk allergy

Abstract

Rationale: The prevalence of many phenotypes of food allergy is increasing. Specific gastrointestinal (GI) phenotype of food allergy (GI allergy) is also increasing but it is difficult to know the prevalence because of many entities.

Methods and Results: A 1 year retrospective study of pediatric patients complaining exclusively gastrointestinal symptoms after cow's milk consumption and at least one positive specific IgE (sIgE) to cow's milk (CM) proteins (CMP) was done (n = 39). The most prevalent symptom was abdominal cramps in 35 patients (90 %), discomfort or abdominal distention in 30 patients (75 %), diarrhea in 10 patients (25 %) and constipation in 5 patients (12 %). IgA anti-transglutaminase antibodies were absent and lactose intolerance was ruled out in all patients. Average of total IgE on this group was 288 UI/ml. sIgE against β-lactoglobulin was the dominant with an average of 4.14 kU/l. sIgE to casein (CAS), which is the dominant protein in systemic anaphylaxis was 1.74 kU/l; sIgE to α-lactoalbumin, the other whey protein, was 0.83 kU/l and sIgE levels to CM were 0.78 kU/l. The quotient sIgE CAS/sIgE β-lactoglobulin in these patients was always lower than 1. Patients experienced an improvement of their symptoms after a CM free diet. An open oral challenge with CM did mimic their initial symptoms in all patients. However, the open oral challenge with dairy products was well tolerated.

Conclusions: Patients with a specific phenotype of GI allergy with CM have specific IgE against β-lactoglobulin, as a dominant sIgE. These patients could beneficiate of a diet with dairy products.

Keywords: Cow's milk allergy, Food allergy, Gastrointestinal allergy, Specific IgE, β-lactoglobulin, Casein, Anaphylaxis, IgE-mediated allergy

Findings

Food allergy is currently recognized as a rising problem worldwide. Specific gastrointestinal (GI) food allergy (GI allergy) is also increasing [1] although it is difficult to know the real prevalence because some entities may overlap and many unclear mechanisms are probably involved [1–3]. GI Allergy could be classified into three types according to the implication of immunoglobulin E (IgE) in their pathogenesis, i.e. classical "IgE-mediated entity"; a "combined IgE- and cell-mediated" type and finally, a "cell-mediated/non-IgE-mediated", consistent with several conditions such as food intolerance or food protein-induced enterocolitis syndrome. Interestingly, those patients afflicted with the GI allergy phenotype develop selective gastrointestinal symptoms exclusively—in contrast to systemic anaphylaxis—hours after the ingestion of offending foods [1, 4]; they probably belong to the cell-mediated/non-IgE-mediated or combined IgE- and cell-mediated food allergy types which are generally diagnosed on the basis of the clinical manifestations [1, 4, 5]. The final pathogenic mechanisms of GI allergy have not been elucidated yet since the diagnostic approaches are not clear and no consensus has been reached either.

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Methods

We performed a 1-year observational retrospective study of patients with selective GI symptoms—i.e. discomfort, abdominal distention, abdominal cramps, flatulence, nausea, vomiting, constipation or intermittent diarrhea only over 30 min after the intake of a glass of cow's milk. The inclusion criteria also comprise a positive (>0.1 kU/l) serum specific IgE (sIgE) to cow's milk proteins (CMP) such as casein (CAS), and the main whey proteins such as α-lactoalbumin (ALA) and β-lactoglobulin (BLG). Those patients with confirmed extra-intestinal-cutaneous, ocu-lar, respiratory and/or cardiovascular-symptoms imme-diately (less than 30 min) occurring after a glass of cow's milk (CM) were excluded of the study group. Patients with food protein-induced enterocolitis syndrome (FPIES) were also excluded A diagnosis of celiac disease by and a diagnosis of lactose intolerance were also con-sidered as exclusion criteria. Two different groups of subjects were used as control groups, both with mark-edly different clinical features (a healthy control group without GI CMP reactions and a CMP anaphylaxis) were compared to the study group.

Skin prick tests (SPT) with commercial extracts of CMP were performed (IPI; Stallergenes, Spain). Measurement of the total concentration of IgE (tIgE) in each serum was obtained by enzime-immunoassay (ImmunoCAP, Phadia AB, Uppsala, Sweden). Specific IgE (sIgE) against whole CM, CAS, ALA and BLG were also measured by the (ImmunoCAP) with a detection limit of 0.1 kIU/L. All patients experienced an open challenge test with cow's milk. Celiac disease and lactose intolerance was ruled out by absence of IgA anti-transglutaminase antibodies [6] by enzyme-linked immunoassay (EliA Celikey IgA, Phadia AB, Uppsala, Sweden) and by hydrogen breath test, respectively. The protocol was approved by the Regional Ethics Committee. Statistical analysis

considered significant differences when $p \leq 0.05$ (Mann–Whitney U test and Kruskall–Wallis test).

Results

A total of 336 subjects referring food reactions, out of 1344 pediatric patients were seen in the Outpatient Office during the 12-months study period. Thirty nine subjects (11 %) referred gastrointestinal symptoms exclusively -no other complain at least 30 min after CM consumption- and showed at least a positive sIgE to CMP. Median of age was 5 years–o and 55 % were girls. The most prevalent symptom was abdominal cramps in 35 patients (90 %), followed by discomfort or abdominal distention in 30 patients (75 %), diarrhea in 10 patients (25 %) and constipation in 5 patients (12 %).

SPT with commercial extracts to CMP were positive in only 40 % (16 patients). IgA anti-transglutaminase antibodies were absent in all patients [6]. Average of tgE on this group was 288 UI/ml (median 144 UI/ml), while sIgE against BLG was dominant among the CMP sIgE profile with an average of 4.14 kU/l (median 1.67 kU/l; range 0,21–33) (Table 1). The other two proteins detected were casein, which is the dominant protein in systemic anaphylaxis [7] with an average of 1.74 kU/l (median 0.18 kU/l) and another whey protein, ALA, with average of 0.83 kU/l (median 0.23 kU/l). Average of sIgE levels to CM were 0.78 kU/l (median 0.27 kU/l). The quotient sIgE casein/sIgE BLG in this selected population was always lower than 1 (average 0.3). The healthy control group without GI CMP reactions had levels of sIgE <0.1 kU/l with every single CM protein.

All patients experienced a clinical improvement of their symptoms after the implementation of CM free diet. The clinical improvement was defined as that gastrointestinal discomfort referred in history was completely yield. A new open challenge with a glass of CM reproduced the initial symptoms in all patients. A subsequent open oral challenge with dairy products (one yogurt) was well tolerated in all patients. Additionally these patients maintained taking dairy products on a daily basis diet (fermented such as yogurt or cheese, and baked such as custard).

Discussion

Bovine BLG, the major whey protein of cow's milk (~3 g/l), is a small protein, with an 18.4 kDa molecular weight. Unlike the other main whey protein, no clear function has been identified to β-lactalbumin, although a role in the transport of some hydrophobic molecules [8] has been suggested. Among bovine whey proteins, BLG is also a known prevalent allergen, as listed in Annex IIIa of Directive 2000/13/EC and demonstrated by both SPT and oral challenges [9].

The patients with the phenotype of GI allergy have shown low, but positive levels of sIgE against BLG. Patients with this specific phenotype are not susceptible to follow regular CMP desensitization protocols [7, 10], which it has been a hot topic in recent years. The current CMP desensitization protocols have been specifically designed for those patients with the classical anaphylaxis phenotype of IgE-mediated reactions [7], although no guidelines have been developed to determine whose patients may benefit. Interestingly, the quotient sIgE CAS/sIgE BLG in the patients with the GI allergy phenotype was always lower than 1 (average 0.3) compared to those subjects with anaphylaxis, with a quotient higher than 1 [7], given that their dominant allergenic CP is casein (Table 1).

Remarkably 10 patients had levels of sIgE against CM lower than 0.1 kU/l and additionally 12 more subjects

Table 1 Figures of levels of specific IgE against some cow's milk protein fractions in the G-I group and Anaphylaxis group

	GI allergy n = 39			Anaphylaxis n = 20			Significance
	Average	Range	Median	Average	Range	Median	p
Total IgE (IU/ml)	288	16–2360	144	457	295–2500	360	<0.01
Allergen specific IgE (kU/l)							
Whole cow's milk	0.74	0–2.48	0.26	189	30–586	146	<0.01
Casein	1.74	0–4.8	0.18	178	26–544	90	<0.01
α-lactoalbumin	0.83	0–3.7	0.23	21	9–34	21	<0.01
β-lactoglobulin	4.14	0.10–33.4	1.67	16.2	2.1–38	7.25	<0.01
Ratio casein/β-lactoglobulin	0.30	0.08–0.99	0.22	20	2.1–99	9.5	<0.01

showed levels between 0.1 and 0.35 kU/l, which has been the traditional cutoff of many immunoassays of specific IgE. This data is interesting, since many of these patients, with sIgE to CM lower than 0.35 kU/l may be under-diagnosed in the past. The current limit of detection, as low as 0.1 kU/l, for sIgE might help to discriminate among different clinical profiles [11] of allergy. Similarly, ten patients with levels of sIgE against CM lower than 0.1 kU/l, had levels of sIgE to BLG higher than 0.1 kU/l. With these findings, we believe that the determination of several proteins including CAS, ALA and BLG, in addition to specific IgE to CM, should be mandatory. The new detection limits of 0.1 kU/l should always be also recommended. It was only noticed that in these subjects with this phenotype of patients, the sensitivity of sIgE quite higher than the SPT with commercial CM extracts, detecting only 40 % of patients with a positive SPT.

A diet including dairy products but free of CM has been useful with strong positive consequences in their nutrition. Dairy products are those obtained by fermentation of cow milk as yogurt. Just as it has been shown that heat diminishes the reactivity of milk [12, 13] or egg [14], fermentation some dairy products may help to decrease some proteins. In the yogurts it has been shown BLG detection greatly diminished compared to fresh milk measured by chromatography [15]. Even many yogurts have shown an absence of BLG; while other proteins such as CAS and ALA remain present in these yogurts. It is not known the cause why this happens. This could be argued because processing of dairy products could stimulate polymerization of BLG, which is the major whey protein of cow's milk, in tetramers that probably changes spatial distribution of allergen epitopes [12] and decreasing allergenicity in children and adults with an IgE-mediated cow's milk allergy [16]. Since there are differences between different products [12], it would also be necessary that manufacturers prove the presence or absence of BLG in natural form to unsure their labeling convinces the requirements to identify BLG in food products. Further studies should be performed to delineate this GI allergy phenotype and if it is suitable to maintain dairy products in these patients. More studies are necessary in defining all possible different milk allergy phenotypes.

Abbreviations

IgE: immunoglobulin E; GI allergy: gastrointestinal food allergy; sIgE: specific IgE; tIgE: total IgE; CM: whole cow's milk; CMP: cow's milk proteins; CAS: casein; ALA: α-lactoalbumin; BLG: β-lactoglobulin; SPT: skin prick tests.

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