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Usefulness of r Ana o 3 assessment before oral food challenge to pistachio

To the Editor

Cashew and pistachio both belong to the *Anacardiaceae* family. Cashew and pistachio allergies may cause severe symptoms such as anaphylaxis with a very low eliciting dose^{1,2}. The cashew 2S albumin r Ana o 3 is the only singleplex component-resolved diagnosis immunoassay currently available in clinical practice³⁻⁵. Its structural similarity with the pistachio 2S albumin may explain the cross-reactivity between cashew and pistachio⁶. The main objective of this study was to assess the performance of specific IgE to r Ana o 3 for the diagnosis of pistachio allergy.

We conducted a retrospective, single-center study in the Pediatric Allergology Department of the Armand Trousseau Children's Hospital in Paris, including children referred for the diagnosis of pistachio allergy using a double-blind, placebo controlled, oral food challenge (OFC) to pistachio between 2014 and 2018 and for whom the levels of specific IgE to r Ana o 3 was available. Any children receiving omalizumab were excluded. Children were suspected of pistachio allergy either because of the existence of allergies to other nuts or legumes, or because of the existence of symptoms suggestive of pistachio allergy. Parental consent before the OFC was systematically collected. Ethics committee approval was not required under French law. Data were anonymized according to good clinical practice and the study was registered with the French *Commission Nationale Informatique et Libertés* (number 215489V0, 20/02/2018).

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Demographic data such as gender, age, time since avoidance of pistachio from the diet, former status towards pistachio (naive or history of reaction), were collected. We also reported the existence of atopic comorbidities (asthma, allergic rhinitis, or eczema), prior status towards cashew (previously known allergy or sensitization, exclusion from the diet), and sensitization to other nuts and legumes. Immunoassays of total IgE and specific IgE to cashew, pistachio, and r Ana o3 were performed the month before OFC (ImmunoCAP; ThermoFisher Scientific, Uppsala, Sweden). Results of the OFC were collected (including the occurrence of any reaction, severity of reaction according to Astier score⁷, epinephrine requirement and cumulative eliciting dose expressed in mg of pistachio protein). Patients with real allergy were differentiated from sensitized-only patients by a positive OFC reaction.

A univariate descriptive analysis was carried out according to the results of the OFC followed by analysis of the receiver-operating characteristic curve to determine the ability of the level of specific IgE to r Ana o 3 to discriminate confirmed pistachio allergy from sensitization, using R-Studio software version 3.6.1 (<http://www.r-project.org>). The correlation between the level of specific IgE to r Ana o 3, Astier score and cumulative eliciting dose was evaluated using the Spearman coefficient.

Twenty-five patients were included. Among these, 15 (60%) were naive to pistachio before the OFC. Nine patients (36%) had excluded cashew from their diet. Four had proven allergy to cashew. Eight patients (32%) had a positive OFC to pistachio (confirmed allergic patients), including one severe reaction requiring adrenaline. Four reactions were classified grade 1 according to Astier score, three grade 2, and one grade 3. The mean cumulative eliciting dose was 90.4 mg ($\pm$ 106.4). The median level of specific IgE to r Ana o 3 was higher in patients with confirmed pistachio allergy compared to sensitized-only patients (0.92 kU / L *versus* 0, $p = 0.02$), while the level of specific IgE to pistachio was not significantly different (Table 1). There was a moderately positive ($\rho = 0.51$) but significant correlation between the level of r Ana o 3 and Astier score ($p = 0.01$), and a trend for a negative correlation ($\rho = -0.35$, $p = 0.09$) between the level of r Ana o 3 and cumulative eliciting dose.

The level of specific IgE to r Ana o 3 (AUC 0.753, CI 0.546-0.961, $p = 0.016$) and the ratio of specific IgE to r Ana o 3 / total IgE (AUC 0.771, CI 0.569-0.973, $p = 0.008$) had better performance characteristics than specific IgE to pistachio (AUC 0.625, CI 0.403-0.847, $p = 0.27$) to predict pistachio allergy. The optimal cutoff point of r Ana o 3 predictive of pistachio allergy

was 0.46 kU/L (sensitivity 87.5%, specificity 78.8 %, $p = 0.8$) (Table 2). A level of r Ana o 3 above 6.1 kU/L had a sensitivity of 95%.

To date, few studies have focused on pistachio allergy⁸. Most of these report predictive values of specific IgE to pistachio . The performance values of specific IgE to pistachio are a matter of debate. Maloney *et al* reported a threshold of specific IgE to pistachio at 88 kU/L for a 90% predicted probability, and 7% of real allergic patients had specific IgE <0.35 kU/L⁹. Couch *et al* also described 31% of patients with real allergy with IgE <2 kU/L¹. Our results confirm those by Savvatanios *et al* who reported that the assay of specific IgE to r Ana o 3 provided a better diagnostic marker of pistachio allergy than specific IgE to pistachio¹⁰. They reported a cutoff point of 0.16 kU/L for a sensitivity of 96.8% and a specificity of 94.4% for pistachio allergy. We confirm here that a relatively low threshold of specific IgE to r Ana o 3 may be predictive of allergy to pistachio. Unfortunately, we were unable to compare the OFC results with skin prick tests as this information was not available in our study. In conclusion, the assay of specific IgE to r Ana o 3 could be more useful than the assay of specific IgE to pistachio to assess the relevance of performing a diagnostic OFC in patients sensitized to pistachio. Prospective studies are needed to confirm these results, and to better understand the cross-reactivity between these two nuts.

Keywords: r Ana o 3; pistachio; IgE; allergy; children; oral food challenge; cross-reactivity

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	Confirmed allergic patients (OFC +) n=8 n (%)	Sensitized- only patients (OFC -) n=17 n (%)	p- value
Demographic data			
Male gender	6 (75)	10 (59)	0.43
Age at the time of OFC, years (median, [25 ^e -75 ^e pc])	8.5 [3-14]	8.9 [3-17]	0.98
Time since exclusion of pistachio from the diet, years (mean, [25 ^e -75 ^e pc])	5.4 [1-8]	6 [2.5 -8]	0.90
Naive from pistachio	4 (50)	11 (54)	0.48
Atopic comorbidities			
Asthma	4 (50)	14 (82)	0.09
Allergic rhinitis	4 (50)	9 (53)	0.89
Eczema	2 (25)	10 (59)	0.11
Other food allergies (apart from known cashew allergy)	7 (87)	16 (94)	0.56
Prior status towards cashew			
Exclusion from the diet	5 (62)	4 (23)	0.05
Previously known allergy	3 (37)	1 (5)	0.04
Sensitization to other nuts	4 (50)	17 (100)	0.01
Almond	2 (25)	9 (53)	0.18
Hazelnut	1 (12)	14 (82)	0.01
Walnut	4 (50)	12 (70)	0.31
Sesame	1 (12)	7 (41)	0.15
Mustard	0	2 (11)	0.31
Sensitization to legumes	6 (75)	100 (17)	0.03
Soy	1 (12)	6 (35)	0.23
Peanut	6 (75)	17 (100)	0.03
Biological data			
Total IgE, kU/L (mean ± SD)	993 ± 935	608 ± 458	0.54
Specific IgE to cashew, kU/L (median [25-75 ^e pc])	1.61	0.6	0.29

pc])	[0.69-3.35]	[0.4-2.2]	
Specific IgE to pistachio, kU/L (median [25-75 ^e pc])	3.02 [1.71-4.2]	1.4 [0.54-2.7]	0.2
Specific IgE to r Ana o 3, kU/L (median [25-75 ^e pc])	0.92 [0.53-4.06]	0 [0-0.3]	0.02

Results from OFC

Cumulative eliciting dose, mg of pistachio protein (mean ± SD)	90.4 (± 106.4)	-	-
Astier score			
1	4 (50)		
2	3 (37.5)	-	-
3	1 (12.5)		
4	0 (0)		
Epinephrine requirement	1 (12.5)	-	-

Table 1 – Descriptive analysis of the cohort

Cut-offs of specific IgE to r Ana o 3	Sensitivity (%)	Specificity(%)	LR+	LR-
0	100	0	1.00	0.00
0.13	87.5	50.0	1.75	0.25
0.17	87.5	55.6	1.97	0.23
0.43	87.5	72.2	3.15	0.17
0.46	87.5	77.8	3.94	0.16
0.60	75.0	77.8	3.38	0.32
0.76	62.5	77.8	2.81	0.48
1.08	50.0	77.8	2.25	0.64
1.97	37.5	77.8	1.69	0.80
2.00	25.0	77.8	1.13	0.96
3.87	25.0	83.3	1.50	0.90
5.00	25.0	88.9	2.25	0.84
6.14	25.0	94.4	4.50	0.79
6.16	12.5	94.4	2.25	0.93
6.82	0.0	94.4	0.00	1.06

Table 2 – Performance characteristics of r Ana o 3 for the diagnosis of pistachio allergy. LR: likelihood ratio