

Diagnostic capacity of commercial extracts versus prick by prick in the study of peanut sensitization. Which technique should we use?

Running title: Skin tests in peanut allergy

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Peanut is a well-known allergen that can cause severe reactions. Skin tests are usually the first step to confirm IgE-mediated sensitization in due to their simplicity and reliability [1]. There is currently no consensus of whether the skin prick test (SPT) using commercialized allergen extracts or the *prick-by-prick* test with the fresh food is preferable to detect allergen sensitization [2-4]. We aimed to describe the diagnostic capacity of SPT versus *prick-by-prick* in the detection of sensitization to peanut and if there is a relation of its results with the molecular sensitization profile.

Fourty two patients (>6 years) were prospectively recruited from five allergy departments of Spain. All patients had history of objective symptoms (digestive, respiratory, urticaria or anaphylaxis) immediately after ingestion of peanut in the past two years and positive SPT with peanut commercial extract (ALK-Abelló®, Bial-Aristegui®, Diater®, or Leti® as used in each allergy service). All participants signed the informed consent [Investigational Ethical Committee (060-2013)]. All patients filled out the study questionnaire regarding symptoms presented with peanut and frequency of consumption of other foods.

Skin prick tests were performed in all patients with four commercial peanut extract (CE) [ALK-Abelló®, Bial-Aristegui®, Diater®, Leti®], peach extract (30 mg/mL of Pru p 3, ALK-Abelló®), profilin (ALK-Abelló®), peanut lipidic fraction extract (Diater®), apple extract (quantified Mal d 1: 10 µg/ml) and *prick-by-prick* with roasted peanut and crunchy peanut butter (Varma Foods SL, Madrid, Spain). Sodium chloride (0,9%) and histamine hydrochloride (10 mg/mL) served as negative and positive controls, respectively. Wheals of ≥ 3 mm of diameter were considered positive as recommended by the EAACI guidelines [5].

Determination of serum specific IgE (sIgE) was performed in all subjects (ImmunoCAP Thermofisher, Uppsala, Sweden) against peanut (whole extract), recombinant(r) peanut allergens (rAra h 1, rAra h 2, rAra h 3, rAra h 9) and rPru p 3. sIgE was considered positive when ≥ 0.10 kUA/L, following manufacturer's recommendations. Statistical analysis was performed with STATA/IC 12.0. Variables were tested for normality by employing the Shapiro-Wilk test. Values for quantitative variables found not to have a normal distribution were described employing median and interquartile range, and comparative analyses were conducted

employing the Mann-Whitney U (Wilcoxon rank-sum) test. ANOVA Test was used to compared the wheal size among the CEs and the Tukey method for pairwise comparison. The comparison between proportion of positive or negative results among the different SPT results was compared by Cochran test.

The mean age of the studied population was 28.3(6; 69) years [26 (62%) females]; 9(21%) were under 18 years [(mean age 11.3 (6-17) years old]. Fourteen patients (33,3%) presented angioedema, 12(28.5%) urticaria, 7(16.7%) anaphylaxis, 5(11.9%) had respiratory symptoms and 4(9.5%) presented gastrointestinal symptoms.

Results of skin tests are shown in Table I. Differences among the different CEs were not statistically significant. Interestingly, all the CEs showed better diagnostic accuracy than the *prick-by-prick*, either with roasted peanut ($p<0.001$) or with peanut butter ($p<0.001$). Statistically significant differences were also detected in the SPT wheal size among the four CEs and the *prick-by-prick* ($p<0.0001$).

Twenty-seven patients (64%) were exclusively sensitized to LTP -not sensitized to storage proteins (SP)-, 6(14%) were sensitized to LTPs and SP; and 4(10%) were exclusively sensitized to SP. Four patients (9.5%) were sensitized to rAra h 1, 10 (23.8%) to Ara h 2 and 1 (2.4%) to Ara h 3; three patients (7.1%) were sensitized to Ara h 8 and 31 (73.8%) to Ara h 9. Thirty-two patients (76.2%) showed sensitization to Pru p 3 (4 patients did not show sIgE to any of these components).

Interestingly, the wheal size was greater in patients sensitized to storage proteins in comparison with those who were not sensitized to these allergens (see online repository Figure 1). The SPT with the CEs yielded greater wheal size than the *prick-by-prick* independently of the molecular sensitization profile ($p<0.001$).

Regarding the size of the resulting wheal, a good correlation was observed among the *prick-by-prick* with peanut butter and roasted peanut (0.80), the lipidic fraction and the roasted peanut (0.72), DIATER CEs and roasted peanut (0.70) and among the DIATER CE and *prick-by-prick* with peanut butter (0.75). Interestingly, all patients that were sensitized to SP had positive *prick-by-prick*.

In the studied sample, the mean value of specific sIgE against peanut was 5,57 kUA/L. Higher concentrations were found in patients with exclusive sensitization to storage proteins in comparison to those who were exclusively sensitized to LTP (median values of sIgE: 14,89 vs. 2,94 KUA/L respectively; Mann Whitney: 0,034).

According to our results, the studied CEs showed better capacity in detecting sensitization to peanut in comparison to the *prick-by-prick* technique, in contrast to the findings of Rancé et al

[6] who found a superior diagnostic capacity of raw extracts over CEs and hypothesized that this difference may be due to the loss of peanut oil and hydrophobic agents in the CEs. However, we only found positive SPT to the lipidic fraction -the isolated oily fraction- in 19% of our population and none of them showed monosensitization nor negative SPT with CEs.

Although the allergenic composition of CEs to foods may be highly variable [6,7], we hypothesize that mechanical or chemical procedures during the preparation of the CEs may favor the availability of the different allergenic components, specially LTP, to which the majority of our patients are sensitized. Moreover, we consider that the presence of other components may be influenced by the cooking or presentation of the food in the *prick-by-prick*, which is often used due to its simplicity and low cost, but it is not standardized [8] for which it is important to consider the possibility of obtaining falsely negative results. Additionally, the performance of *prick-by-prick* may vary according to the total amount of allergen that is tested, its preservation and its exposure to the skin. In our study, the skin tests were performed by allergology nurses to minimize these factors. In our population, the studied available CEs have demonstrated better diagnostic performance in detecting sensitization to peanut in comparison with the *prick-by-prick*, and these results varies according to the molecular profile of sensitization.

A potential limitation of the present study was the impossibility of determining the specific sIgE against all the available peanut allergens and in the fact that not all the patients underwent OFC. According to our data, we suggest to perform SPT to peanut using commercial extracts. However, the standardization of peanut CEs is still needed in order to guarantee the presence of all peanut allergenic components improving their diagnostic accuracy.

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References

1. Keet CA, Wood RA. Food allergy and anaphylaxis. *Immunol Allergy Clin North Am.* 2007;27:193-212.
2. Sicherer SH, Sampson HA. Food allergy: a review and update of epidemiology, pathogenesis, diagnosis, prevention and management. *J Allergy Clin Immunol.* 2018;141:41-58.
3. Valcour A, Jones JE, Lidholm J, Borres MP, Hamilton RG. Sensitization profiles to peanut allergens across the United States. *Ann Allergy Asthma Immunol.* 2017;119:262-6.
4. Heizerling L, Mari A, Bergmann KC, Bresciani M, Burbach G, Darsow U, et al. The skin prick test – European standards. *Clin Transl Allergy.* 2013;3:3.
5. Muraro A, Wefel T, Hoffmann-Sommergruber K, Roberts G, Beyer K, Bindslev-Jensen C, et al. EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy. *Allergy.* 2014;69:1008-25.
6. Rancé F, Abbal M, Lauwers-Cancés V. Improved screening for peanut allergy by the combined use of skin prick tests and specific IgE assays. *J Allergy Clin Immunol.* 2002;109:1027-33.
7. Asero R, Scala E, Villalta D, Pravettoni V, Arena A, Billeri L, et al. Shrimp Allergy: Analysis of Commercially Available Extracts for In Vivo Diagnosis. *J Investig Allergol Clin Immunol.* 2017;27:175-82.
8. Elizur A, Appel MY, Nachshon L, Levy MB, Epstein-Rigbi N, Golobov K, et al. NUT Co Reactivity Acquiring Knowledge for elimination recommendations (NUT CRACKER) study. *Allergy.* 2018;73:593-601.

Table 1. Skin test results in the studied population. CE: commercial extract; SPT: skin prick test.

Extract/Allergen								
	Diater® CE SPT	Leti® CE SPT	ALK- Abelló® SPT	Bial® SPT	Roasted peanut prick by prick	Peanut butter prick by prick	Lipidic fraction (Diater®) SPT	Peach extract (30 mg/ml of Pru p 3, ALK®) SPT
N of positives (%)	30 (90.5)	37 (88)	36(85.7)	33(78.6)	22(52.4)	26(61.9)	8(19)	29(96)
Mean wheal size (min;max) min	32 (8;77)	38.1 (8;103)	35.6 (7;161)	26.6 (7;95)	33.7 (7;143)	31.5 (7;88)	33.7 (7;143)	43.8 (8;102)