



Letter

In vivo and in vitro techniques in the diagnosis of lipid transfer protein sensitization

Sensitization to panallergens, such as lipid transfer proteins (LTPs), causes frequent reactions with taxonomically unrelated plant-derived food. Moreover, patients allergic to LTPs more frequently experience systemic symptoms, including anaphylaxis, compared with other panallergens, such as profilin.¹ Different in vitro techniques and the skin prick test (SPT) are useful in the diagnosis of this sensitization by using peach LTP (Pru p 3)^{2,3} and different extracts that are available for this purpose. The aim of our study was to compare the diagnosis of peach LTP sensitization by SPT and by specific IgE (sIgE) determination.

One hundred six patients were prospectively enrolled. Seventy-two patients (cases) had at least 1 plant-derived food allergic reaction in the previous 2 years and a positive SPT result to peach LTP (Pru p 3) (Bial-Aristegui, Bilbao, Spain), because this was the most concentrated peach LTP extract on the market, and a negative SPT result to profilin and birch pollen (ALK-Abelló, Horsholm, Denmark). Thirty-four patients (controls) with plant-derived food allergy ($n = 25$), pollen allergy ($n = 9$), or both ($n = 24$), with negative SPT results to peach LTP (Bial-Aristegui), were also enrolled. The study was approved by the local ethics committee, and all participants were informed about the protocol and provided written informed consent.

All patients (case and controls) underwent a battery of SPTs, which included testing with 3 peach LTP extracts from different manufacturers: purified 99% natural peach LTP (nPru p 3) (100 $\mu\text{g/mL}$ of nPru p 3; Bial-Aristegui) (SPT-1), peach extract enriched with Pru p 3 (30 $\mu\text{g/mL}$ of nPru p 3; ALK-Abelló, Madrid, Spain) (SPT-2), and purified 99% peach LTP extract (30 $\mu\text{g/mL}$ of nPru p 3; Leti, Madrid, Spain) (SPT-3). SPTs were performed singly by experienced nurses the same day following European Academy of Allergology and Clinical Immunology guidelines.⁴ Results were evaluated by planimetry, centrally by a single allergologist. Wheals of 7 mm^2 or greater were a considered positive result.

sIgE was assessed against recombinant Pru p 3 by 2 different technologies: fluorescence enzyme immunoassay (ImmunoCAP; ThermoFisher, Uppsala, Sweden) and semiquantitative allergen microarray (ImmunoCAP ISAC version CRD 112; ThermoFisher). Values of 0.35 $\text{kU}_\text{A}/\text{L}$ or greater for immunoassay or 0.3 ISU for microarray were considered positive, as recommended by the manufacturer.

Quantitative variables were described as mean (SD) or median (range), depending on whether distribution of the data was normal or not, whereas qualitative variables were described as frequency (percentage). Reliability between qualitative results of SPTs with

different LTP extracts and among SPTs and sIgE determinations by immunoassay and microarray was analyzed by calculating the κ index and interpreting the results based on the Fleiss criteria ($\kappa < 0.4$, poor; κ of $0.4 \leq 0.75$, moderate; $\kappa > 0.75$, excellent)⁵ and as positive agreement (PA) and negative agreement (NA). Quantitative results from the LTP SPTs were compared by analyzing the intra-class correlation coefficient (ICC), interpreting the results using the Fleiss classification⁶ (ICC < 0.3 , poor; ICC of $0.3 \leq 0.50$, mediocre; ICC of $0.51 \leq 0.70$, moderate; ICC, $0.71 \leq 0.90$, good; ICC > 0.91 , excellent). To analyze differences among extracts, the Wilcoxon signed rank test was performed. All data were analyzed using SPSS statistical software, version 20.0 (SPSS Inc, Chicago, Illinois).

All cases ($n = 72$) had a positive result to SPT-1 as an inclusion criteria; 70 of 72 had a positive result to SPT-2, and 63 of 72 had a positive result to SPT-3. The median wheal size was 32 mm^2 (range, 7–319 mm^2) for SPT-1, 42.5 mm^2 (range, 0–240 mm^2) for SPT-2, and 27 mm^2 (range, 0–135 mm^2) for SPT-3. SPT-1 revealed a significantly smaller wheal size than SPT-2 ($P < .001$) and a bigger wheal size than SPT-3 ($P < .05$). SPT-2 revealed a significantly bigger wheal size than SPT-3 ($P < .001$). Agreements between SPTs to peach LTP are excellent (SPT-1 vs SPT-2: $\kappa = 0.96$, PA = 98.6%, NA = 97.1%, ICC = 0.57; SPT-1 vs SPT-3: $\kappa = 0.82$, PA = 93.3%, NA = 88.3%, ICC = 0.50; SPT-2 vs SPT-3: $\kappa = 0.86$, PA = 91.1%, NA = 94.7%, ICC = 0.61).

Regarding sIgE, 69 of the 72 cases had positive sIgE results to Pru p 3 by immunoassay and 63 by microarray. The median levels for sIgE to Pru p 3 were 3.89 $\text{kU}_\text{A}/\text{L}$ (range, 0.08–66.70 $\text{kU}_\text{A}/\text{L}$) by immunoassay and 1.58 ISU (range, 0–21.14 ISU) by microarray. Agreement between peach LTP extracts for SPT and sIgE to peach LTP by immunoassay and microarray is given in Table 1.

Agreement between immunoassay and microarray was excellent ($\kappa = 0.84$, PA = 94.7%, NA = 91.1%). None of the controls had positive SPT results to any of the LTP extracts or sIgE determination to Pru p 3 by either immunoassay or microarray.

The SPT is a simple, accessible, fast, and cheap technique. In our study, the peach LTP SPT also revealed a high concordance with the determination of sIgE to this allergen by immunoassay and microarray. In fact, SPT to peach LTP has previously demonstrated a higher sensitivity than sIgE to Pru p 3 by an immunoassay system (ADVIA Centaur)^{1,7} and by enzyme-linked immunosorbent assay.⁸ Regardless of different technology (ie, allergen-sIgE ratio) to determine sIgE to Pru p 3, excellent agreement was observed between immunoassay and microarray.

Moreover, we observed a similar performance of the peach LTP extracts from the 3 different laboratories in qualitative terms. Nevertheless, some differences in wheal size and in the number of patients whose conditions were diagnosed by LTP extracts were observed, even between extracts with similar protein

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Table 1
Agreement between LTP SPT and sIgE to Pru p 3 by immunoassay and microarray^a

IgE recombinant Pru p 3	SPT-1	SPT-2	SPT-3
Immunoassay			
NA, %	95.8	93.2	86.5
PA, %	97.9	96.4	92.4
κ	0.94	0.90	0.80
Microarray			
NA, %	89.5	93.2	82.4
PA, %	94.1	96.4	88.2
κ	0.82	0.78	0.69

Abbreviations: LTP, lipid transfer protein; NA, negative agreement; PA, positive agreement; SPT, skin prick test.

^aThe Fleiss criteria (1981) were used ($\kappa < 0.4$, poor; κ of 0.4–0.75, moderate; and $\kappa > 0.75$, excellent).

concentrations (SPT-2 and SPT-3), although SPT-2 is an enriched extract in Pru p 3 (other allergens could be present) and SPT-3 is a purified protein. Even more, the highest protein concentration among the extracts used (SPT-1) did not offer the biggest wheal. These disparities may be due to several factors, such as differences in raw material (ie, maturation degree), the manufacturing process, and reagents used, as has been reported by comparative studies with extracts against aeroallergens.⁹ For example, pH of the peach LTP SPT diluents can modify skin response because Pru p 3 solubility is better in acidic solutions.¹⁰ These differences among laboratories highlight the importance of a central system to compare the potency of commercial extracts.

In conclusion, SPTs to peach LTP that are currently marketed have a similar rate of diagnosis of LTP sensitization. The excellent degree of agreement among the different peach LTP SPT solutions and the determination of sIgE to Pru p 3 by immunoassay and microarray highlight the usefulness of peach LTP SPTs for the diagnosis of sensitization to this protein when in vitro techniques are not available.

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