Clinical characterization of peach allergic patients and respective molecular sensitization profile with ALEX®2 macroarray

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This data has been presented on the annual meeting of Sociedade Portuguesa de Alergologia e Imunologia Clínica - 43ª Reunião Anual da SPAIC 2022 and won the 2nd prize of best poster presentation.

To the Editor,

Lipid transfer proteins (LTP) are plant kingdom panallergens, responsible for a significant number of food allergic reactions, particularly in the Mediterranean area. *Pru p3* (peach LTP) is the prototype protein of this family. Symptoms may range from oral allergy syndrome (OAS) to systemic and potentially fatal reactions, like anaphylaxis. Previous studies have found an association between peach allergy and sensitization to pollens from several taxonomically unrelated species, such as *Artemisia*, *Parietaria* or Plane tree and probably, in less extent, with Olive tree, which are quite common in the Mediterranean area. So, it is appropriate to consider the possibility of a link between inhalation sensitization to LTP pollen and LTP food allergy, namely as a primary route of sensitization to LTP (1-8).

Other panallergens have also been well identified for the study of peach allergy: *Pru p1*, a pathogenesis-related protein group 10 (PR-10) (homologous to *Bet v1*); *Pru p4*, a profilin; and *Pru p2*, a thaumatin-like protein (4). More recently, two new proteins considered allergenic were identified: *Pru p7*, a gibberellin-regulated protein (GRP) and *Pru p9*, belonging to the PR-1 group (5,6,9).

The allergens considered *major* are *Pru p3* and *Pru p7*, both resistant to heat and proteolytic digestion, whose typical allergic symptom is anaphylaxis. Similarly to *Pru p3*, sensitization to *Pru p7* is considered a risk factor for severe allergic reactions to fresh fruit and it seems to be particularly important in patients who are peach-allergic but not sensitized to *Pru p1*, *Pru p3* or *Pru p4*, which is especially common in areas with high cypress pollen exposure (9-12).

Skin *prick* tests (SPT) are used in these patient's investigation and follow-up, being validated for reactions' responsible allergens identification and for tolerance acquisition assessment, although sensitivity could vary depending on allergen representation (9). *In vitro* study, using molecular components to characterize the sensitization pattern of patients with different phenotypes of the disease is considered relevant in the investigation (12). The identification of sensitization patterns that are associated with the severity of the disease would allow a better follow-up of these patients, limiting excessive dietary restrictions and an adequate selection for specific immunotherapy with *Pru p3* (12).

The new *Macroarray* Allergy Explorer® (ALEX®2) technique (*Macroarray Diagnostic, Vienna, Austria*) is the first ELISA based *in vitro* multiplex allergy test allowing simultaneous measurement of total IgE (tIgE) and specific IgE (sIgE) for more than 150 total extracts (sIgEte) and more than 100 molecular components (sIgEmc) simultaneously, granting a better interpretation and clinical correlation of the results (13,14). The use of a carbohydrate determinant inhibitor (CCD) reduces the interpretative burden of CCD positive patients (about 25% of patients have anti-CCD IgE), which are known to be clinically insignificant (15).

In this preliminary study, of a small cohort, we aimed to characterize the clinical and molecular sensitization profile of a population of peach allergic patients with the new ALEX®2 technique and identify possible sensitization profiles associated with severe reactions.

We show a single-center cross-sectional study, involving adult patients with a history of peach allergic reaction, positive SPT and sIgE for peach, who were not undergoing immunotherapy with *Pru p3*. Patients were subdivided into 2 groups: systemic reaction (SRG) - 13 patients, and local reaction (LRG) - 7 patients. The study was approved by the hospital ethical committee and every patient signed a written informed consent. Demographic and clinical data were collected through written survey and SPT with fresh fruits (peach, apple, kiwi, grape, plum, pear and tomato), vegetables (celery), nuts (hazelnut), legumes (peanut), cereals (wheat), pollens (*Artemisia* and *Parietaria*), and *macroarray* ALEX®2 were performed. SPT were carried out according to food symptoms and related foods, including as many food groups containing LTP as possible.

For statistical analyses (IBM-SPSS software, v25.0), t-independent and Mann-Whitney tests were used to compare parametric and non-parametric independent samples, respectively. Fisher's exact test or Qui-square test were used to evaluate associations between categorical variables, and Cohen´s K-test was used to assess agreement. P-values <.05 were considered statistically significant.

A total of 20 patients were enrolled in the study, mean age of 28.8±11.1 years old (mean age on the first reaction to peach 18.2±10.3 years old), 16 (80%) were female (Table 1). Almost all patients (n=14; 70%) had a history of atopy, with allergic rhinitis (75%) being the most frequent comorbidity. Median age at first reaction was significantly higher in the SRG (14 years old *vs* 21 years old;p=0.026).

Regarding SPT results, there have been positivity for: LTP (n=20; 100%), peach (n=15; 75%), apple (n=13; 65%), wheat (n=10; 50%), hazelnut (n=8; 40%), peanut (n=7; 35%), *Parietaria* (n=5; 25%), *Artemisia* (n=5; 25%), plum (n=4; 20%), tomato (n=4; 20%), pear (n=3; 15%), celery (n=3; 15%), grape (n=2; 10%), kiwi (n=1; 5%) and Plane tree (n=1; 5%).

The LTP molecular sensitization profile is shown in Table 1; *Tri a 14* and *Ole e7* have not been identified in our cohort, but sensitization to other panallergens has been identified: PR-10 in two patients (*Bet v1*, *Fag s1*, *Mal d1*, *Cor a1*, *Api g1*, *Gly m4, Ara h8*), both belonging to LRG, and profilin

in one patient (*Bet v2*) belonging to SRG. Also, gibberellin-regulated protein *Pru p7* has not been identified in our sample.

sIgEmc mean value was significantly higher in the SRG: *Pru p3* (12.71kUA/L *vs* 0.12kUA/L;p=0.004), *Mal d3* (2.99kUA/L *vs* 0kUA/L;p=0.016), *Ara h9* (5.01kUA/L *vs* 0.12kUA/L;p=0.022) and *Pla a3* (1.48kUA/L *vs* 0kUA/L;p=0.037).

Regarding the molecular sensitization profile, a statistically significant association was found between the presence, individually, of sensitization to *Pru p3*, *Mal d3* and *Pla a3* and systemic reaction, as shown in Figure 1.

Characterization of the molecular profile seems to be relevant as a marker of disease expression. Molecular allergology is increasing in clinical routine worldwide, which will help support the physicians' allergy workup (16,17).

Also in our study, agreeing with previous ones, the most frequent clinical presentation was systemic reaction (65%) and *Pru p3* was the most prevalent LTP (75%) (1-3).

Although the presence of atopy was not significantly related to the severity of the reaction (p=0.354), LTP pollens (*Artemisia*, *Parietaria* and Plane tree) were also identified in our cohort, confirming the existence of cross-reactivity. In these patients, it may be equated the respiratory allergy as possible primary route of sensitization to LTP, according to the literature (3,15). However, the fact that we do not know what were the first symptoms to appear (food *versus* respiratory) and the fact that sIgE values are higher for food allergens compared to respiratory allergens, may refute this theory.

As in other studies, it was verified that sIgEmc for, mainly *Pru p3,* but also *Mal d3, Ara h9* and *Pla a3*, are directly related with the occurrence of systemic reaction and its severity (1,2,18).

The evaluation of agreement between symptoms, SPT and sIgEmc reached statistical significance only for hazelnut and celery (moderate agreement).

Study's limitations include the reduced sample, that could limit extrapolation of results, and the limited assessment of other peach molecular allergens besides *Pru p3* and *Pru p7*, not available on ALEX®2, whose effects on symptoms were missed. Nevertheless, the authors believe that it has the

value of being the first cross-sectional study worldwide characterizing peach allergic patients with ALEX®2, this new technique allowing to reduce the time spent by performing simultaneous IgE measurement of multiple total extracts and molecular components, helping in the identification of sensitization patterns associated with systemic reactions. These findings could improve patients' management in clinical practice, highlighting the importance of evaluating sensitization to other LTP proteins associated with *Pru p3*, instead of only *Pru p3* itself.

Statement of Ethics

Study approval statement: This study protocol was approved by Ethical Committee of Centro Hospitalar Universitário de Lisboa Norte and Centro Académico de Medicina de Lisboa, approval number 128/20.

Consent to participate statement: Written informed consent was obtained from all participants.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Funding Sources: This research did not receive any specific grant for funding agencies in the public, commercial or not-for-profit sectors.

Author Contributions: MIS, CC, MP, FCD and MCPS designed research; MIS, RB, MP and MCPS performed research and analysed data; MIS, CC, RB, MP, CC and FCD collected clinical data; MIS, RB, FCD, MCPS and CC wrote the paper.

Data Availability Statement: All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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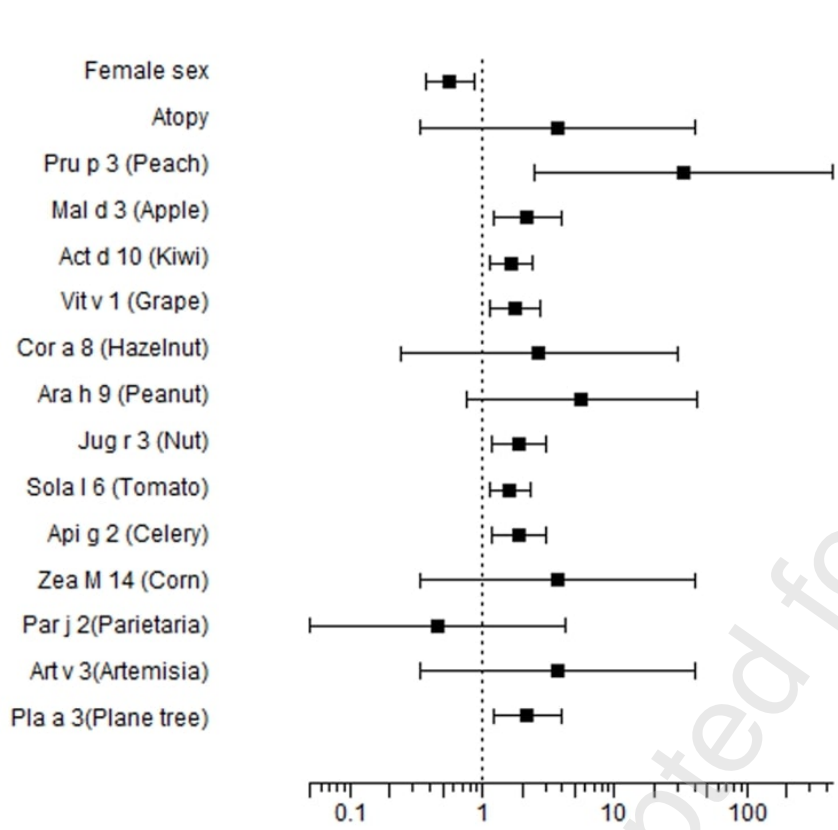
Total number of patients	**20 (100)**
Age, years	28.8±11.1 [18-68]
Gender, male/female	4 (20) / 16 (80)
Age of first reaction to peach, years	18.2±10.3 [1-48]
Allergy to >1 LTP food	18 (90)
Clinical manifestations	
Systemic reaction	13 (65)
Anaphylaxis	7 (54)
Urticaria/Angioedema	4 (31)
Gastrointestinal symptoms	2 (15)
Local reaction (Oral Allergy Syndrome)	7 (35)
Atopy	14 (70)
Rhinitis	15 (75)
Asthma	8 (40)
Eczema	5 (25)
LTP molecular components identified	
Pru p 3 (Peach)	15; 8.7±9
Mal d 3 (Apple)	7; 1.9±2.6
Act d 10 (Kiwi)	2; 0.7±1.3
Vit v 1 (Grape)	4; 0.5±0.8
Cor a 8 (Hazelnut)	5; 1.1±1.6
Ara h 9 (Peanut)	11; 3.3±4.2
Jug r 3 (Walnut)	5; 0.7±1.1
Sola l 6 (Tomato)	1; 0.02±0.03
Api g 2 (Celery)	8; 0.3±0.3
Zea m 14 (Maize)	6; 0.6±0.84
Par j 2 (Parietaria)	4; 1.2±1.9
Art v 3 (Artemisia)	6; 0.4±0.5
Pla a 3 (Plane tree)	7; 1±1.3
Can s 3 (Hemp)	2; 0.78±0.32

Table 1 Epidemiological and clinical characterization of peach allergic patients' cohort.

Data presented as n (%), mean±SD and median (IQR) as appropriate.

LTP, lipid transfer protein; SD, standard deviation.

Fig. 1. Association between LTP proteins and the occurrence of systemic reaction**.**



Legend: t-independent and Mann-Whitney tests were used to compare parametric and non-parametric independent samples, respectively, while Fisher's exact test or Qui-square test were employed to evaluate associations between categorical variables. P-values <.05 were considered statistically significant.

OR, Odds-ratio; CI, Confidence interval.