

SUPPLEMENTARY MATERIAL

METHODS:

Study design, setting, and subjects

In a transversal single-center cohort study, a total of 51 patients with walnut allergy from Fundación Jiménez Díaz Hospital (Madrid, Spain) were recruited. All walnut allergic patients included in the study were diagnosed during the years 2020-2021 and met the criteria of the SEAIC Subcommittee on Adverse Food Reactions [1]. Walnut allergy was diagnosed in patients having a clear history of adverse reactions due to walnut suggestive of IgE-mediated allergy, showing positive skin prick-by-prick tests, or by positive sIgE and/or food challenge tests to walnut. The exclusion criteria were those set out in the EAACI document on standardization of food challenge tests [2]. A serum sample was obtained from each patient and stored at -80° C until use.

The Fundación Jiménez Díaz Ethic Committee approved this study and written informed consent was obtained from all subjects.

sIgE testing

Allergen-sIgE to nuts was measured by ImmunoCAP (ThermoFisher Scientific AB, Uppsala, Sweden) following the manufacturer's recommendations. Besides, the multiplex test system ALEX² (MacroArray Diagnostics GmbH, Wien, Austria) was performed according to the manufacturer's instructions. sIgE levels were expressed in kU/L and kU_A/L, and sIgE values ≥ 0.35 kU/L and ≥ 0.30 kU_A/L were considered positive, respectively.

Skin Prick Tests (SPTs)

SPTs to nuts were performed using commercial nut extracts (C.B.F. LETI S.A; Tres Cantos, Spain), as well as with nuts in prick-by-prick test technique [3]. In addition, skin tests were performed on a battery of commercially available pollen extracts (ALK-Abello, Madrid, Spain). Histamine phosphate at 10 mg/mL and saline solution were used as positive and negative controls, respectively. A weal diameter at least 3 mm larger than the negative control was considered a positive reaction.

Statistical Analysis

Frequencies were expressed by absolute value and/or percentage, quantitative variables by mean and standard deviation (SD), and for sIgE results, medians, and interquartile range (IQR) were given. For statistical analyses, Shapiro-Wilk test, X^2 test, Student test and the Mann-Whitney test were performed using IBM SPSS software (SPSS Inc., Chicago, IL, USA). The Figure S1 was generated using GraphPad Prism 10 (GraphPad Software, Inc., La Jolla, USA). All analysis were 2-tailed and were considered significant at p value < 0.05 .

References:

1. Ibáñez Sandín MDP, De La Hoz Caballer MB, Diéguez Pastor MC, Goikoetxea Lapresa MJ. Metodología diagnóstica en la alergia a los alimentos. In: Dávila, I, Jáuregui, I, Olaguibel, J, Zubeldía, J, SEAIC (Sociedad Española De Alergología E Inmunología Clínica). Tratado de Alergología. Madrid: ERGON, 2016; 1072-92.
2. Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J, et al. Standardization of food challenges in patients with immediate reactions to foods--position paper from the European Academy of Allergy and Clinical Immunology. *Allergy*. 2004 Jul;59(7):690–7.
3. Heinzerling L, Mari A, Bergmann KC, Bresciani M, Burbach G, Darsow U, et al. The skin prick test – European standards. *Clin Transl Allergy*. 2013 Feb 1;3:3.