

C A S E R E P O R T

Sensitivity of prick test with walnut commercial extracts and of prick by prick with raw walnut compared with open food challenge in walnut allergy

Maria Letizia Patti¹, Cristina De Rose¹, Federica Brancato¹, Alessandro Gambacorta¹, Stefano Miceli Sopo²

¹ Department of Woman and Child Health, Policlinico Gemelli University Foundation IRCCS, Catholic University of Sacred Heart, Rome, Italy; ² Allergy Unit, Pediatrics Area, Department of Woman and Child Health, Policlinico Gemelli University Foundation IRCCS, Catholic University of Sacred Heart, Rome, Italy

Abstract. *Background and aim:* Diagnosis of walnut allergy includes the evaluation of IgE sensitization by skin prick tests (SPT) with standardized commercial extracts. When assuming the loss of relevant allergens due to extract preparation and storage, it is possible to perform SPT with fresh foods, i.e., prick by prick (PbP). To our knowledge, there is no published comparison between SPT with commercial extracts and PbP with fresh food about their sensitivity to the diagnosis of walnut allergy. Therefore, we describe our experience. *Methods:* We observed seven children (mean age \pm SD 6.8 years \pm 5.2, range 2 - 15 years; male 85%) with a history of immediate adverse reaction following walnut ingestion. All but one the patients underwent SPT with at least two out of three walnut commercial extracts (Lofarma, Milan, Italy; ALK-Abellò, Milan, Italy; Allergopharma, Rome, Italy). It has also been performed PbP with raw walnut. IgE-mediated walnut allergy was diagnosed based on suggestive history, positivity of PbP and failed open food challenge with walnut. *Results:* The SPT with Lofarma extract was never positive (sensitivity = 0%), that performed with ALK extract was positive in 2/5 cases (sensitivity 40%) and that of Allergopharma extract was positive in 1/5 cases (sensitivity 20%). PbP was positive in 7/7 cases (sensitivity 100%). *Conclusions:* In the specific case of walnut allergy in pediatric age, the execution of SPT alone with commercial extract may not be sufficient and clarifying in the diagnostic iter. We suggest to always associate the execution of PbP test. (www.actabiomedica.it)

Key words: Diagnosis, prick by prick, sensitivity, skin prick test, walnut allergy

Introduction

Walnut allergy is an increasing public health problem. Crucial to the diagnostic process is the collection of clinical history and the evaluation of IgE sensitization by skin prick tests (SPT) with standardized commercial extracts (1). When assuming the loss of relevant allergens due to extract preparation and storage techniques, it is possible to perform SPT with fresh foods, i.e. prick by prick (PbP). To our knowledge, there is no published comparison between SPT with commercial extracts and PbP with fresh food

with regard to their sensitivity to the diagnosis of walnut allergy made by food challenge in pediatric age. Therefore, we describe our experience.

Written and oral consents from the patients and their parents were obtained, and the study was approved by the local ethical committee.

Methods

Between September 2017 to March 2019, we observed seven children (mean age \pm SD = 6.8 years

± 5.2 , range = 2 - 15 years; male 85%) with an history of immediate adverse reaction (cutaneous, respiratory, gastrointestinal, anaphylactic) following walnut ingestion, evaluated in the pediatric allergy unit of the Policlinico Gemelli University Foundation IRCCS of Rome. The diagnosis of anaphylaxis was made according to the definition of Sampson et al (2). All but one the patients underwent SPT with at least two out of three walnut commercial extracts (Lofarma, Milan, Italy; ALK-Abellò, Milan, Italy; Allergopharma, Rome, Italy) according to the standard international methodology (3). It has also been performed PbP with raw walnut, pricked with ALK lancet (at least 20 times in different areas of walnut); then, the patients forearm skin was pricked and raw walnut was pressed and rubbed on patients forearm skin spot. Moreover, PbP were performed twice on both forearms and the mean of the largest wheal diameters was reported. Regardless of the results of SPT and PbP, children were subjected to open food challenges (OFC) with raw walnut (4). We performed an incremental OFC, starting with a very small fragment of walnut, approximately a 68th walnut kernel, and continuing, in the absence of adverse reactions, with doubling the dose every 20 minutes. We considered passed the OFC if the patient tolerated an overall dose of walnut equal to 30 grams (5). When food dependent exercise induced anaphylaxis (FDEIA) was suspected, an OFC followed by physical exercise was performed (6). In addition, SPT have been performed with commercial extract of birch tree (Lofarma, Milan, Italy), peach and palm oil profilin (ALK, Milan, Italy) as paradigms of the pan-allergens respectively pathogenesis-related protein (PR-10), lipid transfer protein (LTP) and profilin. The sensitivity of SPT with commercial extracts and PbP was calculated using the 2 x 2 table and the formula "sensitivity = $a / (a + c)$ ".

Results

The results concerning SPT with commercial extracts of walnut, PbP and OFC with raw walnut are listed in table 1. The case n. 1 did not carry out the SPT with Allergopharma extract, the case n. 2 did not carry out the SPT with ALK extract, and the case n. 5

did not carry out the SPT neither with Allergopharma nor with ALK extracts. Table 1 also shows the results of SPT performed with birch tree, peach and palm oil profilin commercial extract. For the case n. 7 these last SPT were not performed. OFC failed in all seven cases. In particular, case n. 6 completed an incremental OFC up to a dose compatible with its age (20 grams) without adverse reactions. Then, she performed an exercise (free running for 10 minutes) and presented with hives and bronchospasm. The initial suspicion was then confirmed and FDEIA was diagnosed.

The SPT with Lofarma extract was never positive (sensitivity = 0%), that performed with ALK extract was positive in 2/5 cases (sensitivity = 40%) and that of Allergopharma extract was positive in 1/5 cases (sensitivity = 20%). PbP was always positive (sensitivity = 100%). Only in the case n. 6 the positivity to an allergen typically resistant to extractive processes, such as the peach LTP ALK extract, was found.

Discussion

In the 7 cases described, IgE-mediated walnut allergy was diagnosed based on suggestive history, positivity of PbP and failed OFC. The sensitivity of SPT with 3 different commercial walnut extracts was insufficient.

There are no guidelines that prohibits the use of natural food in the execution of the SPT but, at the same time, none explicitly encourages its use in normal clinical practice. Instead, in our experience, the use of PbP was definitely more effective and predictive of the presence of specific walnut IgE compared to SPT with commercial extract. This made it possible to avoid misdiagnosis with possible risks of serious reactions for the patients. This could happen mostly in cases with not very suggestive history, as in case n. 2 described below. The child in question, in fact, had presented only slight perioral erythema in a single occasion when he had ingested a small piece of walnut. The history, therefore, were not sufficient to raise suspicion in the presence of negative SPT with commercial extract.

The walnut allergens known until now, are classified into three main families called respectively prolamine (Jug r 1, Jug n 1, Jug r 3), cupine (Jug r 2, Jug r 6

Table 1. Table shows the symptoms before investigation, the skin prick test with walnut and raw walnut commercial extracts, oral food challenge results and the other food allergens positivity at the skin prick tests.

	Symptoms before investigation	Walnut Lofarma extract	Walnut ALK extract	Walnut Allergo Pharma extract	Raw walnut	Birch Lofarma Peach.ALK Profilin.ALK extracts	Other positive food allergens	OFC	Amount of walnut ingested during OFC Signs and symptoms after OFC
Case n. 1	Labial and palpebral angioedema	negative	negative	not performed	positive (mean diameter = 11 mm)	positive negative negative	Lentils peas	failed	0,5 grams Itchy throat, face erythema, abdominal pain
Case n. 2	Mild perioral erythema	negative	not performed	negative	positive (mean diameter = 6 mm)	negative negative negative	hazelnut	failed	1 grams Urticaria, palpebral angioedema, rhinorrhea and sneezing, vomiting
Case n. 3	Labial and palpebral angioedema	negative	Positive (mean diameter = 8 mm)	negative	positive (mean diameter = 9 mm)	positive negative negative	hazelnut	failed	15 grams Conjunctival hyperemia, lacrimation, persistent rhinorrhea
Case n. 4	Monocular erythema and palpebral angioedema	negative	negative	negative	positive (mean diameter = 9 mm)	positive negative negative	egg	failed	3 grams Abdominal pain, cough, rhinorrhea and sneezing, bronchospasm
Case n. 5	Labial angioedema	negative	not performed	not performed	positive (mean diameter = 8 mm)	negative negative positive	none	failed	8 grams Vomiting, abdominal pain, rhinorrhea and sneezing
Case n. 6	Urticaria, cough, bronchospasm (suspected FDEIA)	negative	positive (mean diameter = 5 mm)	positive (mean diameter = 5 mm)	positive (mean diameter = 5 mm)	negative positive negative	Hazelnut peanuts cashew pistachios	failed	20 grams Generalized urticaria and bronchospasm (FDEIA)
Case n. 7	Angioedema, cough, hoarseness	negative	negative	negative	positive (mean diameter = 5 mm)	not performed	swordfish salmon sole cod hazelnuts egg anchovies tuna fish Kiwi	failed	1 grams Generalized urticaria, itchy throat

Legenda. OFC = oral food challenge; FDEIA = food-dependent exercise-induced anaphylaxis

and Jug r 4), pathogenesis-related protein PR-10 (Jug r 5), profilin (Jug r 7), and a second non-specific lipid transfer protein (nsLTP2, Jug r 8)(4, 7, 8). The major allergen is Jug r 1: its specific IgE have been identified in 50% of the sera of patients with walnut allergy and it is considered the potential responsible for severe allergic reactions and possible cause of anaphylaxis and angioedema as it is particularly resistant to the gastric juices action (9). In the Italian population the main walnut allergen is Jug r 3 and it is associated with serious reactions such as anaphylaxis and glottis edema (10). The profilin family (Jug r 7), instead, is considered responsible for more moderate reactions and relegated to the oral cavity (oral allergy syndrome) (11). Therefore, in the specific case of walnut, the negativity of SPT with commercial extract against the positivity of PbP with fresh food, could be partially related to the degradation of labile allergens in the process of the extract preparation. As evidence of this, of the 2 patients with anaphylaxis after OFC, case 6 was positive for LTP (and therefore the OFC outcome could be so justified), while for case 4 we cannot offer an explanation other than a hypothetical sensitization to oleosins or to seed storage proteins (although the latter should be contained in commercial extracts). We have not performed a component resolved diagnosis (CRD), is a weakness in our study. A better characterization of our patients through CRD would have been appropriate.

Conclusions

From our experience, it could be deduced that, at least in the specific case of walnut and in pediatric age, the execution of SPT alone with commercial extract may not be sufficient and clarifying in the diagnostic iter. We suggest to always associate the execution of PbP test which, instead, showed greater accuracy and allowed us to reinforce the diagnostic suspicion, then confirmed by OFC.

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Correspondence:

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Stefano Miceli Sopo

Allergy Unit, Pediatrics Area, Department of Woman and
Child Health, Policlinico Gemelli University Foundation
IRCCS, Catholic University of Sacred Heart, Rome, Italy

ORCID ID: 0000-0002-8175-6146

Phone: +39 (0)6-30156283, Fax. +39 (0)6-3383211

E-mail: stefano.micelisopo@unicatt.it, stefano.micelisopo@
gmail.com

