**ORIGINAL ARTICLE**

# Evaluation of the safety of multiple nut oral food challenges in the management of nut allergy

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**Summary**

**Background.** Tree nut and peanut allergy are common, and often persistent conditions, that significantly affect quality of life. Multiple nut avoidance is common due to cross-reactivity concerns but may cause unnecessary restrictions. Data regarding multiple nut oral food challenges (MN-OFC) remains limited. Our aim was to evaluate the safety and clinical impact of MN-OFC in the management of tree nut and/or peanut allergy. **Methods.** Retrospective analysis of 25 patients who underwent MN-OFCs between 2022 and 2024 at a tertiary-level Allergy Department. Patients with profilin and/or PR-10 sensitization were excluded. Diagnostic evaluation included clinical history, skin prick tests (SPT), skin prick-to-prick tests (SPPT), and specific IgE measurement (ImmunoCAP® and/or ImmunoCAP™ ISAC). MN-OFC, using incremental dosing and extended observation, were conducted in patients with negative results for nut-specific seed storage proteins and negative PPT with the suspected nut. **Results.** A total of 26 MN-OFC were performed, involving 87 nuts. Sensitization to storage proteins was detected in 54% of patients, and to lipid transfer proteins in 17%.

Despite negative SPPT, 16% had positive SPT. MN-OFC were negative in all patients. Prior to OFC, patients excluded a median of 6 nuts from their diet, which decreased to 2 post-challenge. Use of MN-OFC avoided 261 hours of individual oral food challenge procedures. **Conclusions.** In this population, MN-OFC is a safe, time-efficient, and clinically valuable strategy for ruling out tree nut and/or peanut allergy in selected patients. They support more personalized dietary recommendations and reduce unnecessary avoidance, while optimizing healthcare resources use.

**Key words**

Nut allergy; Multiple nut oral food challenges; Allergy diagnosis.

**IMPACT STATEMENT**

Multiple nut oral food challenges are safe, time-efficient, and clinically valuable, enabling individualized dietary guidance and reducing unnecessary nut avoidance in carefully selected patients.

**Introduction**

Tree nut and peanut allergy are common causes of food-induced anaphylaxis and frequently persist into adulthood, significantly impacting patients' quality of life (1,2) (for simplification purposes, peanut was included in the "nut" category in this study, although botanically classified as a legume (1,3)). Diagnosis is based on clinical history and evidence of sensitization, assessed through skin tests to extracts and raw nuts, and/or specific IgE (4). When clinical relevance is uncertain, oral food challenges (OFC) are necessary to confirm or exclude allergy. However, interpreting sensitization results can be complex due to cross-reactivity amongst nuts, co-sensitization without clinical relevance, and pollen-related cross-reactivity (4). The introduction of molecular component-resolved diagnostics has improved diagnostic precision, enabled risk stratification, and supported personalized management.

In clinical practice, patients are often advised to avoid multiple nuts based on sensitization results or as a precaution due to concerns about cross-reactivity, even in the absence of confirmed clinical allergy (6). While this cautious approach prioritizes safety, it may result in

unnecessary dietary restrictions, heightened anxiety, nutritional inadequacies, and potentially missed opportunities for the development of oral tolerance. In addition, restrictive elimination diets can limit dietary diversity and may increase the risk of sensitization to additional nuts. In this context, multiple nut oral food challenges (MN-OFC) have emerged as a promising diagnostic strategy, though still underused (7-9). This approach may improve diagnostic clarity, minimize unnecessary restrictions, and optimize healthcare resource use by reducing the need for sequential individual OFC. Evidence on the safety and clinical impact of MN-OFC remains limited, and standardized protocols are lacking. However, recent studies suggest that MN-OFC can effectively distinguish multiple nut allergy from isolated sensitization. Additionally, recent evidence suggests that children sensitized to lipid transfer proteins (nsLTP), but without specific IgE to seed storage proteins (SP), may safely tolerate various nuts, including peanut, hazelnut, and walnut, highlighting the importance of individualized tolerance assessment in clinical practice (10). This study aims to evaluate the safety and clinical impact of MN-OFC in the management of nut allergy, based on the experience of a tertiary hospital.

**Material and methods**

A retrospective analysis was conducted on a cohort of patients with suspected nut allergy who underwent MN-OFC between 2022 and 2024 at the Allergy Department of a tertiary-level hospital in the north of Portugal. Patients with nut allergy exclusively sensitized to profilins and/or PR-10 were excluded. Previously to the OFC, patients underwent *in vivo* skin prick testing (SPT) using commercial extracts (Roxall-Aristegui®), skin prick-to-prick tests (SPPT), and *in vitro* assays for serum-specific IgE (ImmunoCAP® and/or ImmunoCAP™ ISAC, ThermoFisher Scientific®). Specific IgE was evaluated with ImmunoCAP™ ISAC for molecular components, and with ImmunoCAP® extracts when molecular allergens were not available or were negative. Written informed consent for the performed procedures was obtained from the patient or legal guardians.

For SPT and SPPT, a wheal diameter ≥ 3 mm than the negative control was considered positive (11). SPT were performed for almond, peanut, cashew, hazelnut, pistachio, walnut, pine nut, peach nsLTP, birch, and profilin. SPPT were performed using the suspected and avoided nuts. Nuts were excluded from SPPT if the patient had a history of severe reaction with positive specific IgE and/or SPT, or if they had previously tolerated the nut without

symptoms. *In vitro* test results were considered positive if specific IgE levels were ≥ 0.35 kU/L (ImmunoCAP®) or ≥ 0.3 ISU-E (ImmunoCAP™ ISAC). In selected cases, when total IgE was very low, values below conventional thresholds were interpreted considering the clinical context and deemed potentially relevant (12).

MN-OFC were conducted in patients with a history of an allergic reaction to at least one nut, and at least one marker of sensitization positive (at least one SP and/or nsLTP, and in cases without a known protein available, the patients should have positive total extract for the nut, namely almond and pine nut). They were also performed in patients with negative SPT, SPPT, and sIgE to SP and/or LTP when home introduction was refused, in cases of very severe reactions, or when the results of SPPT and/or sIgE could have been influenced by immunosuppression. In all patients, SPPT and nut-specific SP IgE to the tested nut were negative. OFC followed a stepwise incremental dosing protocol (Attachment 1); each nut was administered in a cumulative dose comprising an initial half-unit, followed by three incremental whole units. A 20-minute observation period under medical supervision was maintained between doses. After the final dose, patients were monitored for an additional two hours. A negative OFC was defined as the absence of objective or reproducible symptoms during and up to 2 hours after the final dose. Positive reactions were defined by the appearance of reproducible symptoms suggestive of IgE-mediated allergy. Patients were instructed to introduce the tolerated nuts at home and were later evaluated in a follow-up appointment.

Data collected included age, sex, allergic comorbidities, suspected nuts, clinical manifestations, sensitization to SP and/or nsLTP, nuts tested in the OFC, reactions during the OFC, and dietary changes (introduction or continued avoidance) following the OFC. Clinical manifestations were graded using the ordinal Food Allergy Severity Score-5 (oFASS-5) (13). Descriptive statistical analysis was conducted using IBM SPSS Statistics, version 29 (IBM Corp.).

**Results:** A total of 25 patients were included, 56% male (n = 14), with a median age of 7 years (IQR: 21; range: 2-60 years). Of these, 64% (n=16) were under 18 years of age. A personal history of atopy was reported in 76% (n = 19), with allergic rhinitis/rhinoconjunctivitis in 56% (n = 14), atopic dermatitis in 40% (n = 10), and asthma in 36% (n = 9).

Clinical manifestations during the index reaction are reported in Table I; anaphylaxis was documented in 56% (n=14). In 52% (n=12) of the cases, a single nut was identified as the suspected allergen, while in 48% (n=11) two or more nuts were suspected. Among patients with at least two suspected nuts, 8 reported reactions to different nuts in separate episodes, and 3 ingested several nuts simultaneously in the same episode. The most frequently suspectedly involved nuts were walnut (40%, n=10), hazelnut (28%, n=7), and peanut (28%, n=7). SPT were positive for at least one nut in 16% (n=4) of the patients who had negative SPPT. Specifically, patient 4 showed a positive SPT to almond (3.5 mm); patient 9 to cashew (3 mm), hazelnut (3 mm), pistachio (3 mm), and walnut (3 mm); patient 10 to walnut (3 mm); and patient 17 to almond (3 mm) and peanut (3.5 mm), despite all of them having negative SPPT. Additionally, among these patients, none presented specific IgE to SP. The results of the SPPT are presented in Table I.

All patients except one underwent ImmunoCAP™ ISAC testing. Regarding molecular sensitization assessed by ImmunoCAP ISAC™ (Table II), 58% (n=14) were sensitized to SP, 17% (n=4) to nsLTP, 4% (n=1) to both, and the remaining patients showed no sensitization.

A total of 26 MN-OFC were conducted, evaluating 87 different nuts. OFC were performed in patients with suspected and confirmed nut allergy (Table I). The most frequently tested combination, in 19% of cases, was peanut, almond, and cashew. All OFC were negative, confirming tolerance to the tested nuts. The median number of nuts tested per challenge was 3 (IQR: 1; range: 2-8). We excluded allergy to all nuts in 16% (n=4) of the patients; none were sensitized to nsLTP and/or SP.

Before OFC, the median number of nuts excluded from the diet was 6 (IQR 1; range: 2-7), which decreased to 2 (IQR 2; range 0-6) following the challenge. Total outpatient hospital time consumed with this approach was 111h hours and the median duration of each MN-OFC was approximately 4 hours (IQR: 0.8; range: 3.3-6). Assuming one OFC per nut in a traditional individual approach lasts approximately 3 hours, the MN-OFC strategy allowed for a cumulative saving of approximately 150 hours of outpatient hospital, significantly reducing healthcare resource burden.

**Discussion and conclusions**

Our findings align with previous studies that support the feasibility and safety of MN-OFC in selected patients. For instance, Camarda *et al.* demonstrated that MN-OFC are effective in diagnosing IgE-mediated nut allergy in children, allowing for the safe introduction of tolerated nuts into the diet (9). Similar studies have also reported high tolerance rates, reinforcing the diagnostic value of this approach. In our cohort, MN-OFC effectively excluded clinically relevant allergy in all tested patients, including those with previously positive SPT, but negative specific IgE and SPPT tests. By consolidating the evaluation of multiple nuts into a single challenge, this approach reduces the time and resource constraints typically associated with sequential OFC and avoids unnecessary dietary restrictions. The absence of reactions across all OFC reinforces the safety of this method, particularly when nut-specific SP sensitization is excluded. Moreover, the successful reintroduction of the tested nuts into the diet contributes meaningfully to nutritional adequacy, psychosocial well-being, and overall quality of life (14). In addition to saving 150 outpatient hospital hours, this approach may also minimize indirect costs such as time off work or school, reduce patient and caregiver anxiety, and improve overall quality of life by enabling more confident and individualized dietary guidance. This study provides further evidence that MN-OFC are a safe and efficient diagnostic tool in selected patients with nut allergy. In a context where the avoidance of multiple nuts is often based on the potential risk of cross-reactivity, MN-OFC represents a valuable tool to clarify the diagnosis, support food introduction, and guide dietary recommendations based on clinical relevance.

This study is the most extensive cohort of MN-OFC, and it reinforces its applicability and safety in daily practice, when rigorous criteria are used to select the patients who undergo this procedure. By addressing a gap in the literature, this work seeks to support the integration of MN-OFC into clinical practice, helping to clarify diagnosis, prevent unnecessary avoidance, and promote more personalized patient management. Nonetheless, it presents some limitations, including its retrospective design, the relatively small sample size, and the fact that all participants were managed in a tertiary hospital, which may limit the generalizability of our findings. We encourage further validation through multicenter prospective studies to confirm the generalizability and cost-effectiveness of this approach.

In conclusion, our findings highlight that precision in allergy diagnosis does not necessarily require complexity, but rather the thoughtful application of existing tools in ways that prioritize safety, efficiency, and patient-centered care.

**Authors contributions**

MCML: Conceptualization; Investigation; Writing - original draft.

ACMC: Conceptualization; Investigation; Writing - original draft.

LPPD: Conceptualization; Investigation.

SIPC: Writing - review & editing; Validation.

JRQG: Writing - review & editing; Validation; Supervision.

ALRF: Writing - review & editing; Validation; Supervision.

**Conflicts of interest**

The authors declare that they have no conflicts of interest, including financial, political, intellectual, or religious interests, in accordance with the ICMJE recommendations.

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| Patient | Age (years) | Sex | Suspected nut | Clinical manifestations and oFASS-5 | Positive skin prick test(s) | ImmunoCAP™ ISAC and/or sIgE | Skin prick-to-prick tests | Oral food challenge |
|---|---|---|---|---|---|---|---|---|
| 1 | 20 | M | Peanut, Walnut | Cutaneous and AOS - Grade 2 | – | – | Almond (-), Hazelnut (-), Peanut (-), Walnut (-) | Almond, Hazelnut, Peanut, Walnut |
| 2 | 25 | M | Almond | Cutaneous - Grade 2 | – | –* | Almond (-), Cashew (-), Hazelnut (-), Peanut (-), Pine nut (-), Walnut (-) | Almond, Cashew, Hazelnut, Peanut, Pine nut, Walnut |
| 3 | 3 | F | Walnut, Pine nut | Cutaneous - Grade 2 | Walnut | Jug r 1 | Almond (-), Cashew (-), Hazelnut (-), Peanut (-), Pine nut (-), Pistachio (-) | Almond, Cashew, Peanut, Hazelnut, Pine nut** |
| 4 | 2 | F | Peanut | Anaphylaxis - Grade 3 | Almond, Peanut, Profilins | Ara h 2, Ara h 6 | Almond (-), Cashew (-), Hazelnut (-), Walnut (-) | Almond, Cashew, Hazelnut, Walnut |
| 5 | 2 | M | – | Sensitization*** | – | Ara h 1, Ara h 2, Ara h 6, Ber e 1 | Almond (-), **Cashew (+)**, Hazelnut (-), Walnut (-) | Almond, Hazelnut, Walnut |
| 6 | 3 | M | Hazelnut, Walnut | Cutaneous - Grade 2 | Cashew, Hazelnut, Walnut | Jug r 1 | Almond (-), Peanut (-), Pine nut (-) | Almond, Pine nut |
| 7 | 2 | F | Peanut | Anaphylaxis - Grade 3 | Peanut | Ara h 1, Ara h 2 | Almond (-), Cashew (-), Hazelnut (-), Walnut (-) | Almond, Cashew, Hazelnut |
| 8 | 15 | F | Almond | Anaphylaxis - Grade 4 | Almond | – | Hazelnut (-), Cashew (-), Peanut (-), Pistachio (-), Walnut (-) | Cashew, Peanut, Pine nut, Pistachio, Walnut |
| 9 | 5 | F | Peanut | Anaphylaxis - Grade 4 | Cashew, Hazelnut Peanut, Pistachio, Walnut | Ara h 1, Ara h 2, Ara h 6 | Almond (-), Cashew (-), Hazelnut (-), Pistachio (-), Walnut (-) | Almond, Cashew, Hazelnut, Walnut |
| 10 | 23 | M | Almond, Hazelnut, Peanut | Respiratory - Grade 4 | Cashew, Pistachio, Walnut | Ana o 3 | Almond (-), Hazelnut (-), Peanut (-), Walnut (-) | Almond, Hazelnut, Walnut, Peanut |
| 11 | 11 | M | Almond, Pistachio | Anaphylaxis - Grade 3 | Hazelnut, Pine nut, Pistachio, Walnut, Profilins | Cor a 8, Jug r 3, Pru p 3, Ana o 2, Cor a 9, Gly m 6, Gly m 5, Jug r 1 | Almond (-), Peanut (-) | Almond, Peanut |
| 12 | 2 | M | Cashew | Anaphylaxis - Grade 4 | Cashew | Ana o 3 | Almond (-), Hazelnut (-), Peanut (-), Walnut (-) | Almond, Peanut |
| 13 | 7 | F | Cashew, Peanut | Anaphylaxis - Grade 4 | Cashew, Peanut, Pistachio, Profilins | Ana o 3, Ara h 1, Ara h 2, Ara h 6, Ber e 1 | Hazelnut (-), Pine nut (-), Walnut (-) | Hazelnut, Pine nut, Walnut |
| 14 | 26 | F | Almond, Cashew, Pistachio, Walnut | Anaphylaxis - Grade 4 | Cashew, Pistachio | Ana o 3 | Almond (-), Cashew (-), **Hazelnut (+)**, Peanut (-), **Pistachio (+)**, Walnut (-) | Almond, Peanut, Walnut |
| 15 | 2 | M | Hazelnut | Anaphylaxis - Grade 4 | Hazelnut | Cor a 9, Cor a 14 | Almond (-), Cashew (-), **Hazelnut (+)**, Peanut (-), Pine | Almond, Cashew, Peanut, Pine nut |

| # | Age | Sex | Suspected nut | Reaction | Component 1 | sIgE components | OFC results | Confirmed |
|---|-----|-----|---------------|----------|-------------|-----------------|-------------|-----------|
| | | | | | | | nut (-), Pistachio (-), **Walnut (+)** | |
| 16 | 45 | F | Walnut | Anaphylaxis - Grade 4 | – | – | Almond (-), Cashew (-), Hazelnut (-), Peanut (-), Walnut (-) | Almond, Cashew, Hazelnut, Peanut, Walnut |
| 17 | 6 | F | Hazelnut, Walnut | Cutaneous - Grade 2 | Almond, Hazelnut, Peanut, Pistachio, Walnut | Cor a 9, Cor a 14, Jug r 1 | Almond (-), Cashew (-), Peanut (-) | Almond, Cashew |
| 18 | 20 | F | Hazelnut | AOS - Grade 1 | Peanut, nsLTP | Pru p 3, Jug r 3 | Almond (-), Cashew (-), Hazelnut (-), Walnut (-) | Almond, Cashew, Walnut |
| 19 | 17 | M | Pine nut | Anaphylaxis - Grade 4 | Almond, Pine nut | – | Almond (-), Cashew (-), Walnut (-), Peanut (-) | Almond, Cashew, Peanut, Walnut |
| 20 | 60 | F | Almond, Cashew Pistachio, Walnut | Anaphylaxis - Grade 5 | – | – | Almond (-), Cashew (-), Hazelnut (-), Peanut (-), Walnut (-) | Almond, Cashew, Hazelnut, Peanut, Walnut |
| 21 | 3 | M | Walnut | Cutaneous - Grade 2 | Birch, Walnut, nsLTP, Profilins | Cor a 9 | Almond (-), Cashew (-), **Hazelnut (+)**, Peanut (-) | Almond, Cashew, Peanut |
| 22 | 4 | M | Almond, Hazelnut, Pine nut, Walnut | Anaphylaxis - Grade 3 | nsLTP, Pine nut, Profilins | Jug r 3, Pru p 3 | Almond (-), Cashew (-), Hazelnut (-), Peanut (-), Pistachio (-), **Walnut (+)** | Almond, Cashew, Peanut |
| 23 | 41 | M | Hazelnut | Anaphylaxis - Grade 3 | Birch, Hazelnut, nsLTP | Pru p 3 | Almond (-), Cashew (-), Peanut (-), **Walnut (+)** | Almond, Cashew, Peanut |
| 24 | 29 | M | Peanut, Walnut | AOS - Grade 1 | nsLTP | Pru p 3, Jug r 3, Cor a 8 | Almond (-), Cashew (-), Hazelnut (-), Peanut (-), Pine nut (-), Pistachio (-), Walnut (-) | Peanut, Walnut |
| 25 | 4 | M | – | Sensitization*** | – | Jug r 1, Cor a 9 | Almond (-), Cashew (-), Hazelnut (-), Peanut (-), **Walnut (+)** | Almond, Cashew, Peanut |

**Table I. Patients with suspected nut allergy who performed multiple nut oral food challenges**

Legend. OAS = Oral Allergy Syndrome; oFASS-5 score = ordinal Food Allergy Severity Score-5; F = Female; M = Male; nsLTP = Non-specific lipid transfer proteins; sIgE = specific Immunoglobulin E. *ImmunoCAP™ ISAC was not performed – specific IgE to total extracts of almond, peanut, walnut, pine nut, and cashew were negative. **Patient 3 underwent two separate oral food challenges including almond, cashew, peanut, hazelnut, and pine nut. ***Sensitization – Patients without prior introduction of nuts, but with nsLTP and/or SP positivity. Patient 8 – sIgE to almond: 0.37 kU/L. Patient 19 – sIgE to pine nut: 14.7 kU/L.

**Table II. Molecular sensitization to nut allergy assessed by ImmunoCAP ISAC™**

| Patient | LTP | | | 2S albumin proteins | | | | | | 7S globulin proteins | | 11S globulin proteins | | | Total IgE (IkU/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cor a 8 | Jug r 3 | Pru p 3 | Ana o 3 | Ber e 1 | Cor a 14 | Ara h 2 | Ara h 6 | Jug r 1 | Ara h 1 | Gly m 5 | Ana o 2 | Cor a 9 | Gly m 6 | |
| 3 | | | | | | | | | 2.3 | | | | | | 148 |
| 4 | | | | | | | 8.9 | 1.4 | | | | | | | 378 |
| 5 | | | | | 0.3 | | 4.2 | 0.5 | | 1.1 | | | | | 52.2 |
| 6 | | | | | | | | 1.7 | | | | | | | 50.8 |
| 7 | | | | | | | 3.6 | | | 0.5 | | | | | 270 |
| 9 | | | | | | | 2.2 | 5.3 | | 1.6 | | | | | 623 |
| 10 | | | | 0.93 | | | | | | | | | | | 41.30 |
| 11 | 0.4 | 1.0 | 0.6 | | | | | | 5.0 | | 0.4 | 0.7 | 0.8 | 1.4 | 251 |
| 12 | | | | 0.34 | | | | | | | | | | | 43.5 |
| 13 | | | | 7.3 | 17 | | 9.8 | 13 | | 24 | | | | | 1695 |
| 14 | | | | 0.2 | | | | | | | | | | | 211 |
| 15 | | | | | | 4.1 | | | | | | | 0.4 | | 156 |
| 17 | | | | | | 0.3 | | | | 9.4 | | | 0.7 | | 1089 |
| 18 | | 0.4 | 0.7 | | | | | | | | | | | | 373 |
| 21 | | | | | | | | | | | | | 0.2 | | 268 |
| 22 | | 0.5 | 1.2 | | | | | | | | | | | | 122 |
| 23 | | | 0.5 | | | | | | | | | | | | 130 |
| 24 | 3.0 | 2.8 | 1.6 | | | | | | | | | | | | 577 |
| 25 | | | | | | | | | 1.5 | | | | 0.5 | | 1019 |

Legend. Results are expressed in ISU-E (ImmunoCAP Standardized Units for IgE); Patients 1, 8, 16, 19 and 20 presented no sensitization to any nsLTP and/or SP in ImmunoCAP ISAC™.

**Attachmment I. Multiple nut oral food challenges protocol**

| Time (minutes) | Nut | Portion (units) |
|---|---|---|
| 0 | Nut 1 | 1/2 |
| 20 | | 3 |
| 40 | Nut 2 | 1/2 |
| 60 | | 3 |
| 80 | Nut 3 | 1/2 |
| 100 | | 3 |
| 120 | Nut 4 (…) | (…) |
| 140 | | (…) |
| **Discharge 2 hours after the last portion** | | |