

Pine nut allergy in children: A diagnostic test accuracy study

To the Editor,

Allergy to pine nut (seeds of genus *Pinus* trees) causes 3% of paediatric food-induced anaphylaxis in Italy.¹ In contrast to other nuts, where sensitization to cross-reactive antigens is common,² pine nut allergy is usually isolated.³ The main pine nut allergens are 2S albumins and vicilins, the latter triggering mainly local signs and symptoms.³

Diagnosis of pine nut allergy relies on clinical history, examination and specific testing using skin prick test, prick-by-prick test (PbP), and/or serum specific IgE (sIgE) and, where necessary, oral food challenges (OFC).⁴ OFC is time-consuming and can cause severe anaphylactic reactions.⁵ In this study, we evaluated the diagnostic test accuracy of prick-to-prick skin test and sIgE to pine nuts in children.

Charts from patients referred to the Allergy Unit of Meyer Children's University Hospital from December 2015 to March 2020 because of a suspected reaction to pine nuts or who had a positive PbP or sIgE to pine nuts as part of a tree nut allergy assessment were reviewed. All children who underwent an OFC to pine nuts were included in the study, and written informed consent was obtained from the children's parents for all procedures performed.

Prick-by-prick test to pine nut and other nuts (walnut, hazelnut, almond, cashew, peanut and pistachio) were performed with fresh nuts in all included patients.⁶ PbP was considered positive if the weal diameter was equal to or greater than 3 mm at 15 min-reading. Histamine (10 mg/ml; Alk Abellò) and normal saline were used for positive and negative controls. sIgE was determined using a commercial assay (ImmunoCAP system, Thermo Fisher Scientific).

Oral food challenges were open and not placebo-controlled, using 5 mg pine nut (0.7 mg of pine nut protein); then, the dose was doubled every 20 min until a reaction occurred or the total amount of about 20 pine nuts (about 588 mg of pine nut protein) was reached according to a modified protocol.⁷ As soon as any objective clinical manifestations

were observed, the OFC was stopped, and the reaction was treated.⁴ OFC assessors were not blind to PbP and sIgE results. Epinephrine administration was used according to relevant guidelines.⁸

The median values of PbP and sIgE to pine nut were studied in the pine nut tolerant group and in the pine nut allergic group. Thus, we established the cut-off values for PbP and sIgE with the higher sensitivity, specificity, positive predictive values (PPV) and negative predictive value (NPV), as well as sensitivity, specificity, PPV and NPV of other potential cut-off values. Receiver operating characteristic (ROC) curve analyses were performed for PbP and sIgE. Correlation between sIgE and PbP to pine nut was assessed through linear regression and Spearman correlation index. Besides, we studied the role of sex, age, PbP or sIgE values in association with positive OFCs. Finally, we analysed the importance of clinical history in association with positive OFCs with pine nut when it was recorded as the culprit food. A multiple logistic regression analysis was performed that utilized the probability of reaction as an outcome variable and the clinical and demographic variables as covariates. Results were calculated through adjusted odds ratio (aOR) and a 95% Wald confidence interval (CI). Analyses used SAS (version 9.4; SAS Institute, Cary).

Ninety-one children met the inclusion criteria, of whom 66 were pine nut tolerant and 25 pine nut allergic, including 17 with anaphylaxis, of whom 4 received intramuscular adrenaline.

Mean age at OFC was 113.6 months (SD 46.2 months) in pine nut tolerant children and 103.7 months (SD 36.6 months) in those with pine nut allergy. Gender was also similar, with 64% male in both groups.

Most of the children (91%) were atopic, but only 9/60 in the pine nut tolerant group had a previous history of pine nut reaction. Mean time from the reaction to OFC was 49.1 months (SD 42.7 months) in these 9 participants and 64.3 months (SD 24.9 months) in the 15/25 pine nut allergic participants with a previous history of reaction. The

difference in reaction history between allergic and tolerant participants was statistically significant ($p < .0001$).

All children were also tested for other nuts. In the pine nut tolerant group, 35/66 (53%) had a history of reaction to other nuts: 14 hazelnut, 12 walnut, 7 peanut, 7 almond and 2 cashew. In the pine nut allergic group, 9/25 (36%) had a history of reactions to other nuts: 4 hazelnut, 1 walnut, 2 peanuts, 1 cashew and walnut, 1 hazelnut and walnut.

There were differences in PbP and sIgE to pine nut between tolerant and allergic children. PbP median 3.0 mm (IQR 2.0 mm) and sIgE median 0.71 kUA/L (IQR 1.18 kUA/L) in pine nut tolerant group versus PbP median 6.0 mm (IQR 5.0 mm) and sIgE median 1.12 kUA/L (IQR 9.89 kUA/L) in the pine nut allergic group. Sensitivity, specificity, positive and negative predictive values and positive likelihood ratio are shown in Table 1. ROC curves for PbP (area under curve 0.8169, $p < .0001$) and sIgE (area under curve 0.6711, $p = .0089$) are shown in Figure 1. The Spearman correlation coefficient between PbP and sIgE was 0.18, indicating a positive correlation (0.53 for the pine nut allergic group, -0.11 for the pine nut tolerant group).

The multiple logistic regression analysis on risk for reaction to pine nut OFC showed the independent predictors are sIgE, PbP and the history of reaction (for the history of reaction: aOR: 6.23; 95% Wald CI 1.55–25.05, $p = .010$ in the PbP model; aOR: 6.42; 95% Wald CI 1.92–21.43, $p = .003$ in the sIgE model). We found that in children of the same sex, age and history of reaction, odds of reaction increase with each mm increase in pine nuts PbP (aOR: 1.92; 95% Wald CI 1.33–2.77, $p = .0005$), and with each unit increase in sIgE (aOR: 1.21; 95% Wald CI 1.02–1.47, $p = .010$).

KEY MESSAGES

- We evaluated diagnostic accuracy of prick-by-prick test and specific IgE for paediatric pine nut allergy.
- Prick-by-prick test had better diagnostic performance than specific IgE.
- The diagnostic cut-offs identified in our study need validation in other populations.

The diagnostic test accuracy of PbP for pine nut allergy has not previously been evaluated, and to our knowledge, only one study evaluated sIgE.⁹ In our study, pine nut allergy was formally confirmed or excluded by OFC with the culprit allergen.

Limitations include the retrospective, single-centre design, the risk of a selection bias and lack of blinding of OFC interpretation with respect to the result of the PbP and sIgE. Finally, we did not explore the diagnostic test accuracy of skin prick testing using commercial pine nut extract. We recorded a high rate of anaphylaxis at OFC, but a low rate of intramuscular adrenaline use.

In conclusion, we have developed diagnostic cut-offs for pine nut allergy, which may help select patients for OFC in the diagnostic workup of pine nut allergy. PbP test appears to have a better diagnostic performance compared with sIgE for diagnosing pine nut allergy in children. However, the diagnostic cut-offs identified in our study need validation in other populations.

TABLE 1 Sensitivity, specificity, positive and negative predictive values and positive likelihood ratio

	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Positive likelihood ratio (95% CI)
sIgE: Cut-off >0.10 kUA/L	0.80 (0.64–0.96)	0.31 (0.20–0.42)	0.30 (0.19–0.41)	0.81 (0.66–0.96)	1.16 (1.14–1.17)
sIgE: Cut-off >0.60 kUA/L	0.68 (0.50–0.86)	0.56 (0.44–0.68)	0.36 (0.22–0.50)	0.83 (0.72–0.94)	1.54 (1.51–1.57)
PbP: Cut-off >3 mm	0.68 (0.50–0.86)	0.76 (0.66–0.87)	0.52 (0.34–0.69)	0.87 (0.78–0.95)	2.89 (2.82–2.96)
PbP: Cut-off >4 mm	0.64 (0.45–0.83)	0.96 (0.91–1.00)	0.84 (0.68–1.00)	0.88 (0.80–0.95)	14.51 (13.70–15.36)
PbP: Cut-off >7 mm	0.28 (0.10–0.46)	1.00 (1.00–1.00)	1.00 (1.00–1.00)	0.79 (0.70–0.88)	-

Abbreviations: CI, confidence interval; NPV, negative predictive value; PbP, prick-by-prick test; PPV, positive predictive value; sIgE, specific IgE.

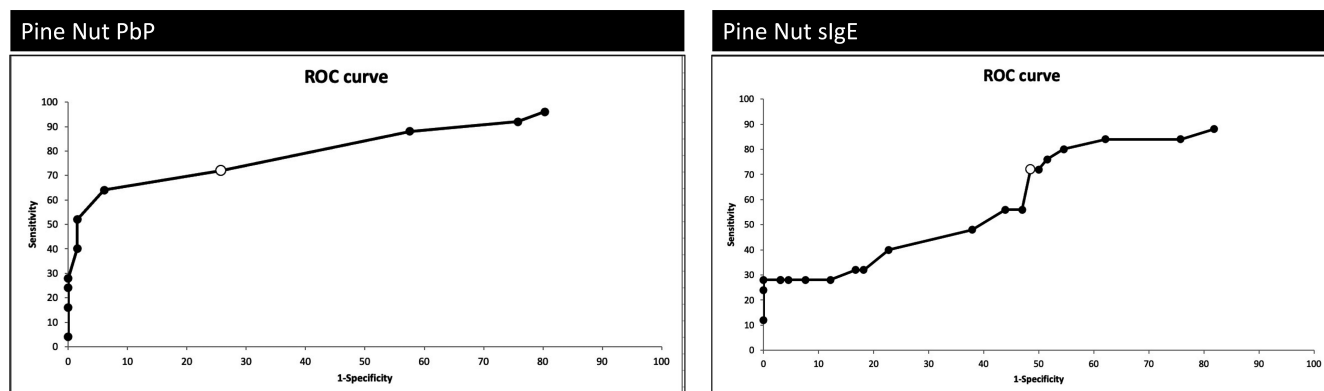


FIGURE 1 ROC curves pine nut PbP and pine nut sIgE. PbP, prick-by-prick test; ROC, receiver operating characteristic; sIgE, specific IgE

KEYWORDS

anaphylaxis, children, cut-off values, oral food challenge, pine nut allergy, prick-by-prick test, specific IgE

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests to disclose in relation to this paper.

AUTHOR CONTRIBUTIONS

CF, EP and TA collected the data. FM, CF, GL, LS, MG, SB and CV performed the investigations. FM, CF and RP analysed the data. FM, CF, RP and MG drafted the initial manuscript. FM, CF, RP, GL, LS, EP, TA, MG, SB and CV interpreted the data and reviewed the manuscript. All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

ETHICAL APPROVAL

Written informed consent was obtained from the children's parents for all procedures performed. The code of the event report issued by Meyer Children's University Hospital is IR904-21-53912.

DATA AVAILABILITY STATEMENT

Aggregate analyses are available on reasonable request to the corresponding author.

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