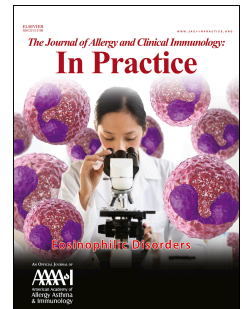


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Clinical predictors and outcomes of oral food challenges illustrate differences among individual tree nuts

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Clinical predictors and outcomes of oral food challenges illustrate differences among individual tree nuts

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Abstract

Background: Although allergy to tree nuts is often considered a single entity, there is heterogeneity in patient reactivity and immune response to different tree nuts.

Objective: We sought to characterize tree nut oral food challenges (OFC) in a pediatric population performed at a single center over a 12-year period and determine differences in OFC outcome to different tree nuts.

Methods: A retrospective chart review was conducted in patients (0- 20 years) who completed an unblinded OFC to any tree nut from 2007-2019 at Lurie Children's Hospital of Chicago. Differences among almond, cashew, hazelnut, and walnut challenges were compared and probability curves were used to estimate positive predictive values (PPV) of specific IgE (sIgE) at OFC.

Results: 531 tree nut OFCs were included. The mean age at OFC was 7.77 years (SD 4.33). Overall, 74.0% of children passed clinically indicated OFCs. Of the four most commonly challenged tree nuts, almost all patients passed OFC to almond (97.3%) and hazelnut (87.9%). Pass rates were lower for cashew (65.3%) and walnut (57.0%), $p < 0.0001$. The odds of failure were 0.83 times lower for patients who were avoiding without a previous reaction compared to those who had previously reacted ($p = 0.0025$).

Conclusions:

The majority of patients pass low risk almond and hazelnut OFCs. PPV at the 50th percentile for walnut (2.84 kU/L) and cashew (3.35 kU/L) were lower than previous studies have suggested.

81

What is already known about this topic? Tree nuts are common food allergens and have a higher likelihood of triggering a severe reaction compared to other food allergens. Little is known regarding differences between tree nut allergenicity and decision point thresholds for individual tree nuts.

What does this article add to our knowledge? Patients are more likely to pass almond and hazelnut challenges than cashew or walnut.

How does this study impact current management guidelines? Almond and hazelnut could be challenged more aggressively and cashew and walnut challenged more conservatively.

82

83 **Key words:** Food allergy; Tree nuts; Oral food challenges; Predictive value; Serum-
84 specific IgE; Diagnosis

85

86 **Abbreviations used:** AUC (Area under the curve), IQR (Interquartile range), OFC (Oral
87 food challenge), PPV (Positive predictive value), ROC (receiver operating
88 characteristic), sIgE (Serum allergen-specific IgE), SPT (skin prick test), TN (tree nut)

89

INTRODUCTION

A tree nut is botanically defined as a dried fruit, sometimes referred to as the seed, with an inedible hard shell. The nine most common tree nuts include almond, brazil nut, cashew, hazelnut, macadamia, pecan, pine nut, pistachio, and walnut.¹ While tree nuts are noted for being nutrient dense and provide various health benefits, they are common food allergens.^{1,2} In a cross-sectional survey administered from 2009-2010, approximately 1% of children in the United States were reported to have a tree nut allergy.² Furthermore, children with an IgE-mediated tree nut or peanut allergy had a higher likelihood of having a severe reaction than children with other food allergies.²

Current methods of tree nut allergy diagnosis use a combination of clinical history, serum-specific IgE, skin prick testing (SPT), and oral food challenges (OFCs).³ Although OFCs remain as the gold standard for diagnosis, they are time-consuming and present inherent and potentially dangerous risk of anaphylaxis.⁴ In the evaluation of tree nut allergy, serum-specific IgE and SPT assist in determining the need for an OFC to confirm whether the diagnosis.⁵ A 2006 study determined that for cashew, hazelnut, and walnut, a SPT ≥ 8 mm had a >95% accuracy for predicting a positive food challenge.⁶ Positive predictive values of SPT for almond, pistachio, pecan, and brazil nut have not been defined. Negative predictive values of SPT < 3 mm are shown to be reliable indicators of excluding the allergy for all tree nuts.⁵ Along with SPT, serum-specific IgE can also guide clinical decisions around challenging a patient. While diagnostic decision points with high positive and negative predictive values have been suggested for some common food allergens such as milk, egg, and peanut, widely

accepted cutoffs have not been determined for tree nuts. A peanut and tree nut study from the UK reported that walnut sIgE ≥ 15 kU/L had a PPV of 99% and hazelnut sIgE < 0.35 kU/L had a NPV of 95%, providing a preliminary threshold for further testing.⁸ Furthermore, sIgE predictive values may indicate the likelihood of reaction, but cannot predict the severity of reaction.⁵

Historically, tree nut allergy has been treated as one entity. Although concordance between specific nuts (ex: walnut and pecan (0.96), cashew and pistachio (0.95), and almond and hazelnut (0.84))⁵ has been shown, little is known regarding differences between tree nut allergenicity and decision point thresholds for challenging individual tree nuts. Determining predictive sIgE levels for passing or failing a challenge for individual tree nuts would provide further nuance to guide clinicians regarding oral food challenges for specific nuts. This will help minimize the risk associated with food challenges, limit excessive dietary restrictions, lifestyle changes, and possible financial demands for families and the healthcare system. Also, providing evidence that tree nuts have different potential cutoffs not only from peanuts but can also vary from nut to nut suggest the possibility for further characterization of storage proteins or other nut components that make one nut more allergenic than another. Thus, we sought to characterize the differences in tree nut specific IgE for almond, cashew, walnut and hazelnut between those who passed and failed their oral food challenges.

METHODS

Study design

An IRB approved retrospective chart review was conducted in children (0 to 20 years) who completed an unblinded standard of care oral food challenge (OFC) to any tree nut (almond, brazil nut, cashew, hazelnut, macadamia, pecan, pine nut, pistachio, and walnut) from 2007 to 2019 at Ann & Robert H. Lurie Children's Hospital of Chicago. Patients are offered food challenges at the discretion of the treating provider in order to definitively diagnosis food allergy or natural tolerance, as part of routine clinical care. In general, food challenges are offered in several scenarios such as: suspected ingestion and subsequent reaction; negative testing with a history of reaction; or positive testing with a questionable reaction or history of tolerating the food. Challenges are typically offered when there was at least a 50% likelihood of passing the food challenge, often using sIgE of 6 kU/L. Of note, these were clinically indicated food challenges performed for routine care (and not for the sake of research). The challenges were exclusively performed in an outpatient setting. Outpatient clinics are located throughout both city and suburban locations. All providers are trained and comfortable treating anaphylaxis.

Open OFCs were performed in a graded fashion, and a total goal dose of 6-8 grams of tree nut protein was divided and administered in increasing doses with a 2 hour observation after the last dose. Dose preparation was standardized across food challenges. Clinicians provided recipes or food options for families to bring to the food challenge. Nurse protocols are standardized with 4 to 5 step dose escalations given in a

graded fashion (typically 10%, 20%, 30%, 40% of the total serving) as tolerated in 15-minute intervals. The goal dose is an age appropriate serving size. Resolution was determined when patients were able to tolerate 6-8 g of the nut protein. Challenge outcomes were determined as pass, fail, or inconclusive by the provider performing the challenge at the time of challenge.

To declare a failed OFC, objective symptoms observed by the provider were required. Food challenges were considered inconclusive if families chose not to proceed when the patient presented with only subjective symptoms. Based off of clinician notes, we determined a score based on number of organ systems involved and objectivity of symptoms. Objective symptoms included urticarial lesions/hives, rash, sneezing, nasal congestion, rhinorrhea, emesis/diarrhea, and cough/wheeze. Subjective symptoms included abdominal pain, nausea, change in behavior, and distress. Challenges with subjective symptoms only were considered inconclusive and were excluded from the analysis.

Demographic data, personal and family history of atopy, details of previous reactions, sIgE levels to tree nuts, and SPT were also collected.

Skin Prick Testing (SPT)

Skin prick testing was performed using a bifurcated needle with Greer (Cambridge, MA) food allergen extracts. A skin prick test was considered positive if the wheal diameter was 3 mm greater than the negative control. In the case of multiple skin

prick tests, the first recorded patient SPT results on file were used for statistical analysis.

Serum-specific IgE testing

Serum-specific IgE testing was performed using the Immunocap fluorescence enzyme immunoassay (Phadia Immunocap, Uppsala, Sweden). Any IgE levels reported as more than 100 kU/L were analyzed as 100.1 kU/L, less than 0.35 kU/L as 0.34 kU/L, and less than 0.1 kU/L as 0.09 kU/L. The first recorded sIgE on file was used as the sIgE at diagnosis. The most recent sIgE to the time of the challenge was used as sIgE at challenge. sIgE testing (and not SPT) was performed routinely at the time of OFC.

Statistical Methods

For statistical analyses, only almond, cashew, hazelnut and walnut were used in the analyses as our power calculations suggested that we did not have sufficient numbers of brazil nut, macadamia, pine nut, pecan, and pistachio challenges to make meaningful conclusions. Using standard deviation estimates derived from Fleischer et al. and using a type 1 error rate of 0.05, our power calculations suggested that we needed approximately 100 challenges per nut to detect a significant difference.¹⁰ To compare demographic variables, the reported p-values are type III p-values from generalized estimating equation models with repeated measures where challenge status (pass/fail) was used as the outcome.

201 Within the subset of food challenges that were either almond, cashew, hazelnut,
202 or walnut, descriptive statistics were calculated for type of food challenge by challenge
203 outcome (pass/fail). A generalized estimating equation model with repeated measures
204 was fit to assess the association between the predictor of food type and outcome of
205 challenge status.

206 Within each subset of tree nut, the box plot distributions used median [IQR] and
207 are reported for IgE at OFC, IgE at diagnosis, and SPT. There were four patients who
208 had multiple walnut challenges. For these four individuals, their most recent walnut
209 challenge was used. Wilcoxon rank sum tests were then utilized to assess the
210 association between each measure (IgE at OFC, IgE at diagnosis, SPT) and challenge
211 outcome (if passed/failed). Within the subset of observations where food challenges
212 were either from almond, cashew, hazelnut, or walnut, ROC curves were created for
213 three models: IgE at OFC predicting a failed challenge, IgE at Dx predicting a failed
214 challenge, and SPT predicting a failed challenge. Each ROC curve was created using
215 the predicted probability values generated from GEE models with repeated measures.
216 Additionally, within the subset of observations from each individual nut (almond,
217 cashew, hazelnut, or walnut), ROC curves were created from a logistic regression
218 model with challenge result as the outcome and IgE at OFC as the predictor. Again, for
219 the four patients that had multiple challenges, only their most recent walnut challenge
220 was used.

221 Within the subset of failed food challenges that were either almond, cashew,
222 hazelnut, or walnut, descriptive statistics were calculated for type of food challenge by

223 epinephrine requirement status. To assess this association, generalized estimating
224 equations models with repeated measures were used. Type III p-value is reported.

225

226 Within the subset of failed challenges, descriptive statistics of allergic comorbidities
227 were reported by epinephrine requirement status. To assess these associations,
228 generalized estimating equations models with repeated measures were used. Type III
229 p-values are reported.

RESULTS

Overall, 531 tree nut (almond, brazil nut, cashew, hazelnut, macadamia, pecan, pine nut, pistachio, and walnut) challenges were reviewed and analyzed. The population was pediatric with 98.5% of patients younger than 18 years (100% younger than 21 years) and a mean age of 7.78 (SD 4.33) years. The study population was slightly more male than female patients (57.5% and 42.5% respectively). Allergic comorbidities were common with nearly half of patients having eczema, 40.2% having wheezing or asthma, and over half (59.5%) with allergic rhinitis.

Of the total 531 tree nut OFCs reviewed, 74.0% passed and 26.0% failed. No significant difference in demographic characteristics was found when comparing challenges that were passed or failed. The demographic characteristics of each group are presented in Table 1.

Almond, cashew, hazelnut and walnut were used in the analyses as the number of challenges for the other nuts (brazil nut, macadamia, pine nut, pecan and pistachio) were small. Approximately 100 challenges for each of the four tree nuts were reviewed to include in the analyses (Figure 1). A significant difference was found between pass and fail rates of the four different tree nuts ($p < 0.0001$, Table 2). Most patients passed OFC to almond (97.3%) and walnut was the least likely OFC passed, although the majority still passed (57.0%).

Using pairwise comparisons, we analyzed the odds ratio of passing one tree nut over another. Most patients did not have more than one challenge. Due to the fact that a few patients had multiple challenges, our statistical analyses required generalized estimating equations models to appropriately take the repeated measures into account.

We found that participants were most likely pass an almond challenge compared to any other tree nut, and less likely to pass a cashew or walnut challenge (Table 3). While there was significance between most pairs of tree nuts, there was no difference in pass rates between cashew and walnut ($p=0.25$).

We next compared participants who passed or failed their tree nut OFC and found an association between specific IgE at OFC for almond ($p = 0.029$), cashew ($p = 0.011$), and walnut ($p = 0.0007$). There was also an association between OFC outcome and specific IgE at diagnosis for cashew ($p = 0.0053$) and walnut ($p < 0.0001$). Additionally, association between SPT at diagnosis and OFC outcomes were found for almond ($p = 0.012$), cashew ($p = 0.0076$), and walnut ($p = 0.012$). There was no association between diagnostic test results and outcome for the hazelnut challenges (Table 4).

The interquartile ranges of sIgE at OFC for almond, cashew, hazelnut, and walnut were plotted with outliers (Figure 2). Patients that passed hazelnut challenges had IgE at OFC values with the largest range. Cashew and walnut appeared to have more narrow ranges for challenges that passed.

We compared epinephrine requirement for failed challenges among the four tree nuts and also separated by allergic comorbidities. We did not find a significant differences among conditions in either analysis (Table E1, E2).

ROC analysis revealed that cashew (AUC, 0.66) and hazelnut (AUC, 0.39) IgE at OFC were poor predictors of failed challenge outcome (Figure 3A and 3B). While there is an association between IgE at OFC and failing an almond challenge (AUC, 0.87), the

IgE at the estimated 95% predicted probability of challenge failure would be 167 kU/L (Table E3), which is much higher than any of the observed almond IgE results in our population (Figure 3C). This could be due to the small number of failed almond challenges used for the analysis (n=3). We also found an association between IgE at OFC and failing a walnut challenge (AUC, 0.7, Figure 3D). The IgE at the estimated 95% predicted probability of walnut challenge failure was slightly lower than 12 kU/L (Table E3). 50th PPV for the nuts are shown in Table 5. As shown, for cashew and walnut, we further specified our analyses to determine PPVs separated by reason for avoidance. "Prior ingestion" accounted for challenges performed after a known ingestion of the allergen. "Preventatively avoiding" accounted for challenges performed without a suspected ingestion, but had positive SPT or sIgE indicating sensitivity to the specific tree nut allergen (Table 5).

DISCUSSION

As common food allergens, tree nut allergy affects 1% of all children in the United States.³ Additionally, children with IgE-mediated nut allergy have been shown to have more severe reactions than those with other food allergies.³ While OFCs remain as the gold standard for food allergy diagnosis, there are various obstacles such as time, resources, and risk of severe reaction. Thus, it is important to develop a clearer idea of the diagnostic utility and accuracy of skin prick tests and sIgE tests to help guide clinical decisions.

We performed an examination of over 500 oral food challenges characterizing the diagnostic testing for tree nut allergy and found that there were differences among tree nuts. Overall, 74.0% of children passed their OFC, and regarding the four most commonly challenged tree nuts (almond, cashew, hazelnut, and walnut), most patients passed OFC to almond (97.3%) and hazelnut (87.9%). Pass rates for cashew and walnut were significantly lower ($p < 0.0003$) with pass rates of 65.3% and 57.0% respectively. Comparing participants who passed or failed their tree nut OFC, we found an association between sIgE at OFC for almond ($p = 0.029$), cashew ($p = 0.011$), and walnut ($p = 0.0007$). Furthermore, the odds of failure were 0.83 times lower for patients who were preventatively avoiding compared to those who were not ($p = 0.0025$).

This study reviewed tree nut oral food challenges performed for standard clinical care. Therefore, oral challenges were performed to confirm or refute a diagnosis of food allergy, or to determine the development of natural tolerance. Fleischer et al suggested that in children > 5 years of age, a sIgE value < 5 kU/L conferred a 58% chance of

passing the challenge.¹⁰ In our study, 459 out of 531 challenges (86.4%) had an sIgE < 5 kU/L at OFC. Looking at only those OFCs with a tree nut sIgE < 5 kU/L, 335 out of 459 challenges (73.0%) passed. In a tree nut study conducted by Couch et al, 76% of 67 TN OFCs passed, even as most challenges took place with recent SPT wheal size <3 mm and/or sIgE <2 kU/L and the median sIgE of the passed challenges was 0.35 kU/L (mean sIgE 0.99 kU/L).¹¹ Thus, our study had a higher median sIgE of 0.61 kU/L (mean 2.56 kU/L) than the Couch study, with a similar pass rate; and a higher pass rate than the Fleischer study.

While using the overall threshold of 5 kU/L shows a high pass rate, our study sought to determine differences between individual tree nuts. Overall, we found that patients challenged to almond or hazelnut were likely to pass, with 97.3% of almond and 87.9% of hazelnut challenges passing. This was similar to results found in Couch's study where 100% of almond challenges and 83% of hazelnut challenges passed--the highest pass rates out of the nuts they examined.¹¹ Of all nine tree nuts that we included in our study, cashew and walnut accounted for the majority of reactions, similar to results found by Fleischer et al.¹⁰ These data suggest that pass rates may be different among the tree nuts. Therefore, we sought to examine differences among the tree nuts more closely.

Patients challenged to cashew and walnut passed with significantly lower rates (65.3% and 57.0% respectively) as compared to almond and hazelnut ($p < 0.0001$). These data suggest that almond and hazelnut challenges may be able to be offered at higher specific IgE levels than cashew and walnut. Additionally, ROC analysis was done to determine the predictive strength of sIgE at OFC for failed challenges. The ROC

curves revealed that almond (AUC, 0.87) and walnut (AUC, 0.71) IgE at OFC were acceptable predictors for failed challenge outcome. However, the calculated almond and walnut IgEs at the estimated 95% predicted probability, 167 kU/L and 12 kU/L respectively, were much higher than observed in our study population. For almond, this could be due to the high passing rate for almond challenges (97.3%). ROC analysis for cashew (AUC, 0.66) and hazelnut (AUC, 0.39) IgE at OFC were insufficient predictors of failed challenge outcome. Overall, the 50th PPV for cashew was 3.35 kU/L and walnut was 2.84 kU/L.

Upon review of hazelnut OFCs, we found that 63.6% of our study population did not have birch sensitization testing. Of the 36.4% total subjects who did have birch testing, 66.7% tested positive to birch. Of those that passed the hazelnut OFC and were tested to birch (28 subjects): 67.9% were sensitized to birch (n=19). Of those that failed the challenge and were tested to birch (8 subjects): 62.5% were sensitized to birch (n=5). Although the numbers are small, this suggests that birch sensitization was common among all children that were tested, but sensitization rates did not differ between children who passed or failed their hazelnut OFC. The high rates of birch sensitization in both groups support the use of component testing for hazelnut in children. Larger studies are needed to confirm these observations.

Reason for avoidance also affected pass rates of tree nut OFC. "Prior ingestion" indicates children that were avoiding the nut due to prior symptoms on ingestion along with positive testing. "Preventative avoidance" is defined as avoiding the nut due to positive testing only (without prior ingestion) or allergy to another nut. Only cashew and walnut are compared between reason of avoidance because so few patients failed

almond and hazelnut challenges; therefore, we had insufficient sample size to compare failed challenges for almond and hazelnut. When challenged after avoiding due to prior reaction (to that specific nut), the predicted probability at the 50th percentile was 0.83 kU/L for cashew and 0.63 kU/L for walnut (Table 5). When challenged after preventative avoidance, the predicted probability at the 50th percentile was noticeably higher-- with a corresponding sIgE of 4.38 kU/L for cashew and 3.46 kU/L for walnut. Furthermore, we found that the overall odds of failing a challenge are 0.83 times lower for those who were avoiding tree nuts due to sensitivity indicated by sIgE results of one or more other nuts (including peanut) compared to those who had a prior ingestion and subsequent reaction to the challenged nut ($p=0.0025$). This suggests that those children preventatively avoiding tree nuts due to another nut allergy (including peanut or other tree nut) pass tree nut challenges at higher sIgE levels. In addition, Couch et al found that 91% ($n=42$) of TN sensitized patients who were challenged after preventatively avoiding tree nuts passed their OFC, further supporting the claim that more aggressive use of OFCs in these patients should be considered.

The positive predictive values (PPVs) at the 50th percentile for cashew and walnut, not taking into consideration reason for avoidance, were 3.35 kU/L and 2.84 kU/L respectively. Since both values fall within the range of observed sIgE at OFC of our study population, it is reasonable to use these two probabilities to assess clinical outcomes for failed OFCs. The PPV at the 50th percentile for almond was calculated to be 92.8 kU/L which reflected the low fail rate we observed. For hazelnut, PPVs were unable to be attained by the statistical model because the values in its corresponding plot fell below a predicted probability of 0.2. Because of the inconclusive values derived

for almond and hazelnut, it would be valuable to study a large cohort of almond and/or hazelnut sensitized patients challenged at a higher sIgE at OFC to develop a better model to estimate PPVs.

Along with analyzing sIgE of individual tree nuts, our study also reviewed epinephrine requirement for failed challenges among the different nuts. We found that epinephrine use for failed OFC was not significantly different based on the specific nut ($p = 0.53$). Since there is provider based variability and subjectivity when epinephrine is used, epinephrine use was looked at independently from severity scoring.

Our study provides a real world examination of people who get offered an oral food challenge. Due to current clinical recommendations, challenges are only offered to patients who have a high likelihood of passing, meaning that sIgE at OFC and SPT are likely to be lower in our study population than if we had offered OFCs to all patients presenting with tree nut allergy. Additionally, challenges are offered to and performed on food allergic or sensitized individuals, and thus may not be generalizable to other populations. All challenges were open and not double blinded, which could make subjective failure a possibility and influence challenge results.

In conclusion, we have shown that differences exist among individual tree nuts. Most children pass almond and hazelnut oral food challenges even with sIgEs at OFC over the current suggested decision point thresholds. Children who are avoiding tree nuts due to sensitization to one or more other nut are also more likely to pass their oral food challenge compared to those who have had a prior ingestion and reaction. While further work is needed on hazelnut and almond challenges to determine more clinically

400 relevant PPVs, our data suggests that almond and hazelnut can be challenged more
401 aggressively and cashew and walnut challenged more conservatively.

References

1. Alsalvar C, Shahidi F. Tree nuts: Composition, Phytochemicals, and Health Effects. Boca Raton: CRC Press; 2008.
2. Gupta RS, Springston EE, Warrier MR, Smith B, Kumar R, Pongratic J, Holl J. The prevalence, severity, and distribution of childhood food allergy in the United States. *Pediatrics*. 2011; 128:e9-17.
3. Weinberger T, Sicherer S. Current perspectives on tree nut allergy: a review. *J Asthma Allergy*. 2018; 11: 41-51.
4. Perry TT, Matsui EC, Conover-Walker MK, Wood RA. Risk of oral food challenges. *J Allergy Clin Immunol*. 2004; 114: 1164-1168.
5. Moloney JM, Rudengren M, Ahlstedt S, Bock SA, Sampson HA. The use of serum-specific IgE measurements for the diagnosis of peanut, tree nut, and seed allergy. *J Allergy Clin Immunol*. 2008; 122(1): 145-151.
6. Ho MH, Heine RG, Wong W, Hill DJ. (2006, Jun). Diagnostic accuracy of skin prick testing in children with tree nut allergy [Letter to the editor]. *J Allergy Clin Immunol*. 117(6): 1506-8.
7. Branum AM, Lukacs SL. Food allergy among US children: trends in prevalence and hospitalizations. *NCHS Data Brief*. 2008; 10:1-8.
8. Steifel G, Anagnostous K, Boyle RJ, Brathwaite N, Ewan P, Fox AT, Luyt D, Till SJ, Venter C, Clark AT. BSACI guideline for the diagnosis and management of peanut and tree nut allergy. *Clinical and Experimental Allergy*. 2017; 47: 719-739.

- 423 9. Bock A, Sampson HA, Atkins FM, Zeiger RS, Lehrer S, Sachs M, Bush RK, Metcalfe
424 DD. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure:
425 A manual. J Allergy Clin Immunol. 1988. 82(6): 986-97.
- 426 10. Fleischer DM, Conover-Walker MK, Matsui EC, Wood RA. The natural history of
427 tree nut allergy. J Allergy Clin Immunol. 2005. 116(5):1087–1093.
- 428 11. Couch C, Franxman T, Greenhawt M. Characteristics of Tree Nut Challenges in
429 Tree Nut Allergic and Tree Nut Sensitized Individuals. Ann Allergy Asthma Immunol.
430 2017. 118(5): 591-596.

431

FIGURE LEGENDS

Figure 1: **Oral Food Challenge Outcome for All Tree Nuts.** *Stacked bars represent the number of OFCs. The light stack represents passed challenges and the dark represents failed challenges. Percentages above the stack represent the pass rate for that tree nut. The p-value indicates that there are significant differences in pass rates among the tree nuts.*

Figure 2: **Specific IgE at time of OFC.** *White boxes represent passed OFCs, grey boxes represent failed OFCs. The line represents the median and boxes represent the 25th and 75th percentiles. Outliers are shown.*

Figure 3: **Predicted probabilities and ROC curves of IgE at OFC for cashew (A), hazelnut (B), almond (C), and walnut (D).**

	level	Overall (n = 531)	Pass (n = 393)	Fail (n = 138)	p-value
Age at visit (mean (SD))		7.78 (4.33)	7.73 (4.33)	7.92 (4.35)	0.6714
Sex(%)	Female	225 (42.5)	164 (41.8)	61 (44.5)	0.6045
	Male	304 (57.5)	228 (58.2)	76 (55.5)	
Race(%)	Non-White	177 (33.8)	140 (36.1)	37 (27.4)	0.0630
	White	346 (66.2)	248 (63.9)	98 (72.6)	
Eczema(%)	No	272 (51.8)	195 (50.3)	77 (56.2)	0.2502
	Yes	253 (48.2)	193 (49.7)	60 (43.8)	
Asthma(%)	No asthma or wheezing	313 (59.7)	224 (57.9)	89 (65.0)	0.2560
	Asthma	182 (34.7)	139 (35.9)	43 (31.4)	
	Wheezing	29 (5.5)	24 (6.2)	5 (3.6)	
Allergic rhinitis (%)	No	210 (40.1)	152 (39.3)	58 (42.3)	0.5518
	Yes	314 (59.9)	235 (60.7)	79 (57.7)	

Table 1: **Demographics** Overall demographics of children (0 to 20 years) who completed an unblinded oral food challenge (OFC) of any tree nut from 2007 - 2019 at Lurie Children's Hospital, overall and by challenges that passed and failed. The p-value compared children who passed to those that failed an oral food challenge. Frequency and percent are reported for all categorical variables, whereas, mean (SD) are reported for numeric variables.

	Tree nut	Pass (n = 319)	Fail (n = 94)	p-value
food (%)	Almond	110 (97.3)	3 (2.7)	<.0001
	Cashew	66 (65.3)	35 (34.7)	
	Hazelnut	86 (87.9)	13 (12.1)	
	Walnut	57 (57.0)	43 (43.0)	
	Overall	319 (77.2)	94 (22.8)	

Table 2: **Tree Nut Challenge by Outcome.** Number (percent) of challenges passed and failed are shown for each nut. The percent represents the percent of challenges that passed out of the total number of challenges for a given tree nut. The global p-value indicates that there is significance between pass and fail rates depending on which tree nut is challenged.

Comparison	Odds Ratio	95% Confidence Interval	P-Value
Almond vs. Cashew	0.73	(0.66, 0.80)	<0.0001
Almond vs. Hazelnut	0.90	(0.84, 0.96)	0.0027
Almond vs. Walnut	0.67	(0.60, 0.75)	<0.0001
Cashew vs. Hazelnut	1.24	(1.10, 1.39)	0.0003
Cashew vs. Walnut	0.92	(0.80, 1.06)	0.2483
Hazelnut vs. Walnut	0.74	(0.66, 0.84)	<0.0001

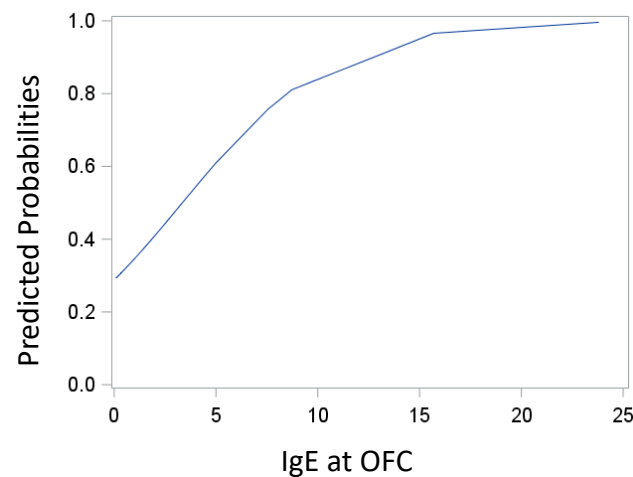
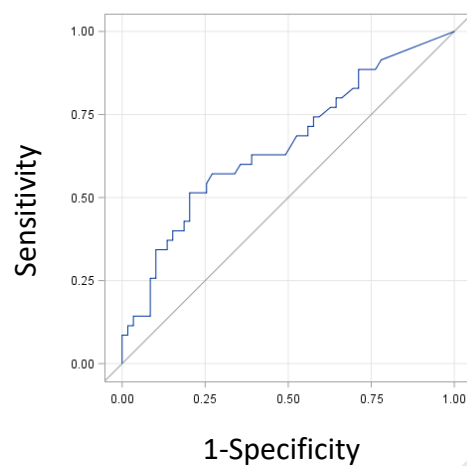
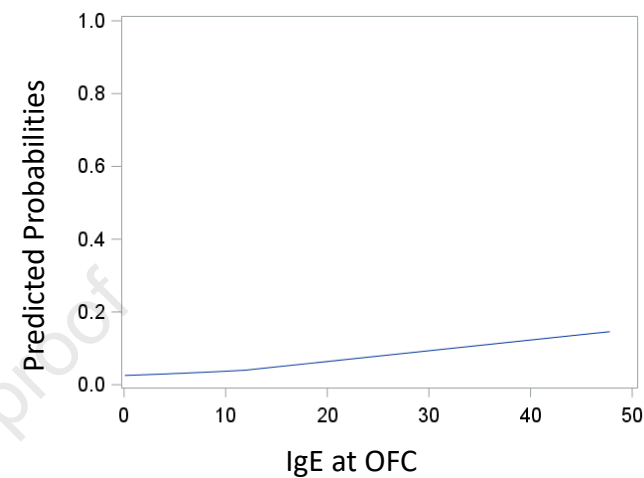
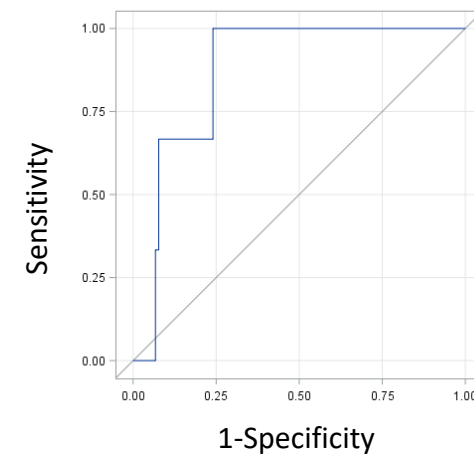
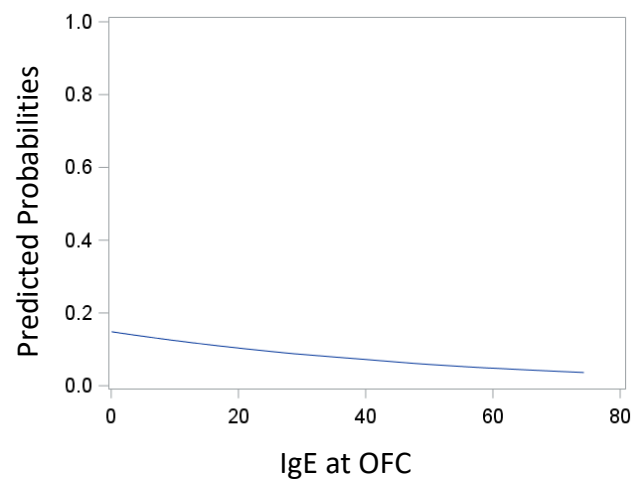
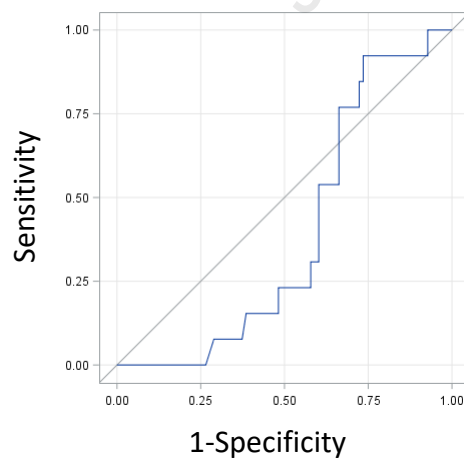
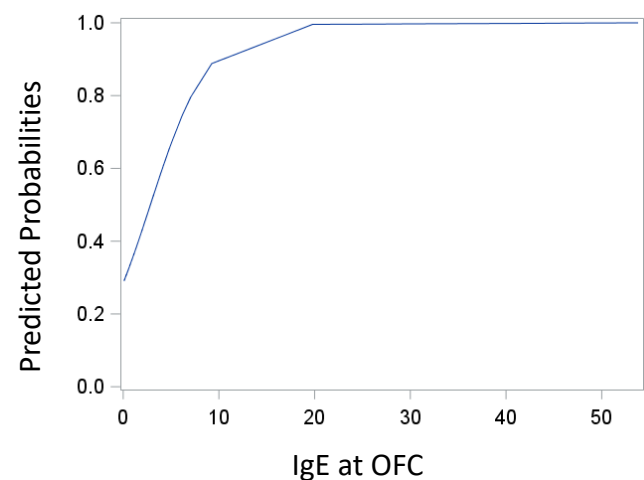
Table 3: **Odds ratio of Passing Individual Nut OFCs.** *Pairwise comparisons were performed between each possible pair of tree nuts. The odds ratio compares the chance of failing the OFC to the first nut compared to the second nut listed.*

	Almond			Cashew			Hazelnut			Walnut		
	Pass (n = 110)	Fail (n = 3)	p-value	Pass (n = 66)	Fail (n = 35)	p-value	Pass (n = 86)	Fail (n = 13)	p-value	Pass (n = 56)	Fail (n = 38)	p-value
IgE at OFC (kU/L)	0.70 [0.20, 1.70]	4.46 [3.23, 4.73]	0.0290	0.34 [0.11, 0.64]	1.06 [0.26, 2.96]	0.0108	1.00 [0.35, 5.46]	2.10 [1.68, 2.97]	0.2160	0.71 [0.15, 1.35]	1.86 [0.64, 3.94]	0.0007
IgE at dx (kU/L)	0.67 [0.20, 2.19]	4.00 [3.00, 4.23]	0.0562	0.37 [0.15, 0.97]	1.14 [0.33, 3.25]	0.0053	0.87 [0.33, 5.46]	2.58 [1.68, 3.48]	0.0582	0.35 [0.14, 1.29]	2.41 [0.67, 3.64]	<.0001
SPT at dx (mm)	4.00 [2.75, 5.00]	8.00 [7.00, 9.00]	0.0122	3.00 [0.00, 5.00]	6.00 [3.00, 8.00]	0.0076	4.00 [1.50, 5.00]	6.00 [3.00, 7.00]	0.1403	3.00 [0.00, 4.00]	4.00 [3.00, 6.00]	0.0115

Table 4: **Diagnostic Test Results.** Allergy test results comparing participants who passed and failed challenges to almond, cashew, hazelnut, and walnut. Median [IQR] are represented in results.

		Prior ingestion	Preventatively avoiding
Tree Nut	Predicted Probability	Corresponding Value of IgE at OFC	Corresponding Value of IgE at OFC
Cashew	0.25	-1.67185	0.5040
	0.50	0.82500	4.3765
	0.95	7.51691	14.7552
Walnut	0.25	-0.08492	0.3056
	0.50	0.62607	3.4553
	0.95	2.53161	11.8969

Table 5: Predicted probabilities for failing a cashew or walnut challenge separated by reason for avoidance

A**Cashew****ROC Curve [AUC = 0.66]****Almond****ROC Curve [AUC = 0.87]****B****Hazelnut****ROC Curve [AUC = 0.39]****D****Walnut****ROC Curve [AUC = 0.71]**