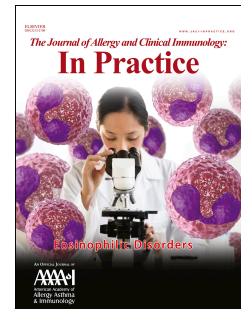


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Added diagnostic value of peanut component testing: a cross sectional study in Australian children

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Added diagnostic value of peanut component testing: a cross sectional study in Australian children

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Abstract:**Added diagnostic value of peanut component testing: a cross sectional study in Australian children**

BACKGROUND: Peanut components are widely used in clinical practice; however, their utility to predict challenge outcome in the Australian children, outside of infants, is not well studied.

OBJECTIVE: Can peanut component testing predict outcome of challenge in peanut allergic children.

METHODS: All children attending peanut challenges, regardless of prior allergic reactions to peanut or sensitization (skin prick testing or peanut-IgE) alone, were recruited. Serum collected before the challenge was analysed for peanut-IgE, Ara h 1, 2, 3, 6, 8 and 9 (ImmunoCap).

RESULTS: Of the 222 children recruited, 89 (40%) were allergic on OFC (oral food challenge). Ara h 2 and 6 performed similarly to peanut-IgE and SPT in predicting challenge outcome (AUC 0.84-0.87). No baseline clinical characteristics, including past history, predicted challenge outcome. By logistic regression, degree of polysensitization to Ara h 1, 2 or 3 increased the odds of allergic reaction at OFC at 0.35 and 1.0 kU_A/L cut-off levels ($p < 0.001$ for both). All 11 children sensitized (> 0.35 kU_A/L) to Ara h 1, 2 and 3 reacted to peanut challenge. Degree of polysensitization at > 1.0 kU_A/L was associated with a lower cumulative eliciting dose ($p = 0.016$) and with severity of allergic reaction on challenge ($p = 0.007$).

CONCLUSION: In our cohort, sensitisation to the combination of Ara h 1, 2 and 3 was highly predictive of peanut allergy. Overall, only Ara h 2 as individual component, most correlated with severity of reaction at challenge and adrenaline use. Ara h 8 and 9 were not useful in predicting challenge outcome.

What is already known about this topic? A number of studies have suggested IgE to peanut component Ara h 2, may be a better predictor to differentiate sensitization and clinical reactivity to peanut.

What does this article add to our knowledge? This study suggests that polysensitization to Ara h 1, 2 and 3 may be better marker to, not only predict the outcome of peanut challenge, but also the severity of reaction at challenge.

How does this study impact current management guidelines? Combination of Ara h 1, 2 and 3, may be a helpful diagnostic test to identify patients with persistent peanut allergy regardless of whether they were sensitized or had clinical reaction in the past.

Key words: Peanut allergy, component-resolved diagnostics (CRD), open peanut challenge, Ara h 1, 2, 3, 6, 8, 9 proteins, skin prick testing (SPT), serum specific peanut IgE (peanut IgE)

Abbreviations used

SPT: Skin prick test

peanut-IgE: Serum specific peanut IgE

CRD: Component-resolved diagnostics

OFC: Oral Food Challenge

MWD: Mean Wheal Diameter

IQR: Interquartile range

AUC: Area under the curve

PPV: Positive predictive value

NPV: Negative Predictive Values

ED: Eliciting dose

The prevalence of IgE mediated peanut allergy in Australian infants and children is estimated to be 2-3%^{1,2}. In Australia, peanut is the most common cause of paediatric anaphylaxis hospital emergency presentations^{3,4,5} and food related anaphylaxis fatalities^{4,5,6}.

Making a correct diagnosis of peanut allergy is a vital part of clinical management; however, the diagnosis of peanut allergy and subsequent determination of tolerance remains problematic due to imperfect tests available to the clinician. Oral food challenges remain the gold standard^{7,8,9} but are time consuming, resource limited and carry the risk of anaphylaxis. Although both skin prick testing (SPT) and serum specific peanut IgE (peanut IgE) have established positive predictive values (PPV) for peanut allergy^{10,11}, the limitations of these tests include the establishment of cut-offs that are often not generalisable to other cohorts and interpreting values which fall below the 95% PPV.

Rather than measuring IgE production against whole peanut, component-resolved diagnostics (CRD) detects specific IgE against individual allergenic peanut protein components^{12,13} and is being increasingly used in clinical practice. Although 13 relevant Ara h proteins have been identified, Ara h 2 appears the most useful diagnostic component to date¹⁴⁻¹⁸. Reports have suggested that higher accuracy and prediction of severity may be improved by also assessing Ara h 1, 3, 8 and 9^{15,20,21}. Similarly, higher epitope diversity has been shown to be more associated with peanut challenge outcome than single parameters, such as peanut-IgE^{22,23,32}.

There appear significant regional variations in prevalence and relevance of Ara h 8 and 9 in particular, depending on the co-sensitization patterns for birch homologues in Northern Europe/US and lipid transfer protein cross reactivity in the Mediterranean region^{19-21, 24-27}. Australia has different patterns of aeroallergen and lipid transfer protein sensitization than Europe/US, and the clinical relevance of sensitization to Ara h 8 and 9 in Australia remains unclear. Although the utility of Ara h 2 for peanut diagnosis in Australia has been reported, this was studied in young infants, and the diagnostic utility of other CRDs was not examined¹⁴.

We aimed to determine the diagnostic accuracy of peanut-IgE to Ara h 1, 2, 3, 6, 8, 9 in predicting the challenge outcome of children, referred by the allergists for OFC to peanut as part of their routine clinical care.

METHODS

Recruitment

The study was approved by Sydney Children Hospitals Network Human Research Ethics Committee HREC: LNR/14/SCHN/516. Children were recruited from May 2016 to March 2018, from specialist allergy services at three large public hospitals located in New South Wales (NSW), Australia, including The Children's Hospital at Westmead, Sydney Children's Hospital at Randwick and Campbelltown Hospital.

Written consent was obtained from carers of all children. All children were eligible to participate if they were referred by their treating Allergist for an oral food challenge (OFC) to peanut as part of their routine clinical care. Children may have had a prior reaction to peanut or sensitized alone on SPT or peanut-IgE. Children were not excluded if they had had a prior history of anaphylaxis, or if their peanut-IgE/SPT was > 95% PPV. Children were only excluded if they had already had peanut-IgE to Ara h components performed.

Prior to the OFC, all children underwent medical evaluation and their history of peanut allergy prospectively documented (Figure E1 Online Repository www.jacionline.org). All children at the time of or within 3 months of the booked OFC, had serum stored and frozen at -20⁰ C, and underwent a standardised panel of SPT to peanut, rye, bahia, bermuda grass, English plantain and birch using standard lancet technique, standardized across sites. SPT equal to or greater than 3 mm, greater than the negative control was considered positive.

The serum samples were analysed using Unicap (Phadia) for total IgE (measured in IU/ml), peanut-IgE, peanut-IgE to recombinant Ara h 1,2,3,6, 8 and 9 (specific IgE titres were measured in kU_A/L with a range of 0.01 kU_A/L to 100 kU_A/L). Sensitization was defined as > 0.35 kU_A/L according to commonly used reporting ImmunoCAP reporting criteria. We also used cut off of >1.0 kU_A/L to compare the test performance characteristics using a higher level.

All children underwent open peanut challenge using a standardised food challenge protocol (ASCI), with reactions scored according to PRACTALL guideline²⁸. In addition to objective symptoms, persistent subjective symptoms of abdominal pain and nausea were sufficient to stop the challenge, however stopping criteria using the PRACTALL scoring system was encouraged. Nursing and medical staff performing the challenge were blinded to peanut SPT. Anaphylaxis was defined as per Australasian Society of Clinical Immunology and Allergy definitions²⁹. The cumulative eliciting dose (ED) was determined as the total dose of peanut (in mg) eliciting an allergic reaction resulting in challenge cessation.

The severity of allergic reaction at OFC was assessed in two ways, firstly by the requirement for adrenaline, and secondly by a modified PRACTALL score, which included the sum of only symptom scores in the categories which were suggestive of reaction severity including all cardiovascular and neurological (0-3), lower respiratory (0-3), laryngeal (0-3), and objective gastroenterological (0-3) symptoms to a total maximum of 12.

Statistical analysis

The diagnostic test accuracy measures included sensitivity, specificity, positive predictive value (PPV), negative predictive values (NPV), receiver operator characteristics (ROC) and area under the curve (AUC). To generate sensitivity and specificity for peanut-IgE and peanut components the following cut-offs were used:

0.01, 0.1, 0.35, 1, 10, 100, 1000 kU_A/L. The nonparametric random forest methods were used to investigate the components' ability to correctly discriminate between the subjects with peanut tolerant and peanut allergic subjects.

Analysis of peanut-IgE data was performed with and without children 0-2 years to assess for possible bias due to lower IgE production in younger children. Poly-sensitization was defined as sensitization to more than one component in 2 separate scenarios: > 0.35 kU_A/L (definition 1) and >1.0 kU_A/L (definition 2).

Due to the high correlation between Ara h 2 and Ara h 6-sensitization to Ara h 2 and or 6 were considered a single sensitization for these scenarios.

RESULTS

Patient characteristics

A total of 222 children were eligible and recruited. Baseline characteristics are shown in Table I. Sixty six percent were male with a median cohort age of 8.0 years (5.0 – 12.0) at OFC. Concurrent atopy at the time of OFC was common. Of the 222 children, 37% had no history of prior peanut exposure and were avoiding peanut based on sensitization alone. Fourteen percent (32/222) of children had past history of anaphylaxis to peanut and 70% (154/221) of the total cohort were avoiding other food/s on the basis of IgE-mediated food allergy by history.

Challenge Outcome

133/222 children (60%) were peanut tolerant and 89 (40%) were peanut allergic at study OFC (Figure 1). Three of 89 children were considered to have reacted to peanut on challenge due to persistent subjective abdominal pain and nausea, without any objective symptoms. Children who reacted at OFC were slightly older than those who were tolerant, however past history or past history of severe allergic reactions to peanut was not predictive of OFC outcome (Table I). Of the 89 children who reacted at OFC, 30/89 (34%) experienced anaphylaxis requiring treatment with adrenaline and the remainder had mild or moderate reactions (Figure I). Twenty of 30 children with anaphylaxis at OFC required one dose of adrenaline, 9 required 2 doses and one child required 3 doses of adrenaline. Of the 30 children with anaphylaxis at OFC, 5 had previous history of anaphylaxis and 7 had history of sensitization only. Of those children who were allergic at OFC, the median cumulative eliciting dose (ED) of peanut was 1075mg (IQR 300-2325mg).

Univariable associations between serum and skin testing and peanut challenge outcome

The most common sensitization was to Ara h 2 and 6. At >0.35 kU_A/L, 72% and 81% of children sensitized to these components were allergic at OFC, respectively. This was followed by Ara h 1, Ara h 9 and 3 sensitization respectively (Table II) 7 (8%) of

children allergic on OFC were not sensitized to any component at >0.35 kU_A/L (Table III).

The median values of peanut SPT, peanut IgE, Ara h 2 and 6 were higher in children who reacted on challenge than those who were tolerant (Table II). Sensitivity, specificity, area under the curve (AUC) and positive and negative predictive values were generated for peanut SPT, peanut-IgE and specific IgE for all peanut components (Table IV). Overall test performance characteristics of peanut SPT, peanut-IgE and Ara h 2 and Ara h 6 were similar at AUC 0.84-0.87, whereas Ara h 1, 3, Ara h 8 and 9 were not predictive of OFC outcome (Figure II).

When the ratios of individual peanut components to peanut-IgE or total IgE were examined, this ratio compared to individual peanut component level, this did not improve performance in predicting peanut challenge outcome (Table E1, E2, Figure E2, E3, Table E2 Online Repository www.jacionline.org). There was a high degree of correlation between levels of Ara h 2 and Ara h 6 ($r=0.79$), (Figure E4 Online Repository www.jacionline.org). Lower correlation between other components was observed.

All above analysis were repeated excluding children aged 2 years and less, to examine for any effects of reduced production of IgE with younger age. The results were similar in this cohort of 210 children (data not shown).

Poly-sensitization to peanut components and OFC outcome

Children were categorized as non-sensitized, monosensitized and polysensitized to peanut components (excluding co-sensitization to Ara h 2 and 6 as polysensitization), separately for cut-offs >0.35 and >1 kU_A/L. For both cut offs, the proportion reacting to OFC increased with the level of sensitization (Table III).

We then examined sensitization patterns restricted to the components Ara h 1, 2 and 3, excluding Ara h 6 (due to high degree of correlation with Ara h 2), Ara h 8 and Ara h 9. Sensitization to an increasing number of components at >0.35 and >1 kU_A/L was associated with peanut allergy at OFC (Table III).

Twenty-one children were sensitized to exactly 2 components of Ara h 1, 2 or 3 at >0.35 kU_A/L and a further 11 were sensitized to all 3 components (Ara h 1, 2 and 3). The most common pattern of sensitization was the combination of Ara h 1 and 2 ($n=14$), with 4 sensitized to Ara h 1 and 3 and only 3 children sensitized to the combination of Ara h 2 and 3. In all combinations, reaction at OFC was high.

All 11 children who were sensitized to Ara h 1, 2 and 3 at >0.35 kU_A/L reacted on OFC (7 had anaphylaxis), Table III. Of these children; 6/11 children had previous sensitization only (of whom 3 had anaphylaxis at study OFC); 5/11 children had history of allergy (of whom 4 had anaphylaxis at study OFC), all with peanut SPT of between 5.5-12mm.

Peanut components and severity of peanut allergic reactions at challenge

We examined whether increasing levels of IgE to peanut components and peanut-IgE were associated with severity of the allergic reaction at OFC using modified PRACTALL severity scores (as outlined in methods). There was a positive correlation between peanut IgE ($r=0.56$), Ara h 1 ($r=0.32$), Ara h 2 ($r=0.61$) and Ara h 6 ($r=0.71$) and severity of allergic reaction at OFC by severity score, whereas little or no correlation between levels of Ara h 3, 8 and 9 and severity was observed (Table V).

In those children who reacted at OFC, the degree of polysensitization (any component) was associated with reaction severity at the >1.0 kU_A/L level ($p=0.0067$) but not at >0.35 kU_A/L ($p=0.19$). However, reaction severity scores were higher in those children who were polysensitized to Ara h 1, 2 and 3 at both >0.35 and >1.0 kU_A/L compared with those who were monosensitized or not sensitized to these ($p=0.012$, $p=0.014$ for >0.35 and >1.0 respectively).

Children who required adrenaline at OFC had higher serum levels of IgE to total peanut, Ara h 1 and 2 than those children who reacted but did not receive adrenaline at OFC ($p=0.0009$, AUC 0.77; $p=0.02$, AUC 0.66 and $p=0.008$, AUC 0.80 respectively, Table V). No other component levels were highly associated with adrenaline use, including Ara h 6 ($p=0.065$, AUC 0.78). Patterns of mono/polysensitization were not predictive of use of adrenaline at OFC in contrast to severity scores.

Peanut components and eliciting dose of peanut protein at challenge

Total amount of Ara h 6 correlated most closely with lower cumulative eliciting dose of peanut protein at OFC ($r=-0.74$), with levels of Ara h 2 and total peanut IgE also highly negatively correlated (Table V). In the children who reacted at OFC, polysensitization (>1.0 kU_A/L of Ara h 1, 2 and/or 3) was associated with a lower cumulative eliciting dose at challenge ($p=0.0186$). The median (IQR) cumulative eliciting dose of peanut protein in children who were non-sensitized, monosensitized and polysensitized at >1.0 kU_A/L was 1270g (1075-4645), 1075mg (300-2325) and 470mg (158-1440) respectively.

Associations between aeroallergen sensitization, peanut components and OFC outcome

In our cohort, 128 children (58%) were sensitized to one or more of the aeroallergens tested by SPT prior to the OFC (grass, weed and tree pollens), primarily to ryegrass, bahia, bermuda and English plantain with only 19% sensitized

to birch pollen. Sensitization to one or more aeroallergens did not correlate with challenge outcome ($p=0.92$) or to Ara h 8 ($p=0.13$) or Ara h 9 (0.26).

There was no relationship between the outcome of peanut challenge and sensitization to Ara h 8. A total of 7 children had Ara h 8 > 0.35 kU_A/L (including 4 children with Ara h 8 > 1.0 kU_A/L). Two of the 7 children with Ara h 8 > 0.35 kU_A/L were monosensitized, and one reacted on challenge and one did not. Likewise, Ara h 9 sensitization was not predictive of challenge outcome, at either >0.35 kU_A/L ($n=23$) or >1 kU_A/L ($n=11$). Eight children were monosensitized to Ara h 9 at >0.35 kU_A/L; 7 of these children were tolerant and one allergic on OFC.

DISCUSSION

It is well established that regional variations in the patterns and clinical significance of production of IgE to peanut components exist^{16, 19-21, 24-27}. Many cohorts around the world have reported Ara h 2 as a superior test to peanut SPT or total peanut IgE in predicting peanut challenge outcome¹⁴⁻¹⁸, however apart from the infants from the HealthNuts cohort in Melbourne¹⁴, Australian data has been lacking. For example, the significance of Ara h 8 sensitization (outside of birch predominant regions) and that of Ara h 9¹⁹, outside of the Mediterranean is unclear.

Our cohort provides potentially useful insights into predictors for clinical peanut allergy within Australia, based upon populations similar to those presenting for clinically indicated peanut food challenges, to establish diagnosis or tolerance in routine clinical practice. Our results may also have some useful globally applicable insights for the use of Ara h 1, 2 and 3 as a more predictive tool than any single peanut component or total peanut-IgE.

In our cohort of Australian children, sensitization to Ara h 2 and 6 was most common, Ara h 1 being the next most common, followed by Ara h 9 and 3. We found that the production of IgE to the combination of Ara h 1, 2 and 3 was highly predictive of peanut challenge outcome, and also was associated with more severe reactions at challenge (those that included laryngeal, lower respiratory, cardiovascular/neurological and/or objective gastroenterological symptoms) and lower cumulative peanut protein eliciting doses. These results were similar to results published by a number of other studies²¹.

Moreover, there was a clear dose effect, such that increasing the degree of polysensitization to Ara h 1, 2 and 3 was associated with increased reaction at challenge, reaction severity (by severity score) and lower eliciting dose. Although the level of IgE to total peanut IgE, Ara h 2 and Ara h 1 were highly correlated with adrenaline use at OFC, we did not find the same strong association between adrenaline use and polysensitisation vs monosensitization as we observed with polysensitisation to Ara h 1, 2 and 3 and reaction severity at OFC, as measured by the modified PRACTALL score. The most common combination of sensitization to 2 of these components was Ara h 1 and 3, a combination also described^{20,22,33}.

Polysensitization to peanut components as a marker for clinical peanut allergy and severity^{17,21,22, 30-32,34} is also consistent with peanut epitope diversity research, which also reported increasing severity of clinical allergic phenotype with production of IgE against more epitopes^{22,23,33,35}.

Individual levels of peanut components, and particularly Ara h 2 have been previously associated with challenge outcome severity and also of a history of more severe clinical allergic reactions to peanut¹⁴⁻¹⁸. This has also been reported to correlate with sensitization to Ara h 1, 3 and 6^{21,30,34,36}. In our cohort Ara h 6 correlated most closely with Ara h 2, not unexpectedly given they are both members of the prolamin protein family, and was the most highly correlated of all variables with challenge severity and eliciting dose. Systematic review by Kim et al, evaluated 3 studies for diagnostic accuracy of Ara h proteins (1, 2, 3, 6, 8 & 9) and reported higher sensitivity and specificity for Ara h 6 (94.9% and 95.1%)^{16,17,37,38,39}. We found specificity of 98% (sensitivity of 0.56) and PPV of 94% for Ara h 6 at ≥ 1.0 kUA/L. We did not find any children who were sensitized to Ara h 6, without Ara h2 sensitization in our cohort, however this pattern has been previously reported to be associated with severe reactions⁴⁰.

In this cohort of children, where 63% had a previous clinical reaction to peanut, we did not find that any single one of peanut SPT, peanut-IgE or Ara h 2 was clearly superior, although all 3 performed relatively well, and in line with most other published cohorts in predicting OFC outcomes. Given peanut SPT was a known variable for clinicians requesting the peanut OFC, the results of the SPT are surprisingly close to those of Ara h 2 and total peanut IgE but should be interpreted with some caution because of this study design. However, one may have expected children with known higher peanut SPT's to have been excluded from this real-life challenge population, so it is interesting that the AUC for SPT was still very similar to Ara h 2 and peanut IgE.

Sensitization to Ara h 9 was not uncommon in our population with 10 % of the cohort having an Ara h 9 >0.35 kUA/L, 35% of whom were sensitized only to this component. Ara h 9 is a lipid-transfer protein (LTP) protein, and generally associated with severe reactions in Southern Europe, particularly Spain where it may be associated with cross sensitization to Pru p 3, and the LTP /mugwort syndromes. However, the clinical relevance of sensitization to this component in the Australian setting is unclear²⁴, with no correlation between any outcome and sensitization to Ara h 9, including severity of reactions in those sensitized or OFC outcome.

A smaller number of children were sensitized to Ara h 8 in this cohort, a sensitization pattern typically associated with food pollen syndromes (oral allergy syndrome or isolated mild oral symptoms) in regions of high birch allergy due to the Bet v 1 homology^{18,24}. Ara h 8 in our population did not correlate with typical Australian NSW grass pollen sensitization, and few children were highly sensitized to birch pollen by SPT.

Limitations of the study include that the OFCs were conducted in an open fashion, not blinded or placebo controlled. This was a consequence of drawing the study population from children attending for peanut challenges as part of their routine clinical care, however all 3 study sites used the same PRACTALL criteria for scoring challenges. The discordance of severity scores and use of adrenaline with respect to polysensitisation may relate to use of adrenaline being at the supervising allergists clinician's discretion and minor variations in practice between the three study sites and to the inclusion of objective gastroenterological symptoms into the severity score.

In conclusion, our results suggest that consideration should be given for performing Ara h 1, 2 and 3 rather than Ara h 2 in isolation prior to conducting peanut challenges, where the pre-test probability of peanut allergy is otherwise relatively low, such as in a typical routine clinical practice for testing for either outgrowth of a peanut allergy, or where the history is unclear. We would suggest that further studies should be conducted to validate these findings before widespread adoption, however it would be highly unlikely that children sensitized to all of Ara h 1, 2 and 3, even at relatively low levels will be shown to be tolerant to peanut at OFC.

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Figure legends:

FIGURE I: Flowchart of outcome of OFC

FIGURE II: Area under the curve (AUC) for peanut SPT, peanut-IgE and peanut components (Ara h 1 to Ara h 9). Peanut SPT, peanut IgE and Ara h 2 and 6 performed similar at AUC 0.84-0.87, whereas Ara h 1, 3, Ara h 8 and 9 were not predictive of OFC outcome

TABLE I: Baseline patient characteristics in the tolerant and the allergic group and OFC outcome

TABLE II: Peanut-IgE and peanut component sensitization and peanut OFC outcome

TABLE III: Degree of sensitisation to peanut components and OFC outcome

TABLE IV: Test performance characteristics of peanut and peanut component IgE

TABLE V: Peanut-IgE and components association with challenge severity, cumulative eliciting dose and adrenaline use at OFC

TABLE I: Baseline patient characteristics in the tolerant and the allergic group and OFC outcome

	All patients (n = 222)	Tolerant at OFC (n = 133)	Allergic at OFC (n = 89)	Tolerant Vs allergic at OFC P-value*
Male (n, %)	146 (66)	87 (65)	59 (66)	0.89
Age at diagnosis, years, median (IQR)	2.0 (1.0-3.0)	2.0 (1.0-3.0)	2.0 (1.0-3.0)	0.34
Age at challenge, years, median (IQR)	8.0 (5.0-12.0)	7.0 (5.0-11.0)	9.0 (6.0-12.0)	0.04
Sensitised only (n, %)	83 (37)	55 (41)	28 (31)	0.14
Previous clinical reaction to peanut (n, %)	139 (63)	78 (59)	61 (69)	
Previous history anaphylaxis to peanut (n, %)	32 (14)	18 (14)	14 (16)	0.65
Other food allergies (n, %)	154 (70) ¹	91 (69) ²	63 (71)	0.77
Current allergic rhinitis (n, %)	108 (49)	66 (50)	42 (47)	0.72
Current asthma (n, %)	89 (41) ³	51 (39) ⁴	38 (45) ⁵	0.43
Current eczema (n, %)	88 (41) ⁶	55 (42) ⁷	33 (39) ⁸	0.64

¹ n = 221, ² n = 132, ³ n = 215, ⁴ n = 130, ⁵ n = 85, ⁶ n = 216, ⁷ n = 131, ⁸ n = 85. * P-value from chi-square test or logistic regression with baseline characteristic as predictor of OFC outcome.

TABLE II: Peanut-IgE and peanut component sensitization and peanut OFC outcome

	All patients (n = 222)	Tolerant at OFC (n = 133)	Allergic at OFC (n = 89)	P value***
Peanut SPT (mm), mean, median (IQR)	4.9, 5.3 (3.0-7.0)	3.4, 3.5 (1.0-5.5)	7.1, 7.0 (6.0-8.0)	<0.001
IgE peanut (kU _A /L), mean, median (IQR)	6.8, 0.9 (0.2-3.1)	1.4, 0.4 (0.1-1.0)	14.9, 2.9 (1.5-10.6)	<0.001
Ara h 1 (kU _A /L), mean, median (IQR)*	1.9, 0.0 (0.0-0.2)	0.2, 0.0 (0.0-0.1)	4.5, 0.1 (0.0-1.5)	0.006
Ara h 1 > 0.35 kU _A /L, n (%)	40	10 (8%)	30 (34%)	
Ara h 1 > 1 kU _A /L, n (%)	29	4 (3%)	25 (28%)	
Ara h 2 (kU _A /L), mean, median (IQR)**	4.3, 0.2 (0.0-1.3)	0.3, 0.1 (0.0-0.3)	10.3, 1.9 (0.5-9.8)	<0.001
Ara h 2 >0.35 kU _A /L, n (%)	95	27 (20%)	68 (78%)	
Ara h 2 > 1 kU _A /L, n (%)	64	11 (8%)	53 (61%)	
Ara h 3 (kU _A /L), mean, median (IQR) *	0.8, 0.1 (0.0-0.1)	0.1, 0.0 (0.0-0.1)	1.9, 0.1 (0.0-0.3)	0.09
Ara h 3 >0.35 kU _A /L, n (%)	22	4 (3%)	18 (20%)	
Ara h 3 > 1 kU _A /L, n (%)	9	1 (<1%)	8 (9%)	
Ara h 6 (kU _A /L), mean, median (IQR) *	2.9, 0.0 (0.0-0.9)	0.1, 0.0 (0.0-0.0)	7.2, 1.4 (0.3-5.3)	<0.001
Ara h 6 >0.35 kU _A /L, n (%)	81	15 (11%)	66 (75%)	
Ara h 6 > 1 kU _A /L, n (%)	52	3 (2%)	49 (56%)	
Ara h 8 (kU _A /L), mean, median (IQR) *	0.1, 0.0 (0.0-0.0)	0.1, 0.0 (0.0-0.0)	0.2, 0.0 (0.0-0.1)	0.38
Ara h 8 >0.35 kU _A /L, n (%)	7	5 (4 %)	2 (2%)	
Ara h 8 > 1 kU _A /L, n (%)	4	2 (2%)	2 (2%)	
Ara h 9 (kU _A /L), mean, median (IQR)	0.4, 0.0 (0.0-0.1)	0.4, 0.0 (0.0-0.1)	0.4, 0.0 (0.0-0.0)	0.96
Ara h 9 >0.35 kU _A /L, n (%)	23	14 (11%)	9 (10%)	
Ara h 9 > 1 kU _A /L, n (%)	11	8 (6%)	3 (3%)	

*n = 221, tolerant 133, allergic 88 (1 result missing). **n = 220, tolerant 133, allergic 87 (2 results missing), *** from logistic regression with component as continuous predictor and tolerance at OFC as outcome variable

TABLE III: Degree of sensitisation to peanut components and OFC outcome

Any component >0.35 kUa/L #	n	OFC tolerant (N=113) n (%)	OFC allergic (N=89) n (%)	p*	OR (95%CI) from logistic regression
Not sensitised	91	84 (92)	7 (8)***	P<0.001	0.06 (0.03 – 0.15)
Monosensitised	86	37 (43)	49 (57)		1.00 (Reference)
Polysensitised**	45	12 (27)	33 (73)		2.1 (0.95 – 4.6)
Any component >1.0 kUa/L#					
Not sensitised	132	112 (85)	20 (15)	P<0.001	0.06 (0.03 – 0.13)
Monosensitised	67	17 (25)	50 (75)		1.00 (Reference)
Polysensitised**	23	4 (17)	19 (83)		1.61 (0.48 – 5.4)
Number of Ara 1, 2 or 3 >0.35 kUa/L	n	OFC tolerant (N=113) n (%)	OFC allergic (N=89) n (%)	p*	OR (95%CI) from logistic regression
none	108	95 (88)	13 (12)	P<0.001	1.00 (Reference)
1 of	82	35 (43)	47 (57)		9.8 (7.7 – 20.3)
2 of	21	3 (14)	18 (86)		43.8 (11.3 – 170)
3 of	11	0	11 (100)		
Number of Ara 1, 2 or 3 >1.0 kUa/L					
none	192	128 (67)	64 (33)	P<0.001	1.00 (Reference)
1 of	22	5 (23)	17 (77)		6.8 (2.4 – 19)
2 of	8	0	8 (100)		
3 of	0	0	0		

#-Any peanut component- including Ara h 1,2 (or 6), 3,8,9

*- two sided Cochran-Armitage trend test

**- more than one component

***- Of these 7 children, 5 had a past history of a clinical reaction to peanut and 2 were avoiding based upon sensitisation alone. 2 received epinephrine/adrenaline due to symptoms at OFC. Median peanut s-IgE was 0.9 kU_A/L (range 0-1.27) and median peanut SPT was 6.5mm (range 0-8.5mm).

TABLE IV: Test performance characteristics of peanut and peanut component IgE

	AUC (95% CI)	Cut off value (kU_A/L)	PPV (95% CI)	NPV (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
IgE peanut	0.84 (0.79-0.90)	≥0.35	0.54 (0.46 - 0.62)	0.91 (0.83 - 0.96)	0.93 (0.86 - 0.97)	0.48 (0.40 - 0.57)
		≥1.00	0.69 (0.60 - 0.77)	0.88 (0.80 - 0.93)	0.84 (0.75 - 0.90)	0.75 (0.67 - 0.82)
Ara h 1	0.70 (0.63-0.77)	≥0.35	0.76 (0.61 - 0.86)	0.68 (0.61 - 0.75)	0.35 (0.26 - 0.46)	0.92 (0.87 - 0.96)
		≥1.00	0.86 (0.69 - 0.95)	0.67 (0.60 - 0.73)	0.28 (0.20 - 0.39)	0.97 (0.93 - 0.99)
Ara h 2	0.87 (0.82-0.92)	≥0.35	0.72 (0.62 - 0.80)	0.85 (0.77 - 0.90)	0.78 (0.68 - 0.86)	0.80 (0.72 - 0.86)
		≥1.00	0.83 (0.72 - 0.90)	0.78 (0.71 - 0.84)	0.61 (0.50 - 0.71)	0.92 (0.86 - 0.95)
Ara h 3	0.63 (0.55-0.71)	≥0.35	0.83 (0.63 - 0.93)	0.65 (0.58 - 0.71)	0.22 (0.14 - 0.31)	0.97 (0.93 - 0.99)
		≥1.00	0.89 (0.57 - 0.98)	0.62 (0.56 - 0.69)	0.09 (0.05 - 0.17)	0.99 (0.96 - 1.00)
Ara h 6	0.86 (0.81-0.91)	≥0.35	0.81 (0.72 -	0.84 (0.77 - 0.89)	0.75 (0.65 - 0.83)	0.89 (0.82 - 0.93)

			0.88)			
		≥ 1.00	0.94 (0.84 - 0.98)	0.77 (0.70 - 0.83)	0.56 (0.45 - 0.66)	0.98 (0.94 - 0.99)
Ara h 8	0.55 (0.48-0.62)	≥ 0.35	0.29 (0.08 - 0.64)	0.60 (0.53 - 0.66)	0.02 (0.01 - 0.08)	0.96 (0.92 - 0.98)
		≥ 1.00	0.50 (0.15 - 0.85)	0.60 (0.54 - 0.67)	0.02 (0.01 - 0.08)	0.98 (0.95 - 1.00)
Ara h 9	0.51 (0.44-0.58)	≥ 0.35	0.39 (0.22 - 0.59)	0.60 (0.53 - 0.66)	0.10 (0.05 - 0.18)	0.89 (0.83 - 0.94)
		≥ 1.00	0.27 (0.10 - 0.57)	0.59 (0.53 - 0.66)	0.03 (0.01 - 0.10)	0.94 (0.88 - 0.97)

TABLE V: Peanut-IgE and components association with challenge severity, cumulative eliciting dose and adrenaline use at OFC

Variable kU _A /L	Severity score* N=222	Maximum dose reached N=222	Cumulative eliciting dose amongst OFC reactors N=89	Adrenaline use at OFC N=222
Peanut IgE	0.56, p<0.0001	-0.58, p<0.001	-0.34, p=0.001	0.77, p=0.001
Ara h 1	0.32, p<0.0001	-0.31, p<0.001	-0.07, p=0.51	0.66, p=0.02
Ara h 2	0.61, p <0.0001	-0.63, p<0.001	-0.38, p<0.001	0.80, p=0.008
Ara h 3	0.22, p=0.0009	-0.21, p=0.002	-0.10, p=0.37	0.63, p=0.09
Ara h 6	0.71, p<0.0001	-0.74, p<0.001	-0.43, p<0.001	0.78, p=0.07
Ara h 8	0.10, p=0.15	-0.07, p=0.32	0.06, p=0.58	0.60, p=0.23
Ara h 9	-0.03, p=0.69	0.03, p=0.61	0.07, p=0.51	0.52, p=0.57

Spearman correlation coefficient, p-value, **AUC, p-value

*Sum of PRACTALL score for lower respiratory, laryngeal, objective gastrointestinal and cardiovascular/neurological **AUC- area under ROC curve from logistic regression with component as predictor of adrenaline use

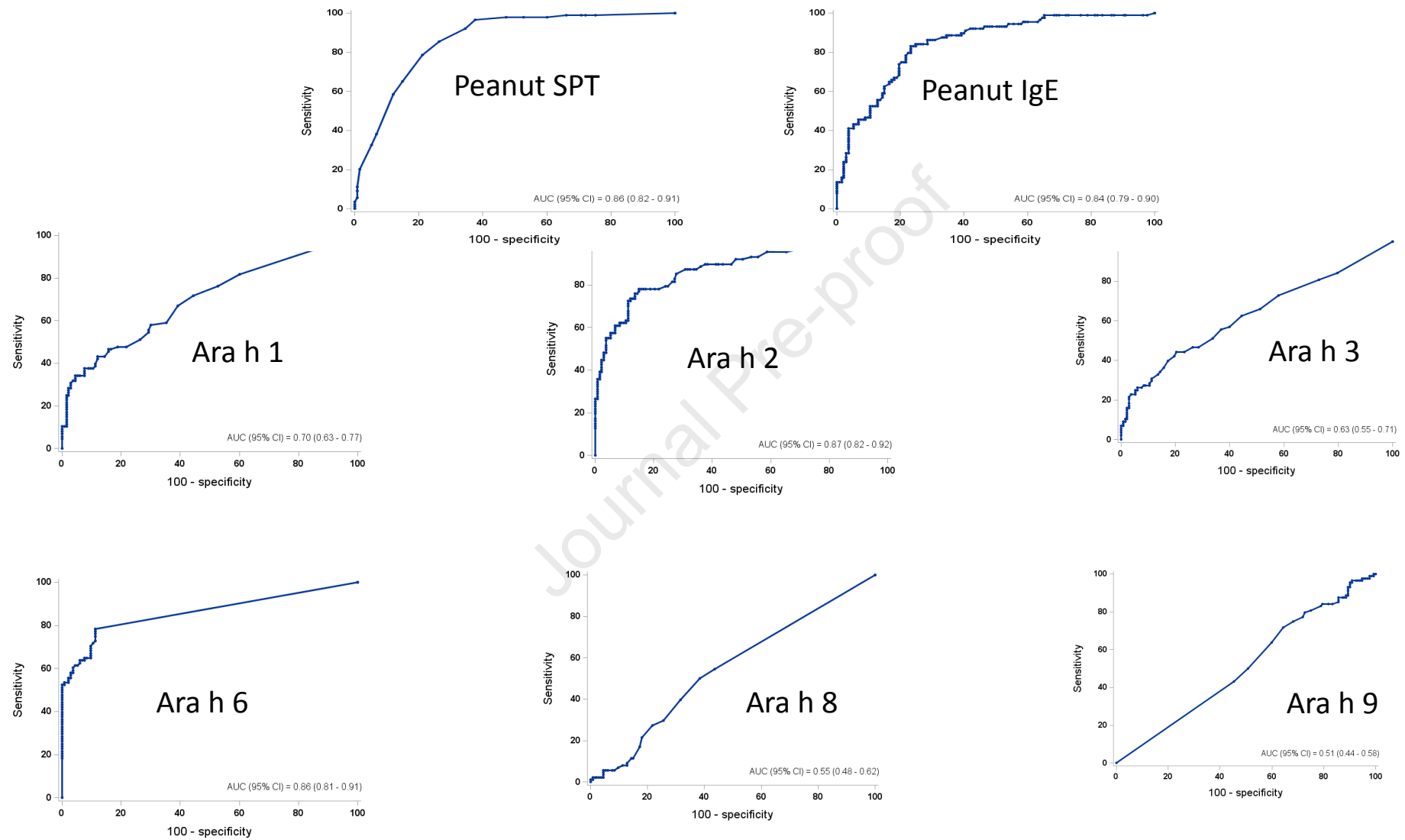


FIGURE II: Area under the curve (AUC) for peanut SPT, peanut-IgE and peanut components (Ara h 1 to Ara h 9). Peanut SPT, peanut IgE and Ara h 2 and 6 performed similar at AUC 0.84-0.87, whereas Ara h 1, 3, Ara h 8 and 9 were not predictive of OFC outcome

Figure 1

Consent Form date signed:	AFFIX patient ID
Assent Form date signed (required if > 14 yrs old)	

A: DEMOGRAPHICS			
Gender:	Male / Female	Age (yrs):	MRN:
B: PEANUT ALLERGY			
Physician Diagnosed IgE- peanut allergy		YES/NO	
Approximate age at diagnosis:		____ Years ☐	
Peanut allergy diagnosis is based on:		☐ Previous reaction ☐ Previous Food challenge ☐ Sensitization alone	
Adrenaline auto-injector prescribed?		YES/NO	
With regards to the <u>previous most severe reaction</u> to peanut:			
Age at occurrence (years):			
Estimated amount eaten (no. approximate teaspoons):			
Time from exposure to symptoms:			
Symptoms:			
Where did reaction occur (Home/Restaurant):			
Other Reactions to peanut			
	Age at reaction	Estimated amount Ingested (teaspoons)	Symptoms
1			
2			
3			
4			
5			
Has the subject ever had an in-hospital/rooms observed food challenge to peanut? YES/NO:			
Does the child have clinical allergic rhinitis? YES/NO		SAR/PAR/BOTH	

Figure 1

LIST OTHER clinical FOOD allergies:		
CURRENT ASTHMA: YES/NO	CURRENT ECZEMA : YES/NO	Family History peanut allergy (first degree relative): YES/NO
C: SPT ALLERGY INVESTIGATIONS: SPT (mm)		
Negative Control		
Histamine		
Peanut		
Birch		
Rye grass		
Bahia grass		
Bermuda grass		
English plantain		
D: Previous INVESTIGATIONS: peanut sslgE		
Date	RESULT	
	_____ KU/L	
	_____ KU/L	
E. CURRENT CHALLENGE OUTCOME and DETAILS		
REACTED/TOLERANT		
Dose (g peanut) at stopping OFC		
Cumulative total challenge dose (g peanut)		
Symptoms triggering stopping (as per PRACTALL scoring sheet)		
Treatment given		
Anti-histamine	Yes/No	
Adrenaline	YES/No number of doses.....	
Other (specify)		
Blood taken (prior to OFC)	YES/NO	Date:

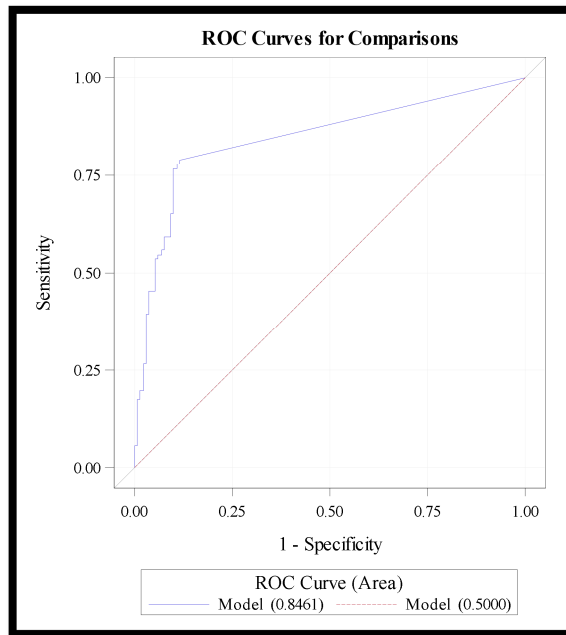


Figure A

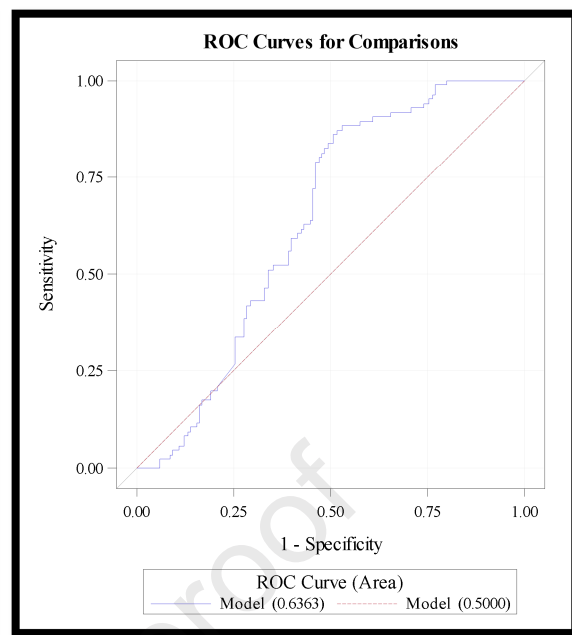


Figure B

FIGURE E2:

A: Ratio of Ara h 6 to peanut specific IgE

B: Ratio of Ara h 2 to peanut specific IgE

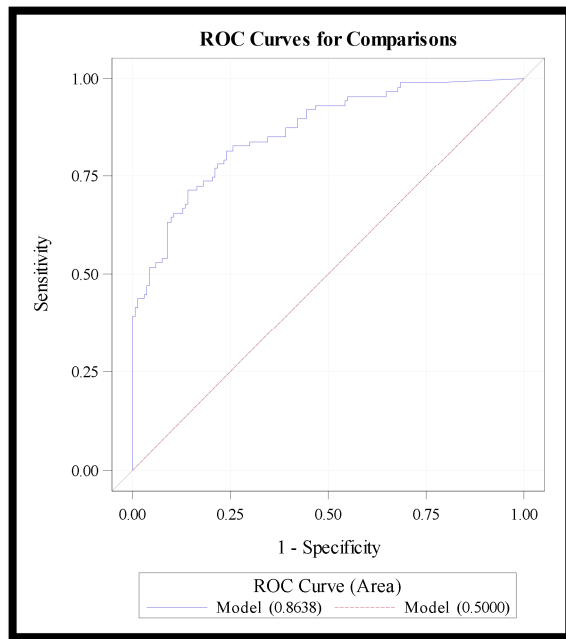


Figure A

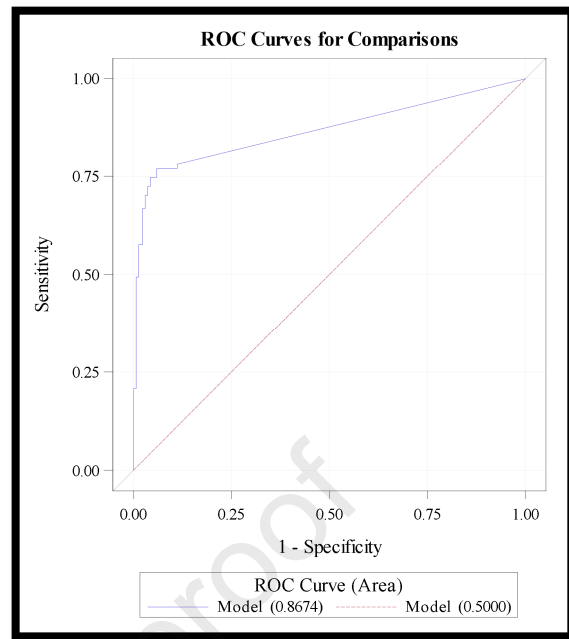


Figure B

FIGURE E3:

A: Ratio of Ara h 2 to total IgE

B: Ratio of Ara h 6 to total IgE

	Ara h 1	Ara h 2	Ara h 3	Ara h 6	Ara h 8
Ara h 1					
Ara h 2	0.46				
Ara h 3	0.70	0.41			
Ara h 6	0.41	0.79	0.37		
Ara h 8	0.49	0.32	0.68	0.25	
Ara h 9	0.36	0.23	0.57	0.15	0.76

FIGURE E4: Spearman correlation between individual peanut components

Repository Legends:

FIGURE E1: Clinical Information Form

TABLE E1: Ratio of each peanut component to peanut IgE

TABLE E2: Ratio of each peanut component to total IgE

FIGURE E2: A: Ratio of Ara h 6 to peanut specific IgE B: Ratio of Ara h 2 to peanut specific IgE

FIGURE E3: A: Ratio of Ara h 2 to total IgE B: Ratio of Ara h 6 to total IgE

FIGURE E4: Spearman correlation between individual peanut components

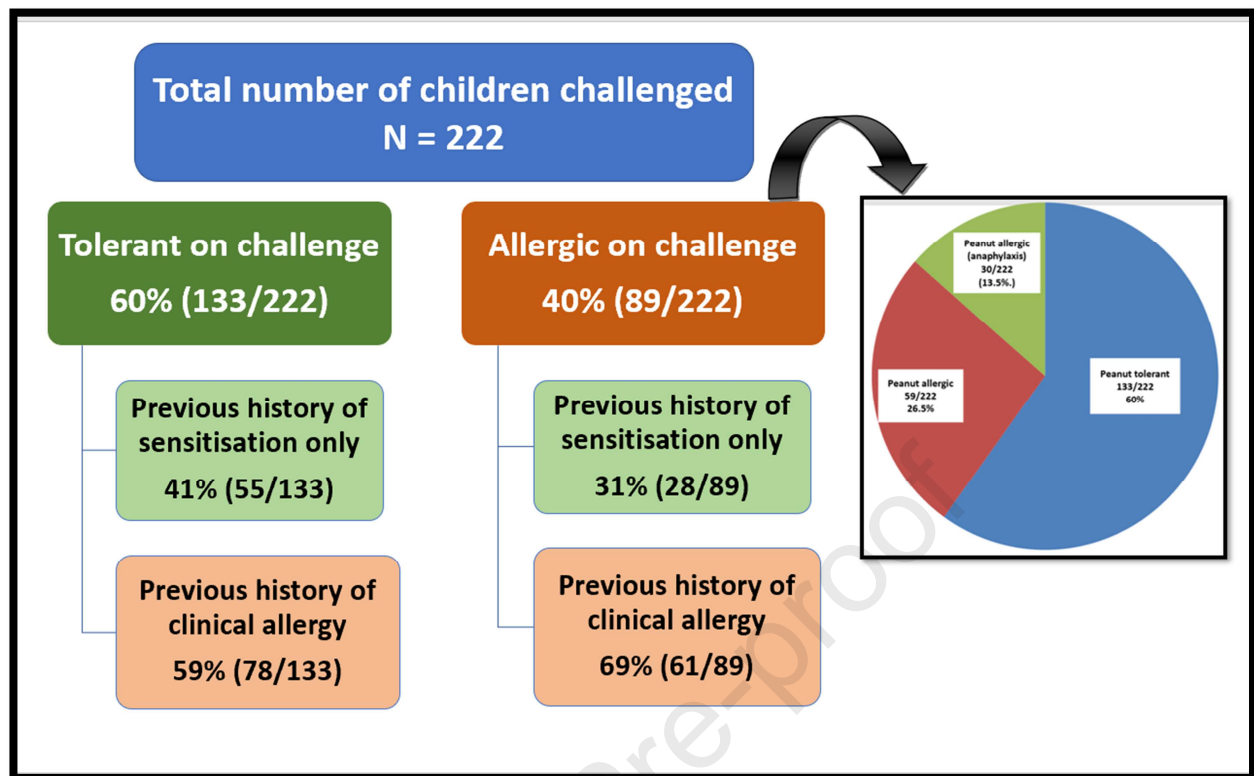


FIGURE I: Flowchart of outcome of OFC