

SPT predictive values for the outcome of cashew challenges in children

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Abstract:

Background: Cashew is a common cause of tree nut allergy in children. To date there have been few studies of diagnostic tests for cashew allergy, and positive predictive values (PPVs) for cashew as well as other tree nuts are largely extrapolated from studies of peanut allergy. How relevant these cut-offs are for cashew has not been formally explored.

Objective: We aimed to establish skin prick test (SPT) wheal sizes that correlated to 95% PPV for a positive food challenge for cashew.

Methods: We included all cashew oral food challenges (OFC) conducted as part of the HealthNuts (n=108, age 4-6 years) and SchoolNuts (n=37, age 10-14 years) studies, both recruited from the community (Population cohort). A second cohort of all cashew OFCs conducted at the Royal Children's Hospital (RCH) allergy centre (n=343) (2011-2016) and a private allergy clinic based at RCH (n=43) was included via electronic medical record review (Clinic cohort). 95% PPV for cashew SPT was calculated for both cohorts.

Results: Among the population cohort (n=145), 62% of cashew OFC were positive compared to 20% of the clinic cohort (n=386). The SPT cut-off for 95% PPV derived from the population cohort was 10mm (95%CI 7.5-12.0). For the clinic cohort the 95% PPV was 14mm (95%CI 9.5-unknown). A SPT wheal size of 8mm had a PPV of 89% (95%CI 79-95) in the population cohort and 62% (95%CI 45-78) in the clinic cohort.

Conclusion: A higher SPT wheal size may be more appropriate than the commonly used 8mm cut-off to guide clinical decisions around when to perform OFC for cashew.

Word count = 258

Key words: food allergy, tree nut allergy, cashew allergy, population, predictive value of tests, skin prick test

Abbreviations:

slgE: serum immunoglobulin E

OFC: oral food challenge

LR: likelihood ratio

NPV: negative predictive value

PPV: positive predictive value

ROC: receiver operating characteristic

AUC: area under the curve

SPT: skin prick test

68 **Introduction:**

69 Tree nut allergies, like peanut allergy, are thought to be increasing in prevalence, affecting up to 3%
70 of Australian children¹. Cashew has been reported as the most common tree nut allergen and the
71 most common cause of adverse food reactions and anaphylaxis ²⁻⁴. It has also been reported that
72 reactions to cashew can be more severe than those to peanut ⁵⁻⁸. Despite these reports, cashew
73 allergy remains understudied.

74 Diagnosis of IgE-mediated food allergy is reliant on a detailed medical history and physical
75 examination, in conjunction with diagnostic testing of immunoglobulin E (IgE) levels either via serum
76 specific IgE measurements (sIgE) or skin prick testing (SPT)⁹. Diagnosis of cashew allergy, like other
77 tree nuts, can be problematic with allergy screening tests often performed for tree nuts for those
78 with allergy to other foods and no history of prior ingestion or reaction to cashew. We have
79 previously reported that up to half of those with egg and peanut allergy can be sensitised on SPT to
80 tree nuts as early as one year of age¹. The diagnostic gold standard is to perform an oral food
81 challenge (OFC), however this is time and labour intensive, limited in availability in many tertiary
82 allergy services and exposes the child to the risk of severe reactions ¹⁰. This can result in patients
83 being advised to avoid multiple tree nuts based on sensitisation alone, which, given recently
84 published evidence of the protective effect of early allergen introduction, may increase their risk of
85 developing tree nut allergy ¹¹.

86 SPT and sIgE thresholds above which the individual has a 95% probability of being clinically allergic
87 (95% positive predictive value (PPV)) have been used as a tool for clinicians to determine when an
88 OFC should be performed. In Australia, SPTs are performed in preference to sIgE in most paediatric
89 allergy clinics. PPVs are dependent on the underlying prevalence of disease (in other words, the pre-
90 test probability of food allergy), which is likely to depend on factors such as age, SPT method and the
91 population in which the allergy testing is being performed ^{12,13}. Clinic-based PPV studies often do not
92 report the reason for challenge, however in our experience most OFC in the clinical setting are
93 undertaken to determine resolution of allergy (tolerance) rather than a diagnostic challenge. Those
94 at higher risk of reaction, such as those with high SPT wheal sizes are not offered an OFC, which
95 impacts the pre-test probability of food allergy.

96 PPVs for SPT have now been widely published for peanut, egg, milk and sesame in both clinical and
97 population cohorts ¹⁴⁻²⁶. There is limited data on tree nut PPVs and no data derived from population
98 cohorts^{16, 27}. With the possibility of increasing prevalence and severity of cashew allergy there is an
99 urgent need for more accurate, timely and cost effective diagnostic methods.

We aimed to establish the SPT wheal size that correlated with 95% positive predictive value to a positive oral food challenge for cashew in a population-based cohort of children and a clinic-based cohort of children. We also aimed to explore if these thresholds differ when stratified by known risk factors for cashew allergy (co-existing peanut or other food allergy, co-existing atopy, previous reaction history, age and sex).

Methods

Study Populations

The population cohort of this study comprised of participants who underwent cashew OFC as part of the HealthNuts and SchoolNuts studies. The HealthNuts and SchoolNuts studies are two large population-based allergy studies done at the Murdoch Children's Research Institute. Both studies recruited participants from the community. Cashew OFCs were performed at 4-year-old and 6-year-old follow-up in the HealthNuts study, whereas the SchoolNuts study assessed students aged 10-14 years. The study populations are outlined in further detail below.

The HealthNuts study is a population-representative longitudinal study of 5276 children recruited at age 1 and followed up to age 6 years. The study methods have been described in detail previously²⁸⁻³⁰.

At age 4 years all participants were followed up via questionnaire (83% participation) and those who reported a new food allergy reaction, and those who had any food allergy at age 1 year, were invited for clinic assessment that included SPT to a predetermined panel of 8 foods (milk, egg, peanut, wheat, sesame, cashew, almond and hazelnut). All those with a SPT \geq 1mm were offered an OFC.

At age 6 years the entire cohort (n=5276) were invited to participate in questionnaire and SPT assessment. Questionnaires were mailed to all participants capturing demographic details, history of food allergy and new food reactions, common allergen exposure information, history of asthma/wheeze and eczema. All participants were invited for an allergy/health assessment that included SPT to the same predetermined panel of 8 foods as age 4 years (60.5% participation). All those with a positive SPT (\geq 1mm) were offered a cashew OFC except those that had a cashew positive OFC at age 4 years and a cashew SPT \geq 8mm at 6 years or a recent history of IgE-mediated reaction.

The SchoolNuts study is a cross-sectional population-based study that aimed to determine the population prevalence of challenge-proven food allergy in early adolescence. The study methods

have been described in detail previously^{2, 31}. Briefly, recruitment was via stratified random population-based sampling of schools in Melbourne (n=9663) from 2011-2014. Questionnaires with phone follow up were used to identify students with potential IgE-mediated food allergy. Students with potential IgE mediated food allergy were invited to attend a clinic assessment for allergy testing (SPT) to a panel of 15 food allergens (egg white, cow's milk, soy, peanut, cashew, almond, hazelnut, walnut, pistachio, macadamia, pecan, brazil nut, pine nut, sesame, shellfish) and a panel of environmental allergens.

Students were eligible for OFC if they had a positive SPT result (≥ 3 mm) to a food that they had a history of reaction to and were currently avoiding, or that they had never eaten. OFC were not performed if participants met any one of the following criteria: 1. were sensitised (≥ 3 mm) and had a past history of a severe reaction requiring multiple doses of epinephrine, 2. Reported an episode of anaphylaxis when older than 10 years of age, 3. reported a food reaction in the past 12 months consistent with an IgE-mediated food allergy reaction, or 4. were highly sensitised (≥ 8 mm) and reported a past history of reaction consistent with IgE-mediated food allergy.

The clinic cohort of this study comprised of patients who had undergone a cashew OFC as part of the allergy clinic at the Royal Children's Hospital (RCH) between 2011 and 2016 and a private allergy clinic-based at RCH, the Melbourne Allergy and Children's Centre (MACCS), that opened in 2015 and had an OFC database established from 2016. We conducted a retrospective analysis of all sequential open cashew OFCs undertaken at both sites. Patients undergoing cashew OFC were identified from the electronic medical records databases at both the RCH and MACCS. Cashew SPT wheal size, co-existing food allergy, current and resolved comorbid allergic disease and food and environmental allergen sensitisation were extracted via chart review. Those with their most recent cashew SPT conducted more than 3 months prior to OFC were excluded.

Skin prick testing

All SPT performed at RCH, MACCS and the HealthNuts study were performed on the child's back, while the SchoolNuts study utilised the volar aspect of the student's forearm. Wheal size was measured after 15 minutes and calculated by subtracting the negative control from the average of the longest diameter and the diameter perpendicular to it. SPTs for MACCS and RCH were performed with Quintip device (Stallergenes, Antony, France) and the HealthNuts and SchoolNuts studies were performed with single-tine lancets (Stallergenes, Antony, France) using cashew extract along with a positive and negative saline control. Extracts for the HealthNuts and SchoolNuts studies

were ALK-Abello SA, Madrid, Spain and RCH and MACCS Hollister-Stier, Stallergenes, Antony, France.
All SPT were performed within 3 months prior to cashew OFC.

Oral food challenges

Criteria for undergoing cashew oral food challenges

HealthNuts participants with a cashew SPT ≥ 1 mm or a parent-reported reaction consistent with an IgE-mediated allergy were invited for a clinic appointment with a specialist allergy nurse and OFC. The low SPT cut-off for sensitisation was applied in the HealthNuts study to ensure that all potential cases of food allergy were detected. Cashew OFC were offered at both the 4-year-old and 6-year-old follow up.

SchoolNuts participants with a parent-reported history of an adverse food reaction consistent with IgE-mediated food allergy were invited to participate in an allergy assessment which included cashew SPT. Those with a cashew SPT ≥ 3 mm were invited for cashew OFC.

Cashew OFC protocols

OFC dosage protocols for all sites were consistent with those of the Australian Society of Clinical Immunology and Allergy (ASCI) using graded, incremental doses administered at 15- to 20-minute intervals with a top dose of 2 teaspoons of crushed cashew. OFC protocols for the HealthNuts and SchoolNuts studies have been previously described^{2, 32}. An OFC was deemed positive if it met at least one of the following predefined criteria: (1) three or more concurrent non-contact urticaria lasting at least 5 minutes; (2) severe persistent vomiting; (3) peri-oral or peri-orbital angioedema; or (4) anaphylaxis (evidence of circulatory or respiratory involvement) within 2 hours of the last challenge dose in the presence of IgE sensitisation. For the SchoolNuts study, additional criteria based on persistent subjective symptoms in the upper airways or the gastrointestinal tract were included due to the older age of participants. In the absence of objective signs, if subjective symptoms (itchy mouth or throat, abdominal pain or nausea, tightness in throat, difficulty talking or difficulty breathing) continued up to the timing of the next dose, the previous dose was repeated. If the above symptoms persisted for a total of more than 40 minutes or reoccurred on 2 doses, the challenge was considered positive.

OFCs were deemed negative at all sites if a patient or participant tolerated a top dose of 2 teaspoons of crushed cashew nut on the day of the OFC and did not report any positive reactions during continued daily intake of 2 teaspoons at home in the week after the OFC.

RCH and MACCS cashew OFCs were performed in a clinical allergy setting with the recommendation to undertake an OFC based on the clinical expertise of the attending allergist. In this setting OFCs are not routinely performed if there is a high likelihood of reaction such as those with high-level sensitisation or a recent history of reaction. Therefore, OFCs are largely performed to determine tolerance rather than diagnose food allergy. Occasionally, an OFC may be performed despite a high positive SPT if there is a recent history of tolerance to a partial ingestion of cashew. All RCH and MACCS OFCs had symptoms retrospectively reassessed and those not meeting OFC stopping criteria as outlined by PRACTALL guidelines were excluded (n=2) ³³.

Statistical Methods:

Continuously-valued variables are summarised using means (and standard deviations) or medians (and range), with frequencies reported as percentages with 95% confidence intervals based on the binomial distribution. A two-sample comparison of the prevalence of positive OFC within the clinic cohorts showed little evidence these samples were drawn from populations with different prevalence of cashew allergy ($p = 0.24$) and similarly for the population-based cohorts ($p = 0.24$).

There was no evidence against the null hypothesis of a linear relationship between the SPT wheal size and the log-odds of a positive OFC based on data from any of the cohorts, so we assumed linear association throughout our analyses.

In light of these results, we combined data for the two clinic cohorts RCH and MACCS to form a clinic cohort and for the two population-based cohorts HealthNuts and SchoolNuts to form a population cohort for all analyses. All analyses were conducted for the population and clinic cohorts separately. The capacity of the cashew skin prick test to diagnose OFC-confirmed cashew allergy was assessed using receiver operating characteristic (ROC) curves and the area under the curve (AUC) was used to quantify the accuracy of the test. Logistic regression was used to model the association between the SPT wheal size in millimetres and the risk of cashew allergy. The assumption that the logarithm of the odds of food allergy was linearly related to the SPT wheal size was assessed using the likelihood ratio test to compare the linear model with a more general “saturated” model that made no assumption about the shape of the relationship between SPT wheal size and the risk of food allergy. A fitted probability of food allergy was produced for each study participant given their SPT wheal size and used to replace the observed binary outcome in the standard formula for the PPV that is, a modelled PPV for each level of SPT wheal size was produced by taking the average of the fitted probability of cashew allergy for all infants with an SPT wheal size of greater than the given level. This method produces a smooth non-decreasing curve for the PPV across the range of SPT wheal sizes therefore overcoming fluctuations (sampling variation) in the observed proportion of infants

with cashew allergy for increasing SPT. To quantify the precision of estimation of the PPVs, we used bootstrapping, a method of deriving SEs and CIs from repeated samples drawn with replacement from the original dataset. One hundred bootstrap replications were used to determine the variability of parameter estimates and to calculate 95% CIs for the thresholds with 95% PPVs to food allergy.

The analysis was stratified on known risk factors for food allergy/positive tree nut challenge: sex, co-existing peanut allergy, co-existing tree nut allergies, co-existing non-nut allergies, other allergic disease and previous reaction history. All analyses were done using Stata 15.0 (release 15.0, StatCorp, College Station, Texas).

Ethics

Approval to conduct the HealthNuts study was obtained from the Victorian State Government Office for Children (reference no. CDF/07/492), the Victorian State Government Department of Human Services (reference no. 10/07) and the RCH Human Research Ethics Committee (reference no. 27047). For the SchoolNuts study, ethics approval was obtained from the RCH Human Research Ethics Committee (HREC number 31079), the Department of Education and Early Childhood and the Catholic Education Office. All parents provided written informed consent.

Ethics approval for the use of de-identified clinic data from RCH and MACCS was obtained from the RCH Human Research Ethics Committee (HREC number 37076A).

Results:

Study Populations

Demographic and clinical characteristics stratified by cashew OFC cohort are outlined in Table 1. A total of 386 cashew OFC were performed in a clinical setting (RCH n=343, MACCS n=43) and 145 cashew OFC were performed as part of the population-based studies (HealthNuts n=108, SchoolNuts n=37). Age stratified demographic and clinical characteristics for the clinic cohort are outlined in Supplementary Table 1.

Results of cashew OFC

Among the clinic cohort, 19.9% (n=77) of cashew OFC were positive compared to 62.1% (n=90) of the population cohort challenges. The most common reason for cashew OFC among both cohorts was cashew sensitisation without a history of cashew ingestion, 46.6% of those in the clinic cohort and 67.7% of those among the population cohort. Among the population cohort, 91% (n=61) of those with an SPT \geq 8mm and no history of cashew exposure had a positive cashew OFC compared to 59% (n=22) of those among the clinic cohort.

Characteristics of the positive cashew OFCs

Characteristics of positive cashew challenges stratified by cohort are outlined in Table 2. Among those in the clinic cohort with a positive cashew challenge (n=77) the most common symptoms were skin (75%), gastrointestinal (48%) and oropharyngeal (38%). Among those in the population cohort with a positive cashew challenge (n=90) the most common symptoms were angioedema (61%), skin (60%) and oropharyngeal (50%). There were marked differences in reported treatments between the clinic and population cohorts. Among those with a positive cashew OFC in the population cohort (n=90), 77% required no treatment, 22% antihistamine and 5.6% salbutamol. Among those with a positive cashew OFC in the clinic cohort (n=77), 19.5% required no treatment, 78% antihistamine, 3.4% salbutamol and 1.3% oral steroid.

Among the 168 positive cashew challenges in the population and clinic cohorts, nine children required epinephrine (5.3%). Interestingly the majority of these had an SPT that was lower than 10 mm and more than half had never ingested cashew. Table 3 summarises the details of the nine patients requiring epinephrine. Five had no history of cashew ingestion and three had a previous history of cashew reaction (one anaphylaxis) and cashew SPT wheal sizes ranged from 1-18mm. Five had co-existing food allergy (milk 2, sesame 2 and another tree nut 1).

The majority of reactions among the population cohort occurred with a crumb (52%, n=47) whereas reaction doses in the clinic population tended to be higher with 29/77 (38%) occurring at the top dose (2 teaspoons) (Table 2).

Diagnostic capacity of cashew SPT for challenge confirmed cashew allergy

There were marked differences in the prevalence of cashew allergy between the population and clinic-based cohorts 62.1% and 19.5% respectively (see Table 1), which we believe is due to differences in the selection of participants for cashew challenge between the 2 cohorts. Patients in the allergy services from which the clinic cohort is drawn are *less* likely to have undergone an OFC if the attending clinician determines that they are *more* likely to have a positive result, such as patients with high level sensitisation (>8mm SPT). This is not the case for the population-based cohorts, where OFC were offered to all participants with a detectable SPT (wheal size ≥ 1 mm except those with a recent history of a reaction consistent with IgE-mediated food allergy (Supplementary Table 2). This is further supported by the difference in median SPT between the 2 cohorts [clinic cohort=3mm (IQR 1-5), population cohort=7.5mm (IQR 4-11)]. The SPT threshold with 95% PPV for cashew allergy also varied between the population and clinic-based cohorts, 10mm or greater (95% CI 7.5-12.0) and 14mm or greater (95%CI 9.5-unknown) respectively. AUC was 0.89 (95% CI 0.83-0.95) for the population cohort and 0.81 (0.76-0.87) for the clinic cohort. SPT thresholds with 95% PPV for cashew allergy, along with sensitivity, specificity, NPV, positive and negative LR at the reported thresholds are presented in Table 4. A 50% NPV could not be generated for the clinic cohort and among the population cohort a 50% NPV of 10mm or less (95%CI 9-14) was determined.

There were 11 participants in the population cohort that were defined as cashew allergic but did not have an OFC performed (n=11, sensitised and recent reaction consistent with IgE-mediated food allergy). We undertook a sensitivity analysis including these participants and determined a 95% PPV of 10mm (95%CI 8-12).

When stratified by risk factors for cashew allergy, 95% PPVs could not be generated for the clinic cohort. For the population cohort some differences were noted but with overlapping confidence intervals there is little evidence to support differences in 95% PPVs between the groups at the population level. (Supplementary Table 4). There was no evidence of differences in population-level 95% PPVs based on analyses after stratification on other known risk factors for food allergy or age (data not shown).

The commonly applied SPT thresholds of less than 3mm and 8mm and above are presented in Table 5. The 3mm threshold performed as expected with high sensitivity and low specificity for the population and clinic-based cohorts.

Discussion:

To our knowledge this is the largest series of oral food challenges for cashew allergy reported and the first study to report 95% PPVs for cashew SPT. We found the SPT threshold for 95% PPV among a clinic cohort of 14mm and a population cohort of 10mm with marked differences in cashew allergy prevalence between the clinic and population cohorts.

The strengths of this study are the large number of cashew OFCs and the development of SPT thresholds with 95% PPVs for cashew allergy using data contributed from population and allergy clinic-based samples of children in the same city. The population-based cohorts provide cashew OFC outcomes based on pre-determined stopping criteria irrespective of SPT wheal size.

There are also a number of limitations. Similar to other studies based on data from allergy clinics, the results of our analyses of data from the clinic-based cohorts are affected by two types of selection bias since patients are likely to have (1) more severe allergic disease when compared to individuals from population cohorts of children; and (2) been offered OFC only if the allergist deemed them to be at low risk of reacting. As a result, the clinical cohort has a limited number of OFCs represented with high-level sensitisation (>8mm), a lower median SPT and the majority of reactions were to the top OFC dose. The clinic cohort data are also likely to have suffered from a type of information bias, since details were obtained from a retrospective record review and OFC outcomes were not standardised. All challenge outcomes were, however, reviewed and those that did not meet the PRACTALL criteria³³ for a positive oral food challenge were excluded from analysis. Both the population and clinic cohorts have a limited number of cashew OFC performed in children <2 years of age which is the age that children typically present with food allergy and when 95% PPV estimates for cashew SPT would be most useful to guide cashew introduction recommendations.

It should also be noted when making comparisons between the two cohorts in our study that there are SPT device and extract differences. Our centre has previously shown that there is little variation between the Quintip and single-tine lancet SPT devices³⁴. However, performance variability between the two extracts is unknown and was not undertaken as part of this study.

Comparison of our thresholds for cashew SPT response to other studies is difficult due to the limited number of studies to date. One study reported that SPT wheal diameters $\geq 8\text{mm}$ predicted a positive

food challenge with >95% accuracy individually for cashew, hazelnut, walnut, and sesame. This study was, however, from a highly selected clinic population and included lower numbers of cashew OFC (n=89)¹⁶. A more recent study that retrospectively reported outcomes of tree nut challenges from the University of Michigan Allergy clinic included 28 cashew challenges and fitted a single predictive model that combined cashew and pistachio OFCs to generate a 50% NPV of 6.5mm.

If the sensitivity and specificity of SPT thresholds for OFC allergy are assumed not to vary between populations, then PPVs and NPVs will be dependent on the underlying prevalence of food allergy. It has been previously argued that PPVs for sIgE derived from clinic populations cannot be meaningfully applied to general populations, although this question has not yet been addressed using relevant data on SPT wheal size¹³. Our data demonstrates a similar variation of PPVs based on different populations and highlights that PPVs based on clinic cohorts are more likely to include those with lower prevalence of food allergy due, most likely, to selection bias related to physician decisions around who is offered an oral food challenge, and therefore generate artificially higher PPV thresholds. Based on our results we propose that a SPT wheal size of 10mm may be more appropriate than the commonly used 8mm cut-off to guide clinical decisions around when to perform OFC for cashew. However, our results also support the limitation of SPT to predict reaction severity with the majority of those requiring epinephrine having an SPT <10mm.

It has been proposed that likelihood ratios, which compare the probability of a positive SPT result in patients with and without food allergy, should be the tool of choice to determine the probability of allergic disease as they have the potential to be independent of disease prevalence³⁵. The calculation and appropriate application of likelihood ratios does, however, require several steps, which may explain why this procedure has received limited use in clinical practice. In the present study, we have included both positive and negative likelihood ratios to assist clinicians with applying our data to their own settings.

Conclusion

Within a population-based cohort we have established a 95% PPV for cashew SPT of 10mm. It is known that 95% PPVs can vary depending on the population they are generated from and this study has demonstrated considerable variability between clinic- and population-based cohorts for the 95% PPV for cashew SPT. To improve tree nut allergy diagnosis and management, further work is required utilising data based on OFC confirmed outcomes with OFC offered to all sensitised individuals irrespective of wheal size to generate 95% PPVs for the additional tree nuts.

372 Word count =4114

373

374 **Figure Legend**

375 Fig 1a. ROC curve for cashew SPT wheal size, population cohort.

376 Fig 1b. ROC curve for cashew SPT wheal size, clinic cohort.

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379 **Table Legend**

380 Table 1: Demographic and clinical characteristics, stratified by cashew OFC cohort

381 Table 2: Characteristics of positive cashew oral food challenges, stratified by cohort

382 Table3: Details of the participants with a positive OFC that required epinephrine (n=9)

383 Table 4: Diagnostic capacity of SPTs to challenge-confirmed cashew allergy

384 Table 5: Diagnostic capacity of commonly applied SPT thresholds (<3mm and > 8mm) to challenge-
385 confirmed cashew allergy

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