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Petteri Röntynen MD , Kaarina Kukkonen MD, PhD ,
Terhi Savinko PhD , Mika J Mäkelä MD, PhD

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Title page

Optimizing tools for evaluating challenge outcome in children with cashew nut allergy

Petteri Röntynen¹, MD, Kaarina Kukkonen², MD, PhD, Terhi Savinko¹, PhD, Mika J Mäkelä¹, MD, PhD

¹University of Helsinki and Helsinki University Hospital, Skin and Allergy Hospital

²University of Helsinki and Helsinki University Hospital, Children's Hospital, and Pediatric Research Center

Corresponding Author Information:

Petteri Röntynen

Iho- ja allergiasairaala,

Meilahdentie 2,

000290, HUS, Helsinki, Finland

Phone: +358405666176

Fax: +358947186416

Email: petteri.rontynen@hus.fi

Fax: +358947186416

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Abbreviations/Acronyms:

AUC: Area under the curve

BAT: Basophil activation test

BAT%: Percentage of activated CD63+ basophils

CBC: Complete blood count

CI: Confidence interval

DBPC: Double-blind placebo-controlled

LR: Likelihood ratio

MCT: Mast cell tryptase

NPV: Negative predictive value

OFC: Oral food challenge

PPV: Positive predictive value

ROC: Receiver operator characteristic

SPT: Skin prick test

sIgE: Specific IgE

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Background: The incidence of cashew nut anaphylaxes is increasing and there is need for accurate diagnostic tests. Age-specific cut-offs in children are lacking. Changes in serum tryptase are not well documented in pediatric food allergy, except in anaphylaxis.

Objective: We aimed to evaluate the ability of various tests to diagnose cashew nut allergy, and to predict reaction severity. We also investigated changes in tryptase, and their correlation to reaction severity.

Methods: We performed an open cashew nut challenge on 106 children (aged 1-16 years), who were sensitized to cashew nut with either previous allergic reaction to cashew nut or no known exposure. We analyzed the accuracy of Ana o 3 -IgE, cashew nut-IgE, skin prick test, basophil activation test (BAT), and combinations thereof to diagnose cashew nut allergy and to predict reaction severity. Tryptase was measured at the baseline and during an allergic reaction.

Results: Seventy-two children had a positive challenge. Ana o 3-IgE seemed to be the best single test to diagnose cashew allergy, with 0.97 kU/L cut-off showing 94.1% specificity and 61.1% sensitivity. While $BAT \geq 22.8\%$ best predicted reaction severity, with 91.7% specificity and 60.7% sensitivity, the cut-offs were age-specific. Tryptase increased significantly 1-2 hours after the first allergic symptoms compared to baseline.

Conclusion: Ana o 3-IgE seems to be the best diagnostic test in pediatric cashew nut allergy, and test combinations do not appear to improve the diagnostics. Cut-offs are age-specific.

BAT is promising in predicting reaction severity. Tryptase should be measured 1-2 hours after initiation of an allergic reaction.

Introduction

There is limited evidence that tree nut allergy is increasing.¹ In Finland the number of anaphylaxis cases caused by cashew nut has increased during last ten years.² According to Finnish customs Uljas-database cashew nut import to Finland has increased over 37-fold between the years 2002 to 2019, probably due to changes in eating and cooking habits.

Cashew nut (*Anacardium occidentale*) belongs to Anacardiaceae-family, along with pistachio nut and mango. Cashew and pistachio nut cross-react both in IgE-testing and clinically.³⁻⁵ Cashew nut is commonly consumed as a snack and is used in Asian cuisine, in pesto, baked goods, butters, and in sweets, ice cream and chocolates.

Cashew nut is a potent allergen. Less than 1 mg of protein can cause allergic symptoms in 5% of children with cashew nut allergy.⁶ In a double-blind placebo-controlled (DBPC) multicenter study, nearly 50% of patients experienced subjective and 11% objective symptoms from the first 1 mg dose,⁷ and in further study some patients had mild transient objective symptoms to 0.3 mg protein.⁸ Davoren reported a 74% anaphylaxis rate to cashew nut, with some reacting even to skin or mucosal contact.⁹ In one case-matched study, cashew nut seemed to cause more severe reactions than peanut.¹⁰

Component-resolved diagnostics has shown better accuracy than skin prick test (SPT) and specific IgE (sIgE) to some culprit foods.¹¹ Sensitization to 2S albumin of cashew nut (Ana o 3) seems to be an accurate predictor for positive cashew nut challenge.¹²⁻¹⁴

The basophil activation test (BAT) measures the increase of the activation marker CD63 on the surface of basophils after incubation with an allergen. BAT is a promising tool in diagnosing food allergy, especially peanut and milk allergies.¹⁵ Basophil reactivity is associated with reaction severity.¹⁶⁻¹⁷ Santos demonstrated high specificity and sensitivity of the BAT in diagnosing peanut allergy.¹⁸ The accuracy of diagnosing pediatric cashew nut allergy was improved by combining BAT to SPT.⁵

Tryptase is a serine protease released from degranulating mast cells in an allergic reaction.¹⁹ Peak mast-cell tryptase and tryptase ratio have been used as markers of mast-cell activation in anaphylaxis.²⁰ An adult study showed a significant increase in tryptase during peanut challenge, and tryptase ratio correlated to reaction severity. The optimal time point to measure tryptase was after two hours from reaction.²¹ An over 20% elevation in serum tryptase during positive hazelnut challenge was seen in 63% of children.²² In pediatric shrimp allergy, a tryptase ratio ≥ 1.5 had high sensitivity and specificity for anaphylaxis.²³

In the ideal patient care setting, one should use tests that predict both the likelihood of an allergic reaction and even more importantly, the severity of the reaction. Here, we examined the utility of sIgE to Ana o 3 and cashew nut, SPT, BAT, serum tryptase measurement, and combinations thereof in evaluating pediatric cashew nut allergy.

Methods

Participants

We recruited 110 children, aged 1 to 16 years, with suspected cashew nut allergy at the Helsinki University Skin and Allergy Hospital between January 2017 and January 2020. Patients were either sent to hospital due suspected cashew nut allergy or followed up for other allergic conditions. Inclusion criteria were sensitization to cashew nut or its storage protein (SPT ≥ 3 mm or sIgE against cashew nut or Ana o 3 ≥ 0.35 kU/L) and either a history of an allergic reaction to cashew nut or no known exposure. Patients with earlier anaphylactic reaction or high sIgE to cashew nut or Ana o 3 were also included. We excluded sensitized patients who tolerated cashew nut and those with uncontrolled asthma or any major chronic illness. Natural course of early food allergy usually leads to tolerance in the majority of children by the age of 5 years, and therefore the study population was also analyzed between two subgroups, children $<$ and ≥ 5 years of age.²⁴

Procedures

A blood sample was drawn from each participant before the challenge. ImmunoCap© system (Thermo Fisher) immunoassays were used to measure sIgE to birch, cashew nut, and Ana o 3. Complete blood count (CBC), serum tryptase and total IgE were also assessed.

Baseline tryptase and CBC were assessed before challenge, and 60, 120 and 240 minutes after occurrence of significant allergic symptoms. The 240-minute sample was taken when possible within clinic hours. In the negative challenge, CBC and tryptase were reassessed two hours after the last cashew nut dose. Tryptase was measured using ImmunoCAP© system (Thermo Fisher) immunoassay, which has a detection limit of 1 $\mu\text{g/L}$; 13.5 $\mu\text{g/L}$ was considered as upper limit of normal (95th percentile). Tryptase ratio (peak tryptase divided by baseline) and delta-tryptase (peak tryptase minus baseline) were

determined to investigate mast-cell activation. Peak mast cell tryptase (MCT), with $\geq 120\%$ of baseline tryptase plus 2 $\mu\text{g/L}$, was considered as a marker of confirmed mast-cell activation.²⁵

Skin prick tests were performed on each child's forearm with a single-tine lancet assessing cashew nut, pistachio nut, peanut, hazelnut, walnut, pecan, macademia nut, coconut, brazil nut, almond, and birch. (10 HEP; ALK-Abelló, Hørsholm, Denmark). Patients aged 1 to 5 years were also tested for raw egg, milk, and wheat. Histamine chloride (10 mg/ml) was the positive control and the solvent was the negative control. SPT to all nuts was performed with in-house allergen extract from raw nuts. The nuts were grounded with a mortar and then incubated with saline before performing SPT by using prick-prick technique. A wheal size ≥ 3 mm was considered positive.

We performed BAT to cashew nut (BÜHLMANN Laboratories, Switzerland) before each challenge. BAT was performed with a Flow CAST® kit (BÜHLMANN Laboratories). A blood sample was collected into K2EDTA (Greiner Bio-one) tube and 50 μl of whole blood was incubated with cashew nut allergen (BÜHLMANN Laboratories) for 25 minutes at 37 °C in the presence of stimulation buffer containing IL-3 and fluorescently labelled antibodies against CCR3 and CD63. Red blood cells were lysed and cells suspended in wash buffer for flow cytometry (Accuri C6, Becton Dickinson). Basophils were gated SSClowCCR3⁺ and CD63 was used as an activation marker. To determine the basal value for the test, cells were incubated with stimulation buffer containing IL-3. Anti-Fc ϵ RI antibody and N-formyl-methionyl-leucyl-phenylalanine (fMLP) were used as positive controls. The BAT result is presented as percentage of activated (CD63⁺) basophils (BAT %). BAT% could not be assessed in four patients; three did not react to positive control, and one had hyperreactive basophils at baseline.

IgE and BAT measurements, as well as SPTs were performed either on the challenge day or within a month before the challenge.

Open cashew nut challenges were performed as follows: each patient received 5, 25, 50, 100, and 500 mg raw cashew nut protein at 30-minute intervals. The cumulative dose was 680 mg cashew protein, corresponding to 3.5 cashew nuts. The challenge was stopped with emergence of significant subjective or objective symptoms according to PRACTALL guidelines.²⁶ Oral allergy symptoms, nausea and stomach ache, and itching were regarded as subjective symptoms. All other observed symptoms or signs of an allergic reaction concerning skin, upper or lower respiratory tract, gastro-intestinal system, cardiovascular system, or central nervous system were regarded as objective symptoms. Reaction severity was assessed by the following modified threshold-adjusted score: 1 to 5 indicated mild, 6 to 13 moderate, and 14 to 23 severe reactions, presented in eTable 1.²⁷⁻²⁸ The results were analyzed in three reaction groups (negative, mild, and moderate-to-severe). Anaphylaxis was defined according to EAACI 2014 guidelines with rapid onset (minutes to several hours) of allergic symptoms from at least two organs (involvement of the skin, respiratory compromise, reduced blood pressure or related symptoms, persistent gastro-intestinal symptoms), or low age-specific systolic blood pressure, or ≥ 30 mmHg decrease in systolic blood pressure.²⁹ All challenges were performed by the same pediatric allergist who examined each patient before the challenge and recorded patient and family asthma, atopic eczema, allergic rhinitis, and food allergies. Earlier history of avoidance or allergic reaction to cashew nut was inquired. Information regarding time from the latest reaction and associated symptoms was obtained. Pistachio nut allergy was defined as proven sensitization and clinical history of an allergic reaction to pistachio.

Statistical Analyses

Primary outcome measures were the correlation between BAT% and challenge reaction severity in three categories (negative, mild, and moderate-to-severe), the correlation of sIgE to Ana o 3 with reaction severity, and the correlation between severity of reaction and change

in tryptase levels. Secondary outcome measures were sensitization to Ana o 2, 3, and to cashew nut whole extract and changes in tryptase levels at different time points. We applied receiver operator characteristic (ROC) curves to estimate optimal cut-off levels for diagnostic tests, and calculated sensitivity, specificity, and likelihood ratio (LR) with 95% confidence intervals (95% CI). We defined positive (PPV) and negative predictive values (NPV) for the sIgE to cashew nut and Ana o 3, SPT and BAT%. We used the Mann-Whitney-U and Kruskal-Wallis tests to compare non-normally distributed data and Pearson's Chi-square or Fisher's exact test for categorical data. Pairwise comparison of tryptase levels at different time points was analyzed by using Wilcoxon signed-rank test. Correlations were investigated by Spearman's correlation test. Binary logistic regression was used to compare the combinations of various diagnostic tests. We used SPSS Inc. (Chicago, IL) version 25 software in our analyses.

Ethics

Food challenge is the gold standard in diagnosing food allergy. For safety reasons, each patient had an i.v. catheter inserted before challenge. The ethics committee at the Helsinki University Hospital of Children and Adolescents approved the study protocol, according to which one of the parents and patients ≥ 6 years signed written informed consents. The study followed the principles of Helsinki declaration.

Results

Study population

110 patients participated in the study, of which four were excluded due to negative sIgE and SPT to cashew nut. Median age was 5.04 (range 1.41-16.66) years; 50 (48%) patients were male. Twenty-three children (21.7%) were aged <3 years. Baseline characteristics and baseline values by challenge outcome are presented in **Table 1**.

Challenge outcomes

Seventy-two (68%) patients developed symptoms to cashew protein in oral food challenge (OFC). Fourteen (19%) of the reactions were graded as mild, 26 (36%) moderate, and 32 (44%) severe. Eighteen (25%) patients had cardiovascular symptoms, which were most often drowsiness. No patients experienced fall in blood pressure >30%. Thirty-four (47%) positive-challenge reactions were defined as anaphylactic. Forty-two (58%) patients received epinephrine, of which 8 (11%) received epinephrine twice, one three times and one patient four times. Symptoms and medication used in challenges are presented in **eTable 2 A-B** in this journal's eSupplement. Cashew nut challenge was negative in 19 (29 %) children despite reliable history of earlier reaction, of whom seven patients had their latest reaction <1 year before the challenge. In three patients, the earlier reaction was severe (multiorgan symptoms requiring emergency visit, epinephrine, or both). Among those with negative challenge despite earlier reaction, median sIgE to Ana o 3 was 0.07 kU/L (range 0.01-31.20). Out of the children allergic to pistachio, 6 (67%) had a positive cashew nut challenge. Sixty-six (62%) children had an earlier reaction to cashew nut, and 9 (8%) to pistachio nut, respectively.

Cumulative reactive dose and correlations

The first (5 mg) dose caused objective symptoms in 20 (28%), and subjective symptoms in 32 (44%) children, which led to a severe reaction in 7 and 16 patients, respectively. Median cumulative reactive dose was 80 mg protein. There was a moderate negative correlation between the cumulative reactive dose and Ana o 3-IgE (Spearman's rho -0.583), and cashew nut SPT (Spearman's rho -0.545), while cashew nut-IgE and BAT% showed a weak negative correlation to the cumulative reactive dose (-0.493 and -0.469 respectively). The skin prick test to cashew correlated strongly to pistachio SPT (Spearman's rho 0.725). The severity score correlated to cashew nut SPT and to BAT% (Spearman's rho 0.412 and 0.448, respectively) and also with Ana o 3-IgE and cashew nut-IgE (Spearman's rho 0.338 and 0.379, respectively).

Sensitization

Median sIgE to cashew nut in the entire study population was 1.02 (range 0.03-81.30), and 0.69 (0-96.90) to Ana o 3. Median SPT to cashew nut was 8 mm (0-18). Ten children with positive challenge had Ana o 3-IgE <0.35 kU/L. Specific IgE to Ana o 3 and cashew nut, SPT and BAT% by reaction severity are presented in **Figure 1 A-D**.

Tryptase

Median baseline tryptase was 4.1 µg/L (range 1.3-15.0). Tryptase was ≥11.40 µg/L in seven patients already at baseline. In one patient, the value was elevated after experiencing a severe reaction. Three children with a baseline tryptase ≥11.4 kU/L had a negative challenge and four experienced a severe reaction. Six (5.7%) patients had elevated peak MCT (≥120% of the baseline tryptase plus 2 µg/L), in all cases reactions were graded as severe. Tryptase ratio elevated both in mild and moderate-to-severe reactions, demonstrated best in the subgroup of children aged <5 years. Baseline tryptase and tryptase ratio by reaction severity in the study population and in the two subgroups (children <5 and ≥5 years) are presented in **Figure 2 A-**

D. Changes in tryptase levels at four different time points are shown in **Figure 3**. Optimal cut-off levels for tryptase ratio and delta-tryptase for a positive challenge were 1.12 and 0.7 µg/L, respectively. The ability of tryptase ratio and delta-tryptase to support the positive challenge outcome and the suspicion of a moderate-to-severe reaction in the study population and in the two subgroups are presented in **Figure 4 A-D**. Delta-tryptase levels by reaction severity in the study population and in the two subgroups are presented in **eFigure 1 A-C** (available in this article's eSupplement).

Cut-off levels and combinations of diagnostic tests

Ana o 3-IgE had the largest AUC (94.1% specificity and 61.1% sensitivity) and seemed to be the best predictor for a positive challenge, although 95% CIs for all different tests were overlapping. Various test combinations did not improve the diagnostic values. ROC curves with 95% CI for each diagnostic test and combinations thereof are presented in **Figure 5 A-B**. Optimal cut-offs for sIgE to cashew nut and Ana o 3, SPT and BAT% were higher in children aged <5 years compared to older children. The optimal cut-off levels, sensitivity, specificity, positive (PPV) and negative predictive values (NPV), and LRs for sIgE to cashew nut and Ana o 3, SPT, and BAT% in predicting challenge outcome in the study population and in the two subgroups (children <5 and ≥5 years) are shown in **Table 2**. While BAT ≥22.8% best predicted reaction severity in the study population, with 91.7% specificity and 60.7% sensitivity, the cut-offs were age-specific. In the subgroup of children aged ≥5 years, the specificity and sensitivity of BAT were better, the cut-off ≥14.5% demonstrated 100% specificity and 76.5% sensitivity. The optimal cut-off levels for diagnostic tests in predicting moderate-to-severe reaction compared to mild reaction in the study population and in the two subgroups (children <5 and ≥5 years) are presented in **Table 3**. In ROC-analysis BAT showed the largest AUC (0.812) in predicting moderate-to-severe reaction, data is presented in **eFigure 2** in this journal's eSupplement.

Discussion

Ana o 3-IgE seems to bring major additional value in predicting cashew nut allergy.

Combining these tests does not improve diagnostic accuracy, although BAT in particular may be useful in predicting risk for moderate-to-severe reaction and tryptase elevation is related to reaction severity.

With regard to the challenge outcome, Ana o 3 -IgE seemed to work better compared to SPT, BAT% and sIgE to cashew nut in the study population. However, the differences between diagnostic tests were not statistically significant due to overlapping of the confidence intervals, possibly as a result of too small sample size. In earlier studies sensitization to Ana o 3-IgE was considered the best predictor for positive challenge outcome.¹²⁻¹⁴ Various combinations of these tests did not seem to improve the diagnostic accuracy. Earlier studies have not examined all these test combinations in diagnosing cashew nut allergy.

We show here that children <5 years have significantly higher cut-off values for sIgE, SPT and BAT% compared to older children, which should be considered when performing the cashew nut challenge. A Japanese study of 95 participants defined 2.2 kU/L as a cut-off level for Ana o 3-IgE for positive challenge with 96% specificity. The median age was 7 years (range 2-26 years), but only 26 patients were allergic,¹² limiting the value of the study. Another study on 61 children sensitized to cashew nut (median age of 80 months), revealed 2.0 kU/L as a cut-off point for Ana o 3-IgE in defining 95% probability for cashew allergy. However, allergy diagnosis was confirmed by OFC in only 14/42 children; the cut-off level was 4.8 kU/L among these patients.¹³ The lower cut-off value for Ana o 3-IgE observed in our study could be due to the larger number of allergic children, lower median age compared to earlier studies, and challenge-confirmed diagnosis. The different ethnic

background may also have an effect on cut-off value. In two different study populations SPT ≥ 10 and ≥ 14 mm, respectively, were determined as cut-offs in predicting positive challenge with PPV of 95%³⁰ being in line with our results. Cashew-IgE combined with SPT showed larger AUC for challenge outcome than either single test alone, optimal thresholds were 1.2 kU/L and 5 mm, respectively.³¹

BAT exhibited a rather low sensitivity and specificity by challenge outcome among younger children but had superior specificity and sensitivity in children ≥ 5 years when compared to other tests used. Previous studies have demonstrated over 90% specificity and sensitivity for BAT by challenge outcome for peanut, hazelnut and milk allergy.^{18,22,32} However, in these studies median age was higher and in some studies not all patients were challenged to confirm their current allergy status. In the DBPC hazelnut study, only eight patients reacted in the challenge.²² The clinical outcome of cashew nut allergy was matched in 97.6% of cases when cut-off levels for BAT and wheal size in SPT to cashew were $\geq 5.0\%$ and ≥ 3 mm, respectively.⁵ We suggest that BAT may be valuable in predicting the challenge outcome in cashew nut allergy especially among children ≥ 5 years.

This study revealed a novel finding regarding that BAT seems to be superior in predicting the severity of a reaction to cashew nut compared to other diagnostic tools used. Similar results have been reported in peanut allergy.^{17,33} The usefulness of BAT was best demonstrated in the subgroup of children aged ≥ 5 years. Our results suggest that BAT could help to decide the optimal time for cashew nut challenge and thus avoid severe allergic reactions. The utility of BAT in this regard should be further studied with a larger sample size and in different age groups. The skin prick tests associated well with severity analysis, especially among older children. The advantages of SPT are lower expenses and better availability compared to BAT. The limited availability and greater costs of BAT are

restricting the use of BAT to special cases in order to estimate the optimal time for a cashew nut challenge for example after a history of a severe reaction.

Tryptase elevation in anaphylaxis has been observed in many studies.²⁰ We showed that the tryptase ratio and delta-tryptase were higher among those with reactions; both parameters were elevated in mild and moderate-to-severe reactions and not just in anaphylaxis. Children <5 years in particular had a more elevated tryptase ratio in moderate-to-severe reaction group compared to the mild reaction group, which was not seen in older children. This could suggest a greater role of mast-cell driven mechanism in cashew allergy among younger children. Interestingly, based on our data, BAT seems to work much better in older children, which suggests a more prominent role of basophils in allergic reaction with increasing age. Peak MCT showed high specificity but low sensitivity to detect even severe reactions. We defined 1.12 as an ideal cut-off for tryptase ratio and 0.7 µg/L elevation in delta-tryptase to suggest an allergic reaction. One pediatric study revealed $\geq 20\%$ elevation in serum tryptase 30 to 60 minutes after allergic reaction in 6/8 children with positive challenge.²² A British study stated a 30% tryptase elevation from baseline as a cut-off to identify an allergic reaction, and tryptase ratio correlated to severity in adult peanut allergy. The ideal time point to assess tryptase seemed to be two hours from the beginning of an allergic reaction.²¹ By comparing baseline tryptase to tryptase levels at multiple time points during a reaction, we observed that the optimal time point to measure tryptase in children would be 1 to 2 hours after beginning of symptoms. In unclear cases, measuring tryptase during emergency or during challenge within 1 to 2 hours from the first symptoms and comparing to a baseline tryptase level assessed later might be useful in confirming diagnosis of suspected allergic reaction. We provide novel information regarding the timing and the degree of tryptase elevation in pediatric cashew nut allergy, both in mild and moderate-to-severe reactions and in different age groups.

Previous studies revealed a resolution of peanut allergy of approximately 20%³⁴ and in tree nut allergy 9-20%.^{7,35,36} In this study, the resolution of cashew nut allergy based on earlier reliable history was greater than in previous studies, nearly 30%. More interestingly, out of patients with earlier reaction and negative challenge, nearly 40% had experienced their latest reaction within a year; some of these reactions were severe. In many studies, patients with a positive reaction history in the previous year were automatically considered as allergic. Our findings emphasize that some patients with recent, and even severe reaction might not necessarily be allergic and their current allergy status should always be confirmed with OFC.

One of the strengths of this study was the large sample size and low median age. Both analyzed subgroups included the same number of patients. This was beneficial for defining age-specific cut-off levels for diagnostic tests. Every patient was challenged, which helped define their correct current allergy status. All challenges were supervised by same pediatric allergist and challenges were based on PRACTALL guidelines, thus improving diagnostic accuracy and estimation of reaction severity. Children with high sIgE and previous anaphylaxis history and children with rather recent reaction history were included. On the other hand, many patients had no known exposure to cashew nut. In this regard, we believe we have avoided patient selection bias.

This study had some shortcomings. The study was not double-blind and a greater number of patients in mild reaction group may have been beneficial for statistical analysis. A larger sample size in different reaction groups would have better enabled multivariable analysis.

In conclusion, Ana o 3-IgE seems to provide the most value in predicting clinical reactivity and a combination of several tests does not improve sensitivity or specificity. However, the cut-off levels for single diagnostic tests are age-specific, suggesting a need for different tests and combinations in various age groups both in predicting challenge outcome and in severity

analysis. We propose further studies with a larger sample size to evaluate more profoundly the age-specific cut-offs, also beyond subgroups analyzed in this study. Serum tryptase is also increased in milder allergic reactions, not just in anaphylaxis, and optimal time to measure serum tryptase seems to be 1 to 2 hours after onset of allergic symptoms.

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References

1. McWilliam VL, Perrett KP, Dang T, Peters RL. Prevalence and natural history of tree nut allergy. *Ann Allergy Asthma Immunol* 2020 May;124(5):466-472.
2. Edelman SM, Kukkonen AK, Makela MJ. Eliciting allergens and treatment of anaphylaxis: Report of the finnish national anaphylaxis registry. *Allergy* 2019 Oct;74(10):2010-2013.
3. Maloney 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 Jul;122(1):145-151.
4. van der Valk JPM, Bouche RE, Gerth van Wijk R, de Groot H, Wichers HJ, Dubois AEJ, et al. Low percentage of clinically relevant pistachio nut and mango co-sensitisation in cashew nut sensitised children. *Clin Transl Allergy* 2017 Mar 20;7:8-017-0145-z. eCollection 2017.

5. Elizur A, Appel MY, Nachshon L, Levy MB, Epstein-Rigbi N, Golobov K, et al. NUT Co Reactivity - ACquiring Knowledge for Elimination Recommendations (NUT CRACKER) study. *Allergy* 2018 Mar;73(3):593-601.
6. Divaret-Chauveau A, Clerc-Urmes I, Renaudin JM, Cordebar V, Dumond P, Schweitzer C. Threshold dose distribution for cashew nut allergy in children. *Clin Exp Allergy* 2018 Jul;48(7):902-904.
7. van der Valk JP, Gerth van Wijk R, Dubois AE, de Groot H, Reitsma M, Vlieg-Boerstra B, et al. Multicentre Double-Blind Placebo-Controlled Food Challenge Study in Children Sensitised to Cashew Nut. *PLoS One* 2016 Mar 11;11(3):e0151055.
8. van der Valk JP, Gerth van Wijk R, Baumert JL, Nordlee JA, Vlieg-Boerstra BJ, de Groot H, et al. Threshold dose distribution and eliciting dose of cashew nut allergy. *Ann Allergy Asthma Immunol* 2016 Dec;117(6):712-714.
9. Davoren M, Peake J. Cashew nut allergy is associated with a high risk of anaphylaxis. *Arch Dis Child* 2005 Oct;90(10):1084-1085.
10. Clark AT, Anagnostou K, Ewan PW. Cashew nut causes more severe reactions than peanut: case-matched comparison in 141 children. *Allergy* 2007 Aug;62(8):913-916.
11. Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, Valenta R, Hilger C, Hofmaier S, et al. EAACI Molecular Allergology User's Guide. *Pediatr Allergy Immunol* 2016 May;27 Suppl 23:1-250.
12. Sato S, Moverare R, Ohya Y, Ito K, Nagao M, Borres MP, et al. Ana o 3-specific IgE is a predictive marker for cashew oral food challenge failure. *J Allergy Clin Immunol Pract* 2019 Nov - Dec;7(8):2909-2911.e4.

13. Lange L, Lasota L, Finger A, Vlajnic D, Busing S, Meister J, et al. Ana o 3-specific IgE is a good predictor for clinically relevant cashew allergy in children. *Allergy* 2017 Apr;72(4):598-603.
14. van der Valk JP, Gerth van Wijk R, Vergouwe Y, Steyerberg EW, Reitsma M, Wichers HJ, et al. sIgE Ana o 1, 2 and 3 accurately distinguish tolerant from allergic children sensitized to cashew nuts. *Clin Exp Allergy* 2017 Jan;47(1):113-120.
15. Santos AF, Lack G. Basophil activation test: food challenge in a test tube or specialist research tool? *Clin Transl Allergy* 2016 Mar 15;6:10-016-0098-7. eCollection 2016.
16. Song Y, Wang J, Leung N, Wang LX, Lisann L, Sicherer SH, et al. Correlations between basophil activation, allergen-specific IgE with outcome and severity of oral food challenges. *Ann Allergy Asthma Immunol* 2015 Apr;114(4):319-326.
17. Santos AF, Du Toit G, Douiri A, Radulovic S, Stephens A, Turcanu V, et al. Distinct parameters of the basophil activation test reflect the severity and threshold of allergic reactions to peanut. *J Allergy Clin Immunol* 2015 Jan;135(1):179-186.
18. Santos AF, Douiri A, Becares N, Wu SY, Stephens A, Radulovic S, et al. Basophil activation test discriminates between allergy and tolerance in peanut-sensitized children. *J Allergy Clin Immunol* 2014 Sep;134(3):645-652.
19. Vitte J. Human mast cell tryptase in biology and medicine. *Mol Immunol* 2015 Jan;63(1):18-24.
20. Passia E, Jandus P. Using Baseline and Peak Serum Tryptase Levels to Diagnose Anaphylaxis: a Review. *Clin Rev Allergy Immunol* 2020 Jun;58(3):366-376.

21. Dua S, Dowey J, Foley L, Islam S, King Y, Ewan P, et al. Diagnostic Value of Tryptase in Food Allergic Reactions: A Prospective Study of 160 Adult Peanut Challenges. *J Allergy Clin Immunol Pract* 2018 Sep - Oct;6(5):1692-1698.e1.
22. Brandstrom J, Nopp A, Johansson SG, Lilja G, Sundqvist AC, Borres MP, et al. Basophil allergen threshold sensitivity and component-resolved diagnostics improve hazelnut allergy diagnosis. *Clin Exp Allergy* 2015 Sep;45(9):1412-1418.
23. Wongkaewpothong P, Pacharn P, Sripramong C, Boonchoo S, Piboonpocanun S, Visitsunthorn N, et al. The utility of serum tryptase in the diagnosis of food-induced anaphylaxis. *Allergy Asthma Immunol Res* 2014 Jul;6(4):304-309.
24. Saarinen KM, Pelkonen AS, Makela MJ, Savilahti E. Clinical course and prognosis of cow's milk allergy are dependent on milk-specific IgE status. *J Allergy Clin Immunol* 2005 Oct;116(4):896-75.
25. Valent P, Akin C, Arock M, Brockow K, Butterfield JH, Carter MC, et al. Definitions, criteria and global classification of mast cell disorders with special reference to mast cell activation syndromes: a consensus proposal. *Int Arch Allergy Immunol* 2012;157(3):215-225.
26. Sampson HA, Gerth van Wijk R, Bindslev-Jensen C, Sicherer S, Teuber SS, Burks AW, et al. Standardizing double-blind, placebo-controlled oral food challenges: American Academy of Allergy, Asthma & Immunology-European Academy of Allergy and Clinical Immunology PRACTALL consensus report. *J Allergy Clin Immunol* 2012 Dec;130(6):1260-1274.

27. Hourihane JO, Grimshaw KE, Lewis SA, Briggs RA, Trewin JB, King RM, et al. Does severity of low-dose, double-blind, placebo-controlled food challenges reflect severity of allergic reactions to peanut in the community? *Clin Exp Allergy* 2005 Sep;35(9):1227-1233.
28. Kukkonen AK, Pelkonen AS, Makinen-Kiljunen S, Voutilainen H, Makela MJ. Ara h 2 and Ara 6 are the best predictors of severe peanut allergy: a double-blind placebo-controlled study. *Allergy* 2015 Oct;70(10):1239-1245.
29. Muraro A, Roberts G, Worm M, Bilo MB, Brockow K, Fernandez Rivas M, et al. Anaphylaxis: guidelines from the European Academy of Allergy and Clinical Immunology. *Allergy* 2014 Aug;69(8):1026-1045.
30. McWilliam V, Peters RL, Allen KJ, Dharmage SC, Ponsonby AL, Tang MLK, et al. Skin Prick Test Predictive Values for the Outcome of Cashew Challenges in Children. *J Allergy Clin Immunol Pract* 2020 Jan;8(1):141-148.e2.
31. Sindher S, Long AJ, Purington N, Chollet M, Slatkin S, Andorf S, et al. Corrigendum: Analysis of a Large Standardized Food Challenge Data Set to Determine Predictors of Positive Outcome Across Multiple Allergens. *Front Immunol* 2020 Nov 30;11:625796.
32. Rubio A, Vivinus-Nebot M, Bourrier T, Saggio B, Albertini M, Bernard A. Benefit of the basophil activation test in deciding when to reintroduce cow's milk in allergic children. *Allergy* 2011 Jan;66(1):92-100.
33. Santos AF, Du Toit G, O'Rourke C, Becares N, Couto-Francisco N, Radulovic S, et al. Biomarkers of severity and threshold of allergic reactions during oral peanut challenges. *J Allergy Clin Immunol* 2020 Aug;146(2):344-355.

34. Peters RL, Allen KJ, Dharmage SC, Koplin JJ, Dang T, Tilbrook KP, et al. Natural history of peanut allergy and predictors of resolution in the first 4 years of life: A population-based assessment. *J Allergy Clin Immunol* 2015 May;135(5):1257-66.e1-2.
35. Gupta RS, Lau CH, Sita EE, Smith B, Greenhawt MJ. Factors associated with reported food allergy tolerance among US children. *Ann Allergy Asthma Immunol* 2013 Sep;111(3):194-198.e4.
36. Fleischer DM, Conover-Walker MK, Matsui EC, Wood RA. The natural history of tree nut allergy. *J Allergy Clin Immunol* 2005 Nov;116(5):1087-1093.

Figure legends

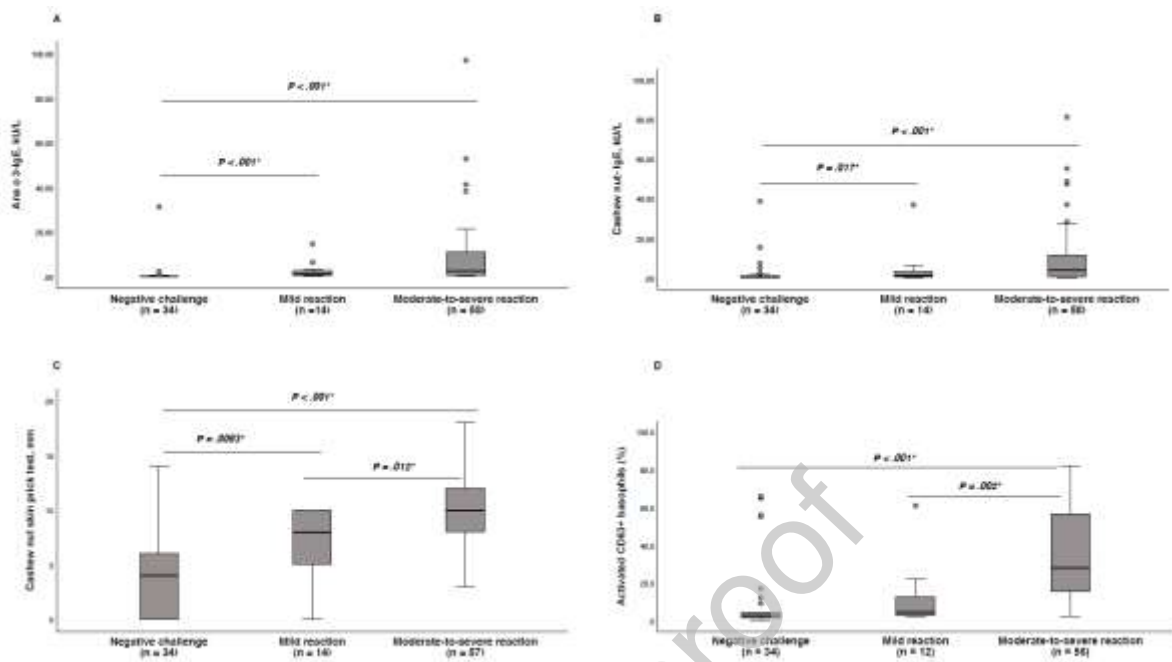


Figure 1 A-D. Ana o 3-IgE (A), cashew nut-IgE (B), skin prick test to cashew nut (C), and percentage of activated CD63+ basophils (D) by reaction severity. P-values are Bonferroni corrected. Kruskal-Wallis and Mann-Whitney-U tests used for statistics.

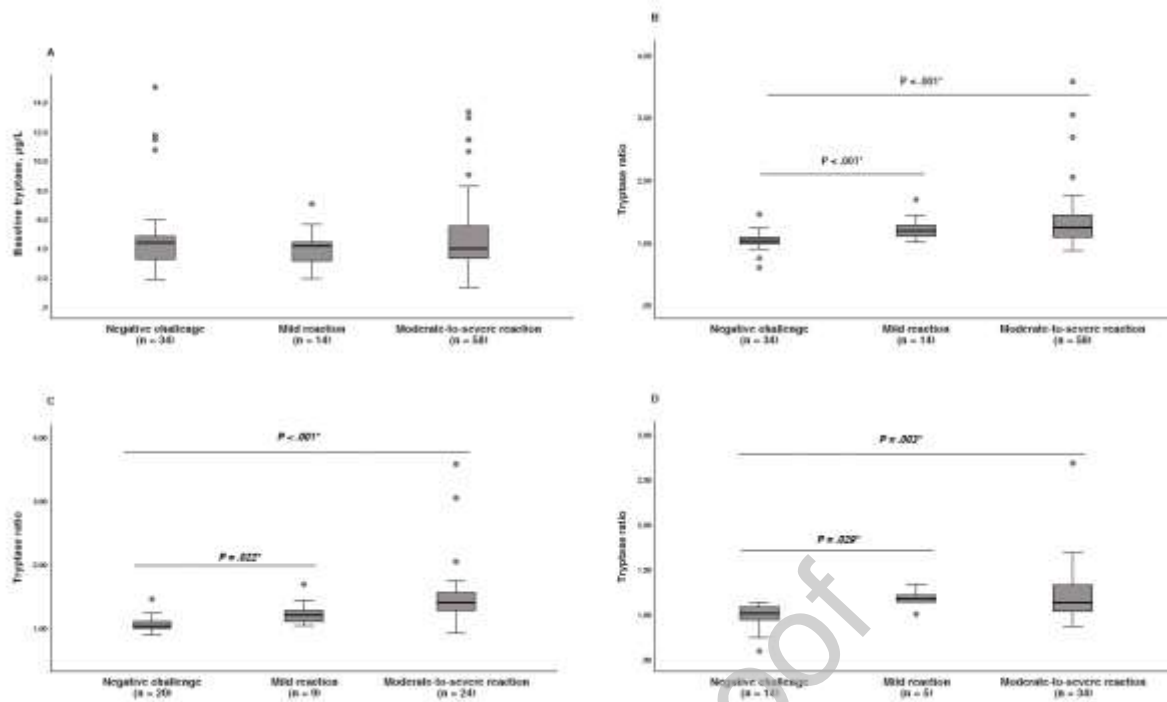


Figure 2 A-D. Baseline tryptase (A) and tryptase ratio in the study population (B), and tryptase ratio in children aged <5 years (C) and ≥5 years (D) by reaction severity. P-values are Bonferroni corrected. Kruskal-Wallis and Mann-Whitney-U tests used for statistics.

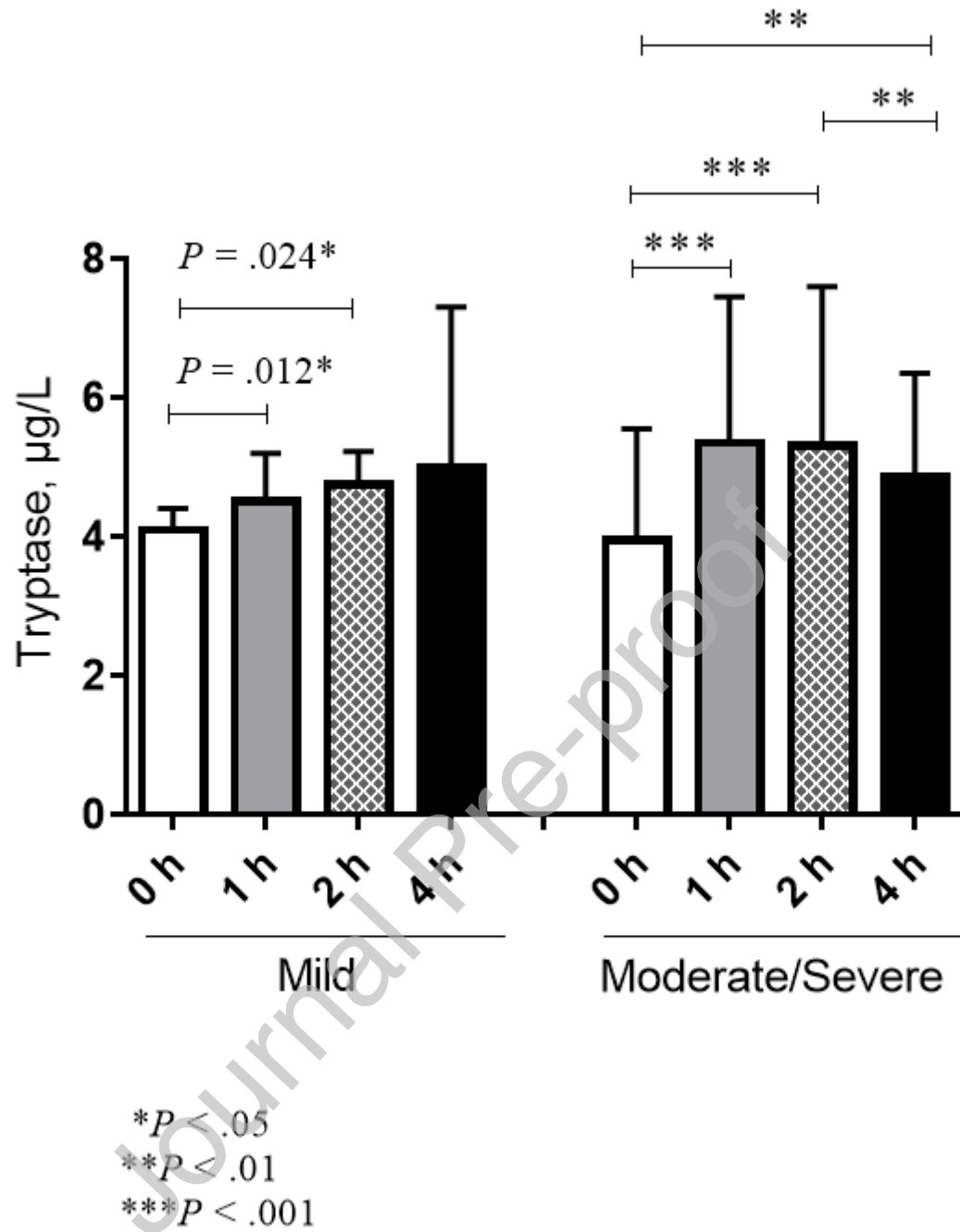


Figure 3. Serum tryptase levels (median, interquartile range) by reaction severity. Among patients with a mild reaction ($n=14$), there were five with measurements at all four time points, the respective figure in those with a moderate-to-severe reaction ($n=58$) was 42. Results are Bonferroni corrected.

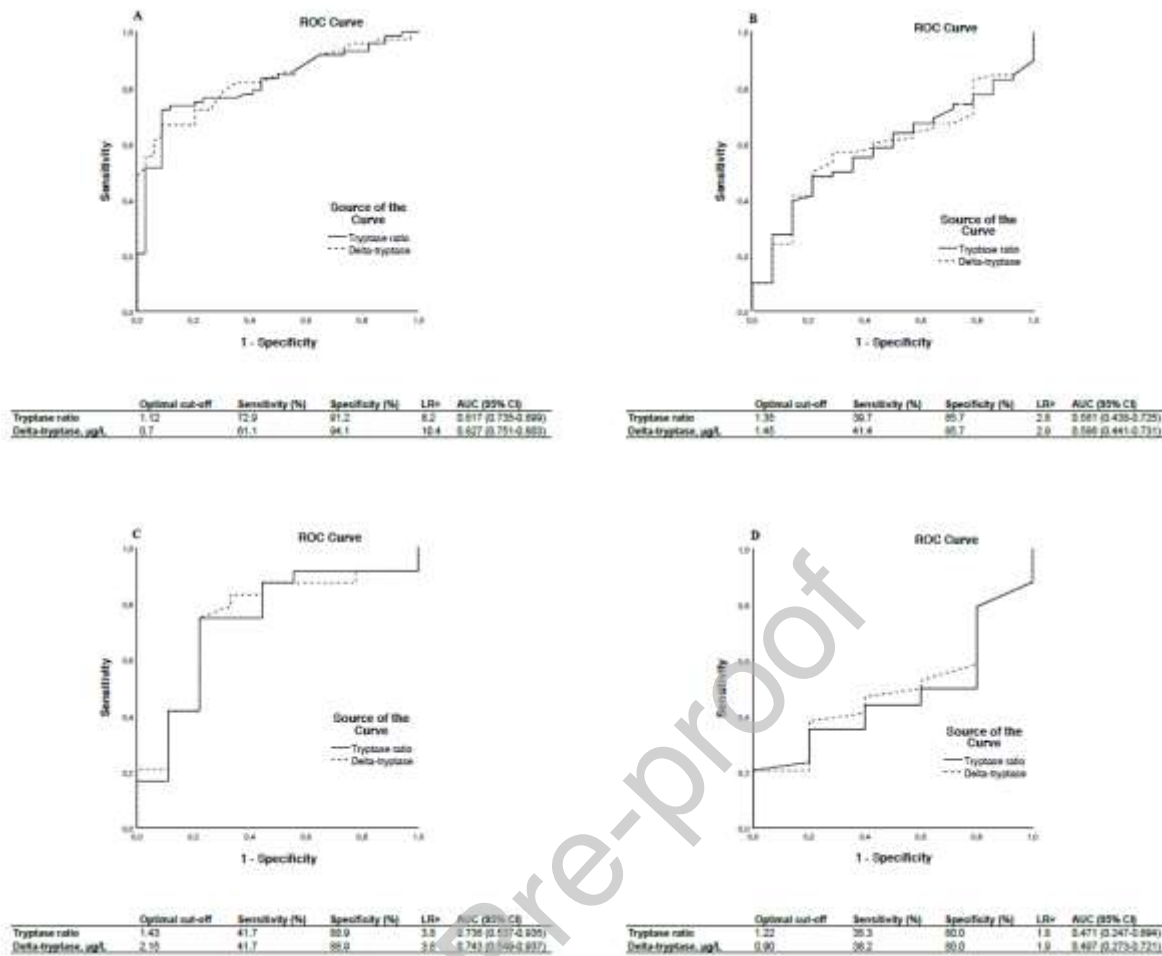


Figure 4 A-D. Tryptase ratio and delta-tryptase by challenge outcome (A), and in supporting the suspicion of moderate-to-severe reaction compared to mild reaction in the study population (B), and in children <5 (C) and ≥ 5 years (D). AUC, area under the curve; CI, confidence interval; LR+, positive likelihood ratio; ROC, receiver operator characteristic

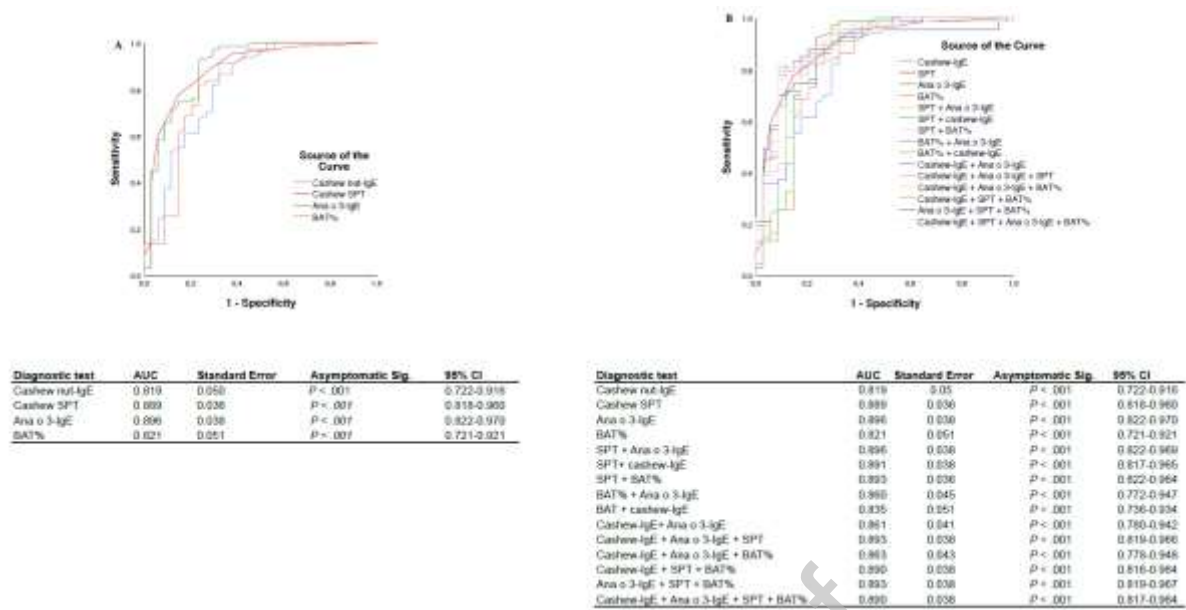


Figure 5 A-B. ROC curves for diagnostic tests (A), and for diagnostic tests and test combinations thereof (B) in predicting positive challenge outcome. AUC; area under the curve; BAT%, percentage of activated CD63+ basophils; CI, confidence interval; ROC, receiver operator characteristic; SPT, skin prick test. Binary logistic regression used for statistics. n=101

Table 1. Baseline clinical characteristics and baseline values by challenge outcome

Clinical characteristics	Positive n = 72 (68%)	Negative n = 34 (32%)	P-value
Male (%)	38 (52.7)	12 (35.3)	.09
Age, yrs., median (range)	5.5 (1.4-16.7)	4.5 (1.6-15.1)	.10
History of reaction to cashew nut (%)	47 (65.2)	19 (55.9)	.35
Latest reaction to cashew nut (%)			
< 1 years	15 (20.8)	7 (20.6)	.98

1-3 years	16 (22.2)	4 (11.8)	.20
> 3 years	16 (22.2)	8 (23.5)	.88
Convincing pistachio allergy (%) ^b	6 (8.3)	3 (8.8)	.93
History of IgE-mediated milk, wheat, egg or other nut allergy, n (%) ^b	45 (62.5)	18 (52.9)	.35
Atopic eczema, ever (%)	57 (79.1)	24 (70.6)	.33
Asthma, ever (%)	28 (38.9)	7 (20.6)	.06
Allergic rhinitis, ever (%) ^b	37 (51.4)	11 (32.4)	.07
Total serum IgE, kU/L, median (range)	208 (18-6226)	175 (9-1598)	.35
Cashew nut-IgE, kU/L, median (range)	2.85 (0.16-81.30)	0.20 (0.03-38.70)	< .001
Ana o 3-IgE, kU/L, median (range)	1.82 (0.11-96.90)	0.08 (0.00-31.20)	< .001
Cashew nut SPT, mm, median (range) ^a	10 (0-18)	4 (0-14)	< .001
Pistachio nut SPT, mm, median (range)	7 (0-15)	4 (0-8)	< .001
Sensitized to birch (%) ^b	47 (65.2)	14 (41.2)	.019
Baseline blood eosinophil count, E9/L, median (range)	0.37 (0.02-1.01)	0.34 (0.03-1.46)	.66
Baseline serum tryptase on a challenge day, µg/l, median (range)	4.0 (1.3-13.3)	4.35 (1.8-15.0)	.54
Cumulative reactive dose, mg, median (range) ^c	80 (5-730)	680 (430-680)	< .001

a. n=105, b. Skin prick test ≥ 3 mm or specific IgE $\geq .35$ kU/L. c. In patients with a negative challenge median and range demonstrate cumulative dose protein. Significant P-value < .05 calculated by Pearson Chi-square, Fisher's exact or Mann-Whitney-U test

Table 2. Cut-offs for diagnostic tests in predicting challenge outcome in the study population, and in children < and ≥ 5 years

Diagnostic test with optimal cut-off by challenge outcome	Positive challenge	Sens	Spec	PPV	NP V	LR+	LR-	AUC (95% CI)
	n	%	%	%	%			
Study population (n=106)								
Cashew SPT ≥ 11 mm	27	38.0	97.1	96	43	12.9	0.6	0.889 (0.818-0.960)
Cashew-IgE ≥ 2.33 kU/L	39	54.2	88.2	91	48	4.6	0.5	0.819 (0.722-0.916)
Ana o 3-IgE ≥ 0.97 kU/L	45	61.1	94.1	96	54	10.4	0.4	0.896 (0.822-0.970)
BAT $\geq 17.15\%$	42	61.8	85.3	89	53	4.2	0.4	0.821 (0.721-0.921)
Children <5 y (n = 53)								
Cashew SPT ≥ 9 mm	22	68.8	85.0	88	63	4.6	0.4	0.863 (0.748-0.977)
Cashew-IgE ≥ 5.07 kU/L	18	54.5	90.0	90	55	5.5	0.5	0.847 (0.724-0.970)
Ana o 3-IgE ≥ 2.47 kU/L	21	63.6	95.0	96	61	12.7	0.4	0.902 (0.803-1.000)
BAT $\geq 18.2\%$	17	60.0	80.0	81	55	3.0	0.5	0.779 (0.633-0.925)
Children ≥ 5 y (n = 53)								
Cashew SPT ≥ 8 mm	29	71.8	92.9	94	55	10.1	0.3	0.907 (0.827-0.986)

Cashew-IgE $\geq$ 2.61 kU/L	15	38.5	92.9	94	35	5.4	0.7	0.814 (0.671-0.958)
Ana o 3-IgE $\geq$ 0.48 kU/L	27	69.2	92.9	96	52	9.7	0.3	0.917 (0.828-1.000)
BAT $\geq$ 9.6%	28	73.7	92.9	97	57	10.3	0.3	0.880 (0.753-1.000)

AUC, area under the curve; BAT, basophil activation test; CI, confidence interval; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; SPT, skin prick test

Table 3. Cut-offs for diagnostic tests in predicting moderate-to-severe reaction compared to mild reaction in the study population, and in two age-groups.

Diagnostic test with optimal cut-off by reaction severity	Sens	Spec	PPV	NP V	LR+	LR-	AUC (95% CI)
	%	%	%	%			
Study population (n=72)							
Cashew SPT ≥ 10 mm	64.9	71.4	90	33	2.3	0.5	0.749 (0.629-0.868)
Cashew-IgE ≥ 6.47 kU/L	39.7	92.9	96	27	5.6	0.6	0.682 (0.530-0.833)
Ana o 3-IgE ≥ 6.47 kU/L	31.0	92.9	95	25	4.3	0.7	0.638 (0.492-0.783)
BAT $\geq 22.8\%$	60.7	91.7	97	33	7.3	0.4	0.812 (0.678-0.946)
Children <5 y (n = 33)							
Cashew SPT ≥ 11 mm	47.8	100	100	43	N/A	0.6	0.717 (0.538-0.896)
Cashew-IgE ≥ 4.50 kU/L	75.0	88.9	95	57	6.8	0.3	0.824 (0.684-0.965)
Ana o 3-IgE ≥ 4.00 kU/L	62.5	88.9	94	47	5.6	0.4	0.771 (0.613-0.928)
BAT $\geq 23.6\%$	59.1	87.5	93	44	4.7	0.5	0.801 (0.605-0.997)
Children ≥ 5 y (n = 39)							
Cashew SPT ≥ 9 mm	67.6	100	100	31	N/A	N/A	0.821 (0.663-0.978)
Cashew-IgE ≥ 1.11 kU/L	55.9	80.0	95	21	2.8	0.6	0.671 (0.364-0.977)
Ana o 3-IgE ≥ 1.30 kU/L	47.1	80.0	94	18	2.4	0.7	0.612 (0.341-0.883)
BAT $\geq 14.50\%$	76.5	100	100	33	N/A	N/A	0.860 (0.740-0.980)

AUC, area under the curve; BAT, basophil activation test; CI, confidence interval; LR, likelihood ratio; N/A, not applicable; NPV, negative predictive value; PPV, positive predictive value; SPT, skin prick test

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eSupplementary material (clean copy)

eTable legends and eTables

eTable 1. The modified severity scoring of allergic reactions at a food challenge.

Symptoms	Cumulative dose (mg) of protein at which the symptoms appear (mg)		
	>100	6-99	≤5
Localized urticaria <5 lesions on the face or extremities or pruritus	1	2	3
Mild stomach pain <30 min and resolves without medication	3	4	5
Generalized urticaria	6	7	8
Abdominal pain >30 min, vomiting, lethargy, diarrhea, or angioedema	9	11	13
Laryngeal edema or mild wheezing: hoarseness of voice, stridor, cough, mild wheezing, pain in the throat	14	15	17
Severe respiratory symptoms or a collapse: drop in the blood pressure, severe bronchial obstruction, oxygen saturation <92%, pallor, collapse	21	22	23

Modified from Hourihane (26).

Severity of the reaction is the highest, not cumulative, score: 1-5 indicate mild, 6-13 moderate, and 14-23 severe allergic reactions

eTable 2 A. Medication used during the challenge.

Data is presented in number of patients and the percentage.

Antihistamine, n (%)	71 (99)
Cortisone, n (%)	46 (64)
Epinephrine, n (%)	42 (58)
Albuterol, n (%)	3 (4)
Rasemic epinephrine, n (%)	1 (1)
Oxygen, n (%)	2 (3)
Intravenous fluids, n (%)	15 (21)

eTable 2 B. Symptoms during the challenge.

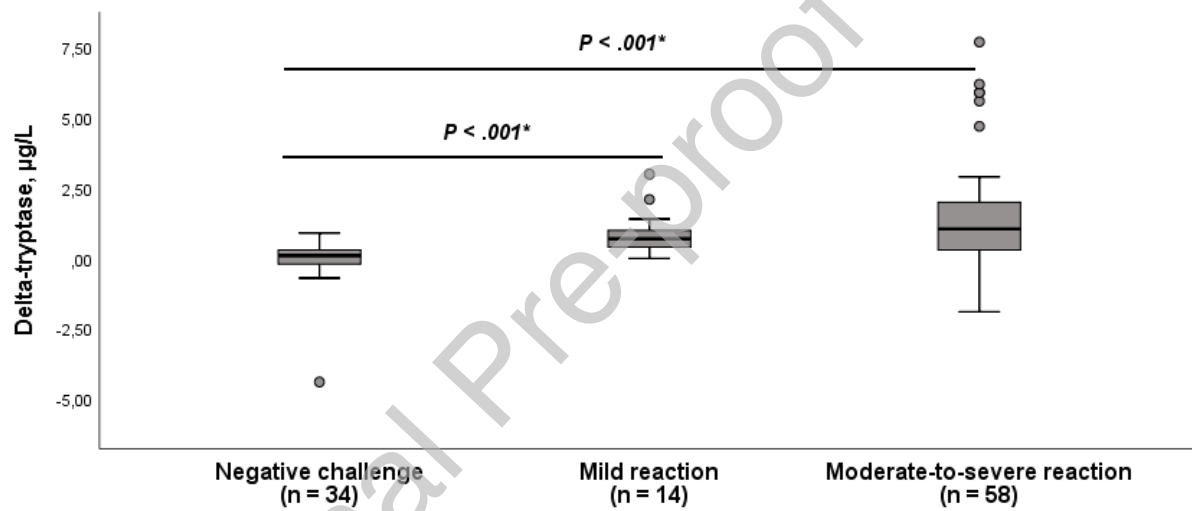
Data is presented in number of patients and the percentage.

Gastro-intestinal, n (%)	51 (71)
Skin, n (%)	47 (65)
Oral allergy syndrome, n (%)	41 (57)
Upper respiratory, n (%)	22 (31)
Lower respiratory, n (%)	13 (18)
Cardio-vascular, n (%)	18 (25)

eFigure legends and eFigures

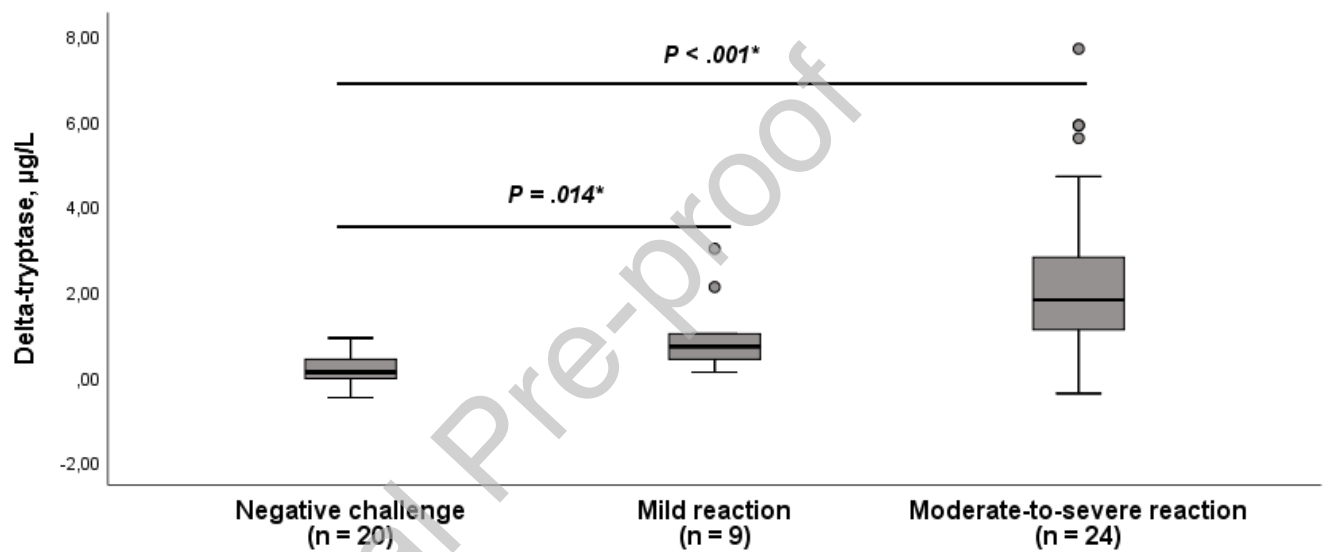
eFigure 1 A. Delta-tryptase by reaction severity in the study population (n = 106).

P-values are Bonferroni corrected. Kruskal-Wallis and Mann-Whitney-U tests used for statistics



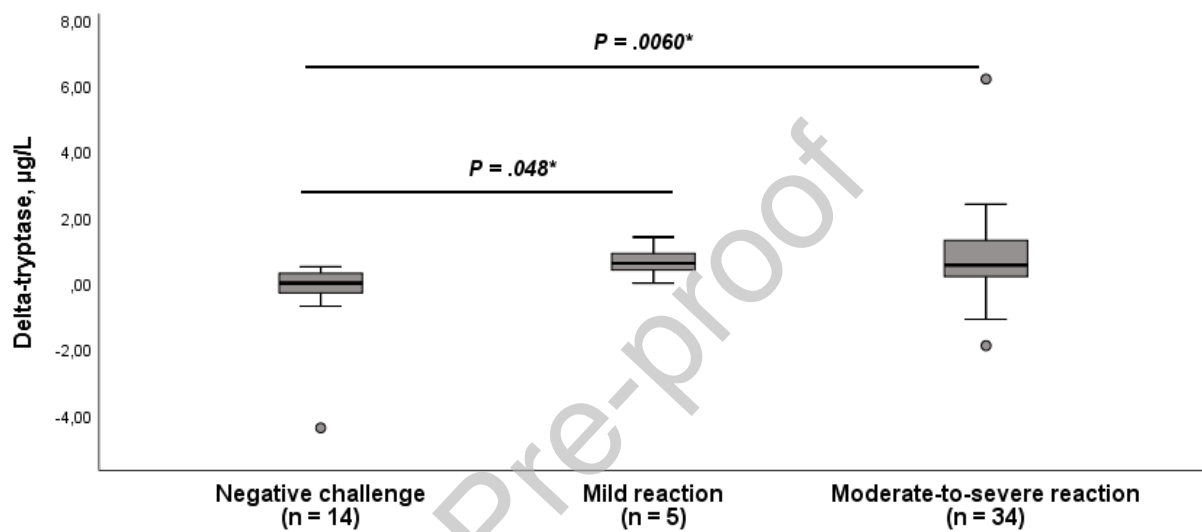
eFigure 1 B. Delta-tryptase by reaction severity in children <5 years (n = 53).

P-values are Bonferroni corrected. Kruskal-Wallis and Mann-Whitney-U tests used for statistics

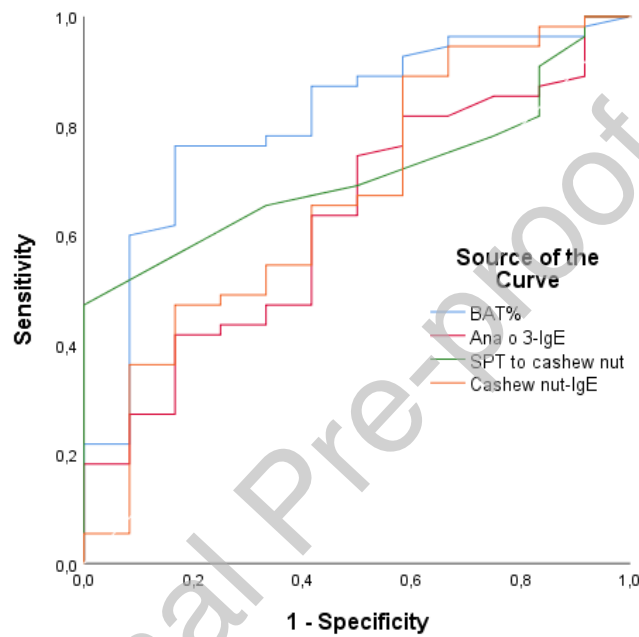


eFigure 1 C. Delta-tryptase by reaction severity in children >5 years (n = 53).

P-values are Bonferroni corrected. Kruskal-Wallis and Mann-Whitney-U tests used for statistics



eFigure 2. ROC curves for BAT%, Ana o 3-IgE, SPT to cashew nut, and cashew nut-IgE in predicting moderate-to-severe reaction compared to mild reaction with 95% confidence intervals. AUC, area under the curve; BAT%, percentage of activated CD63+ basophils; CI, confidence interval; SPT, skin prick test.



Diagnostic test	AUC	Standard Error	Asymptomatic Sig.	95% CI
BAT%	0.810	0.069	P = .001	0.676-0.944
Ana o 3-IgE	0.630	0.085	P = .160	0.464-0.796
SPT to cashew nut	0.712	0.064	P = .022	0.587-0.837
Cashew nut-IgE	0.668	0.089	P = .070	0.493-0.844