

Conjunctival provocation test in diagnosis of peanut allergy in children.

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Abstract

Background: Peanut allergy frequently cause severe allergic reactions. Diagnosis include detection of IgE to peanuts in serum or by skin prick tests. While children may have allergic sensitisation without having clinical peanut allergy, oral peanut challenge is often required for accurate diagnosis. The conjunctival provocation test is used for diagnosis and evaluation of treatment effect in inhalant allergies, but it has not been evaluated as a tool for diagnosing peanut allergy.

Objective: To investigate if the conjunctival provocation tests may be feasible, accurate and safe in diagnosing clinically relevant peanut allergy in patients with suspected peanut allergy.

Methods: This cross-sectional case-control study in children with clinical or laboratory suspected peanut allergy included 102 children recruited from the regional paediatric departments and specialist practices during one year from April 2011. A peanut tolerant control group of 28 children of similar age was recruited locally. A double-blind placebo-controlled

conjunctival provocation test with peanut extract was performed in all children, while oral peanut provocation was performed as double-blind placebo-controlled challenge in children with suspected peanut allergy and as an open challenge in the control children.

Results: All 81 children with a positive double-blind placebo-controlled oral food challenge also had a positive conjunctival provocation test. None of the children with negative conjunctival provocation test had a positive oral food challenge. The sensitivity and the specificity of the conjunctival provocation test was 0.96 and 0.83 respectively. No children had severe adverse reaction caused by the conjunctival provocation test, whereas 23 children suffered an anaphylactic reaction to the oral food challenge.

Conclusion & Clinical Relevance: Conjunctival allergen challenge appears to be a feasible, accurate and safe in diagnosing children referred for suspected peanut allergy.

Key words: conjunctival provocation test, oral food challenge test, food allergy, anaphylaxis, pediatrics

Abbreviations used:

CPT: Conjunctival provocation test

DBPCFC: Double-blind placebo-controlled food challenge

OFC: Oral food challenge

SPT: Skin prick test

FEV₁: Forced expiratory volume in 1 second

FEF₅₀: Forced vital capacity

FVC: Forced vital capacity

FeNO: Fractional exhaled nitric oxide

CI: Confidence interval

SD: Standard Deviation

LR+: Positive likelihood ratio

LR-: Negative likelihood ratio

PPV: Positive predictive value

NPV: Negative predictive value

ROC: Receiver operating characteristic

AUC: Area under the curve

i.m.: Intramuscular

Introduction

Peanut allergy has increased in prevalence during the last decades (1, 2), and might have reached a plateau in some areas (3). It is a frequent cause of severe and fatal food allergic reactions (4, 5).

Allergic reactions to peanut vary from mild oral reactions, often related to cross reactions to birch pollen (6, 7) or by exposure to small doses of peanut to experiencing severe anaphylactic reactions (8). A positive skin prick test (SPT) and identification of increased peanut-specific IgE levels demonstrate allergic sensitization, but not necessarily clinical peanut allergy. An oral food challenge test is often needed to confirm the diagnosis, with the double-blind placebo-controlled oral food challenge (DBPCFC) as the gold standard (9). However, food challenge tests may cause severe allergic reactions and are time consuming (10), and are subsequently often not performed. The

uncertainty of clinical reactions in many subjects sensitized to peanuts may result in unnecessary dietary restrictions and in unnecessary fear of allergic reactions (11). An optimal diagnostic test to establish clinical peanut allergy is essential for appropriate management.

The conjunctival provocation test (CPT), described by Blackley in 1870 for diagnosing pollen allergy (12), is a reliable diagnostic test of inhalant allergies (13, 14), found to be reproducible and safe (15).

To our knowledge, only two studies have assessed the CPT as a potential diagnostic tool for food allergy (16, 17). Performing CPT requires small amounts of allergens, potentially reducing the risk of severe allergic reactions compared to the allergen load of oral food challenge tests (18). A safe test with little risk of severe allergic reactions is mandatory for accurate diagnosis in a large number of children, also in outpatient clinics.

The objective of the present study was therefore to investigate if CPT may be an alternative to oral food challenge in the diagnosis of peanut allergy in children with suspected peanut allergy. The primary aim was to investigate the diagnostic value of CPT in peanut allergy. Secondly, we aimed to investigate if CPT could predict anaphylactic reactions to peanuts and also to investigate the safety of CPT.

Material and Methods

Study design:

In the present cross-sectional study in children with suspected peanut allergy and a control group of peanut tolerant children, CPT with peanut extract was compared to oral food challenges with peanut, the latter used as the gold-standard. The inclusion criteria for the peanut allergy suspected children was a positive SPT and/or detection of peanut-specific IgE, with or without a history of possible clinical reactions and age 4-18 years of age were chosen to reflect the common clinical

dilemma in paediatric outpatient clinics of whether or not a positive allergy test (SPT or specific IgE) may indicate clinically relevant allergy. The children were recruited from the paediatric departments in and near Oslo and from paediatric specialist practices during one year from April 2011. Inclusion criteria of the control children were children of similar age with known clinical tolerance to peanut. They were recruited locally from research group relatives, neighbours or friends of their children. Exclusion criteria (all children) were poorly controlled asthma or atopic eczema, or other chronic illness that could interfere with the tests or interpretation of the results.

Patients attended three visits, the controls the first visit day only. Interviews, clinical examination including eye examination, SPT, blood sampling, lung function and a double-blinded CPT with peanut extract were performed during the first visit. The food challenge consisted of a two-day DBPCFC for the patients and an open oral challenge for the controls.

The study was performed at the Children's Department at Oslo University Hospital.

Written informed consent was obtained from all parents and from all children 12 years and older.

The study was approved by the Regional Ethical Committee for South-East Norway, and registered by the Norwegian Biobank register (reference number 2010/2362a).

Subjects

The study included 102 children with suspected peanut allergy and 28 control children of similar age (9.9 years (4.9 – 18.6 years) and 10.5 years (5.6 – 14.9 years), respectively) (Table 1, and Figure 1), but with significantly more boys and more pronounced allergic disease related characteristics in the patient group. Previous allergic reaction to peanut was reported in 53 children (Table 1).

Methods

Children should be free of symptoms of asthma, rhinoconjunctivitis, respiratory or ocular infection for at least one week prior to each study visit. Prior to investigations, the following medications should be withheld; systemic corticosteroids for at least 14 days, antihistamines, topical ophthalmic solutions and nasal steroid fluticasone fuorate for at least 7 days, long acting β 2-agonists, leukotriene antagonists and theophyllamines for at least 72 hours, and short acting β 2-agonists, sodium di-cromoglycate nasal spray and inhaled corticosteroids for at least 12 hours, respectively.

Conjunctival provocation test

The CPT was performed double-blind with commercially available defatted, powdered and dried peanut extract from GREER[®] (Greer Laboratories, Lenoir, DC). The extract was reconstituted and diluted with NaCl 0.9%, initially 1:80, then 1:40, 1:20, 1:10 and finally 1:1 without preservatives. In children with a previous anaphylactic reaction to peanut the test solutions were initially diluted with NaCl 0.9 % to 1:160. The peanut concentration in the extract was 17 mg/ml and after dilution 1:1 with NaCl 0,9% 8.5 mg/ml. One drop per eye contained approximately 1 ml and thus resulting peanut exposure of 8,5 mg per eye in the maximum dose. One drop of the test solution or placebo (NaCl 0.9 %,) randomly assigned to the left and the right eye, respectively per patient, was placed in the lower conjunctival pouch of each eye at every test step with two hours' observation after the last challenge concentration. Redness, itching, chemosis and tearing were recorded in a modification of the scoring system by Abelson et al (15). Abelson et al graded hyperemia and itching on a scale ranging from none (0) to extremely severe (4) and chemosis from none (0) to severe (3). They performed post challenge evaluations 20 minutes after challenge. In the present study the test was considered positive and discontinued if two or more symptoms appeared; the concentration was otherwise increased every 30 minutes. Photos were taken before and after the CPT and analysed by

an ophthalmologist (JN), ignorant to the test solution containing peanut or placebo. The eye examination with slit lamp was performed to exclude ocular diseases, particularly current active conjunctivitis or keratitis prior to applying eye-drops with allergen or placebo.

Oral food challenge

Food challenges were not performed in children who had experienced anaphylaxis due to peanut exposure in the last 2 years. The DBPCFC was performed with peanut in chocolate balls (19), starting with 3 mg peanut and increasing the dose to 10 mg, 30 mg, 100 mg, 300 mg, 1 g and at last 5 g using 30 minutes intervals between the doses. The placebo chocolate balls were identical except containing no peanut. The DBPCFC was considered positive and discontinued after the occurrence of objective symptoms. The children were observed in the ward at least 3 hours after the last challenge dose. Appropriate treatment was given in case of symptoms. The open oral food challenges consisted of eating 8 peanuts, with an observation phase of ½ hour.

Randomization procedures for CPT and food challenges:

For the CPT all children were randomized to whether peanut extract or placebo were placed in the right or left eye, and children with suspected peanut allergy were also randomized for the DBPCFC. The control children were subjected to open oral food challenges, thus not randomized for this test. For the CPT two bottles were assigned to each child (code number), marked right and left eye, respectively. The bottles were randomized to contain peanut extract or placebo to the right or left eye. Likewise, each child was randomized to chocolate balls with peanut or placebo on day one or two of the DBPCFC. Randomization was performed using Statistical Analysis System (SAS version 9.3) in block sizes of eight for both challenge tests. The CPT randomization list produced by the research group statistician was given to personnel not involved in the study, who labelled the bottles. For the

DBPCFC a cook in an allergy-specialized kitchen prepared the chocolate balls with peanut or placebo and assigned them according to the randomization list to the first or second food challenge test day. Thus, the investigator was blinded to the assignments to peanut or placebo for both challenge tests.

Blood sample

Serum was sampled and analysed for total IgE and, specific IgE to peanut and to inhalant allergens, using the UniCAP fluoroenzyme immunoassay (FEIA) according to the manufacturer's instructions (Thermo Fisher, Uppsala, Sweden). The lower detection level was 0.10 kU/l.

Skin prick test

Skin prick tests were performed with Soluprick® allergens (ALK Abello, Denmark) for peanut, hazelnut, almond, soy 1:20 W/V, and birch pollen, grass pollen, mugwort, cat, dog, dermatophagoides pteronyssinus, and cladosporium herbarium 10 HEP; with pea 5000 PNU/ml from (Allergopharma, Reinbek, Germany), and with peanut extract from GREER® (Greer Laboratories, Lenoir, DC). The tests were performed on the volar side of the underarm, according to European standards (20). A positive SPT result was defined as a wheal with a diameter (mean of longest and the midpoint orthogonal diameter) ≥ 3 mm larger than the negative control.

Lung function tests and exhaled nitric oxide

Lung function was measured by forced expiratory flow volume loops according to European guidelines (21, 22) using Sensormedics V-max (Sensormedics Diagnostics, Yorba Linda, CA, USA) spirometer. The FEV₁, FEF₅₀ and FVC measures are reported as % predicted, using the reference values of Stanojevic (23).

Fractional exhaled nitric oxide was measured with an Eco Medics CLD 88 Exhalyzer® (Eco Medics, AG 8635 Duernten, Switzerland) according to international recommendations (24).

Outcome definitions

The *primary outcome* was a positive CPT versus a positive oral food challenge.

Anaphylaxis was defined as having at least one of three criteria after peanut exposure (25): acute onset involving the skin and/or mucosal tissue together with respiratory compromise or reduced blood pressure; two or more of the following occurring rapidly: involvement of the skin and/or mucosa, respiratory compromise, reduced blood pressure or persistent gastrointestinal symptoms; or reduced blood pressure only.

Severity of the allergic reactions during the food challenge is reported as the Astier score from 0 (no signs) to 5 (cardiac and respiratory symptoms requiring hospitalization in the intensive care unit) (26).

Adverse reactions were defined as mild (local allergic reactions that resolved without treatment), moderate (reactions that required medical treatment, but not i.m. adrenaline) or severe (reactions that required treatment with i.m. adrenaline).

Statistical analysis

Normally distributed variables are given as mean and standard deviation (SD). Comparisons were performed by using two-sample t-tests or in case of non-normality by use of logarithmic transformation and geometric mean with a 95% confidence interval (CI) for continuous variables and Pearson Chi-Square test for categorical variables, respectively. Receiver operating characteristic

(ROC) curves were used to establish the optimal cut-off value for CPT as a diagnostic test for a positive food challenge test and for identifying anaphylaxis. We applied Wilson's method (27) to calculate CI based upon the ROC curves for sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). Kendall's tau test was applied to assess the relationship between the CPT and the DBPCFC tests whereas a loess nonparametric smoother was used to draw the relationship between the CPT and the DBPCFC tests.

Statistical power analyses based upon our sample of 102 suspected peanut allergic patients, showed that a control group of 28 would achieve at least 80% power to detect a difference of 0.17 between the area under the ROC curve (AUC) under the null hypothesis of 0.50 and an AUC under the alternative hypothesis of 0.67 using a two-sided z-test at a significance level of 0.05. The data are discrete (rating scale) responses. The ratio of the standard deviations for the two groups is assumed to 1.00. The level of significance was set to 0.05 and analyses were performed using Statistical Analysis System (SAS, version 9.3, SAS Institute Inc, Carey, NC, USA) and IBM SPSS, Version 19.

Results

The baseline characteristics are shown in Table 1. The CPT was performed in all 130 children. The DBPCFC was successfully performed in 94/102 children with suspected peanut allergy and open food challenge in all 28 control children. Eight children with suspected peanut allergy were excluded from analyses; either they did not perform the DBPCFC due to reported anaphylactic reaction to peanut prior to the food challenge (n=1), unstable asthma (n=1), because of withdrawal before the food challenge (n=2), or they did not manage to ingest sufficient amount of the challenge food to complete the challenge test (n=4). Their baseline characteristics were largely similar to the 94 children with successful DBPCFC except for less allergic sensitisation (significantly fewer positive peanut SPT ($p=0.02$), smaller SPT reactions to peanut ($p=0.03$) and more sensitisation to inhalant allergens) ($p<0.05$). Thus 122 children were included in the final analysis.

Diagnosis of peanut allergy by CPT and oral food challenge

The CPT was positive in 93/122 children, 92 (98%) among the children with suspected peanut allergy and in one (4%) control child. The oral food challenge was positive in 81/122 children; in 86% of children with suspected peanut allergy and in none of the controls (Figure 2). The CPT was positive in 12 children with a negative oral food challenge at dilutions 1:1 (n=5), 1:10 (n=4), 1:20 (n=2), 1:40 (n=1). No child with a negative CPT had a positive oral food challenge test (Figure 2).

The dilution 1:10 had the highest diagnostic value for having a positive oral food challenge test, combining sensitivity and specificity (Figure 3A), giving positive and a negative predictive values of 0.92 (CI: 0.84 , 0.96)) and 0.92 (CI: 0.79 , 0.97). The sensitivity and specificity of the CPT varied from 0.15 – 1.00, and 0.71 – 1.00, respectively with the dilutions of 1:1 giving the best sensitivity and 1:40 - 1:160 giving the best specificity, respectively (Table 2A). The positive likelihood ratio (LR+) for having positive oral food challenge with a positive CPT was 5.64 (CI: 2.87 , 11.10), and the negative likelihood ratio (LR-) was 0.05 (CI: 0.02 , 0.14).

Prediction of anaphylactic or severe allergic reactions by CPT

Anaphylactic reaction during the DBPCFC occurred in 23 children, all with a positive CPT tests at dilutions between 1:160 and 1:10. The median concentration 1:40 for children, gave the best combination of sensitivity and specificity for having anaphylactic reactions (Figure 3B). The sensitivity and specificity of the CPT varied from 0.17 – 1.00 and 0.29 – 0.92, respectively with the conjunctival dilution of 1:1 giving the highest sensitivity of 1.0, and the dilutions 1:40 - 1:160 giving the highest specificity of 0.74 (Table 2B). Anaphylaxis was not observed in children with a positive CPT at dilution 1:1 (n=8) or a negative CPT (n=29) (Figure 4).

Anaphylaxis was observed after a cumulative peanut dose of 285-999 mg peanut in nine patients and after a cumulative peanut dose >1000 mg peanut in 15 children. The association between cumulative peanut dose giving anaphylaxis and the dilution of peanut extract giving a positive CPT is given in Figure 5.

Safety and adverse reactions by CPT and oral food challenge test

The safety of the CPT and oral food challenge were significantly different ($p < 0.001$), with severe reactions observed only during oral food challenge. No or mild adverse local reactions in relation to the CPT were observed in 97%. Moderate reactions were observed in 3.3% after the CPT, whereas moderate or severe reactions in 25.7% after food challenge. A ten-fold higher risk (35%) for moderate or severe adverse reactions was observed with oral food challenges, requiring adrenaline injections in 17% (Table 3).

Discussion

In this first study to test the appropriateness of a conjunctival peanut allergen provocation test in diagnosing clinical peanut allergy among patients assessed as being highly likely to be peanut allergic, the CPT was feasible, had high specificity and sensitivity at the optimal concentration of 1:10 and was associated with significantly less moderate and no severe allergic reactions compared to the oral food challenge test.

The diagnostic potential of the CPT in peanut allergy among patients with suspected peanut allergy has to our knowledge not been reported previously. Our finding that this double-blind peanut CPT was positive in all children with a positive oral peanut challenge, and negative for most, but not all

(9.8%) children with a negative oral challenge is in line with the high sensitivity and specificity (97.1 and 90.5% respectively) compared to nasal provocation test found in mite-sensitized children with allergic rhinitis (28). Similar sensitivity and specificity (92% and 100%, respectively) was reported for diagnosing latex allergy (29), and in grass pollen rhinitis in some (83-96%) (30), but not all studies (31). Kvenshagen et al. reported 100% sensitivity and specificity using a CPT score from 0 (no reaction) to score II (rubor, itching and oedema occurring within 5 minutes after challenge) for diagnosing food allergy (16).

All 12 children in the present study with a positive CPT and negative oral food challenge test were sensitized to birch pollen and the majority (10/12) reacted at the least concentrated dilutions (1:10 and 1:1). Thus, CPT may be less precise in diagnosing peanut allergy in children sensitized to birch pollen, and a confirmatory oral challenge test may be warranted in such patients.

Importantly, all children in the present study with a negative peanut CPT also had a negative oral food challenge, in line with what was found by Kvenshagen et al (16).

The dilution giving a positive CPT correlated with the severity of the allergic symptoms using the Astier score (26) at the oral food challenge test (Figure 4). We were unable to distinguish children with and without anaphylaxis among children with a positive CPT reaction within the lowest doses. Nevertheless, although the prediction ability of the CPT for severity of peanut reaction was low as judged by the LR, all of the patients who experienced anaphylaxis during food challenge also reacted to the first steps of CPT, ie the lowest concentrations, and none of those who had a negative CPT or reacted only at the last step (highest concentration) of CPT, had anaphylactic reactions. We therefore suggest that the CPT may have some potential in assessing severity of symptoms at peanut

challenge. We are unaware of other studies comparing CPT to the severity of food allergy, whereas Radcliff et al found no correlation between CPT and symptom severity in patients with seasonal allergic rhinoconjunctivitis (32).

The safety of the CPT was superior to the food challenges, with moderate local adverse reactions in less than 5% of subjects after the CPT, compared to the 10-fold risk of anaphylaxis after oral peanut challenge, requiring adrenaline injections in 25% of the subjects. The CPT thus appears to be safe (16, 29) although severe reactions have been reported (15), is less cumbersome to the patient and less time- and resource demanding than an oral food challenge. However, CPTs may not be feasible during pollen seasons in patients with pollen allergy due to conjunctival reactions related to pollen exposure.

The CPT was relayed to the study personnel as being preferable compared to the DBPCFC, although this was not studied systematically. Some children were anxious prior to the first CPT dose, but with the lack of discomfort, quickly accepted the subsequent doses. The DBPCFC was relayed as more uncomfortable for the patients due to severe allergic reactions with urticaria, nausea, stomach pain, rhinitis and asthma and more time consuming than the CPT for patients who had extended hospital stay due to severe allergic reactions.

Strengths and limitations

The study is strengthened by including all relevant diagnostic tests, including a DBPCFC, as well as the novel Peanut CPT in a large number of children who are well characterised, as well as in control children who were tolerant to peanuts. Also, consistency of therapeutic interventions with severe

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reactions is strengthened by the study involving the use a pre-defined treatment algorithm based upon symptoms and signs during the challenge tests, as well as only one paediatrician, highly experienced within food allergy, being responsible for all investigations in all children. However, the recruitment of children to the present study was skewed towards including patients highly likely to have peanut allergy, with relatively few peanut-tolerant children with sensitisation to peanut, as might be typical in most allergy clinics. The PPV and NPV are therefore likely to be higher with the present design, and not generalizable to a general allergy patient population or to a general population per se. Nevertheless, the study showed that the concept of adding a CPT to a diagnostic armamentarium for suspected peanut allergy may be valuable. The diagnostic value of CPT needs confirmatory studies including patients who may be sensitised, but without clinical reactions to peanuts, and in less selected patient groups before the CPT is considered a relevant alternative to oral food challenges. Also, the possibility to identify a cut-off point within the CPT for predicting severe clinical reactions during food challenge needs further studies.

Another potential limitation of the study is that one paediatrician performed all tests in the study, and she would know the result of the CPT prior to the DBPFC. However, as the randomisation code (placebo or active peanut) for both tests were not broken until completion of all tests, this is likely to have had only limited effect on the results of the DBPCFC test.

In the present study the CPT was compared to the DBPCFC, as this is considered the gold standard in diagnosing food allergy. Based upon our study results the CPT appears to be easily and safely performed as a diagnostic supplement to SPT and peanut-specific IgE, also in outpatient clinics possibly reducing the need for referral to hospitals for oral food challenges. However, future studies should also assess the potential diagnostic value of the CPT compared to the traditional diagnostic tools as the patient history, peanut specific IgE and SPT.

Conclusion: The conjunctival provocation test appears feasible, accurate and safe and may be a promising supplement to oral food challenges for diagnosing clinically relevant peanut allergy in patients with suspected peanut allergy.

Acknowledgements, conflict of interest

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Magnus P. Borres is employed by Thermo Fisher Scientific, the other authors declare no conflict of interest.

Table 1. Baseline characteristics of the study participants

Baseline characteristics of the 130 study participants, including 102 patients with suspected peanut allergy and 28 control children. Eight children with suspected peanut allergy were excluded from the final analyses by reasons given in the result section. The final analyses thus included 122 subjects with complete data of conjunctival provocation tests and oral peanut allergen challenges.

	Patients with suspected peanut allergy (n=102)	Control children (n=28)	P value
Age (mean, (min, max)) years	9.8 (4.9 , 18.6)	10.5 (5.6 ,14.9)	0.28
Gender (male) (N, %)	59 (57.8)	9 (32.1)	0.02
Reported previous clinical reaction to peanut (N, %)	53 (40.8)	0 (0.0)	<0.001
Total IgE kU/l (geometric mean, 95% CI)	382.9 (311.8 , 470.4)	38.2 (21.1 , 69.0)	<0.001
Specific IgE peanut kU/l (geometric mean, 95% CI)	29.1 (19.4 , 43.5)	0.3 (0.0 , 0.1)	<0.001
Peanut SPT positive (N,%)	95 (94.1)	1 (3.6)	<0.001
Peanut SPT mm (mean)	7.5	0.2	n.a.
Specific IgE birch pollen kU/l (N, %)	69 (69.7)	6 (23.1)	<0.001
Birch pollen SPT positive (N, %)	52 (92.9)	4 (7.1)	<0.001
Birch pollen SPT mm (mean)	5.1	4.5	0.54
Sensitisation to at least one allergen (IgE ≥0,35 kU/l or SPT ≥3 mm):			
Seasonal allergens (birch, timothy, mugwort, chladosporium)	69 (88.3)	7 (25.0)	<0.001
In-door allergens (cat, dog, mite)	63 (62.4)	4 (14.3)	<0.001
Asthma* (N,%)	80 (78.4)	5 (17.9)	<0.001
Allergic rhinoconjunctivitis* (N, %)	74 (72.5)	5 (17.9)	<0.001
Food allergy (other than peanut) * (N, %)	80 (78.4)	1 (3.6)	<0.001
Atopic eczema* (N, %)	84 (82.4)	10 (35.7)	<0.001
Adrenalin autoinjector previously prescribed (N, %)	68 (66.7)	0 (0.0)	<0.001

FEV ₁ % (mean, 95% CI)	102.2 (99.0 , 103.5)	101.2 (95.0 , 107.3)	0.50
FEV ₁ /FVC (mean, 95% CI)	0.86 (0.85 , 0.89)	0.87 (0.85 , 0.87)	0.33
FEF ₅₀ % (mean, 95% CI)	94.9 (90.3 , 99.5)	107.0 (98.1 , 116,0)	0.01
FVC % (mean, 95% CI)	101.6 (98.9 , 104.2)	97.4 (91.8 , 103.0)	0.14
FeNO (ppb) (mean, 95% CI)	29.9 (24.4 , 35.3)	13.0 (7.66 , 18.28)	0.002

*Reported by participants and/or parents. A positive skin prick test is defined as ≥ 3 mm. A positive IgE is defined as IgE $\geq 0,35$ kU/l

SPT: Skin prick test

FEV₁%; Percentage predicted value of the forced expiratory volume after 1 second

FEF₅₀%; Percentage predicted of mean forced flow at 50% of forced vital capacity

FVC%; Percentage predicted of forced vital capacity

FeNO: Fractional exhaled nitric oxide

kU/l: kilo Units per litre

CI: Confidence interval

Table 2A. Reliability of the Conjunctival provocation test in diagnosing peanut allergy

Conjunctival provocation test in predicting clinical peanut allergy demonstrated by oral food challenge test in 94 children with suspected peanut allergy and 28 children without peanut allergy. The number of children refers to subjects with a positive CPT, and the number of children with positive CPT that had negative DBPCFC at each dilution.

CPT dilution	Number of children with positive CPT	Number of children with positive CPT and negative DBPCFC	Sensitivity	Specificity	LR+	LR-	PPV	NPV
1:1	8	5	1.00 (0.96 , 1.00)	0.71 (0.56 , 0.82)	3.42 *	0.00 *	0.87 (0.79 , 0.93)	1.00 (0.88 , 1.00)
1:10	15	4	0.96 (0.90 , 0.99)	0.83 (0.69 , 0.92)	5.64 (2.87 , 11.09)	0.05 (0.02 , 0.14)	0.92 (0.84 , 0.96)	0.92 (0.79 , 0.97)
1:20	28	2	0.83 (0.73 , 0.89)	0.93 (0.81 , 0.98)	11.86 (3.79 , 33.76)	0.18 (0.12 , 0.30)	0.96 (0.88 , 1.00)	0.73 (0.60 , 0.83)
1:40 – 1:160	42	1	0.51 (0.40 , 0.61)	0.98 (0.88 , 1.00)	20.75 (2.46 , 145.56)	0.51 (0.40 , 0.63)	0.98 (0.88 , 1.00)	0.50 (0.40 , 0.63)

* Not feasible to calculate confidence intervals because of zero cells.

Table 2B. Reliability of the Conjunctival provocation test in diagnosing peanut anaphylaxis

Conjunctival provocation test in predicting anaphylactic reaction to peanut observed in 23 children demonstrated by oral food challenge test in 94 children with suspected peanut allergy and 28 children without peanut allergy. The number of children refers to subjects with anaphylaxis during the DBPCFC at each dilution of CPT.

CPT dilution	Number of children with positive CPT	Number of children with positive CPT and no anaphylaxis	Sensitivity	Specificity	LR+	LR-	PPV	NPV
1:1	0	70	1.00 (0.86 , 1.00)	0.29 (0.21 , 0.40)	1.41 (1.31 , 1.64)	0.00 (0.00 , 0.50)	0.25 (0.17 , 0.34)	1.00 (0.88 , 1.00)
1:10	3	62	1.00 (0.86 , 1.00)	0.37 (0.29 , 0.47)	1.59 (1.43 , 1.89)	0.00 (0.00 , 0.39)	0.27 (0.19 , 0.37)	1.00 (0.91 , 1.00)
1:20	4	50	0.87 (0.68 , 0.96)	0.49 (0.40 , 0.59)	1.71 (1.28 , 2.20)	0.27 (0.09 , 0.67)	0.29 (0.19 , 0.40)	0.94 (0.84 , 0.98)
1:40	16	26	0.70 (0.49 , 0.84)	0.74 (0.64 , 0.81)	2.65 (1.68 , 4.00)	0.41 (0.21 , 0.70)	0.38 (0.25 , 0.53)	0.91 (0.83 , 0.96)
1:160								

LR+: Positive likelihood ratio

LR-: Negative likelihood ratio

PPV: Positive predictive value

NPV: Negative predictive value

Criteria for positive conjunctival allergen challenge: two of four symptoms (redness, itching, chemosis and/or tearing).

Criteria for positive oral food challenge: occurrence of objective symptoms: rhinitis, conjunctivitis, upper or lower airway obstruction, cough, dyspnoea, urticaria, angioedema, stomach cramping, vomiting, diarrhoea or hypotension.

Criteria for anaphylactic reactions are defined in reference 25.

Table 3. Safety of the conjunctival provocation test

Safety of the CPT versus oral food challenge test in 94 children with suspected peanut allergy and 28 children without peanut allergy

	Conjunctival provocation test	Oral food challenge	<i>P</i> value<0.001
No adverse effects	29 (23.8%)	40 (32.8%)	
Mild adverse effects	89 (73.0%)	39 (32.0%)	
Moderate adverse effects	4 (3.3%)	22 (18.0%)	
Severe adverse effects	0 (0%)	21 (17.2%)	

Grading of adverse effects:

Mild adverse effects: local symptoms, such as allergic rhinitis, conjunctivitis and/or oral allergy syndrome that resolve without treatment.

Moderate adverse effects: moderate allergic symptoms that require medical treatment with antihistamines, corticosteroids and/or beta2 agonists, but not intramuscular (i.m.) adrenaline.

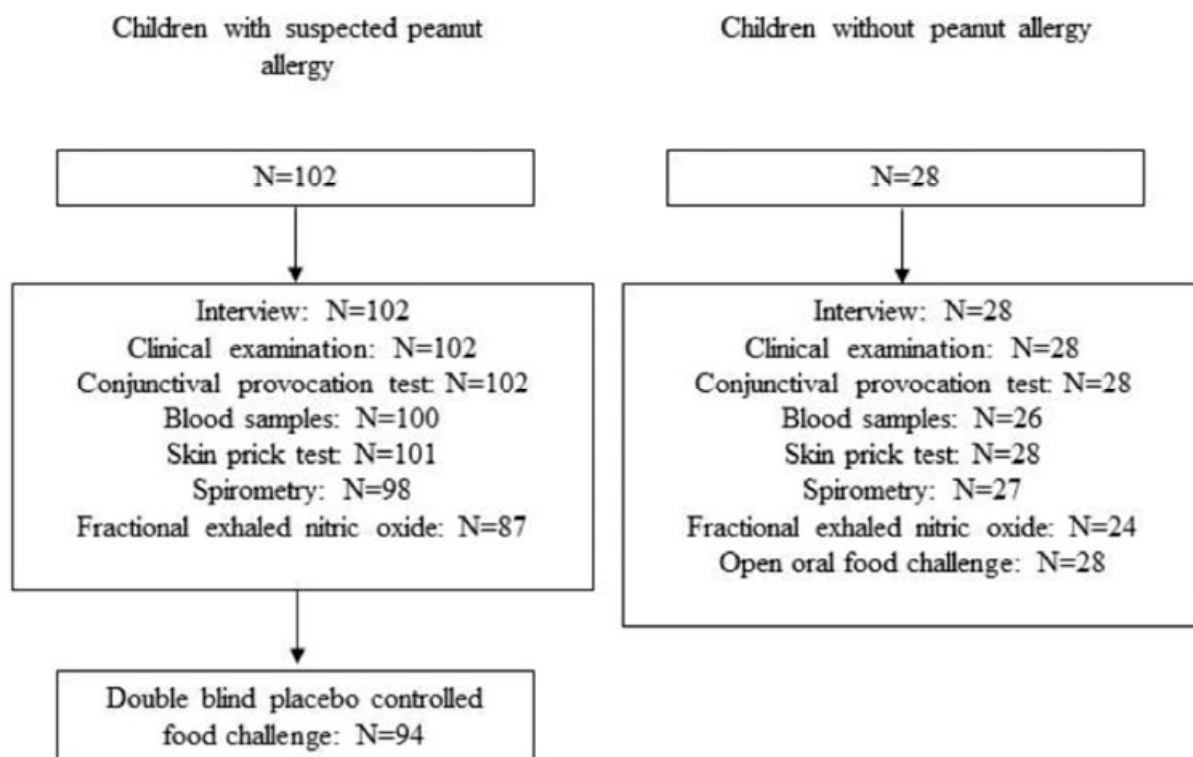
Severe adverse effects: severe allergic reactions with symptoms requiring treatment with adrenaline injections.

References

1. Grundy J, Matthews S, Bateman B, Dean T, Arshad SH. Rising prevalence of allergy to peanut in children: Data from 2 sequential cohorts. *The Journal of allergy and clinical immunology*. 2002 Nov;110(5):784-9.
2. Sicherer SH, Munoz-Furlong A, Sampson HA. Prevalence of peanut and tree nut allergy in the United States determined by means of a random digit dial telephone survey: a 5-year follow-up study. *The Journal of allergy and clinical immunology*. 2003 Dec;112(6):1203-7.
3. Venter C, Hasan Arshad S, Grundy J, Pereira B, Bernie Clayton C, Voigt K et al. Time trends in the prevalence of peanut allergy: three cohorts of children from the same geographical location in the UK. *Allergy*. 2000 Jan;65(1):103-8.
4. Anagnostou K, Clark A. The management of peanut allergy. *Archives of disease in childhood*. 2014 Aug 25.
5. Bock SA, Munoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. *The Journal of allergy and clinical immunology*. 2001 Jan;107(1):191-3.
6. Vereda A, van Hage M, Ahlstedt S, Ibanez MD, Cuesta-Herranz J, van Odijk J, et al. Peanut allergy: Clinical and immunologic differences among patients from 3 different geographic regions. *The Journal of allergy and clinical immunology*. 2011 Mar;127(3):603-7.
7. Niggemann B, Schmitz R, Schlaud M. The high prevalence of peanut sensitization in childhood is due to cross-reactivity to pollen. *Allergy*. 2011 Jul;66(7):980-1.
8. Burks AW. Peanut allergy. *The Lancet*. //;371(9623):1538-46.
9. Muraro A, Werfel T, Hoffmann-Sommergruber K, Roberts G, Beyer K, Bindslev-Jensen C, et al. EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy. *Allergy*. 2014 Aug;69(8):1008-25.
10. Niggemann B. When is an oral food challenge positive? *Allergy*. 2010 Jan;65(1):2-6.
11. Primeau MN, Kagan R, Joseph L, Lim H, Dufresne C, Duffy C, et al. The psychological burden of peanut allergy as perceived by adults with peanut allergy and the parents of peanut-allergic children. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology*. 2000 Aug;30(8):1135-43.
12. Blackley CH. Experimental researches on hay fever. 1873. Bailliere Tindall and Cox.
13. Tuft L, Ettelson LN, Gupta K, Krueger C. Eye tests with inhalant allergens; their feasibility, indications, and clinical value. *The Journal of allergy*. 1959 Nov-Dec;30:492-505.
14. Moller C, Bjorksten B, Nilsson G, Dreborg S. The precision of the conjunctival provocation test. *Allergy*. 1984 Jan;39(1):37-41.

15. Abelson MB, Chambers WA, Smith LM. Conjunctival allergen challenge. A clinical approach to studying allergic conjunctivitis. *Archives of ophthalmology*. 1990 Jan;108(1):84-8.
16. Krane Kvenshagen B, Jacobsen M, Halvorsen R. Can conjunctival provocation test facilitate the diagnosis of food allergy in children? *Allergologia et immunopathologia*. 2010 Nov-Dec;38(6):321-6.
17. Sastre J, Lluch-Bernal M, Quirce S, Arrieta I, Lahoz C, Del Amo A et al. A double-blind, placebo-controlled oral challenge study with lyophilized larvae and antigen of the fish parasite, *Anisakis simplex*. *Allergy*. 2000 Jun;55(6):560-4.
18. Kvenshagen BK, Jacobsen M. The value of mucosal allergen challenge for the diagnosis of food allergy. *Current opinion in allergy and clinical immunology*. 2013 Jun;13(3):268-72.
19. van Odijk J, Ahlstedt S, Bengtsson U, Borres MP, Hulthen L. Double-blind placebo-controlled challenges for peanut allergy the efficiency of blinding procedures and the allergenic activity of peanut availability in the recipes. *Allergy*. 2005 May;60(5):602-5.
20. Dreborg S, Frew A. Position paper. Allergen standardization and skin tests. *Allergy*. 1993;48(Suppl. 14):48S-82S.
21. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. *The European respiratory journal*. 2005 Aug;26(2):319-38.
22. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *The European respiratory journal Supplement*. 1993 Mar;16:5-40.
23. Stanojevic S, Wade A, Stocks J, Hankinson J, Coates AL, Pan H, et al. Reference ranges for spirometry across all ages: a new approach. *American journal of respiratory and critical care medicine*. 2008 Feb 1;177(3):253-60.
24. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *American journal of respiratory and critical care medicine*. 2005 Apr 15;171(8):912-30.
25. 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):1026-45.
26. Astier C, Morisset M, Roitel O, Codreanu F, Jacquenet S, Franck P, et al. Predictive value of skin prick tests using recombinant allergens for diagnosis of peanut allergy. *The Journal of allergy and clinical immunology*. 2006 Jul;118(1):250-6.
27. Wilson EB. Probable inference, the law of succession, and statistical inference. *J Am Stat Assoc*. 1927;22:209-12.

28. Anantasit N, Vilaiyuk S, Kamchaisatian W, Supakornthanasarn W, Sasisakulporn C, Teawsomboonkit W, et al. Comparison of conjunctival and nasal provocation tests in allergic rhinitis children with *Dermatophagoides pteronyssinus* sensitization. *Asian Pacific journal of allergy and immunology* / launched by the Allergy and Immunology Society of Thailand. 2013 Sep;31(3):227-32.
29. Chelminska M, Niedozytko M, Jassem E. Clinical value of conjunctival allergen challenge in diagnosing allergic conjunctivitis related to latex. *International archives of allergy and immunology*. 2011;154(2):149-54.
30. Bokanovic D, Aberer W, Hemmer W, Heinemann A, Komericki P, Scheffel J, et al. Determination of sIgE to rPhl p 1 is sufficient to diagnose grass pollen allergy. *Allergy*. 2013 Nov;68(11):1403-9.
31. Huss-Marp J, Darsow U, Brockow K, Pfab F, Weichenmeier I, Schober W, et al. Can immunoglobulin E-measurement replace challenge tests in allergic rhinoconjunctivitis to grass pollen? *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology*. 2011 Aug;41(8):1116-24.
32. Radcliffe MJ, Lewith GT, Prescott P, Church MK, Holgate ST. Do skin prick and conjunctival provocation tests predict symptom severity in seasonal allergic rhinoconjunctivitis? *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology*. 2006 Dec;36(12):1488-93.



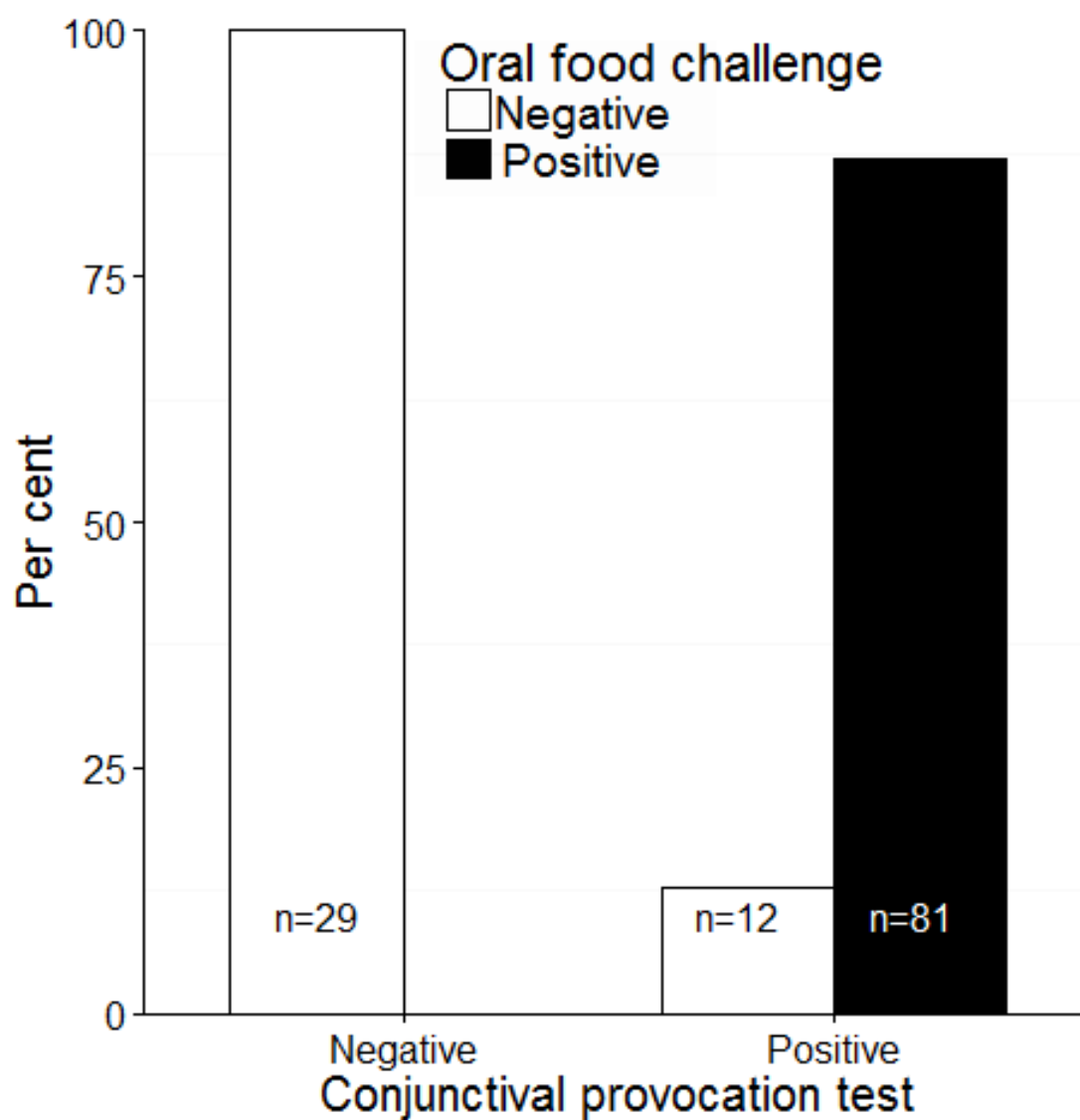


Figure 3A.

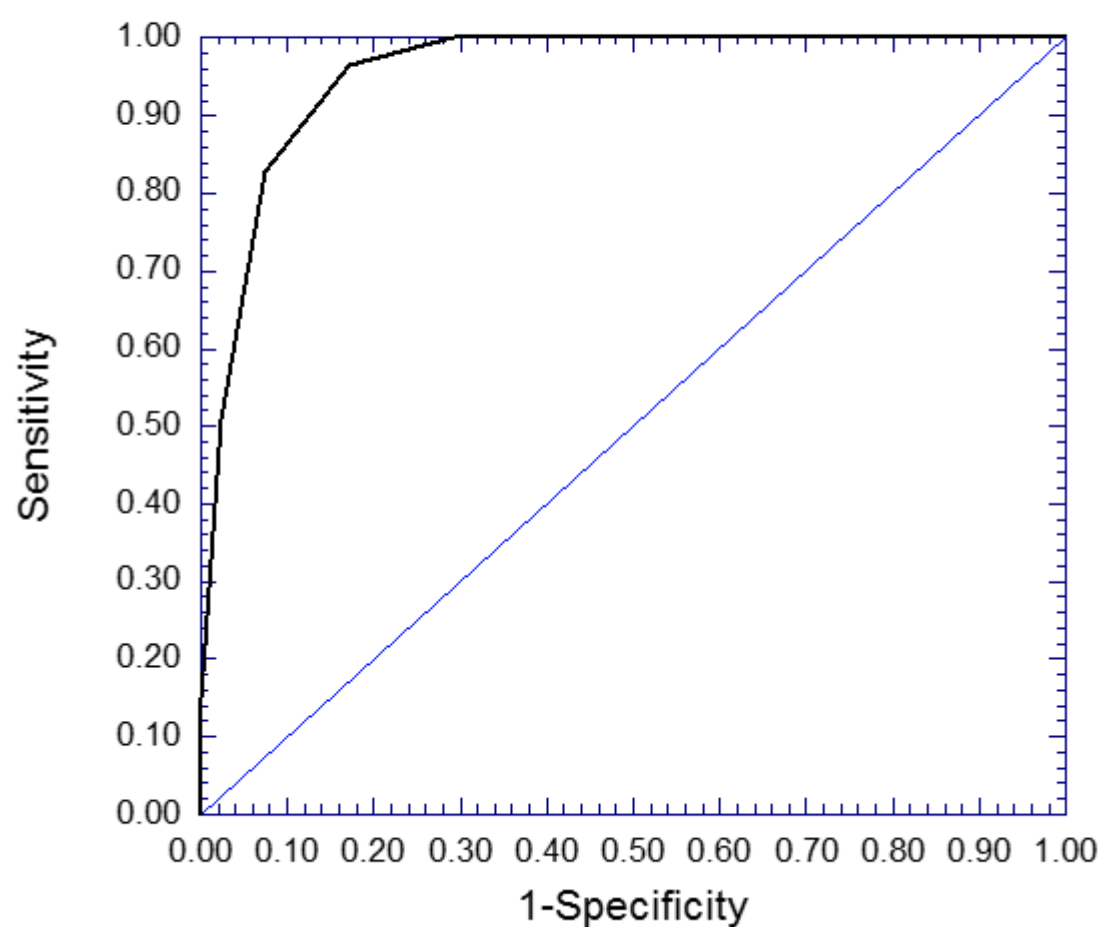


Figure 3B.

