

RESEARCH ARTICLE

Multicentre Double-Blind Placebo-Controlled Food Challenge Study in Children Sensitised to Cashew Nut

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Abstract

Background

Few studies with a limited number of patients have provided indications that cashew-allergic patients may experience severe allergic reactions to minimal amounts of cashew nut. The objectives of this multicentre study were to assess the clinical relevance of cashew nut sensitisation, to study the clinical reaction patterns in double-blind placebo-controlled food challenge tests and to establish the amount of cashew nuts that can elicit an allergic reaction.

Methods and Findings

A total of 179 children were included (median age 9.0 years; range 2–17 years) with cashew nut sensitisation and a clinical history of reactions to cashew nuts or unknown exposure. Sensitised children who could tolerate cashew nuts were excluded. The study included three clinical visits and a telephone consultation. During the first visit, the medical history was evaluated, physical examinations were conducted, blood samples were drawn and skin prick tests were performed. The children underwent a double-blind placebo-controlled food challenge test with cashew nut during the second and third visits. The study showed that 137 (76.5%) of the sensitised children suspected of allergy to cashew nut had a positive double-blind placebo-controlled food challenge test, with 46% (63) manifesting subjective symptoms to the lowest dose of 1 mg cashew nut protein and 11% (15) developing objective symptoms to the lowest dose. Children most frequently had gastro-intestinal symptoms, followed by oral allergy and skin symptoms. A total of 36% (49/137) of the children experienced an anaphylactic reaction and 6% (8/137) of the children were treated with epinephrine.

Competing Interests: Technology Foundation STW (STW number 11868), Food Allergy Foundation, Siemens Healthcare Diagnostics, HAL Allergy, Intersnack the Netherlands B.V., ALK-Abelló B.V., and the Netherlands Anaphylaxis Network funded this project. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. STW along with any other relevant declarations relating to employment, consultancy, patents, products in development, marketed products, etc. has no competing interest to report. This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials.

Abbreviations: DBPCFC, Double-blind placebo-controlled food challenge; HEP, Histamine equivalent prick; sIgE, Specific IgE; SPT, Skin prick test.

Conclusion

This prospective study demonstrated a strikingly high percentage of clinical reactions to cashew nut in this third line population. Severe allergic reactions, including anaphylaxis requiring epinephrine, were observed. These reactions were to minimal amounts of cashew nut, demonstrated the high potency of this allergens.

Trial Registration

[www.ncbi.nlm.nih.gov/pubmed NTR3572](http://www.ncbi.nlm.nih.gov/pubmed/NTR3572)

Introduction

Only a limited number of clinical studies have been published on cashew nut allergy. Five relevant studies have been performed examining clinical symptoms [1]. All of these studies were based on a limited number of patients, varying between 16 and 47 participants. Cashew-allergic patients most frequently show skin symptoms, followed by respiratory and gastro-intestinal symptoms. Studies have shown that a small amount of cashew nut allergen may cause severe clinical reactions, suggesting a high potency of this nut, comparable to that of other tree nuts and peanuts [2]. The study by Davoren et al. reported that 30% of the peanut and 74% of the cashew nut sensitised patients with peanut and tree nut allergy developed an anaphylactic reaction after allergen ingestion. Moreover, in this study, 5 of 27 patients with cashew nut allergy experienced an allergic reaction after only skin or mucosal contact. One of these five patients developed anaphylaxis [2].

Clinical history, combined with the outcome of a skin prick test (SPT) and/or specific IgE (sIgE) test, is often used to establish the diagnosis of cashew nut allergy. The gold standard, however, is the double-blind placebo-controlled food challenge (DBPCFC) test.

The objectives of this study were to assess the clinical relevance of cashew nut sensitisation, to study the clinical reaction patterns and the severity of symptoms during the DBPCFC tests with cashew nut and to establish the amount of cashew nut that can elicit an allergic reaction.

Material and Methods

Study design and patient selection

This study was a collaboration of three tertiary care centres for food allergy and the Research Centre Wageningen, the Netherlands. Consecutive new children and children known to have a sensitisation to cashew nut (sIgE and/or SPT) and a history of previous reaction(s) to cashew nut or unknown exposure were asked to participate in this study. More than 1000 children from this tertiary care population between 2 and 17 years of age were asked to participate. Approximately 1 in 3 parents of children who responded to the invitation (40%), agreed. Children with high sIgE (≥ 100 kU/l) to cashew nut and/or anaphylactic reactions after cashew nut ingestion in the past were also included. Sensitised children who could tolerate cashew nuts were excluded. All children were included in the study between May 2012 and March 2015. The last enrolled child finished the study in May 2015. The inclusion and exclusion criteria of the study are shown in Table 1 and a flowchart of the patient inclusion is shown in Fig 1.

Table 1. Inclusion and exclusion criteria.

Inclusion criteria
Age between 2 and 17 years.
Positive skin prick test (mean wheal diameter ≥ 3 mm $\varnothing$ and HEP-index area ≥ 0.4 and/ or detectable sIgE (> 0.35 kU/L) to cashew nut.
History of previous positive reaction to cashew nut or unknown exposure.
Written informed consent from parents (2–12 years old), or parents and child (≥ 12 years old).
Exclusion criteria
History of severe or uncontrolled asthma (according to the physician's assessment).
Autoimmune diseases, cardiovascular diseases or cancers.
Severe psychosocial problems.
The patient is allergic to one or more of the ingredients of the test food, unless a suitable substitute for the ingredient in question can be found.
Unable to stop taking antihistamine medication for a short period.
Use of beta-blockers.
Unable to speak and understand the Dutch language.

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Study procedure

The study program consisted of three clinical visits and a telephone consultation, as shown in [Table 2](#). During the first visit, written informed consent was obtained (from parents of children (2–12 years old) and from parents and children (≥ 12 years old) and two medical history questionnaires were completed. Blood samples were drawn, SPT and physical examinations were conducted by a nurse and a physician. All children underwent a DBPCFC test with cashew nut in the second and third visits. The results of the challenge tests were discussed with the parents of the children by phone within a week after the DBPCFC test.

Medical ethical approval for this study was obtained on 19 April 2012 and the study was registered in the Dutch trial register on 10 August 2012 (registered with administrative delay).

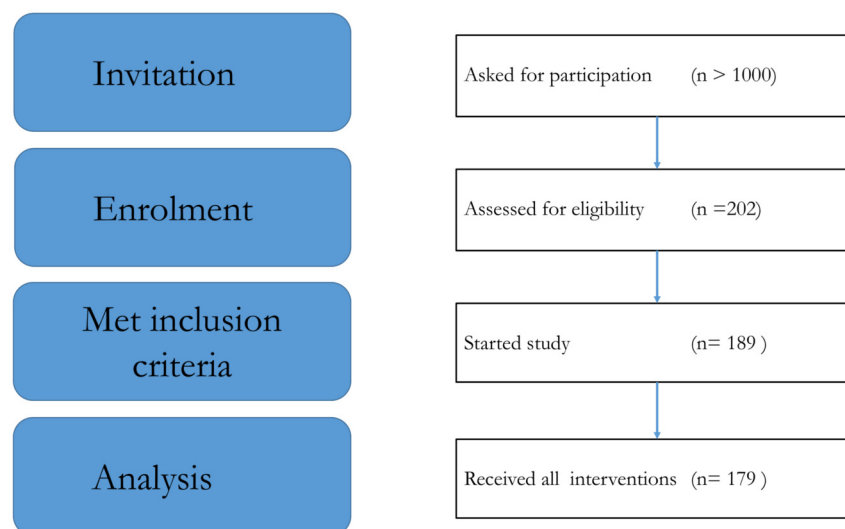


Fig 1. Flowchart of patient inclusion.

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Table 2. Study program.

Visit 1	Visit 2	Visit 3	Telephone consultation	Final visit (optional)
Week 1	Week 4	Week 6	Week 7	Week 8
Written informed consent	DBPCFC test	DBPCFC test	Result DBPCFC test	Dietary advice
	Session 1	Session 2		
Medical history (2 questionnaires)				
Physical examination				
Blood samples (19 ml)				
Skin prick test				

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Questionnaires

We used two questionnaires, which were specifically designed for this study. The medical history questionnaire contained 54 questions about general health, asthma and eczema. Also food allergies other than cashew nut allergy were evaluated by questions about the symptoms, the time between exposure and reaction, and the amount of allergenic food that caused the reaction. The dietary history questionnaire (12 questions) was used to identify allergic reactions in the past caused by cashew nut consumption. The type and severity of reaction was extensively evaluated and the amount of cashew nut causing the reaction was determined. The time between the reaction in history and the intake was also checked.

Skin prick test

The children underwent a SPT with cashew nut, pistachio nut, hazelnut, peanut, mango and birch pollen extracts, a positive control (histamine 10 mg/ml ALK-Abello, Nieuwegein, the Netherlands) in duplicate and a negative control. All the extracts, except birch pollen (ALK 10.000 BU), were made according to a previously described method [3]. Cashew nuts (roasted, unsalted) and pistachio nuts, hazelnuts and peanuts (fresh, not roasted, unsalted nuts) were homogenised mechanically, ground with a mortar and pestle, defatted by ether extraction, and subsequently the extracts were air-dried. A 10% w/v extract in PBS (phosphate-buffered saline) with the pre-treated material was made and stored at -20°C in small aliquots. Before testing the aliquots were defrosted and mixed. Mango juice was prepared from small pieces of ripe mango fruit pulp, without skin or kernel.

SPTs were performed by applying a drop of the allergen extract on the skin of the volar part of the forearm. The extract was pierced through the skin barrier with a lancet. Twenty minutes after the skin tests, the contours of the wheal were encircled with a fine-tip pen and transferred to a record sheet by translucent tape. The area of the wheals was determined by using a scanner device (Hewlett Packard 2400c) in combination with software previously developed in our centre: Precise Automated Area Measurement of Skin Test (PAAMOST). The area of the allergen-induced wheal was divided by the mean area of the two positive histamine-induced wheal controls. This ratio was defined as the Histamine Equivalent Prick (HEP)-index area. The average wheal diameter was measured as well. An average wheal diameter ≥ 3 mm and a HEP-index area ≥ 0.4 was considered positive[4].

In vitro tests

Serum samples were analysed for sIgE using the Siemens IMMULITE 2000 XPi Immunoassay System (Med. Imm. Laboratory; Reinier de Graaf Groep (RdGG)). Levels above 0.35 kU/L were

considered positive. sIgE against cashew nut, pistachio nut, hazelnut, peanut, mango and tree pollen were determined.

Food challenge test

Procedure and recipe challenge test. Each patient underwent a DBPCFC test with cashew nut. The test food was administered in increasing amounts of 8 doses at time intervals of 30 minutes. Placebo and cashew nut challenges were randomly administered on separate days with at least a one-week interval. Validated and standardised food challenge material was used in the DBPCFC tests [5]. Roasted cashew nuts were provided by Intersnack, Doetinchem, the Netherlands. NIZO Food Research, Ede, the Netherlands prepared the low-fat food matrix (muffin dough). The food matrix predominantly consisted of wheat, sugar, gingerbread spice mix and coconut. The total volume/weight of the cashew nut gingerbread was 120 grams. The starting dose consisted of 1 mg cashew protein, followed by increasing doses of 3, 10, 30, 100, 300, 1000, 1736 mg cashew protein. Dose 8 consisted of the remainder of the 120 grams cashew nut gingerbread recipe. In children below the age of 4 years the challenge was stopped at step 7 (1000 mg cashew protein), because of the large amount of challenge material. The challenged doses are shown in Table 3.

Assessment and DBPCFC tests. The DBPCFC test was discontinued and considered positive when objective symptoms occurred, or when subjective symptoms re-occurred twice after the same dose of challenge material had been administered, three times consecutively [6], or when severe subjective symptoms persisted for more than one hour. If the child presented with the same symptoms on the placebo as on the verum day, the DBPCFC test was considered as undetermined. Anaphylaxis was defined as described in the EAACI Guidelines for Food Allergy and Anaphylaxis [7].

Procedure after the outcome of the DBPCFC test. In negative challenge test results, the child was advised to introduce cashew nuts at home. If the parents or child expected to experience problems with the introduction, a home introduction schedule developed by Vlieg-Boerstra et al. [8] and made available online [9] with increasing amounts of cashew nuts was recommended. These introduction schedules comprise instructions for parents and photographs with information on the required amounts of specific food for home introduction. The schedules were advised to improve the safety of the cashew nut introduction at home. Children with a positive DBPCFC test were advised to strictly avoid cashew nuts. If necessary, the participant was referred to a dietician after the DBPCFC test for extensive information and advice.

Table 3. Challenge dosage DBPCFC test with cashew nut [5].

Dose steps	Cashew nut protein (mg)	Cashew nut protein cumulative (mg)	Cashew nut cumulative (number)*
1	1	1	0.01
2	3	4	0.03
3	10	14	0.10
4	30	44	0.30
5	100	144	1
6	300	444	3
7	1000	1444	10
8	1736	3180	22

* 1 cashew nut = approximately 700 milligrams.

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Statistical analysis

In this descriptive study, the patient and the study characteristics were reported in median, ranges and proportions. All analyses were done using SPSS software, 20th edition.

Results

Study population

The study included a total of 179 children. The most commonly cited reason for not participating was that it was time consuming, burdensome for the child to undergo allergy testing (SPT and sIgE), and the fear of a reaction during the challenge test. The median age was 9.0 years (range 2–17 years), with 106 boys (59%) and 73 girls (41%). The children came from all over the Netherlands because the three participating medical research centres were spread across the country. All patients' demographic and clinical characteristics are summarised in [Table 4](#).

Questionnaires

Symptoms consistent with eczema were reported by 70 children (39%) and those for asthma by 55 children (31%). 94 children (53%) had symptoms consistent with hay fever. In 112 children (63%) consumption of, or contact with cashew nuts had elicited an allergic reaction before study entrance. These symptoms consisted mostly skin symptoms after cashew nut consumption in their history, followed by gastro-intestinal symptoms, respiratory symptoms, oral allergy symptoms and eye symptoms. The majority of the children reacted to cashew nuts as a single food ingested and not incorporated in other foods and to an amount of approximately one cashew nut.

Twenty-three percent (42 of 179 children) reported pistachio nut consumption. 21 of 42 children (50%) reported allergic symptoms to pistachio nut. of these children. Mango was consumed by 116 of 179 children (65%) and allergy was reported in 8 of 116 children (7%). Hazelnuts were consumed by 143 of 179 children (80%) and 32 of these 143 children (22%) reported a hazelnut allergy. Peanuts were consumed by 151 of 179 children (84%) and peanut allergy was reported in 52 of these 151 children (34%) (see [Fig 2](#)).

Table 4. Demographic and clinical characteristics.

Total	179	
Gender		
Male	106	(59%)
Female	73	(41%)
Median age (years)	9.0	(range 2–17)
Atopic disease symptoms		
Asthma	55	(31%)
Eczema	70	(39%)
Hay fever	94	(53%)
Diagnostics		
Median sIgE cashew nut (kU/l)	3.72	(range 0–100)
Median SPT (HEP-index area)	3.02	(range 0–15.16)
Diagnosis DBPCFC test		
Positive	137	(76.5%)
Negative	36	(20.1%)
Undecided	6	(3.4%)

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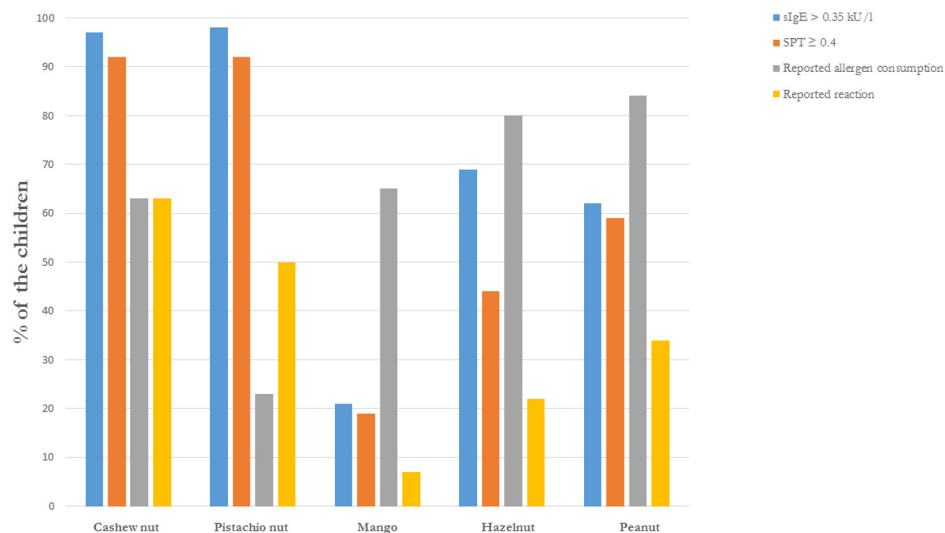


Fig 2. History and sensitisation to tested allergens. This figure shows the history and sensitisation to cashew nut and the history and co-sensitisation to pistachio nut, mango, hazelnut and peanut.

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Sensitisation to cashew nut

173 children had a positive sIgE cashew value (> 0.35 kU/l) and 164 had a positive SPT (diameter ≥ 3 mm and HEP-index area ≥ 0.4). The median sIgE cashew was 3.72 kU/l (range 0– ≥ 100 kU/l). The median HEP-index area of cashew SPT was 3.02 (range 0–15.16).

Food challenge test

A total of 179 children were challenged with cashew nuts and 137 of the challenges were considered positive (76.5%), 36 negative (20.1%) and 6 undecided (3.4%). Most children experienced gastro-intestinal symptoms (nausea, vomiting, stomach pain and diarrhea), followed by oral allergy symptoms, skin symptoms (redness and itchiness), urticaria and angioedema (Table 5). A total of 49 (36%) of the children had an anaphylactic reaction as defined by the EAACI Guidelines for Food Allergy and Anaphylaxis (7). The most commonly observed type of anaphylactic reaction was a combination of skin and gastro-intestinal symptoms (Table 6). A total of 8 children (6%) with an positive reaction were treated with epinephrine. A single dose ($0.15\text{ml} < 25$ kg and $0.30\text{ml} > 25$ kg) of epinephrine was sufficient to treat the child. None of the children had a life-threatening reaction.

Only objective symptoms were seen in 16 children (12%), 47 children reported only subjective symptoms (34%) and 74 (54%) of the children showed both. After the first dose (1 mg cashew protein) 63 (46%) children experienced subjective symptoms and objective symptoms were observed after the first dose in 15 children (11%). Fig 3 shows the threshold distribution curve for objective and subjective symptoms. Anaphylaxis was observed in 23 (17%) children with the start of the reaction to the first dose. Cashew nut allergy could not be confirmed with the DBPCFC test in almost 20% of the children with a positive history.

Co-sensitisation

In cashew nut sIgE-sensitised children, sIgE co-sensitisation to pistachio nuts was observed in 98% of the cases (169/173), to hazelnut in 69% of the cases (119/173), to peanut in 62% of the cases (107/173), to mango in 21% of the cases (37/173) and in 77% of the cases (134/173) to

Table 5. Clinical symptoms during positive DBPCFC tests.

Symptoms	Number of children with positive DBPCFC test	
	Total N = 137 (76.5%)	
Gastro-intestinal	Number	%
Oral allergy	87	64
Nausea, stomach pain, vomiting, diarrhea	98	72
Skin		
Urticaria	29	21
Redness, itchiness	38	28
Angioedema	37	27
Eye symptoms	26	19
Upper airway symptoms	20	15
Lower airway symptoms	9	7
Cardio-vascular symptoms	0	0
Indefinite symptoms		
Change in behavior	18	13
Pallor/ feeling weak	9	7

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tree pollen. SPT cashew co-sensitisation to pistachio nut was seen in 92% (151/164). Lower percentages of SPT co-sensitisations were seen for hazelnut 71/163 (44%), to peanut 97/164 (59%) to mango 31/164 (19%), and to birch pollen 99/163 (61%).

Discussion

Here, we present a diagnostic study in children sensitised to cashew nuts, carried out in three pediatric food allergy expertise centres in the Netherlands. We performed a DBPCFC test with cashew nut in this group, to measure the clinical relevance of sensitisation, to investigate the severity of the allergic reaction to cashew nut and the dosage of cashew nut to which they react.

More than 75% of the children sensitised to cashew nuts showed a clinical response in the DBPCFC test. This percentage is much higher than that observed in a previous other cashew nut study that analysed the clinical features of 42 children with a clinical history suggestive of cashew nut allergy, and a positive skin prick test (SPT) and/or a positive specific sIgE and/or a previous positive food challenge test [10]. Only in 8 (19%) of these children a cashew nut

Table 6. Anaphylactic reactions during positive DBPCFC tests.

Anaphylaxis	Total N = 49/137 (36%)
Skin and respiratory	3 (2%)
Skin and decrease of blood pressure*	0
Skin and gastro-intestinal	40 (30%)
Respiratory and decrease of blood pressure*	0
Respiratory and gastro-intestinal	6 (4%) **
Decrease of blood pressure and gastro-intestinal	0
Decrease of blood pressure > 30% SB	0

*Or associated symptoms such as syncope, incontinence and collapse

** Children had also skin symptoms.

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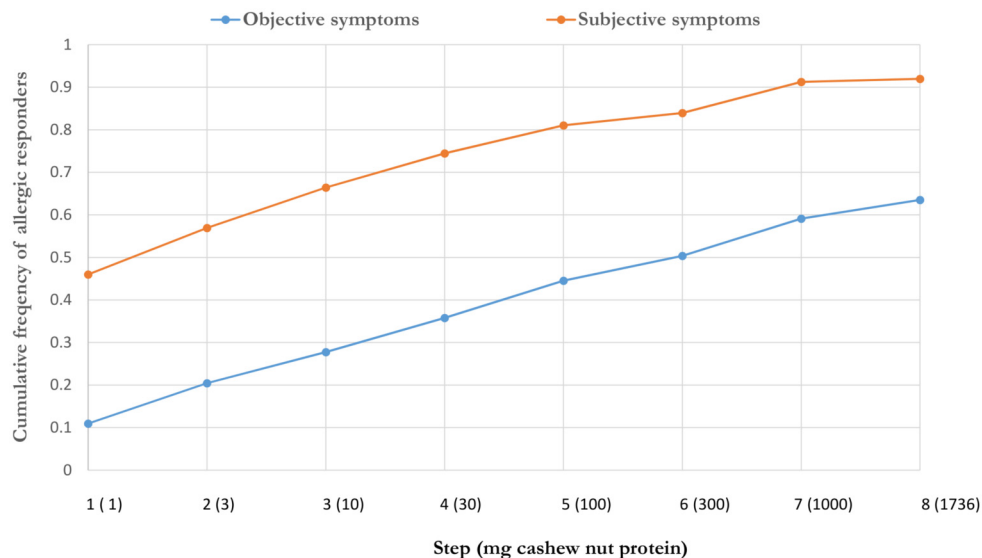


Fig 3. Threshold distribution curve for objective and subjective symptoms in cashew nut allergic children.

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allergy could be confirmed with a positive challenge test. The percentage of a clinically relevant sensitisation to cashew nut in our study was also higher compared to the results of studies with other food allergens such as hazelnut. A study by Flinterman et al. showed that of the 28 children sensitised to hazelnut, a DBPCFC test could only confirm a hazelnut allergy in half of the patients [11]. During the challenge test in our study, most patients experienced gastro-intestinal symptoms, with skin manifestations as the second most prevalent symptom. This is in contrast to other studies on cashew-allergic patients in which skin symptoms were observed more frequently than respiratory and gastro-intestinal symptoms [10]. Sixty-three percent of the children tested reported a history of allergic reactions to cashew nuts in our study. However, a cashew nut allergy could not be confirmed with the DBPCFC test in almost 20% of the children with a positive history. Half of these children experienced the last allergic symptoms to cashew nut between one month and two years ago. A negative oral food challenge test after positive testing and/or positive history is reported between 9% and 38% for peanut allergy [12–16]. Children with a positive history and negative testing may have outgrown their allergy or may have an unreliable history. In addition personal co-factors or differences in exposure may account for this discrepancy. Accidental ingestion of cashew nut is not very likely as they are incorporated in products in an unrecognisable form less often than peanut or hazelnut.

With the first dosage of only 1 mg of cashew protein, 46% of the children experienced subjective and 11% objective symptoms. The food allergy threshold study by Blom et al. with 363 DBPCFC tests showed that the number of patients with any type of symptoms caused by 1 mg cashew nut-, hazelnut-, egg-, milk- and peanut protein varied between 5 and 20% [17]. The number of patients with objective symptoms to 1 mg hazelnut protein was reported in 10% of the patients and was comparable with our results for cashew nut. The number of patients reported with objective symptoms to 1 mg egg-, milk- and peanut protein was lower compared with cashew nut in our study. This confirms the potency and thus the potentially dangerous nature of cashew nut compared to other allergens.

Almost 40% of the children in our study showed anaphylactic reactions and 6% of these children was treated with epinephrine. Anaphylaxis was observed in 17% of the children with

the start of the allergic reaction to the first dose (1 mg cashew nut protein). A previous threshold study demonstrated that approximately 5% of 257 peanut allergic patients reacted to 1 mg peanut protein with severe symptoms [18]. Therefore, our study supports previous observations, showing that minimal amounts of cashew nut are sufficient to cause these severe allergic reactions.

Cashew nut, pistachio nut and mango belong to the *Anacardiaceae* family and are thus botanically related. In line with previous reports, this study shows a high rate of co-sensitisation between cashew nuts and pistachio nuts in SPT and sIgE (respectively 92% and 98%) [19–22]. Almost 50% (21/42) of our pistachio nut sensitized and exposed children reported allergic reactions to pistachio nuts.

Many cashew nut and pistachio nut sensitised children reported no consumption of cashew nut and/or pistachio nut. In most cases, these children were previously advised to eliminate cashew nuts and also pistachio nuts from the diet because of the possibility of cross-reactions. We advise, however, in these cases to perform a DBPCFC test to avoid unnecessary eliminations.

Mango is also botanically related to cashew nut, but our study shows only 19% co-sensitisation with mango in cashew positive SPT children and 21% in sIgE positive children. In this study, almost all children have consumed mango and only 7% reported a history of reactions due to the consumption of mango. Cross-reactivity has not been reported between cashew nut and mango [1] and oral challenges with mango are necessary to confirm the histories we obtained.

Strengths and weaknesses of this study

The strength of this study was the prospective multicentre study design and its size compared to previous studies on cashew nut allergy [1]. This relatively large number of children enabled us to estimate the rate of clinical reactions in sensitised children and to determine the severity of cashew nut allergy. All children underwent a DBPCFC test with cashew nut and thus, the diagnosis was based on this gold standard to establish food allergy, and consequently the clinical relevance of sensitisation and potency of the cashew nut allergen could be accurately examined. A limitation of this study was that we were unable to use the well-accepted scoring system to assess DBPCFC tests as proposed by a PRACTALL consensus group, as this was published after the start of the study [23]. Furthermore, there might be a selection bias in this study because a lot of children or parents of children refused to participate in this study because of e.g. fear for severe allergic reactions during the DBPCFC test. However, there was also a large group of children with an unknown history of cashew nut ingestion and among this group were children with a severe cashew nut allergy. Furthermore, many children experienced an anaphylactic reaction in this study. Therefore, the selection bias seems to be small.

Conclusion

This is the largest prospective clinical study reported in children, sensitised to cashew nut so far. The study demonstrates a high percentage of clinical reactivity to cashew nut in sensitised children. Cashew nuts may cause severe allergic reactions, including anaphylaxis. A minimal amount of cashew nut (1 mg comparable with 1/100 part of a cashew nut) may be sufficient to cause clinical symptoms.

Supporting Information

S1 TREND Checklist.
(PDF)

S1 Database IDEAL-Study.
(PDF)

S1 Study protocol.
(DOC)

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Author Contributions

Conceived and designed the experiments: HJW NWJ. Performed the experiments: JPMV AEJD HG. Analyzed the data: JPMV. Contributed reagents/materials/analysis tools: JPMV AEJD HG NWJ. Wrote the paper: JPMV RGW AEJD HG MR BVB HFJS HJW NWJ.

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TREND Statement Checklist

Paper Section/ Topic	Item No	Descriptor	Reported?	
				Pg #
Title and Abstract				
Title and Abstract	1	• Information on how unit were allocated to interventions		
		• Structured abstract recommended		
		• Information on target population or study sample		
Introduction				
Background	2	• Scientific background and explanation of rationale		
		• Theories used in designing behavioral interventions		
Methods				
Participants	3	• Eligibility criteria for participants, including criteria at different levels in recruitment/sampling plan (e.g., cities, clinics, subjects)		
		• Method of recruitment (e.g., referral, self-selection), including the sampling method if a systematic sampling plan was implemented		
		• Recruitment setting		
		• Settings and locations where the data were collected		
Interventions	4	• Details of the interventions intended for each study condition and how and when they were actually administered, specifically including:		
		○ Content: what was given?		
		○ Delivery method: how was the content given?		
		○ Unit of delivery: how were the subjects grouped during delivery?		
		○ Deliverer: who delivered the intervention?		
		○ Setting: where was the intervention delivered?		
		○ Exposure quantity and duration: how many sessions or episodes or events were intended to be delivered? How long were they intended to last?		
		○ Time span: how long was it intended to take to deliver the intervention to each unit?		
○ Activities to increase compliance or adherence (e.g., incentives)				
Objectives	5	• Specific objectives and hypotheses		
Outcomes	6	• Clearly defined primary and secondary outcome measures		
		• Methods used to collect data and any methods used to enhance the quality of measurements		
		• Information on validated instruments such as psychometric and biometric properties		
Sample Size	7	• How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules		
Assignment Method	8	• Unit of assignment (the unit being assigned to study condition, e.g., individual, group, community)		
		• Method used to assign units to study conditions, including details of any restriction (e.g., blocking, stratification, minimization)		
		• Inclusion of aspects employed to help minimize potential bias induced due to non-randomization (e.g., matching)		

TREND Statement Checklist

Blinding (masking)	9	Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to study condition assignment; if so, statement regarding how the blinding was accomplished and how it was assessed.		
Unit of Analysis	10	Description of the smallest unit that is being analyzed to assess intervention effects (e.g., individual, group, or community)	✓	
		If the unit of analysis differs from the unit of assignment, the analytical method used to account for this (e.g., adjusting the standard error estimates by the design effect or using multilevel analysis)		
Statistical Methods	11	Statistical methods used to compare study groups for primary methods outcome(s), including complex methods of correlated data		
		Statistical methods used for additional analyses, such as a subgroup analyses and adjusted analysis		
		Methods for imputing missing data, if used		
		Statistical software or programs used	✓	
Results				
Participant flow	12	Flow of participants through each stage of the study: enrollment, assignment, allocation, and intervention exposure, follow-up, analysis (a diagram is strongly recommended)	✓	
		- Enrollment: the numbers of participants screened for eligibility, found to be eligible or not eligible, declined to be enrolled, and enrolled in the study	✓	
		- Assignment: the numbers of participants assigned to a study condition	✓	
		- Allocation and intervention exposure: the number of participants assigned to each study condition and the number of participants who received each intervention	✓	
		- Follow-up: the number of participants who completed the follow-up or did not complete the follow-up (i.e., lost to follow-up), by study condition		
		- Analysis: the number of participants included in or excluded from the main analysis, by study condition	✓	
		Description of protocol deviations from study as planned, along with reasons		
Recruitment	13	Dates defining the periods of recruitment and follow-up	✓	
Baseline Data	14	Baseline demographic and clinical characteristics of participants in each study condition	✓	
		Baseline characteristics for each study condition relevant to specific disease prevention research		
		Baseline comparisons of those lost to follow-up and those retained, overall and by study condition		
		Comparison between study population at baseline and target population of interest		
Baseline equivalence	15	Data on study group equivalence at baseline and statistical methods used to control for baseline differences		

TREND Statement Checklist

Numbers analyzed	16	Number of participants (denominator) included in each analysis for each study condition, particularly when the denominators change for different outcomes; statement of the results in absolute numbers when feasible	✓	
		Indication of whether the analysis strategy was “intention to treat” or, if not, description of how non-compliers were treated in the analyses	🗨️	
Outcomes and estimation	17	For each primary and secondary outcome, a summary of results for each estimation study condition, and the estimated effect size and a confidence interval to indicate the precision	✓	
		Inclusion of null and negative findings	🗨️	
		Inclusion of results from testing pre-specified causal pathways through which the intervention was intended to operate, if any	🗨️	
Ancillary analyses	18	Summary of other analyses performed, including subgroup or restricted analyses, indicating which are pre-specified or exploratory	🗨️	
Adverse events	19	Summary of all important adverse events or unintended effects in each study condition (including summary measures, effect size estimates, and confidence intervals)	🗨️	
DISCUSSION				
Interpretation	20	Interpretation of the results, taking into account study hypotheses, sources of potential bias, imprecision of measures, multiplicative analyses, and other limitations or weaknesses of the study	✓	
		Discussion of results taking into account the mechanism by which the intervention was intended to work (causal pathways) or alternative mechanisms or explanations	🗨️	
		Discussion of the success of and barriers to implementing the intervention, fidelity of implementation	✓	
		Discussion of research, programmatic, or policy implications	✓	
Generalizability	21	Generalizability (external validity) of the trial findings, taking into account the study population, the characteristics of the intervention, length of follow-up, incentives, compliance rates, specific sites/settings involved in the study, and other contextual issues	🗨️	
Overall Evidence	22	General interpretation of the results in the context of current evidence and current theory	✓	

From: Des Jarlais, D. C., Lyles, C., Crepaz, N., & the Trend Group (2004). Improving the reporting quality of nonrandomized evaluations of behavioral and public health interventions: The TREND statement. *American Journal of Public Health*, 94, 361-366. For more information, visit: <http://www.cdc.gov/trendstatement/>

Johanna van der Valk

Data IDEAL-study Trial number NTR3572

	Patient_ID	Age	Gender	Hay_Fever	Asthma	Eczema	Atopy	Outcome_challenge	slgE_Cashew	slgE_Pistachio	SPT_Cashew	SPT_Pistachio	Anaphylaxis
1	1110001	11	Female	Yes	No	No	Yes	Negative	15,70	20,60	1,82	1,91	No reaction
2	1110002	9	Female	Yes	No	Yes	Yes	Positive	3,18	5,89	4,60	2,97	Anaphylaxis
3	1110004	5	Male	No	No	No	No	Positive	8,96	13,70	2,36	,50	Mild reaction
4	1110005	8	Female	Yes	No	No	Yes	Positive	23,70	29,20	4,48	1,77	Mild reaction
5	1110006	5	Male	Yes	No	Yes	Yes	Negative	,85	,60	,37	,14	No reaction
6	1110007	13	Male	Yes	No	Yes	Yes	Negative	1,30	1,35	,00	,00	No reaction
7	1110008	11	Female	No	Yes	Yes	Yes	Negative	,00	,00	,49	1,14	No reaction
8	1110009	14	Male	Yes	Yes	Yes	Yes	Positive	21,80	26,90	1,25	2,04	Mild reaction
9	1110010	11	Female	No	No	No	No	Positive	10,90	8,39	1,11	3,14	Anaphylaxis
10	1110011	12	Male	Yes	Yes	Yes	Yes	Positive	11,10	19,90	3,28	4,77	Mild reaction
11	1110012	4	Female	No	No	Yes	Yes	Positive	13,00	18,50	3,98	3,14	Mild reaction
12	1110013	8	Female	No	Yes	Yes	Yes	Positive	2,63	6,63	1,68	1,37	Mild reaction
13	1110014	17	Female	Yes	Yes	Yes	Yes	Negative	5,28	11,50	,59	,00	No reaction
14	1110015	9	Male	Yes	Yes	Yes	Yes	Positive	38,60	37,60	2,11	7,48	Mild reaction
15	1110016	13	Female	Yes	No	No	Yes	Negative	2,16	1,10	,39	,27	No reaction
16	1110019	7	Female	Yes	Yes	Yes	Yes	Negative	,65	,00	,30	,99	No reaction
17	1110020	4	Female	No	No	No	No	Positive	6,03	7,92	3,21	1,44	Anaphylaxis
18	1110021	6	Male	No	Yes	Yes	Yes	Negative	3,76	3,55	4,37	,19	No reaction
19	1110022	16	Female	Yes	Yes	No	Yes	Undetermined	,38	,59	3,28	5,68	No reaction
20	1110023	14	Male	Yes	No	Yes	Yes	Positive	1,25	2,33	3,38	6,04	Anaphylaxis
21	1110024	9	Female	No	Yes	No	Yes	Negative	1,17	2,36	3,19	,97	No reaction
22	1110025	6	Female	No	No	No	No	Positive	1,79	4,61	5,89	1,35	Mild reaction
23	1110027	8	Male	No	Yes	Yes	Yes	Positive	27,00	79,50	1,36	,78	Mild reaction
24	1110028	3	Female	No	No	Yes	Yes	Positive	100,00	100,00	5,54	9,39	Anaphylaxis
25	1110029	11	Male	Yes	Yes	Yes	Yes	Positive	7,39	4,37	5,13	8,29	Mild reaction
26	1110030	10	Female	No	No	No	No	Positive	44,70	47,10	9,33	9,19	Anaphylaxis
27	1110031	9	Male	Yes	Yes	No	Yes	Positive	13,10	29,80	2,16	2,75	Anaphylaxis
28	1110032	6	Female	No	No	Yes	Yes	Positive	10,80	16,20	,65	2,31	Anaphylaxis
29	1110033	2	Male	No	No	No	No	Negative	,00	,00	14,25	,00	No reaction
30	1110034	4	Male	Yes	No	Yes	Yes	Positive	1,38	3,81	3,14	4,92	Mild reaction
31	1110035	5	Female	No	No	No	No	Positive	3,06	5,16	2,28	6,29	Anaphylaxis
32	1110037	13	Female	No	No	No	No	Positive	6,42	10,90	2,49	1,95	Mild reaction
33	1110038	9	Male	Yes	Yes	Yes	Yes	Positive	,46	,27	3,57	,91	Anaphylaxis
34	1110039	3	Male	No	No	Yes	Yes	Positive	47,40	51,40	6,01	12,30	Anaphylaxis
35	1110040	2	Male	No	Yes	Yes	Yes	Negative	5,11	4,51	,53	,71	No reaction
36	1110041	7	Male	Yes	No	Yes	Yes	Negative	,63	,41	,50	2,59	No reaction
37	1110042	2	Male	No	No	No	No	Negative	2,14	2,50	1,32	,84	No reaction
38	1110043	15	Female	Yes	No	Yes	Yes	Negative	,63	,97	,11	,13	No reaction
39	1110045	9	Male	Yes	No	Yes	Yes	Positive	1,02	1,74	,89	1,13	Anaphylaxis
40	1110046	17	Female	No	No	No	No	Positive	,57	,90	3,15	2,64	Anaphylaxis
41	1110048	15	Male	No	No	Yes	Yes	Positive	,41	,37	,90	,54	Mild reaction
42	1110049	5	Female	No	No	No	No	Positive	7,28	11,50	3,17	1,91	Mild reaction
43	1110052	13	Male	Yes	Yes	No	Yes	Positive	3,72	8,28	2,35	8,85	Mild reaction
44	1110053	3	Male	No	Yes	Yes	Yes	Positive	51,90	61,80	5,54	2,19	Mild reaction
45	1110054	9	Male	Yes	Yes	No	Yes	Positive	22,10	36,30	4,53	4,23	Anaphylaxis
46	1110055	6	Male	Yes	Yes	Yes	Yes	Positive	10,80	16,20	1,48	2,43	Anaphylaxis
47	1110056	4	Male	No	Yes	Yes	Yes	Positive	12,70	10,70	5,48	2,98	Anaphylaxis
48	1110057	6	Female	No	No	No	No	Positive	9,00	7,17	2,61	3,97	Mild reaction
49	1110058	4	Female	Yes	Yes	Yes	Yes	Positive	48,70	92,90	2,65	2,63	Anaphylaxis
50	1110059	4	Female	No	No	Yes	Yes	Positive	3,10	3,06	5,43	3,07	Mild reaction
51	1110060	13	Female	No	No	No	No	Positive	9,11	7,98	3,31	2,73	Mild reaction
52	1110061	10	Female	Yes	Yes	Yes	Yes	Positive	1,01	1,99	1,41	2,02	Mild reaction
53	1110062	6	Male	No	No	Yes	Yes	Positive	30,20	44,50	9,41	5,39	Mild reaction
54	1110063	8	Male	No	Yes	No	Yes	Positive	2,57	1,54	2,41	2,25	Mild reaction
55	1110064	12	Male	No	No	No	No	Positive	7,65	7,25	1,95	1,66	Mild reaction
56	1110065	7	Male	No	No	No	No	Positive	,68	,91	5,36	2,80	Anaphylaxis
57	1110066	4	Female	.	No	Yes	Yes	Positive	2,00	3,02	1,86	1,89	Anaphylaxis
58	1110067	5	Male	No	No	Yes	Yes	Positive	1,04	,83	,58	,21	Mild reaction
59	1110069	5	Female	No	No	No	No	Positive	2,76	2,13	4,95	5,00	Anaphylaxis
60	1110070	14	Male	No	No	Yes	Yes	Positive	1,94	3,93	3,87	7,67	Mild reaction
61	1110071	16	Male	No	No	No	No	Positive	7,08	14,40	3,34	2,42	Mild reaction
62	1110072	2	Male	No	No	No	No	Positive	3,23	4,80	3,93	5,58	Mild reaction
63	1110073	3	Male	No	No	Yes	Yes	Positive	1,69	1,03	,39	,71	Mild reaction
64	1110075	3	Male	No	No	No	No	Negative	,48	,21	,46	,26	No reaction
65	1110076	4	Female	Yes	No	No	Yes	Positive	,73	,71	5,80	2,26	Anaphylaxis
66	1110079	10	Male	No	No	No	No	Positive	100,00	82,00	2,50	2,05	Mild reaction
67	2220001	8	Male	No	Yes	No	Yes	Positive	4,49	8,79	4,34	3,74	Mild reaction
68	2220002	8	Male	Yes	Yes	Yes	Yes	Undetermined	1,62	3,49	2,31	7,18	No reaction
69	2220003	6	Male	No	No	No	No	Negative	1,57	1,46	,82	2,08	No reaction

	Patient_ID	Age	Gender	Hay_Fever	Asthma	Eczema	Atopy	Outcome_challenge	slgE_Cashew	slgE_Pistachio	SPT_Cashew	SPT_Pistachio	Anaphylaxis
70	2220004	11	Female	Yes	No	No	Yes	Positive	13,20	26,40	5,02	8,72	Anaphylaxis
71	2220005	6	Male	No	No	Yes	Yes	Undetermined	,83	,98	3,37	2,40	No reaction
72	2220006	14	Male	No	Yes	Yes	Yes	Positive	100,00	100,00	9,66	9,37	Mild reaction
73	2220007	13	Male	No	Yes	No	Yes	Positive	1,77	3,98	7,48	5,13	Mild reaction
74	2220008	10	Female	Yes	Yes	No	Yes	Positive	4,81	9,33	1,16	2,70	Mild reaction
75	2220009	12	Male	Yes	No	No	Yes	Positive	37,70	50,10	1,37	1,96	Mild reaction
76	2220010	7	Male	Yes	Yes	Yes	Yes	Positive	3,53	5,12	3,38	2,80	Anaphylaxis
77	2220011	10	Male	No	No	Yes	Yes	Positive	1,08	1,08	4,80	2,88	Mild reaction
78	2220012	12	Male	Yes	Yes	Yes	Yes	Positive	100,00	100,00	1,16	2,52	Mild reaction
79	2220013	8	Female	Yes	No	Yes	Yes	Positive	,39	,93	2,68	1,65	Mild reaction
80	2220014	14	Male	No	No	No	No	Negative	17,80	12,10	2,73	2,40	No reaction
81	2220015	7	Male	No	No	No	No	Positive	6,55	5,89	3,50	5,50	Mild reaction
82	2220016	8	Male	Yes	Yes	No	Yes	Positive	41,20	44,80	5,61	5,89	Anaphylaxis
83	2220017	4	Female	No	No	Yes	Yes	Negative	2,70	2,42	1,24	,80	No reaction
84	2220018	6	Male	Yes	No	No	Yes	Positive	32,00	21,30	3,57	3,97	Mild reaction
85	2220019	8	Male	No	Yes	No	Yes	Positive	4,62	5,72	6,51	8,80	Mild reaction
86	2220020	12	Male	No	No	No	No	Negative	10,50	7,29	,00	1,47	No reaction
87	2220021	8	Female	No	Yes	No	Yes	Positive	8,75	9,29	14,36	12,00	Anaphylaxis
88	2220022	7	Female	Yes	No	Yes	Yes	Positive	1,56	2,41	8,76	6,26	Mild reaction
89	2220023	10	Male	Yes	Yes	Yes	Yes	Positive	21,20	30,10	2,94	3,77	Mild reaction
90	2220024	14	Male	No	No	No	No	Positive	15,20	11,70	6,85	9,35	Anaphylaxis
91	2220025	9	Male	No	Yes	No	Yes	Positive	82,00	100,00	4,25	3,91	Mild reaction
92	2220026	15	Male	Yes	Yes	Yes	Yes	Positive	3,58	5,26	4,76	1,64	Mild reaction
93	2220027	10	Male	Yes	Yes	No	Yes	Negative	5,48	5,05	1,47	1,37	No reaction
94	2220028	6	Female	Yes	No	No	Yes	Positive	17,50	25,80	13,00	7,98	Mild reaction
95	2220029	6	Female	No	No	No	No	Positive	9,34	10,00	3,29	5,23	Mild reaction
96	2220033	3	Male	Yes	No	No	Yes	Negative	3,01	3,66	,00	,77	No reaction
97	2220034	14	Female	Yes	Yes	Yes	Yes	Positive	8,29	13,40	10,70	12,19	Mild reaction
98	2220035	7	Male	No	No	No	No	Negative	,97	1,16	3,38	,00	No reaction
99	2220036	10	Female	Yes	Yes	Yes	Yes	Undetermined	4,33	10,20	2,70	3,93	No reaction
100	2220037	11	Male	Yes	No	No	Yes	Negative	2,97	7,51	4,22	2,66	No reaction
101	2220038	12	Female	Yes	No	Yes	Yes	Positive	16,90	19,60	1,90	6,77	Mild reaction
102	2220039	15	Male	No	No	No	No	Positive	4,76	8,60	4,68	5,47	Mild reaction
103	2220040	6	Male	No	No	No	No	Negative	,97	,97	,00	,71	No reaction
104	2220041	10	Female	No	No	No	No	Positive	,13	1,02	1,38	1,08	Anaphylaxis
105	2220042	9	Female	Yes	Yes	Yes	Yes	Positive	2,59	1,80	5,75	4,41	Anaphylaxis
106	2220043	10	Male	No	No	Yes	Yes	Positive	8,17	9,01	6,02	4,39	Mild reaction
107	2220044	6	Male	No	No	Yes	Yes	Negative	2,05	2,25	99,00	99,00	No reaction
108	2220045	13	Male	Yes	No	Yes	Yes	Positive	8,54	17,10	5,95	6,45	Mild reaction
109	2220048	7	Male	Yes	Yes	Yes	Yes	Positive	17,30	15,10	12,92	7,62	Mild reaction
110	2220052	7	Male	Yes	Yes	Yes	Yes	Negative	,89	,85	,00	,82	No reaction
111	2220053	5	Male	Yes	No	No	Yes	Positive	50,70	52,80	4,46	4,56	Anaphylaxis
112	2220055	5	Male	No	No	No	No	Positive	2,22	4,82	5,52	5,11	Anaphylaxis
113	2220057	6	Male	Yes	No	No	Yes	Positive	3,89	10,80	1,31	2,36	Mild reaction
114	3330001	13	Male	Yes	No	No	Yes	Positive	,92	1,83	9,60	10,73	Mild reaction
115	3330002	12	Male	Yes	No	Yes	Yes	Positive	3,53	7,92	2,90	3,97	Mild reaction
116	3330003	12	Male	Yes	No	No	Yes	Negative	,78	1,28	1,32	2,36	No reaction
117	3330004	6	Male	Yes	No	Yes	Yes	Negative	,51	,31	,37	,27	No reaction
118	3330005	6	Female	Yes	Yes	Yes	Yes	Positive	,59	,91	1,88	1,15	Anaphylaxis
119	3330006	15	Male	Yes	No	No	Yes	Negative	1,13	2,23	3,22	3,59	No reaction
120	3330007	14	Female	Yes	Yes	No	Yes	Negative	,52	,40	,28	,00	No reaction
121	3330008	4	Female	No	Yes	No	Yes	Positive	58,30	45,00	1,74	1,53	Mild reaction
122	3330009	9	Male	Yes	No	No	Yes	Positive	,83	1,36	3,53	2,40	Mild reaction
123	3330010	16	Male	Yes	No	No	Yes	Positive	9,90	2,02	3,46	2,61	Mild reaction
124	3330011	15	Male	No	No	Yes	Yes	Positive	100,00	100,00	5,64	9,91	Mild reaction
125	3330012	8	Male	Yes	No	Yes	Yes	Undetermined	11,80	16,90	6,27	6,39	No reaction
126	3330013	7	Female	Yes	No	No	Yes	Positive	,00	,00	,54	,42	Mild reaction
127	3330014	9	Male	Yes	No	Yes	Yes	Positive	10,00	14,10	1,73	,72	Anaphylaxis
128	3330015	4	Female	No	No	No	No	Positive	32,00	22,40	,82	4,22	Mild reaction
129	3330016	5	Male	Yes	No	Yes	Yes	Positive	1,50	2,02	6,75	7,42	Mild reaction
130	3330017	13	Male	No	No	No	No	Positive	3,81	5,15	5,45	3,27	Mild reaction
131	3330018	10	Male	No	No	No	No	Positive	100,00	100,00	6,01	10,93	Anaphylaxis
132	3330019	10	Male	Yes	No	No	Yes	Negative	1,52	2,26	1,78	,79	No reaction
133	3330020	10	Female	Yes	No	No	Yes	Positive	,43	1,14	,93	,94	Mild reaction
134	3330021	12	Male	No	No	No	No	Positive	1,13	1,17	2,09	2,44	Mild reaction
135	3330023	16	Male	Yes	No	No	Yes	Positive	3,31	7,35	6,36	7,46	Anaphylaxis
136	3330024	10	Female	Yes	No	No	Yes	Positive	,90	3,66	10,75	4,72	Anaphylaxis
137	3330025	10	Male	No	Yes	No	Yes	Positive	48,10	41,90	3,04	3,70	Mild reaction
138	3330027	7	Male	Yes	No	No	Yes	Positive	32,60	30,90	5,33	5,44	Mild reaction

	Patient_ID	Age	Gender	Hay_Fever	Asthma	Eczema	Atopy	Outcome_challenge	slgE_Cashew	slgE_Pistachio	SPT_Cashew	SPT_Pistachio	Anaphylaxis
139	3330028	8	Male	Yes	No	No	Yes	Negative	,72	,60	,43	,00	No reaction
140	3330029	15	Male	Yes	No	Yes	Yes	Positive	,25	,22	,67	,08	Mild reaction
141	3330030	14	Female	No	Yes	No	Yes	Positive	4,66	10,30	5,46	7,86	Mild reaction
142	3330031	7	Female	No	No	No	No	Positive	3,28	7,76	15,16	3,24	Anaphylaxis
143	3330032	5	Female	No	No	Yes	Yes	Positive	2,33	2,94	,77	,15	Mild reaction
144	3330033	15	Female	Yes	No	No	Yes	Positive	32,90	47,20	5,29	,00	Anaphylaxis
145	3330034	2	Male	No	Yes	No	Yes	Positive	2,12	1,57	3,09	1,36	Mild reaction
146	3330035	10	Female	No	No	No	No	Negative	5,26	2,75	,87	,91	No reaction
147	3330036	10	Male	No	No	No	No	Negative	,43	,46	,30	,00	No reaction
148	3330037	15	Female	No	No	No	No	Positive	1,08	1,03	1,11	2,59	Anaphylaxis
149	3330038	11	Female	Yes	No	No	Yes	Positive	,47	,84	1,22	1,57	Mild reaction
150	3330039	3	Female	Yes	No	Yes	Yes	Positive	48,40	42,40	4,53	7,54	Mild reaction
151	3330040	7	Male	No	No	No	No	Positive	2,35	3,82	4,95	1,93	Mild reaction
152	3330041	9	Female	Yes	No	No	Yes	Undetermined	10,10	16,00	1,62	6,76	No reaction
153	3330042	5	Female	Yes	No	No	Yes	Positive	18,40	20,10	2,21	,00	Anaphylaxis
154	3330043	11	Female	No	Yes	Yes	Yes	Positive	22,50	30,20	5,49	4,66	Mild reaction
155	3330044	11	Male	Yes	No	No	Yes	Positive	2,33	2,67	3,53	,00	Anaphylaxis
156	3330045	10	Male	Yes	No	No	Yes	Positive	12,10	42,50	1,46	2,83	Anaphylaxis
157	3330047	11	Female	Yes	No	Yes	Yes	Positive	5,13	10,90	2,29	1,55	Mild reaction
158	3330048	9	Female	Yes	No	Yes	Yes	Positive	5,04	3,85	1,78	4,14	Mild reaction
159	3330049	7	Male	No	No	No	No	Positive	2,17	3,13	4,77	2,07	Anaphylaxis
160	3330050	4	Female	Yes	Yes	No	Yes	Positive	2,55	5,18	3,73	3,27	Anaphylaxis
161	3330051	11	Male	Yes	Yes	No	Yes	Positive	11,20	8,96	5,10	9,19	Anaphylaxis
162	3330052	10	Female	Yes	Yes	No	Yes	Positive	49,00	34,20	1,16	,99	Mild reaction
163	3330053	11	Male	No	No	No	No	Negative	,21	,46	2,92	1,16	No reaction
164	3330054	4	Female	Yes	No	Yes	Yes	Positive	17,70	10,50	3,86	5,22	Mild reaction
165	3330055	5	Female	Yes	No	No	Yes	Positive	21,10	37,30	,77	3,77	Anaphylaxis
166	3330056	14	Male	Yes	No	No	Yes	Positive	1,44	1,75	3,86	3,75	Mild reaction
167	3330057	13	Male	Yes	Yes	No	Yes	Positive	11,90	23,20	1,47	1,13	Anaphylaxis
168	3330058	8	Female	Yes	No	No	Yes	Positive	1,41	,65	2,54	,72	Mild reaction
169	3330059	6	Male	No	No	No	No	Positive	3,22	9,20	3,00	3,50	Mild reaction
170	3330060	12	Male	Yes	Yes	No	Yes	Positive	,86	1,70	2,43	1,07	Mild reaction
171	3330061	16	Female	No	No	No	No	Positive	1,33	1,67	2,68	2,04	Anaphylaxis
172	3330062	6	Female	Yes	Yes	No	Yes	Positive	37,40	68,30	3,37	7,23	Anaphylaxis
173	3330063	9	Female	Yes	No	No	Yes	Positive	7,89	18,50	3,88	1,28	Mild reaction
174	3330064	12	Male	No	No	No	No	Positive	2,02	2,44	3,98	1,81	Mild reaction
175	3330065	2	Female	Yes	No	No	Yes	Positive	1,71	,74	1,49	,00	Mild reaction
176	3330066	7	Male	Yes	No	No	Yes	Positive	87,90	100,00	2,33	2,92	Mild reaction
177	3330067	2	Male	Yes	No	No	Yes	Positive	5,83	14,50	4,74	4,47	Mild reaction
178	3330068	11	Female	No	No	No	No	Positive	,42	,93	1,70	1,47	Anaphylaxis
179	3330070	14	Male	No	No	No	No	Negative	2,76	2,17	,15	,49	No reaction

Clinical Trial Protocol

Improvement of Diagnostic mEthods for ALlergy assessment. Cashew allergy in children as a showcase (IDEAL study)

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Improvement of Diagnostic mEthods for ALlergy assessment. Cashew allergy in children as a showcase.

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ACQ	Asthma Control Questionnaire
BAT	Basophil Activation Test
CCMO	Centrale Commissie Mensgebonden Onderzoek (Dutch competent authority)
CH	Case History
CRD	Component Resolved Diagnosis
DBPCFC	Double Blind Placebo Controlled Food Challenge
EAACI	European Academy of Allergy and Clinical Immunology
EC	Ethics Committee
ELISA	Enzyme Linked Immuno solvent Assay
FA	Food Allergy
FAQLQ-TF	Food Allergy Quality of Life- Questionnaire- Teenager Form
FAQLQ-PF	Food Allergy Quality of Life- Questionnaire- Parent Form
FAQLQ-CF	Food Allergy Quality of Life- Questionnaire- Child Form
FcεR	Fcε-receptor
HEK	Human Embryonic Kidney 293 cells
HEP	Histamine Equivalent Prick
IgE	Immunoglobuline E
LOAEL	Lowest Observed Allergic Event level
ml	Milliliter
MRA	Mediator Release Assays
NOAEL	No Observed Allergic Event Level.
OFC	Oral Food Challenge
PMNs	Polymorphonuclear cell family
RBL	Rat Basophil Leukemia
SPT	Skin Prick Test

Summary

Rationale: There is an urgent need for improvement of diagnostic procedures in food allergy (FA) testing, as insufficient sensitivity and false-positivity and false-negativity hamper the currently available tests. To optimise the diagnosis of FA this study aims to improve two existing types of Mediator Release Assays (MRAs), the basophil activation test (BAT) and the rat basophil leukemia test (RBL-test). To achieve improvement of these MRAs, cashew nut allergy will serve as a model to test and validate the assays. This model is chosen because there are strong indications that cashew allergy prevalence is rapidly increasing, and is particularly affecting children.

Objective: The hypothesis pertinent to this proposal is that an accurate history, conventional markers of sensitization, IgE antibodies in the serum and positive SPT, and more advanced techniques (MRAs and component resolved diagnosis (CRD)) might predict clinical allergy to cashew nuts. Thereby, the aim of this study is to improve diagnostic procedures for food allergies.

Study design:

Interventional study. Non-drug study. Double-blind randomized food challenge. Duration per patient approx. 8 weeks. After 6 months the FAQLQ questionnaire will be completed once more.

Study population:

The aim is to recruit at least 200 participating children (age 2- 17 (inclusive) years) sensitized to cashew nut. Three clinics will include patients and they will carry out the clinical part of the study: Erasmus MC Rotterdam, location: Kinderhaven, Havenziekenhuis; Reinier de Graaf Gasthuis Delft, University Medical Center Groningen.

Study intervention (if applicable): 1 additional skin prick test, 1 additional blood sampling 12 ml, completion of questionnaire. NB: the food challenge tests are part of the current regular diagnostic procedures.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

- The volunteers will visit the hospital for 4 times, 3 questionnaires will be filled in by either the parents (age 2-6 years) or the children (age 6-12), skin prick tests will be performed (total of 8) and 2 blood samples will be drawn. Furthermore the child will undergo a Double Blind Placebo Controlled Food Challenge (DBPCFC) on 2 different days.
- The burden and risks will be minimal due to extensive experience with DBPCFCs. Nevertheless, we are aware, that the outcome of the DBPCFC is unpredictable and reactions may occur in the patients. Therefore, the DBPCFC will take place at the children's department, at the medium care unit, provided with all necessary rescue medication.
- The study can only be done in this group of children because we find high numbers of sensitization to cashew nut allergens in very young children and the DBPCFC is the only diagnostic tool we have available till now.
- The above described method is the normal diagnostic protocol (golden standard) we use to prove or reject an IgE mediated food allergy in children and adults. The only extra tests we perform for the study is are extra questionnaires, 2 extra blood samples for the Mediated Release Assay's (MRA's) and 6 extra skin prick tests to measure cross- reactivity with other foods.

Possible benefit:

The possible benefit for the patient is a clearer diagnosis concerning their possible IgE mediated cashew nut allergy and thus better dietary and lifestyle counselling.

1 Introduction and rationale

1.1. Current status of allergy diagnosis

In food allergy (FA) diagnosis, the double blind placebo controlled food challenge test (DBPCFC) is considered as the 'golden standard'. The 'simpler' test types, such as *in vitro* tests based on IgE-binding (ELISA, CAP, RAST) often lead to false-positive or false-negative outcomes and do not correlate well with clinical observations, which leads to a limited reliability of their outcomes for e.g. clinical evaluations. Therefore, DBPCFC is usually not only required for initial diagnosis, but also for follow-up diagnostic procedures. DBPCFC is, however, very costly, time consuming, labour intensive, burdensome to patients and not without danger due to the chance of anaphylactic reactions. In addition, it gives no information on the responsiveness of patients to separate allergenic with different allergenic characteristics proteins in food matrices, whereas, particularly in many type of food allergens, a food contains multiple allergenic proteins.

So far, a careful side-by-side comparison of patient samples in *in vitro* assays, skin prick tests and mediator release assays has not been performed with newly emerging and potentially serious allergies, like cashew nut allergy.

Cost-effective, less-burdensome diagnostic procedures with increased relevance for clinical practice can fill an existing gap in current allergy diagnosis, and gain a considerable share in the diagnostic market for allergy.

1.2. Cashew nut allergy as a model to evaluate diagnostic procedures

In 1998, investigators found that 40% of 142 French peanut-allergic children were sensitized to cashew nut [1], whose intake can cause anaphylactic reactions in sensitized subjects [2-3]. At the Royal Children's Hospital in Melbourne, in a five year period 117 anaphylactic reactions occurred in children, where peanut (18%) and cashew nut (13%) were the most common cause of anaphylaxis.[4]

There are several explanations for the occurrence of an allergic reaction at the first intake of cashew nut. First, cashew nut allergies can develop 'in utero' or during breast-feeding when the mother is eating cashew nuts. Secondly, cashew nut, pistachio and mango (*Anacardium* family) are botanically related, which causes cross-reactions. Several companies incorporate mango in food products for children from 6 months old. Finally, allergy to hazelnut might lead to allergic reactions to cashew nuts in some patients. Investigators [5] described 55% homology of allergens from hazelnut and cashew nut. It is known from literature that allergic reactions caused by cross-reactivity are less severe than reactions caused by primary sensitization [6]. It remains unclear if this is also true for sensitization to cashew nut, as most studies on cashew allergy report severe i.e. systemic reactions [3, 7].

1.3. Preliminary results

In Kinderhaven hospital, a multidisciplinary outpatient clinic for allergic children (department of the Erasmus MC), a retrospective study was performed in children with possible cashew nut allergy. In an 18 month survey we identified and tested 94 children with a suspected cashew nut allergy (mean age 7.1 years, range 15 months – 17 years) out of 290 subjects with a possible FA. Of these 94 children 69 (73%) were sensitized to cashew nut. Examining the case histories, we found a striking high number of "never eaten cashew nut" and "unknown" (n = 46; 65%) cases of cashew nut allergy. In 6/69 (9%) children, anaphylactic reactions occurred after eating cashew nuts. Furthermore, 17/69 (24%) reported oral allergy symptoms (OAS) and/or angio-edema (AO). In 26/ 69 cases with positive sensitization to cashew nut, the parents reported possible symptoms caused by pistachio nuts. Skin test with pistachio nut were positive in 24/ 26 of these cases. Co-sensitization was seen with hazelnut: 80% and peanut: 65%. This indicates the presence of possible cross reactivity between different nut allergens. Only 4 children were mono-sensitized to cashew nut.

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In summary, from the literature and preliminary results it can be seen that sensitivity to cashew nuts is highly prevalent, representing a mixed population in terms of disease severity. Cross-reactivity can be a severity modifying factor. The preliminary results indicate that these patients are frequently seen. With this background cashew nut allergy provides an attractive model to validate diagnostic tools.

2. Optimization of the diagnosis of cashew nut allergy

2.1. Components of diagnostic procedures

Mediator Release Assays (MRAs) may circumvent, in particular in the setting of food allergies, the drawbacks of on the one hand IgE-based assays ('overly' simple leading to an unacceptably high level of erroneous outcomes), Skin Prick Test (SPT) (confounding cross-reactions, instability of allergens, thus erroneous outcomes) and on the other hand DBPCFC (expensive, burdensome, no resolution at level of allergenic protein, potentially not without risk):

- In MRAs, allergen cross-linking of IgE (allergen binds to two distinctive IgE's) that is bound to effector cells is required to achieve a positive response. This strongly enhances relevance of such tests in comparison to tests that are based on a single IgE-binding only (as seen in ELISA), as only biologically functional IgE and allergens are detected, and thus potentially discriminates between patients that are only sensitized and patients that have a clinically relevant allergy
- Only a, relatively small, blood sample from test persons is required, thereby avoiding ingestion of food in DBPCFC and the associated risks of strong reactions. Interference of components from skin, as may occur in SPT, is avoided
- This test can provide physiologically relevant information to the level of individual allergenic proteins. This information is relevant for the clinical interpretations, but can also serve to develop physiologically relevant methods to evaluate the allergenic potential of nutritional products, thus creating safer food products for consumers
- The MRA can be utilized as a safe and easy method to establish threshold levels for clinical relevant allergen content, needed to define the "may contain labelling" directive of food allergens in processed nutrition, incurring no risk for patients.
- The MRA-assays are much more cost-effective than DBPCFCs.

Basically, two types of MRAs exist, both of which will be included in this research program:

The basophil activation test (BAT) is performed with patient's own IgE-loaded basophilic cells, which are subsequently exposed to functional, cross-linking-capable, allergen preparations. The rat basophil leukemia test (RBL) makes use of a (rat-derived) cell line that expresses the human Fcε-receptor (FcεR). These cells can be 'loaded' with IgE from a patient's serum, an operation that can be done in a stage at which a certain number of sera (that can stably be stored for long intervals) can be analysed simultaneously. RBL-cells can be used as a 'vehicle' to analyse any type of food allergy, provided suitable allergen preparations are available.

The BAT is hampered by operational logistics being a cost-increasing factor. The RBL-test has limited sensitivity. Both aspects will be addressed in this project.

Currently, it is not known to what extent the outcome of an oral challenge with cashew nuts can be predicted by less invasive procedures. This project will present an unique opportunity to investigate the potential of less invasive allergy diagnostic tests, in particular cell-based assays, and validate these tests against the clinical background. A new approach of diagnosing food allergy is the use of component resolved diagnosis (CRD), which comprises the determination of IgE by ELISA that recognises isolated allergenic proteins, which are either produced by recombinant expression of allergen-encoding cDNAs or by purification from natural allergenic foods.

However, such CRD can also be performed, and be more relevant, with MRAs such as rat basophil leukemia cells (RBL) [8] or basophile activation tests (BAT) [9-10] which analyse the cross-linking ability, and thus the functionality, of allergens. This circumvents potential false-positive outcomes which may result from IgE-binding to single epitopes without the necessity to provoke cross-linking to create a response in the assay.

From a clinical point of view but also to evaluate the importance of MRAs, there is a need to estimate how accurately different components of the FA diagnosis (i.e. history, conventional markers of sensitization and more advanced techniques (cell-assays and CRD) in [11] can predict clinical allergy to cashew nuts as measured by DBPCFC.

Assuming that MRAs will be important in the diagnosis of FA, the question should be answered to what extent an accurate diagnosis of FA decreases the burden of disease. For that reason food related

quality of life will be measured with instruments developed by the UMCG [12]. If a definite diagnosis has a beneficial impact on quality of life, this observation will underwrite the importance of using non-invasive diagnostic procedures (i.e. MRAs).

2.2. Cross reactivity between cashew nuts and other allergens

As primary and secondary sensitizing allergens may have a differential effect on the severity of allergic reactions, further research to cross reactive cashew nut allergens is warranted.

To date 3 major allergenic proteins have been identified in cashew nut and these are available as recombinant proteins. [13-14] Ana o 1, one of these allergens, is a vicilin (a 7S-seed storage protein). Previous research failed to show a significant cross-reactivity of Ana o 1 with the 7S-storage protein in peanut (Ara h 1), in hazelnut (Cor a 11), and walnut (Jug r 2) [15]. In addition Ana o 2 (a 11S globulin) and Ana o 3 (2S Albumin) have been identified, which are seed storage proteins as well. Recently, several 11S globulins with reactivity to patient IgE were found [16-17].

Although the major allergenic proteins are identified, cross reactivity between recombinant allergens from cashew nut and the allergens from pistachio nut and mango (*Anacardium* family) is hardly examined [18]. Pallawid Tawde et al. [5] found that the identified N-terminal amino acid sequences of cashew 11S globulin (*Ana o 2*) possessed significant homology (45-58% identity and 63-74% similarity) to the 11S globulins from diverse tree nuts, seeds, and legumes. These findings contribute to the hypothesis that hazelnut might be the primary sensitizer in some cases. It is known from literature that allergic reactions caused by cross-reactivity are less severe than reactions caused by primary sensitization.

MRAs will offer improved possibilities to analyse the molecular mechanisms underlying these cross-reactive phenomena and their physiological relevance. This will contribute to improved clinical diagnosis and improved patient information, without the need to involve clinical test procedures for this.

3. Study objectives

3.1. Primary objectives

The hypothesis pertinent to this proposal is that an accurate history, conventional markers of sensitization, IgE antibodies in the serum and positive SPT, and more advanced techniques (MRAs and component resolved diagnosis, CRD) might predict clinical allergy to cashew nuts. However, the diagnostic value of these predictors has to be determined by DBPCFCs, which is the gold standard for the diagnosis of food allergy. Thereby, the aim of this study is to improve diagnostic procedures for food allergies. The following research lines are therefore implicated:

- Optimization of the diagnosis of cashew nut allergy; development of new diagnostic tools
Improved Mediator Release Assays (MRAs) will be developed e.g. basophil activation test (BAT) and rat basophil leukaemia test (RBL). The researchers aim to optimize the diagnosis of nut allergy, by using the different components: 1) history, 2) conventional markers of sensitization, 3) MRA's and 4) CRD i.e. IgE-levels of Ana o 1, Ana o 2 and Ana o 3, to finally predict the outcome of the DBPCFC. Measure the effect of improved diagnostic procedures on quality of life.
- Develop a diagnostic model with new Mediator Release assays to predict the outcome of the DBPCFC
Predictive modelling and regression techniques will be used to accomplish and identify the optimal set of diagnostic procedures for the diagnosis of cashew nut allergy. In fact, these models might be useful for other nuts as well. In that way it will accomplish a breakthrough in the diagnosis of food allergy.

3.2. Secondary objective

- Cross reactivity between cashew nuts and other allergens
The question will be addressed as to whether cashew nut is the primary sensitizing agent in cashew nut allergy. For that purpose a profile of co-sensitizing allergens (e.g. peanut, other nuts, and mango) needs to be accomplished. Because most reported cashew allergies are severe, the mechanisms of cashew nut and cross reactivity will be studied in more detail.

4. Investigational plan

4.1. Study design

Interventional study. Non-drug study. Double-blind randomized food challenge. Duration per patient approx. 8 weeks. After 6 months the FAQLQ questionnaire will be completed once more.

The aim is to recruit at least 200 participating children (age 2- 17) sensitized to cashew nut. In this study this number should be sufficient to measure prevalence, investigate cross-reactivity, develop and validate MRAs, and to build models identifying a set of predictors up to 10 for the outcome of DBPCFC's. This assumes, based on our elaborative experience in similar studies, that 1600 children are needed to obtain 200 sensitized children undergoing a DBPCFC (see flow chart, Attachment 1). Based on recruitment from 3 medical centres this should be realistically feasible in 36 months.

Questionnaires need to be filled in by the participants in cooperation with their parents, concerning a possible food allergy, and actual diet. A blood sample will be taken. Additional skin prick tests will be performed with birch pollen, hazelnut, pistachio nuts, peanuts, and mango. The participating children will undergo a DBPCFC with cashew. Selected experiments in blood will be performed to study the mechanism of primary sensitization and potential cross-reactivity, e.g. by ELISA, immunoblot inhibition experiments, BAT, RBL, and the use of recombinant allergens, like Ana o 1, Ana o 2, Ana o 3.

We anticipate to improve the sensitivity of the RBL-2H3-assay by genetically increasing the expression of the FcεR type I receptor via the use of different expression plasmids and by transfecting more suitable cell lines (human HEK293 cells with which we have ample experience) with this receptor under control of a strong constitutive promoter.

In addition, the BAT assays will be modified by using optimized conditions for the patient-derived basophiles, the determination of the *in vivo* IgE loading of the cells, and the preparation of the allergens or fractions thereof for stimulation. The consortium has ample experience in handling these cells.

In total the children will visit the hospital for 4 sessions after the first visit. In one session the skin prick test, questionnaires and blood samples will be taken, in the following 2 sessions DBPCFC's will take place. In a final visit the patient (parents) will be informed whether or not a clinical relevant cashew nut allergy is confirmed and advice will be given concerning the diet in case the DBPCFC is positive. In case the challenge is negative an open challenge will be performed to confirm the absence of cashew nut allergy. For skin tests in house manufactured extracts [19] will be prepared and distributed by the Erasmus MC. Skin prick test results will be scanned and index calculations will be made.

4.2. Existing infrastructure

The scientific challenges require a multidisciplinary approach and the input of two PhD students with different skills. Expertise is required on the following fields: allergen purification and characterization, cell culture of human cells, different cell lines and knowledge of cellular transfection and basic molecular biological techniques (like cloning and expression analysis by real time quantitative PCR), and *in vitro* IgE analysis, skin prick testing and DBPCFC, recognition and interpretation of clinical disease expression. This task is enormous and can be accomplished only by intensive collaboration between centres of excellence in this field. Three clinics, experienced in the field, will include patients and will carry out the clinical part of the study, whereas *in vitro*-analysis will be carried out in one laboratory.

Three clinics will perform the clinical part of the study:

- Erasmus MC Rotterdam, location: Kinderhaven Havenziekenhuis, food challenge in Sophia Kinderziekenhuis
- Reinier de Graaf Gasthuis Delft
- University Medical Center Groningen.

Improvement of diagnostic methods for allergy assessment (IDEAL study)

Immunoblot experiments, recombinant allergens, BAT and RBL assays:

- Wageningen University.

Prognostic modelling and the use of regression techniques:

- Erasmus MC Rotterdam, Centre for Medical Decision Making.

5. Population

All children (2 to 17 years) visiting the outpatient department of the participating clinical centres will be screened for sensitization for cashew nut by skin prick test and/or determination of sIgE to cashew nut.(CAP-FEIA, ALK- Abello)

5.1. Inclusion criteria

- Age: 2-17 year.
- Children with a positive skin prick test (HEP > 0.21) and/ or detectible sIgE (>0.35) to cashew nut.
- History of positive reaction to cashew nut or an unknown reaction (because of never ingested).
- Written informed consent parents and child (≥ 12 year old).

5.2. Exclusion criteria

- History of severe or uncontrolled asthma (investigator's opinion).
- Severe eczema defined as TIS (Three Item Severity) eczema score (> 6).
- Immunological diseases, cardiovascular diseases or malignity.
- Severe psychosocial problems.
- Not able to stop anti-histamine medication for a short period.
- Use of beta-blockers.
- The patient is allergic to one or more of the ingredients of the food matrix, unless a suitable substitute for the ingredient in question can be found.
- Unable to speak and understand the Dutch language properly.
- Not willing to comply with the study procedures.

5.3. Sample size calculation

The aim is to recruit 200 children (age 2-17). In the three year period, all children who are sensitised to cashew nuts enter the study. We expect that 200 children will enter the study. Since the proportion of children that will have a clinical allergy to cashew nuts is unknown, we assume that 50% of the sensitised children experience a clinical allergy. With approximately 100 cases (50% of 200), a reliable prediction model can be constructed when we consider not more than 10 candidate predictor variables, using the 1:10 rule as a guidance [43,44].

This assumes, based on our elaborative experience in similar studies, that 1600 children are needed to obtain 200 sensitized children undergoing a DBPCFC. Which means that: 10 refuse extra skin test, 77% is not sensitized, 25% refuse BDPCFC and 20 is not eligible for analysis. (flow chart; Attachment 1).

Based on recruitment from 3 different medical centres this seems feasible in 36 months.

6. Methods

6.1. Visit schedule and assessments

Patient who meet all the criteria will be asked to participate in the study by the investigator during the normal visit to the physician. The patient/ parents will be given the Patient information form (PIF). After two weeks the patients/ parents will be approached by telephone by the investigator whether or not they are willing to take part in the study. If positive the patient will be invited for the first visit. For this study the patient/parents will come to the hospital for 3 sessions. Inclusion will be stopped when a total of 200 children is included in the three clinics.

6.2. Patient numbering

Every patient is uniquely identified by a combination of his/her centre number (Erasmus MC nr 111; Reinier de Graaf Gasthuis nr 222; University Medical Centre Groningen nr 333), and patient number. In each centre, the first patient is assigned patient number 1, and subsequent patients are assigned consecutive numbers. All results will be collected in the Case Report Form (CRF).

6.3. Data to be collected/assessments

6.3.1. History

Patient demographics/other baseline characteristics

Patient demographics and baseline characteristics will be collected in the CRF at Visit 1.

Data collected will include date of birth, sex, race, ethnicity, height and weight, relevant medical history and current medical conditions, prior concomitant medications.

A questionnaire (CRF) will be used to score on allergic complaints. Inhalation and food allergy complaints will be scored in the CRF, with time of onset of complaints.

6.3.2. Dietary history on consumption of cashew nut

Additionally, the dietary history will be entered in the CRF. In this way patients are allocated to one of the following categories [20], according to the ingestion of cashew nut:

- o Only skin contact, no reaction
- o Only skin contact, allergic reaction
- o Mucosal contact only, no reaction
- o Mucosal contact only, allergic reaction
- o Cashew nut present in diet , no reaction:
 - o when incorporated in other foods
 - o $< \frac{1}{4}$ cashew nut (< 175 mg cashew nut) : < 39 mg E
 - o $\frac{1}{4} - \frac{3}{4}$ cashew nut (175 mg – 525 mg): 39 - 111 mg E
 - o $> \frac{3}{4}$ cashew nut (> 525 mg cashewnut) : > 117 mg E
 - o on a regular basis
 - o unknown
- o Cashew nut was present in diet, most severe allergic reaction:
 - o when incorporated in other foods
 - o $< \frac{1}{4}$ cashew nut (< 175 mg cashew nut) : < 39 mg E
 - o $\frac{1}{4} - \frac{3}{4}$ cashew nut (175 mg – 525 mg): 39 - 111 mg E
 - o $> \frac{3}{4}$ cashew nut (> 525 mg cashew nut) : > 117 mg E
 - o On a regular base
 - o Unknown
- o Presence of cashew nut is unknown
- o Cashew nut is not present

6.3.3. FAQLQ Questionnaire

Food Allergy Quality of Life Questionnaire – Child form 6-12 years (FAQLQ-CF). 12-18 years (FAQLQ-TF) and Parent form 2-6 years (FAQLQ-PF) [12, 21-22]

The FAQLQ is a disease specific instruments that evaluate the impact of food allergy on patients' health-related quality of life along 4 domains: allergen avoidance and dietary restrictions, emotional impact, risk of accidental exposure, and food allergy related health. The FAQLQ is a 29-item questionnaire, self-administered and will be completed by the patient at the clinic at screening (visit 1), and 6 months after the DBPCFC. The hypothesis is, that patient QOL is importantly increasing after a clear cut diagnosis of food allergy. Thus, patients will have time to get used to the new diagnosis and consequently, the Questionnaires will be repeated a half year after the DBPCFC's [23].

The total FAQLQ score is the mean score of all items with a range of 1 'no impairment' to 7 'maximal impairment'. The total FAQLQ score is calculated by adding up the scores and dividing by the number of answered questions at each time point. Within diagnostic evaluation of the cashew nut allergy comparison will be performed for the change from the screening total score to the end of the 5th visit. The statistical significance and 95% confidence intervals will be calculated using paired t-test. For each treatment arm, estimated mean change from baseline with associated 95% confidence interval and p-value will be displayed.

6.3.4. Physical examination

A physical examination will be performed during visit 1.

6.4. Time table

Visit 1 week 1	Visit 2 week 4	Visit 3 week 6	Telephone Week 7	Final visit Week 8
Written informed consent Medical history Physical examination Skin prick tests Blood samples (12 ml) FAQLQ questionnaire(s) *)	DBPCFC Session 1	DBPCFC Session 2	Result DBPCFC	Dietary advice
			When positive	
			When negative	Advice introduction cashew nut at home
*) Patients will be asked to fill in the FAQL Questionnaire at the first visit and the second time will be a half year after the DBPCFC. This second Questionnaire will be filled in at home, so the patients will receive the Questionnaire at home with an answering envelope.				

6.5. Skin prick tests

Skin prick tests with 2 positive controls, one negative control, birch pollen, cashew nut pistachio, mango and peanut will be carried out according to international guidelines and performed by a dedicated allergy nurse. Fifteen minutes after performing the skin tests, the contours of the reaction will be encircled with a fine-tip pen and transferred to a record sheet using transparent tape. ("Skin Prick test result form", Attachment 2) Mean weal of the surface within the encircled area, the Histamine Equivalent Prick index (HEP-index) will be calculated. This will be done by dividing the area of the SPT of the allergen by the area of the mean of two histamines of the SPT using a scanning device (Hewlett Packard 2400c, Houston, TX, USA) and software that was earlier developed in the Erasmus MC hospital [www.PAAMOST.nl]. This method showed higher accuracy and reproducibility than the manually determined mean weal diameter [24]. The cut-off value of 0.21 is used when interpreting the HEP-index. This corresponds to a weal size of 3mm, the internationally

accepted cut-off level for SPT for the mean weal diameter. This classification is a modification of the grading system described by Niemeijer et al. [25].

6.6. Double blind placebo controlled food challenge (visit 2 and 3)

6.6.1. Background

Guidelines recommend using oral food challenges for diagnosing Food Allergy (FA). While a single-blind or an open-food challenge may be considered diagnostic under certain circumstances the double blind placebo controlled food challenge (DBPCFC) is the gold standard approach [26]. Investigators agree that verification of clinical reactivity requires well-designed oral food challenge testing [27]. Because of the inherent risk of serious reactions, an oral food challenge must be conducted at a medical facility with medical supervision and appropriate medication and devices on hand. The challenge test is carried out while the patient is on minimal or no symptomatic medication. The test should be designed and performed under medical supervision primarily for the patient's safety, but also to document the dose that provokes the reaction and to administer symptomatic treatment, which may require management of anaphylaxis. The medical personnel should have experience in carrying out such challenges. Ideally the oral food challenge begins with a low dose (intended to be lower than a dose that can induce a reaction [28]. While monitoring for any allergic symptoms, the dose is gradually increased, until a cumulative dose at least equivalent to a standard portion for age is consumed. Because of the risk of a severe reaction, intentional challenge should be avoided in patients who have recently experienced a life-threatening reaction to cashew nut, particularly if it occurred more than once and if the patient is sensitised for cashew nut. Studies differ in starting doses, typically in the range of the quantity of the food for the most sensitive subjects to produce an objective, but mild reaction. Studies also differ in challenge procedures, the form of the food used, the matrix in which the allergen was presented and the weight accorded to subjective and objective manifestations. Though there is currently no internationally accepted, standardized protocol for performing and interpreting DBPCFCs, several Oral Food Challenge Protocols have been published. Efforts to standardize OFC began in 2004 with the position paper from the European Academy of Allergology and Clinical Immunology [29] and more recently the Adverse Reactions to Food Committee of the American Academy of Allergy, Asthma & Immunology published a review of Oral Food Challenge tests [30].

In a recent study [42], the results of double blind, placebo controlled food challenges with cashew nut were examined in order to generate threshold distribution curves for cashew. This study included the results from 31 challenges with cashew which resulted on positive allergic reactions. Children were included in the test regardless of the severity of the reaction to cashew by history. Children and/or their parents had to consent to the test and the children had to be medically stable at the time of the test. The recipe for these challenges includes a starting dose of 1,75 mg cashew protein (corresponds to 12 mg of cashew) with a final total dose of 4 grams of cashew, divided over 6 incremental doses given at 30 minute intervals. There were no life-threatening reactions and no patients required epinephrine administration or in patient care.

6.6.2. Patients

Preparation:

In case the patient presents with a concurrent illness, e.g. common cold, the DBPCFC should be postponed until the patient has recovered. Before the DBPCFC, the patient may consume his/ her normal breakfast.

On the day of the Challenge a standardized Questionnaire needs to be filled in by the physician whether challenge may be performed. ("Starting Form DBPCFC day", Attachment 3).

The patient is scheduled for a DBPCFC with cashew nut in one of the participating centres of the study. The patient is to remain to stay on a cashew nut free diet prior to the challenge, if indicated (only when clinical symptoms to cashew nut are suspected). The challenge is performed no longer than 6 months after the skin prick tests and the taking of blood samples. Patients must be in good health, and any concomitant allergic diseases e.g. asthma, allergic rhinitis, or atopic dermatitis should be under optimal control.

In case the patient presents with a concurrent illness, e.g. common cold, the OFC should be postponed until the patient has recovered. Before the OFC, the patient may consume his/ her normal breakfast.

6.6.3. Performance

The personnel involved in the food challenge procedure must be trained in the management of acute allergic reactions and equipment for resuscitation (including adrenaline for injection and oxygen) must be readily available. A crash cart must be readily available on site during all food challenges and study drug administrations.

Clinical symptoms and overall condition have to be stable and antihistamines have to be stopped at least 72 hours prior to the test, if possible. Weight and height of the child are measured and the exact doses of the drugs which might be necessary (rescue medication: adrenaline auto-injector (e.g. Epipen), adrenaline, clemastine, other antihistamines, xylometazoline and salbutamol, short acting bronchodilator) are computed and prepared:

Oral

Medication	Age	Concentration	Dose	Note
Fenistil oral solution		1 drop/ kilogram	... drops	
Xyzal oral solution	2-3 year	0.5 mg/ml	1.25mg = 2.5 ml	
Xyzal oral solution	4-6 year	0.5 mg/ml	2.50 mg = 5 ml	
Aerius melt tablet	4-6 year		2.5mg	
Xyzal tablet	≥ 6 year		5 mg	
Aerius melt tablet	≥ 6 year		5mg	
Semprex capsule	≥ 12 year		8 mg	
Prednisone			1-2mg/kg	Max 40 mg

Inhaled

Medication	Age	Concentration	Dose	Note
Xylometazoline	2-6 year	0.05%-solution	1-2 drops or 1 spray/nostril	Max 3x/day
	>6 year	0.1%-solution	2-3 drops or 1 spray/nostril	Max 6x/day
Salbutamol	<4 year	2.5mg/2.5 ml	2.5 mg	Nebulize
	>4 year	5mg/2.5 ml	5 mg	
Atrovent	<4 year	500mcg/ml	250 mcg= 1ml	Nebulize
	>4 year	500mcg/ml	500 mcg= 2ml	

Parenteral Medication	Weight	Concentration	Dose	i.m./i.v.	Note
Adrenaline		1mg/ml	0.01mg/kg = ... ml	i.m.	
Epipen junior	< 30 kg		0.15 mg	i.m.	
Epipen	> 30 kg		0.30 mg	i.m.	
Tavagyl		1mg/ml	0.025mg/kg = ... ml	i.m./i.v.	
Hydrocortisone		50mg/ml	10mg/kg = ... ml	i.v.	Max 200 mg Application: slow
Dexamethasone			0.1mg/kg = ... mg	i.m./i.v.	Max 8mg

6.6.4. Randomization, blinding and preparation

The active test food recipe and placebo recipe for cashew nut are provided monthly, deep frozen by the (NIZO Processing centre; Ede, the Netherlands). The verification code and the preparation of the recipe (ingredients used) are verified by a second individual. A randomization list will be prepared centrally for each participating centre. In each centre an unblinded nurse will be responsible for the preparation of the challenge material in accordance with the randomization list.

The placebo and active test food challenges are administrated on separate days with at least two weeks interval in between. The child is tolerant to the ingredients of the food matrix, as ascertained for by the dietician. A validated recipe “cashew nut in gingerbread” is used as challenge material [31]. Cashew nuts will be provided by Intersnack; Doetinchem. The challenge procedure includes an eight-step incremental design in which progressively greater quantities of the same allergenic food are administrated. The interval between the challenge doses is 30 minutes.[32]

The starting dose will be 1 mg of cashew protein equivalent. As the LOAEL (lowest observable adverse effect level) of peanut appears to be 3 mg of peanut protein [33], we decided to choose 1 mg of cashew. We will use an incremental schedule of 3 fold increase by which 1, 3, 10, 30, 100, 300, 1000 and 3000 mg of cashew protein (or placebo) will be administered. (“Challenge doses Cashew DBPCFC”, Attachment 4)

In a recent study [34], the results of double-blind, placebo-controlled food challenges with cashew were examined in order to generate *threshold distribution curves* for cashew. This study included the results from 31 challenges with cashew which resulted on positive allergic reactions. Children were included in the test regardless of the severity of the reaction to cashew by history. Children and/or their parents had to consent to the test and the children had to be medically stable at the time of the test. The recipe used for these challenges use a starting dose of 1,75 mg cashew protein (corresponds to 12 mg of cashew) with a final total dose of 4 grams of cashew, divided over 6 incremental doses given at 30 minute intervals. There were no life-threatening reactions and no patients required epinephrine administration or in patient care.

Schedules using semi-logarithmic increases are associated with good safety and are more often used in other centres. [35]. Smaller increments (e.g. doubling) may further enhance safety but would also significantly increase the time necessary to complete the challenge procedure. Therefore, the proposed scheme is expected to represent a suitable challenge regimen, balancing safety and feasibility.

Dose	Protein (mg)	Protein cum (mg)	Calculation	Administered amount of food (g)	Administered amount of food cum (g)
1	1	1	$(120*/3180) \times 1$	0,04	0,04
2	3	4	$(120*/3180) \times 3$	0,11	0,15
3	10	14	$(120*/3180) \times 10$	0,38	0,53
4	30	44	$(120*/3180) \times 30$	1,13	1,66
5	100	144	$(120*/3180) \times 100$	3,77	5,43
6	300	444	$(120*/3180) \times 300$	11,32	16,75
7	1000	1444	$(120*/3180) \times 1000$	37,74	1 – 4 years 54,49
8	1736	3180	$(120*/3180) \times 1736$	remaining 65,51	≥ 4 years 120,00

For children of 4 years and above, a total amount of 120 grams of test food (containing 3180 mg protein or 15 grams of cashew nut or ~22 cashew nuts) will be administered.

For children younger than 4 years of age, this will be half of the total amount, i.e. 60 grams of test food (containing 1444 mg protein or 7,5 grams of cashew nut or ~11 cashew nuts).

On both placebo and active test food challenge, a total of 120 grams of test food is administered.

6.6.5. Assessment protocol for the outcome

Challenge sessions in which children consume less than 75% of the maximum challenge dose are considered invalid. The challenge is discontinued when objective allergic symptoms occur, or subjective allergic symptoms occur twice on two successive administrations of the challenge material [28].

Judgments about proceeding must balance safety against the certainty of the challenge outcome. Objective symptoms and signs are defined as (angio) edema, urticaria, exacerbation of atopic eczema, vomiting, diarrhoea, lip or tongue swelling, rhino-conjunctivitis, stridor, coughing, wheezing, hoarseness, collapse, tachycardia and hypotension.

Subjective symptoms are defined as exacerbation of generalized itch (in case of atopic eczema), abdominal pain, nausea and/or cramp, oral allergy symptoms, itchy throat or sensation of throat swelling, difficulty in swallowing and other symptoms such as drowsiness and irritability. Immediate symptoms are defined as symptoms occurring during the challenge or within two hours after the last challenge dose.

A standardized scoring system will be used to score objective and subjective symptoms in the patients. (“Scoring system DBPCFC Cashew study” Attachment 5)

Symptoms

Objective	Subjective
Skin	Skin
Urticaria and angioedema	Itching
Redness of the skin	
Exacerbation of atopic eczema	
Respiratory	Respiratory
Rhinitis	Feeling of dyspnea
Wheezing	
Coughing	
Stridor	
Hoarseness	
Eye symptoms	Eye symptoms
Conjunctivitis	Itching
Gastro-intestinal	Gastro-intestinal
Vomiting	Cramps
Diarrhoea	Stomach ache
	Oral allergy symptoms
	Itching throat
	Sensation of throat swelling
	Dysphagia
Cardiovascular	Cardiovascular
Hypotension	Weakness
Tachycardia	Dizziness
Collaps	
Lose consciousness	
Anafylaxis	
Other	Other
Extreme crying	Psychological
Change of behaviour	

Two hours after the last dose, or when all symptoms have disappeared and the patient is in a stable condition, the patient is sent home. The patient is requested to record late onset symptoms on a scoring sheet (“Scoring system 48 hours after DBPCFC”, Attachment 6) and to contact the hospital in case of severe symptoms. Two days after the challenge session late onset reactions are recorded by telephone using the same Questionnaire. Late onset symptoms are defined as symptoms occurring between two and 48 hours after the last challenge dose [29]. Forty-eight hours after the second challenge session, the code is broken and the outcome of the DBPCFC is assessed as follows:

Active food challenge	Placebo	Assessment of DBPCFC
Positive (or clearly more positive/ more objective than placebo)	Negative	Positive
Positive	Positive	Negative
Questionable	Questionable	Negative
Negative	Negative	Negative

Negative (or positive, but clearly less positive than placebo)	Positive	Negative
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In exceptional cases, a challenge session or total DBPCFC may be assessed as inconclusive, e.g. in case of mild symptoms at the last dose. Inconclusive DBPCFCs are repeated within 6 months.

6.6.6. Results, telephone contact

Patient will be informed about the result of the DBPCFC by telephone by the investigators. Appointment for the last visit will be made. During a final visit the patient (parents) will be informed by the investigator whether or not a clinical relevant cashew nut allergy is established and the diagnosis cashew nut allergy is confirmed. When a cashew nut allergy is confirmed, the patient (parents) will be advised to continue the cashew nut free diet. When the patient has no cashew nut allergy, the patient will be advised how to reintroduce the cashew nut at home [36].

6.7. Blood collection

6.7.1. IgE

For IgE measurement 1 vial of whole blood (8ml) will be drawn, centrifuged (2000 rpm, 10 min.) Serum will be stored at -20 °C for further examination. Specific IgE will be measured against: Cashew allergens: Ana o 1, Ana o 2 and Ana o 3, and to possibly cross-reacting allergens from mango and from pistachio. Rest of the serum will be stored at -20 °C for the use in RBL analysis.

6.7.2. BAT

For the basophil activation test (BAT), 1 vials of EDTA blood (4ml) will be drawn at the first visit and sent to Wageningen immediately. From these blood samples, plasma and granulocytes are isolated via Ficoll-centrifugation. Plasma samples will be stored at -20 °C. Polymorphonuclear cell family (PMNs) will be used to improve the BAT-test to facilitate clinical application in allergy diagnosis.

6.7.3. RBL

For RBL analysis rest of the serum which is stored at -20 °C will be used to study how these RBL-tests, or tests making use of Human Embryonic Kidney 293 (HEK) cells, can be improved to increase their sensitivity and application for clinical testing.

6.8. Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

7. Data collection and statistical analysis

We hypothesize that MRAs are superior to other tests in predicting clinically important allergy to cashew nuts. Superiority will justify their use and may lead to displacement of other diagnostic procedures. To this purpose, predictive modelling and regression techniques will be used to accomplish and identify the optimal set of diagnostic procedures for the diagnosis of cashew nut allergy. In fact, these models might be useful for other nuts as well. In that way it would accomplish a breakthrough in the diagnosis of food allergy.

We will use logistic regression analysis to study the association between candidate predictors (diagnostic tests) and cashew nut allergy. The shape of associations between continuous test results and cashew nut allergy will be studied with restricted cubic splines [37] with three knots (two degrees of freedom). If the association is non-linear, an appropriate transformation will be selected, such as the natural logarithm, square, and square root [38].

The regression coefficients in the final model will be multiplied with a shrinkage factor that is estimated with bootstrapping. Shrinkage is applied to obtain accurate predictions for new patients; without shrinkage, predictions are in general too extreme resulting in low predictions being too low and high predictions too high.

The final model with the shrunken regression coefficients will be presented in an easy to use format to facilitate clinical application, for instance a score chart or monogram.

This analytical strategy to develop a diagnostic model is according to the current state of the art [39]. The strategy aims to develop a diagnostic model that can provide accurate predictions for future patients rather than predictions that are correct for the patients of the development dataset. Therefore, the selection of predictors in the model is based on multivariable modelling only, with a backward selection procedure and liberal p value (0.157 in this study). The use of higher p-values, results in inclusion of relatively weaker predictors, in the model, on the cost of possible selection of a nuisance variable. Such model performs well in new [40-41].

8. Ethical considerations

8.1. Regulation statement

The study will be conducted in accordance with the principles of the current version of the Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act (WMO).

8.2. Recruitment and consent

Patients will be recruited from the patient population of the participating clinical sites. The interest of parents and children for participation will be checked by the treating physician. In case of potential interest the children and parents will be referred to the study staff for the formal informed consent procedure. In case the treating physician is also the investigator, the informed consent procedure will be handled by another member of the study staff. An independent physician will be available.

An information letter will be available for the parents and for the children ≥ 12 years of age. For participation of a child a signed consent form signed by both parents (or legal guardian) is mandatory. If a child is 12 years or above, the child must give written consent as well. Sufficient time will be allowed to consider participation, with a maximum of 2 weeks. This may be extended if deemed justified by the person handling the informed consent procedure.

8.3. Minors

In case the minor patient does not cooperate or shows signs of resistance, the investigator should discontinue the study immediately. The guideline of the Dutch Society for Pediatric Medicine (Nederlandse Vereniging voor Kindergeneeskunde (NVK)) for the protection of minor research subjects will be observed.

8.4. Benefits and risks assessment

Burden/risks:

- The volunteers will visit the hospital for 4 times, 3 questionnaires will be filled in by either the parents (age 2-6 years) or the children (age 6-12), skin prick tests will be performed (total of 8) and 2 blood samples will be drawn. Furthermore the child will undergo a Double Blind Placebo Controlled Food Challenge (DBPCFC) on 2 different days.
- The burden and risks will be minimal due to extensive experience with DBPCFCs. Nevertheless, we are aware, that the outcome of the DBPCFC is unpredictable and reactions may occur in the patients. Therefore, the DBPCFC will take place at the children's department, at the medium care unit, provided with all necessary rescue medication.
- The study can only be done in this group of children because we find high numbers of sensitization to cashew nut allergens in very young children and the DBPCFC is the only diagnostic tool we have available till now.
- The above described method is the normal diagnostic protocol (golden standard) we use to prove or reject an IgE mediated food allergy in children and adults. The only extra tests we perform for the study is are extra questionnaires, 2 extra blood samples for the Mediated Release Assay's (MRA's) and 6 extra skin prick tests to measure cross-reactivity with other foods.

Possible benefit:

The possible benefit for the patient is a clearer diagnosis concerning the possible IgE mediated cashew nut allergy.

8.5. Compensation

A liability insurance which is in accordance with article 7, subsection 6 of the WMO and an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003) has been arranged. The latter insurance provides cover for damage to research subjects through injury or death caused by the study:

1. € 450.000,-- (i.e. four hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 3.500.000,-- (i.e. three million five hundred thousand Euro) for death or injury for all subjects who participate in the Research;
3. € 5.000.000,-- (i.e. five million Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as ‘verrichter’ in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

The insurance policies of the participating clinical sites will be valid for this study.

8.6. Incentives

There will be no incentives for participation. Extra travel expenses due to participation will be reimbursed.

9. Administrative aspects and publication

9.1. Handling and storage of data and documents

The CRF will contain all the study related information of the patient including the completed questionnaires, results of SPT and CAP FEIA and DBPCFC. The CRF will be stored at the site under the responsibility of the investigator. After the last visit the CRF will be sent to the coordinating investigator, and all data will be entered in the database. This database is kept under the responsibility of the coordinating investigator. A data manager will review the entries and will take care of back-up several times a day.

Every patient is uniquely identified by a combination of his/her centre number (Erasmus MC nr 111; Reinier de Graaf Gasthuis nr 222; University Medical Centre Groningen nr 333), and patient number. In each centre, the first patient is assigned patient number 1, and subsequent patients are assigned consecutive numbers (2, 3 etc.). The code list is kept by the local investigator.

The study documents provided to the coordinating investigator will be coded with the centre/patient number. Information directly referring to the patient's identity will not be supplied.

The patient's study data will be stored for 15 years after completion of the study. The blood samples will be destroyed when the study is concluded.

9.2. Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All substantial amendments will be notified to the EC that gave a favourable opinion.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

9.3. Annual progress report

The coordinating investigator will submit a summary of the progress of the trial to the accredited EC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

9.4. End of study report

The investigator will notify the accredited EC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit. In case the study is ended prematurely, the investigator will notify the accredited EC, including the reasons for the premature termination.

Within one year after the end of the study, the coordinating investigator will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited EC.

9.5. Public disclosure and publication policy

An outline of the study protocol and a summary of the study results will be placed on the publically accessible website of the Dutch Trial Register (www.trialregister.nl).

The publication policy of the CCMO (March 2002, www.ccmo.nl) will be adhered to in full.

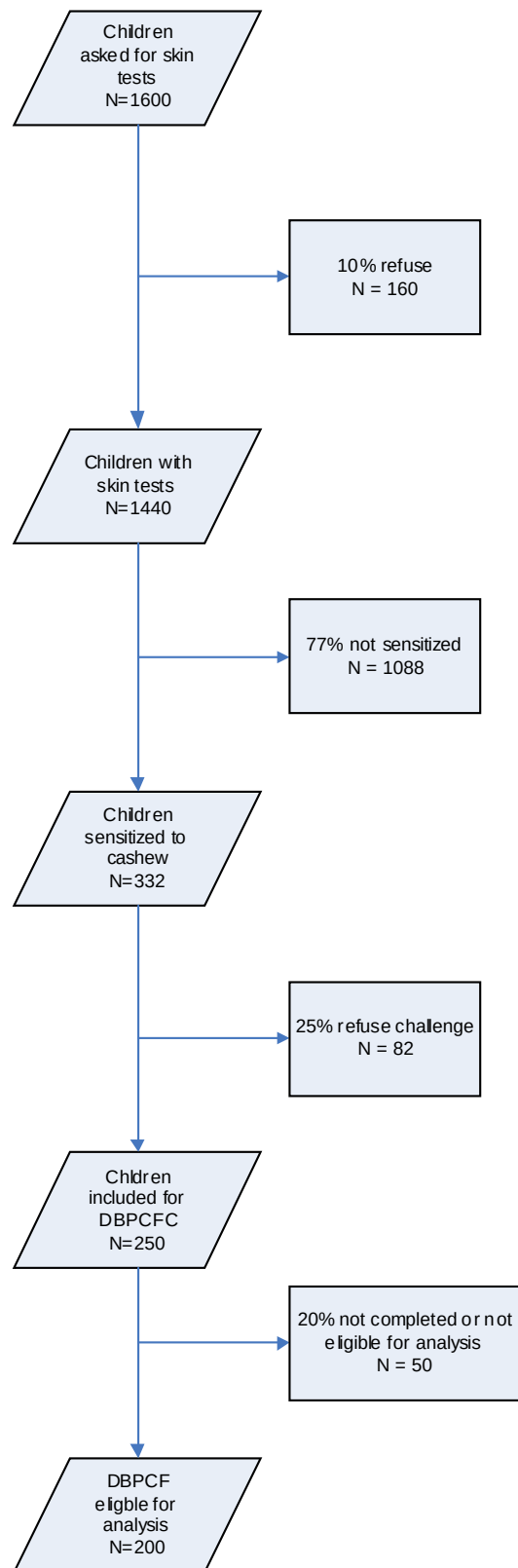
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Attachment 1: Flow Chart



Attachment 2: Skin Prick test result form

SPT formulier

Patient ID: ___ ___ (expl. 111 0001)

Initialen: ___ ___ (voorbeeld: NJ)

Geslacht

Positive control	Mango sap		
Positive Control	Pistache nut		
Negative control	Peanut		
Birch pollen			

Attachment 3: Preparation of the DBPCFC day

Vorbereitung van de Dubbelblinde placebogecontroleerde voedselprovocatie met cashew noot (in te vullen door verpleegkundigen)
2^e en 3^e lijn

Patiëntgegevens:

Naam:
Patiëntnummer:
Geboortedatum:

Naam uitvoerende:

Verantwoordelijke arts:

Naam: Seinnummer:.....

Arts-Achterwacht

Naam;..... Seinnummer;

Anamnese

Wordt cashew noot nog steeds vermeden?

0 ja

0 nee (Indien nee: overleg met arts over indicatie van provocatie)

Medicatie

Medicatie gestaakt	0	ja, te weten
	0	nee
Medicatie door gebruikt	0	ja, te weten.....
	0	nee

NB: Indien antihistaminica de laatste 72 uur of lokale corticosteroïden de laatste 24 uur zijn door gebruikt kan de provocatie NIET doorgaan!

Gebruikte voeding

Het kind heeft de laatste 3 uur niet meer gegeten of gedronken:

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- 0 ja
- 0 nee, namelijk
(zo nee, overleg met de arts)

Allergieonderzoek :

- 0 niet verricht
- 0 wel verricht : 0 Specifiek IgE koemelk: kU/l datum:.....
 - 0 Huidtest met koemelk mm datum
 - 0 huidtest niet verricht

Infuus

Dient de patiënt voor de provocatie een infuus te krijgen?

- 0 Ja
- 0 nee

Reden voor infuus:

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Noodmedicatie

Lengte:

Gewicht:

Bloeddruk:

Hartfrequentie:

Adrenaline (Ampul 1mg =1ml)

0,01 mg/kg intramusculair, max. 0,5 mg

..... mg = ... ml i.m. (dijbeen anterior-lateraal)

Clemastine® (Tavegil))

0,025 mg/kg intraveneus of intramusculair (max. 2 mg)

..... mg i.v.

*Fenistil*druppels (1mg/ml)

1–3 jaar: 15 druppels p.o.

Xyzal (tablet 5 mg, drank 0,5 mg/ml)

2-6 jaar: 1,25 mg = 2,5 ml

Aerius (tablet 5 mg, stroop 0,5 mg/ml)

1-5 jaar: 1,25 mg = 2,5 ml

Dexamethason 0,1 mg/kg (max. 8 mg) intraveneus of intramusculair

..... mg i.v./i.m.

Hydrocortison 4 mg/kg (max. 100 mg) intraveneus of intramusculair

..... mg i.v. /i.m.

Salbutamol® (Ventolin) zie 1^e lijn dosisaerosol met voorzetkamer (nebule 2,5 mg/2,5 ml of 5 mg/2,5 ml)
en

Atrovent (nebule van 500 mcg/2ml) dosisaerosol 20 ug (op indicatie aantal pufs ophogen)

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via Sidestream vernevelaar met 8 liter zuurstof

<4 jaar: Ventolin 2,5 mg (nebule 2,5 mg/2,5 ml)
 Atrovent 250 mcg (1 ml van nebule 500 mcg/ml)

>4 jaar Ventolin 5 mg (nebule 5 mg/2,5 ml)
 Atrovent 500 mcg (2 ml van nebule 500 mcg/ml)

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Beoordeling conditie van de patiënt bij aanvang van de provocatie

Naam arts/verpleegkundige:

Bij aanvang van de provocatie moet het kind in een goede conditie zijn.

Afgelopen 2 weken klachten gehad? (indien ja, specificeren)

Neus/ogen	0	nee	0	ja (verbeterd, wisselend, verslechterd)
Longen	0	nee	0	ja (verbeterd, wisselend, verslechterd)
Huid	0	nee	0	ja (verbeterd, wisselend, verslechterd)
Maagdarmkanaal	0	nee	0	ja (verbeterd, wisselend, verslechterd)
Andere	0	nee	0	ja (verbeterd, wisselend, verslechterd)

Huidige medicatie	naam	dosis	laatste gebruik
Antihistaminica			
Longmedicatie:			
ICS			
Luchtwegverwijder			
Overig			
Huid			
Vette zalf			

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Steroïdcrème

Overig

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Lichamelijk onderzoek voor provocatie

Algemene indruk (verkoudheden, griep, vaccinatie gehad, overig):

Hals/Hoofd:

Longen:

Huid: (*vul TIS score in*)

Temperatuur°C

Piekstroom l/min

Saturatie (O₂)%

Overig:

Tabel 2. Samenhang tussen de ernst van het eczeem, de scores van de afzonderlijke componenten van het eczeem en de 'three item severity' (TIS)-score.

Ernst eczeem	Erytheem	Oedeem	Excoriaties	TIS-score
mild	geen-licht (0, 1)	geen-weinig (0, 1)	geen-gering (0, 1)	1, 2
matig	geen-matig (0-2)	geen-matig (0-2)	krabeffecten (0-2)	3-5
ernstig	fors (2, 3)	oedeem/papels (2, 3)	zeer duidelijke krabeffecten (2, 3)	6-9
Toelichting: mild eczeem=maximale TIS-score kan 2 zijn, matig eczeem=maximale TIS-score kan 5 zijn, ernstig eczeem=maximale TIS-score kan 9 zijn. Allerlei varianten per component als onderdeel van de TIS-score zijn mogelijk, afhankelijk van de ernst (0-3) met steeds 3 als maximum.				

Totaal TIS score:

Kan de provocatie vandaag doorgaan?

0 ja 0 nee, want

Te ondernemen acties:

- 0 IC gewaarschuwd
- 0 Noteren in patiëntenstatus dat provocatie vandaag plaatsvindt
- 0 Informed consent innemen en in status opbergen

Attachment 4: Dosing schedule food challenge

Doseer schema voedselprovocatie cashew in kruidkoek 15 gram cashew (21,2% eiwit)

Uitgangspunten:

Doseerschema in eiwit equivalenten

Cashewnoot bevat 21,2% eiwit, dus 100 g cashew levert 21,2 g = 21.200 mg eiwit.

Recept met 15 gram cashewnoot bevat dus $21,2/100 \times 15 = 3,18$ g eiwit = 3180 mg

Totale recept weegt tussen ca. 120 en 147 g (deel van het vocht verdampt).

1 cashewnoot weegt ca. 700 mg.

Totale recept (**ca. 120 g**) bevat 15 gram cashewnoot (ca. 22 cashewnoten) met **3180** mg eiwit (≥ 4 jaar)

Halve recept (ca. 60 g) bevat ca. 7,5 gram cashewnoot (ca. 11 cashewnoten) met **1590** mg eiwit (1-4 jaar)

Hierbij is uitgegaan van ca.

Dosis	Eiwit (mg)	Eiwit Cum mg	Omrekening	Hoeveelheid Recept (g)	Hoeveelheid recept (Cum g)
1	1	1	$(120*/3180) \times 1$	0,04	0,04
2	3	4	$(120*/3180) \times 3$	0,11	0,15
3	10	14	$(120*/3180) \times 10$	0,38	0,53
4	30	44	$(120*/3180) \times 30$	1,13	1,66
5	100	144	$(120*/3180) \times 100$	3,77	5,43
6	300	444	$(120*/3180) \times 300$	11,32	16,75
7	1000	1444	$(120*/3180) \times 1000$	37,74	1 – 4 jaar 54,49
8	1736	3180	$(120*/3180) \times 1736$	restant 65,51	≥ 4 jaar 120,00

* NB: Dit moet elke keer opnieuw worden berekend, bij elke provocatie, afhankelijk van het totaal gewicht van de kruidkoek, uitgaande van 15 g cashewnoot met 3180 mg eiwit in totaal recept.

Als het gewicht van de kruidkoek afwijkt van 120 gram: dan zelf de porties uitrekenen als volgt:

Voor 1^e dosis: totale gewicht/3180 x 1 = ... gram recept

Voor 2^e dosis: totale gewicht/3180 x 3 = ... gram recept

Voor 3^e dosis: totale gewicht/3180 x 10 = ... gram recept

Voor 4^e dosis: totale gewicht/3180 x 30 = ... gram recept

Voor 5^e dosis: totale gewicht/3180 x 100 = ... gram recept

Voor 6^e dosis: totale gewicht/3180 x 300 = ... gram recept

Voor 7^e dosis: totale gewicht/3180 x 1000 = ... gram recept

Voor 8^e dosis: totale gewicht/3180 x 1736 = ... gram recept

Attachment 5: Scoring system DBPCFC

SYMPTOOM REGISTRATIE FORMULIER DUBBELBLINDE PLACEBOGECONTROLEERDE PROVOCATIE MET CASHEW NOOT

IDEAL STUDIE - TEST DAG 1

Patient ID: ___ ___
(expl. 111 0001)

Initialen: ___ ___
(voorbeeld: NJ)

Geslacht

Testdatum:.....

- 0 Type testvoeding :
- 0 Dubbelblinde test, testdag 1
- 0 Infuus hoeft niet ingebracht
- 0 Infuus ingebracht

Voorbeeld registratie van symptomen:

ernst score 1 = mild

 2 = matig

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3 = ernstig

	Dosis 1 Tijd:	Reactie Tijd:	Dosis 2 Tijd:	Reactie Tijd:	Dosis 3 Tijd:	Reactie Tijd:	Dosis 4 Tijd:	Reactie Tijd:	Dosis 5 Tijd:	Reactie Tijd:	Dosis 6 Tijd:	Reactie Tijd:	Dosis 7 Tijd:	Reactie Tijd:	Dosis 8 Tijd:	Reactie Tijd:
Tijdstip	9.00		9.30		10.00											
Symptomen																
Jeuk						10.20 2										

	Dosis 1 Tijd:	Reactie Tijd:	Dosis 2 Tijd:	Reactie Tijd:	Dosis 3 Tijd:	Reactie Tijd:	Dosis 4 Tijd:	Reactie Tijd:	Dosis 5 Tijd:	Reactie Tijd:	Dosis 6 Tijd:	Reactie Tijd:	Dosis 7 Tijd:	Reactie Tijd:	Dosis 8 Tijd:	Reactie Tijd:
Tijdstip																
Symptomen																
Jeuk																
Roodheid																
Lokale urticaria																
Gegenerali- seerde urticaria																
Zwelling																
Overig																
Gastro- intestinaal																

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Angio-oedeem van de lippen, tong en gehemelte																
Jeuk in de mond																
(Krampende) buikpijn																
Misselijkheid																
Spugen (eenmalig)																
	Dosis 1 Tijd:	Reactie Tijd:	Dosis 2 Tijd:	Reactie Tijd:	Dosis 3 Tijd:	Reactie Tijd:	Dosis 4 Tijd:	Reactie Tijd:	Dosis 5 Tijd:	Reactie Tijd:	Dosis 6 Tijd:	Reactie Tijd:	Dosis 7 Tijd:	Reactie Tijd:	Dosis 8 Tijd:	Reactie Tijd:
Herhaald spugen																
Diarree																
Overig																
Respiratoir																
Neusobstructie																
Niezen																
Conjunctivitis																
Loopneus																
Gevoel van zwelling keel																
Stridor/ Heesheid																
Hoesten																

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[illegible]

Dosis herhaald?

0 nee 0 ja, namelijk dosis:
 tijdstip:

Laatst gegeven testvoeding:

Volledig gegeten: 0 ja 0 nee

Indien nee: hoeveel testvoeding gegeten : g

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Hoeveel testvoeding over: g

Toelichting:

.....
.....
.....
.....
.....

Samenvatting reacties op de testdag

0 geen verschijnselen

0 Verschijnselen optredend na dosis nr.: ...

Medicatie gegeven? 0 nee, 0 ja, namelijk

0 antihistaminicum per os/iv

0 luchtwegverwijders

0 adrenaline

0 anders, nl.:

Lichamelijk onderzoek aan einde testdag

Naam beoordelaar:

Algemene indruk:

Hals/Hoofd:

Longen:

Huid: (*vul TIS score in*)

Bloeddruk:

Hartfrequentie:

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Temperatuur°C

Piekstroom l/min

Saturatie (O2)%

Overig:

Te ondernemen acties:

- 0 Belafspreek met ouders voor over 48 uur
- 0 Symptoomscore formulier voor thuis meegeven
- 0 Check bij de ouders of ze weten wat ze moeten doen bij reacties thuis (contact gegevens – zie Patiënteninformatie)
- 0 Check bij de ouders dat ze weten dat ze het cashew noot nog moeten vermijden
- 0 Voorlopige uitslag provocatie in status noteren

(Telefonische) Controle na 48 uur

Datum:

Naam arts/verpleegkundige:

Sinds provocatie klachten veranderd? Indien ja, specificeren.

Neus/ogen	0 nee	0 ja, namelijk
Longen	0 nee	0 ja, namelijk
Huid	0 nee	0 ja, namelijk
Andere	0 nee	0 ja, namelijk

Wanneer zijn deze klachten opgetreden (hoeveel uren na provocatie)?

Medicatie veranderd sinds laatste provocatie? Indien ja, specificeren.

Objectivatie symptomen (bij optredende symptomen binnen 48 uur)

Datum:

Tijdstip:

Naam arts/verpleegkundige:

Neus/ogen	0 nee	0 ja, te weten
-----------	-------	----------------------

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Longen	☐ nee	☐	ja, te weten
Huid	☐ nee	☐	ja, te weten
Andere	☐ nee	☐	ja te weten.....

☐ Symptoomscore formulier voor thuis opgeborgen in het dossier van de patiënt.

Conclusie provocatie dag

Provocatie is reglementair afgerond (tenminste 75% van de laatst gegeven dosis gegeten of gedronken): ☐ ja ☐ nee

Indien ja:

- ☐ Er is een objectieve reactie opgetreden
- ☐ Er zijn herhaalde of persisterende subjectieve reactie opgetreden
- ☐ Er zijn geen reacties opgetreden

Indien nee:

- ☐ het kind weigerde de voeding volledig te eten
- ☐ De provocatie is gestaakt op verzoek op de ouders
- ☐ Anders, te weten

Beoordeling provocatie dag:

- ☐ De provocatiedag is positief
 - ☐ De provocatie dag is negatief
 - ☐ De provocatie dag is dubieus of onbeslist
- Toelichting:

Een provocatie is geslaagd als tenminste t/m 75% van de laatste provocatiedosis is ingenomen.

Een provocatie is negatief of onbeslist bij milde objectieve of aanhoudende subjectieve klachten op de laatste provocatie dosis.

SYMPTOOM REGISTRATIE FORMULIER DUBBELBLINDE PLACEBOGECONTROLEERDE PROVOCATIE MET CASHEW NOOT

IDEAL STUDIE - TEST DAG 2

Naam kind:

Patiënt nummer

Geboorte datum:

Testdatum:.....

0 Type testvoeding :

0 Dubbelblinde test, testdag 1

0 Infuus hoeft niet ingebracht

0 Infuus ingebracht

Voorbeeld registratie van symptomen:

ernst score 1 = mild

2 = matig

3 = ernstig

	Dosis 1 Tijd:	Reactie Tijd:	Dosis 2 Tijd:	Reactie Tijd:	Dosis 3 Tijd:	Reactie Tijd:	Dosis 4 Tijd:	Reactie Tijd:	Dosis 5 Tijd:	Reactie Tijd:	Dosis 6 Tijd:	Reactie Tijd:	Dosis 7 Tijd:	Reactie Tijd:	Dosis 8 Tijd:	Reactie Tijd:
Tijdstip	9.00		9.30		10.00											
Symptomen																
Jeuk						10.20 2										

Improvement of diagnostic methods for allergy assessment (IDEAL study)

	Dosis 1 Tijd:	Reactie Tijd:	Dosis 2 Tijd:	Reactie Tijd:	Dosis 3 Tijd:	Reactie Tijd:	Dosis 4 Tijd:	Reactie Tijd:	Dosis 5 Tijd:	Reactie Tijd:	Dosis 6 Tijd:	Reactie Tijd:	Dosis 7 Tijd:	Reactie Tijd:	Dosis 8 Tijd:	Reactie Tijd:
Tijdstip																
Symptomen																
Jeuk																
Roodheid																
Lokale urticaria																
Gegenerali- seerde urticaria																
Zwelling																
Overig																
Gastro- intestinaal																
Angio-oedeem van de lippen, tong en gehemelte																
Jeuk in de mond																
(Krampende) buikpijn																
Misselijkheid																
Spugen (eenmalig)																

Improvement of diagnostic methods for allergy assessment (IDEAL study)

	Dosis 1 Tijd:	Reactie Tijd:	Dosis 2 Tijd:	Reactie Tijd:	Dosis 3 Tijd:	Reactie Tijd:	Dosis 4 Tijd:	Reactie Tijd:	Dosis 5 Tijd:	Reactie Tijd:	Dosis 6 Tijd:	Reactie Tijd:	Dosis 7 Tijd:	Reactie Tijd:	Dosis 8 Tijd:	Reactie Tijd:
Herhaald spugen																
Diarree																
Overig																
Respiratoir																
Neusobstructie																
Niezen																
Conjunctivitis																
Loopneus																
Gevoel van zwellend keel																
Stridor/ Heesheid																
Hoesten																
Moeilijk slikken																
Benauwdheid																
Astma aanval																
Overige																
Cardio- Vasculair																
Tachycardie																

Improvement of diagnostic methods for allergy assessment (IDEAL study)

	Dosis 1 Tijd:	Reactie Tijd:	Dosis 2 Tijd:	Reactie Tijd:	Dosis 3 Tijd:	Reactie Tijd:	Dosis 4 Tijd:	Reactie Tijd:	Dosis 5 Tijd:	Reactie Tijd:	Dosis 6 Tijd:	Reactie Tijd:	Dosis 7 Tijd:	Reactie Tijd:	Dosis 8 Tijd:	Reactie Tijd:
Slap worden																
Hypotensie																
Verminderd bewustzijn																
Acute gedrags- verandering																
overig																

Dosis herhaald?

0 **nee** 0 **ja, namelijk dosis:**
tijdstip:

Laatst gegeven testvoeding:

Volledig gegeten: 0 ja 0 nee

Indien nee: hoeveel testvoeding gegeten : g

Hoeveel testvoeding over: g

Toelichting:

.....
.....
.....
.....
.....

Improvement of diagnostic methods for allergy assessment (IDEAL study)

Samenvatting reacties op de testdag

0 geen verschijnselen

0 Verschijnselen optredend na dosis nr.: ...

Medicatie gegeven? 0 nee, 0 ja, namelijk

0 antihistaminicum per os/iv

0 luchtwegverwijders

0 adrenaline

0 anders, nl.:

Lichamelijk onderzoek aan einde testdag

Naam beoordelaar:

Algemene indruk:

Hals/Hoofd:

Longen:

Huid: (*vul TIS score in*)

Bloeddruk:

Hartfrequentie:

Temperatuur°C

Piekstroom l/min

Saturatie (O₂)%

Overig:

Improvement of diagnostic methods for allergy assessment (IDEAL study)

Te ondernemen acties:

- 0 Belafspreek met ouders voor over 48 uur
- 0 Symptoomscore formulier voor thuis meegeven
- 0 Check bij de ouders of ze weten wat ze moeten doen bij reacties thuis (contact gegevens – zie Patiënteninformatie)
- 0 Check bij de ouders dat ze weten dat ze het cashew noot nog moeten vermijden
- 0 Voorlopige uitslag provocatie in status noteren

(Telefonische) Controle na 48 uur

Datum:

Naam arts/verpleegkundige:

Sinds provocatie klachten veranderd? Indien ja, specificeren.

Neus/ogen	0 nee	0 ja, namelijk
Longen	0 nee	0 ja, namelijk
Huid	0 nee	0 ja, namelijk
Andere	0 nee	0 ja, namelijk

Wanneer zijn deze klachten opgetreden (hoeveel uren na provocatie)?

Medicatie veranderd sinds laatste provocatie? Indien ja, specificeren.

Objectivatie symptomen (bij optredende symptomen binnen 48 uur)

Datum:

Tijdstip:

Naam arts/verpleegkundige:

Neus/ogen	0 nee	0 ja, te weten
Longen	0 nee	0 ja, te weten
Huid	0 nee	0 ja, te weten
Andere	0 nee	0 ja te weten.....

0 Symptoomscore formulier voor thuis opgeborgen in het dossier van de patiënt.

Conclusie provocatie dag

Provocatie is reglementair afgerond (tenminste 75% van de laatst gegeven dosis gegeten of gedronken): 0 ja 0 nee

Indien ja:

- 1 Er is een objectieve reactie opgetreden
- 1 Er zijn herhaalde of persisterende subjectieve reactie opgetreden
- 1 Er zijn geen reacties opgetreden

Indien nee:

- 0 het kind weigerde de voeding volledig te eten
- 0 De provocatie is gestaakt op verzoek op de ouders
- 0 Anders, te weten

Beoordeling provocatie dag:

- 1 De provocatiedag is positief
- 1 De provocatie dag is negatief
- 1 De provocatie dag is dubieus of onbeslist

Toelichting:

Een provocatie is geslaagd als tenminste t/m 75% van de laatste provocatiedosis is ingenomen.

Een provocatie is negatief of onbeslist bij milde objectieve of aanhoudende subjectieve klachten op de laatste provocatie dosis.

0	Verbreek de code:	0	Provocatie dag 1 was verum / placebo
		0	Provocatie dag 2 was verum / placebo

Eindbeoordeling provocatie test

Toelichting

* Overleg met een gespecialiseerd centrum: Bij duidelijke verum reacties is de uitslag onbeslist en wordt de DBPCFC herhaald.
* Bij minder duidelijke of vertraagde verum reacties is de uitslag negatief of onbeslist.

FOLLOW-UP DUBBELBLINDE PLACEBOGECONTROLEERDE PROVOCATIE MET CASHEWNOOT

Vervolg advies en te ondernemen acties:

- 0 Cashewnootvrij dieet handhaven (bij positieve test)
- 0 Cashewnoot in dieet introduceren (bij negatieve test)
- 0 Informeer de huisarts, consultatiebureau arts, kinderarts en diëtiste

Acties

- Verwijs zo nodig naar de diëtiste
- Advies thuis introductie Cashew
- Schrijf rapportage

Attachment 6: Symptom score 48 h post challenge

Symptoom score formulier voor thuis (Voor 48 uur na de provocatie)

IDEAL studie

Patient ID: ____
(expl. 111 0001)

Initialen: ____
(voorbeeld: NJ)

Geslacht

Na de voedselprovocatie:

48 uur na afloop van de test vragen wij u contact met ons op te nemen om te bespreken hoe het thuis is gegaan. Hiervoor maken wij een telefonische afspraak.

Wij vragen u om eventueel optredende klachten te noteren op het klachten registratie formulier (zie hieronder) dat u na de provocatie mee krijgt.

Als uw kind binnen 48 uur na de provocatie duidelijke klachten krijgt vragen wij u contact op te nemen met de arts- onderzoeker:

....., telefoon

Na de voedselprovocatie houdt u zich nog strikt aan het dieet. Na afloop van de hele provocatietest wordt de uitslag met u besproken. Afhankelijk van de test uitslag wordt hierna het cashewnoot vrije dieet voortgezet, of dat cashewnoot weer wordt geïntroduceerd in het dieet. Hiervoor komt u nog 1 x naar de kliniek. U ontvangt hiervoor nadere mondelinge en schriftelijke richtlijnen.

Bij twijfel, eventuele reacties thuis of vragen kunt u altijd eerder contact opnemen met het de arts/verpleegkundigen op tel nr ... of bel de huisarts.

Noteer hieronder zo nauwkeurig mogelijk welke verschijnselen er gedurende de 48 uur (2 dagen) na de provocatie op traden, en of u medicatie heeft gegeten:

Datum	Tijdstip	Verschijnselen/allergische klachten:	Medicatie gegeven? Naam, dosering, tijdstip