Cashew Nut Allergy in Children

Hanna Kuiper – van der Valk

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Cashewnootallergie bij kinderen

Hanna Kuiper-van der Valk

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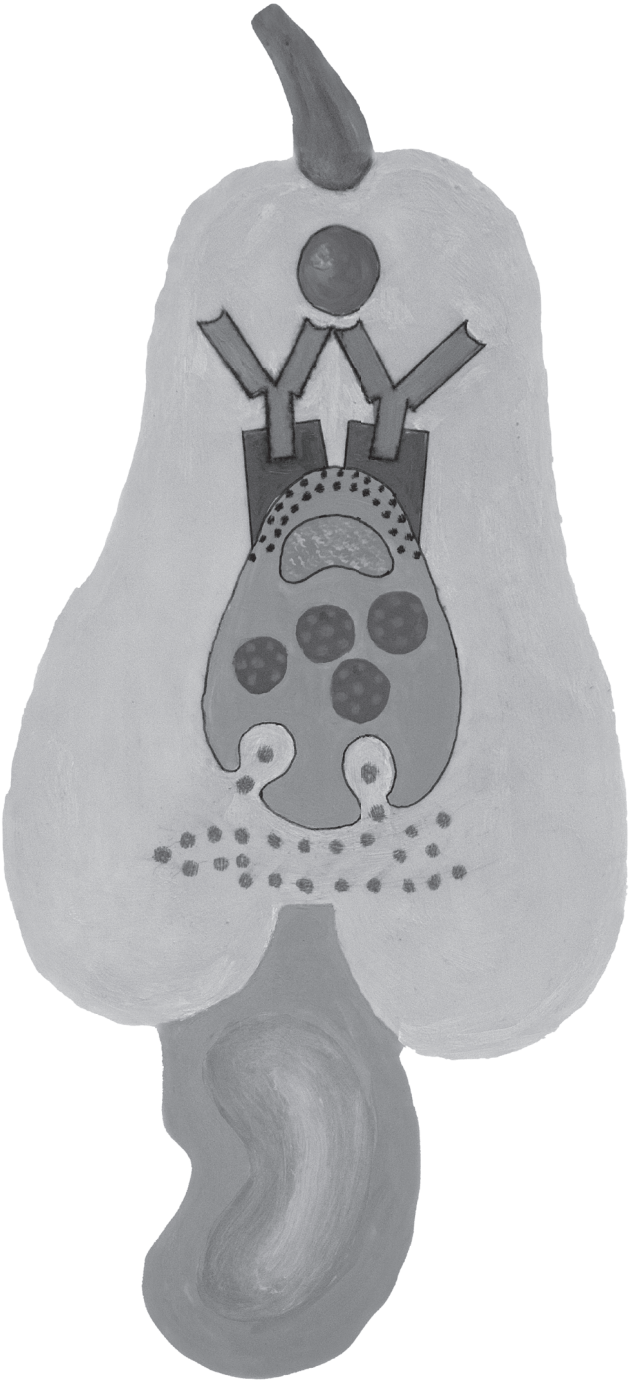
CONTENTS

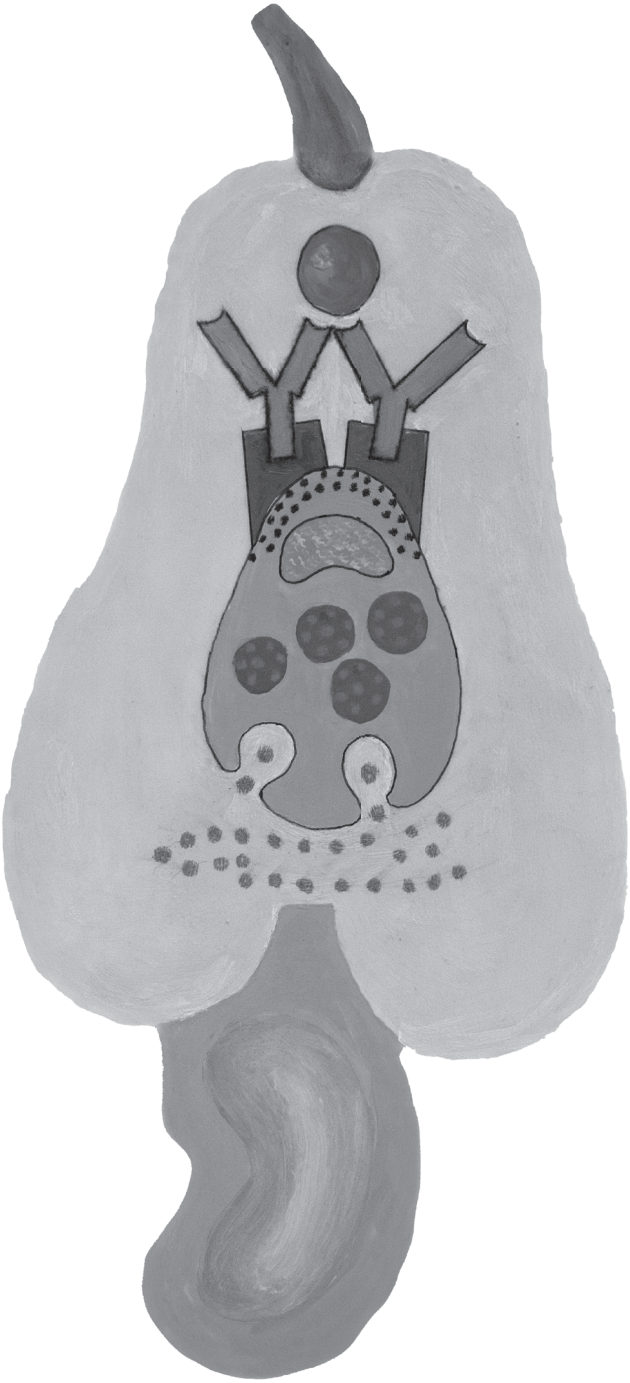
ABBREVIATIONS

AUC	Area Under the Curve
CF	Child Form
C-index	Concordance-index
CRD	Component Resolved Diagnosis
HEP	Histamine Equivalent Prick
ED	Eliciting Dose
FAIM	Food Allergy Independent Measure
FAQLQ	Food Related Quality of Life Questionnaire
FC	Food Challenge
HRQL	Health Related Quality of Life
IDEAL	Improvement of Diagnostic mEthods for ALlergy assessment
DBPCFC	Double-Blind Placebo-Controlled Food Challenge
LOAEL	Lowest Observed Adverse Effect Level
LR	Likelihood Ratio
NOAEL	No Observed Adverse Effect Level
OR	Odds Ratio
OFC	Open Food Challenge
TF	Teenager Form
PF	Patient Form
ROC	Receiver Operating Characteristic
sIgE	Specific IgE
SPT	Skin Prick Test



Chapter 1

General introduction



Chapter 1.1

Food allergy

PATHOPHYSIOLOGY

Food allergy is an unintentional reaction of the immune system to an innocuous foreign substance (food allergen) with relevant clinical symptoms as a result. The patient has to be previously in contact with the allergen or a similar allergen from another source to become sensitised. This sensitisation may occur by consumption, however, also by skin contact, inhalation or in utero (1). Patients with an atopic constitution have a predisposition to develop specific IgE (sIgE) to food or inhalation allergens and to becoming sensitised. The patient is allergic if the sensitisation results in typical allergic symptoms. This typical allergic reaction is called an immediate or anaphylactic type-I reaction (Gell and Coombs classification)(2).

During the first exposure to an allergen, activation of Th2-cells takes place, which in turn activates the B- cells to produce sIgE. This sIgE binds during re-exposure to the antigenic determinant (epitope) of the allergen and to the Fc-receptors, present on the surface of the effector cells (mast cells and basophils). Cross-linking between two sIgE molecules on the effector cell by the allergen causes activation of the basophils and mast cells, which results in signal transduction and receptor mediated exocytosis of the mediator vesicles (3).

The phenomenon of allergen cross-reactions occurs when antibodies responsible for the allergic reaction bind to the original allergen and to similar allergens e.g. in case of botanically related allergens (4). However, these allergens bind often with different affinity. The cross-reaction can occur between food allergens mutually and food allergens and inhalation allergens. Allergic reactions based on cross-reactivity are in general mild.

CLINICAL PRESENTATION

The clinical presentation of food allergy can encompass gastro-intestinal symptoms (oral allergy, nausea, vomiting, stomach pain and vomiting), cutaneous symptoms (urticaria, redness, itchiness and angioedema), eye symptoms, upper airway symptoms, lower airway symptoms, cardio-vascular symptoms and indefinite symptoms (change in behaviour and pallor/feeling weak). In particular in children, the allergic reaction can manifest with indefinite symptoms as tiredness, listless and crying (5). The symptoms can be divided into objective and subjective symptoms. Subjective symptoms are symptoms according to the patients and the physician could not objectify this. Objective symptoms can be measured or observed. The most severe immediate type-I allergic reaction is called anaphylaxis and is defined in the guidelines for food allergy and anaphylaxis (6).

DIAGNOSTIC PROCEDURE

The first step in the diagnostic procedure of food allergy is taking the history. The reaction type and the time interval between exposure to the allergen and onset of symptoms are important in determining whether there may be an allergy. Allergy testing starts with demonstrating sensitisation by either a skin prick test (SPT) or specific IgE (sIgE). The oral food challenge (FC) test could determine the clinical relevance of sensitisation. The double-blind placebo-controlled (DBPCFC) test is considered as the gold standard. The DBPCFC is been carried out in two days. The placebo and active challenge material are randomly administered on these days with at least a one-week interval. The doctor and patient are not informed on which day the active challenge material (verum) or placebo is given. Blinding would be opened after the challenge test. The interpretation of the challenge test results is important to diagnosis food allergy or tolerance (7).

The diagnostics of food allergy has been improved recently with the use of component resolved diagnosis (CRD). CRD measures sIgE against individual allergens utilising native or recombinant allergens (8). Native allergens are purified from allergen extracts and recombinant allergens are biotechnology produced by bacteria or yeasts.

CRD could distinguish between a genuine sensitisation and a sensitisation based on cross-sensitisation of food allergens mutually or food allergens and inhalation allergens (pollen- food syndrome). CRD gives insight in the sensitisation pattern and the risk of an allergic reaction (9). There are relatively harmless, unstable allergens e.g. profilines (10) and potential dangerous allergens that are extreme resistant to proteolysis, heat denaturation and pH changes as the allergens of the Prolamin superfamily e.g. Lipid Transfer Proteins (LTPs)(11) and 2S albumines (12). Pathogen related proteins, like PR-10 proteins play an important role in pollen- food syndrome and cause usually mild to moderate symptoms as 'oral allergy' (13).

CRD is a relatively new in the diagnostic work-up of food allergy, but is rapidly incorporated into clinical use. Therefore, the clinician must have knowledge on the chemical, physical and immunological characteristics of the different allergenic families to understand the CRD test results (9). Next to this single-plexed allergen assays, multi-plexed allergen assays are also available as ImmunoCAP ISAC. The ImmunoCAP ISAC provided sIgE results for 112 components from more than 51 allergens simultaneously, using only 30 µl serum (14). This test method is useful if there is enquiry for many components and as consequence high costs or in patients with high complex sensitisation patterns of food and inhalation allergens (9).

CASHEW NUT ALLERGY AND CASHEW NUT COMPONENTS

There are indications that the cashew nut is an upcoming important allergen (15–16). The cashew nut (*Anacardium occidentale*) belongs to the *Ancardiaceae* family and is botanically related to pistachio (*Pistacia Vera*) nut and the mango (*Mangifera indica)*. The rapid increase in processing of cashew nuts in food product and the change in eating and cooking habits may be responsible for the increasing significance of cashew nut allergy (16). Previous studies demonstrated that a minimal amount of cashew nut can cause severe reaction in cashew sensitised patients (17). Therefore, research on cashew nut allergy is important to make clinician aware of this allergy.

The major allergen components of the cashew nut are Ana o 1, Ana o 2 and Ana o 3 (18–21). Ana o 1 is a vicilin-like protein, resistant to heat and proteolysis. The other two allergens are Ana o 2, a legume-like protein and Ana o 3, a 2S albumin. All three allergens are classified as seed storage proteins. A previous already demonstrated that sensitisation to Ana o 3 is highly predictive of clinical reactivity in cashew nut sensitised patients (22).

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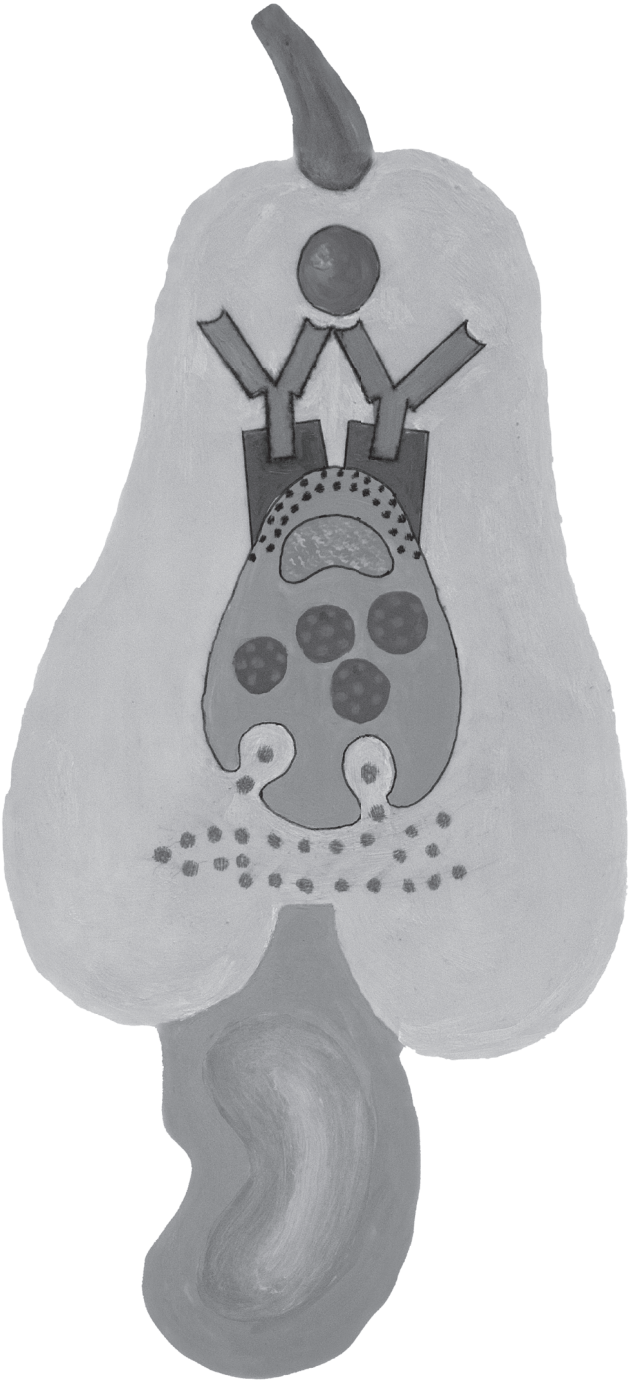


Chapter 1.2

Aim and outline of the thesis

It is important for the patient to diagnose cashew nut allergy in a safe and efficient way with less false negative and false positive test results. The SPT, sIgE determination and the open and DBPCFC challenge test fall short of this. The DBPCFC as gold standard is costly, time-consuming and a potentially stressful test for the patient. Therefore, the study Improvement of Diagnostic mEthods for ALlergy assessment (IDEAL) was developed with cashew nut allergy as a show-case.

The aim of this study is:

1) To investigate *the clinical presentation of cashew nut allergy* i.e the clinical relevance of cashew nut sensitisation and the clinical reaction patterns during the DBPCFC tests with cashew nut.
2) To *optimise the diagnostic procedures of cashew nut allergy* by improvement of the measurement and interpretation of the SPT and to improve the diagnostic procedure of food allergy by combining different diagnostic tools: history, standard diagnostics (SPT and sIgE) and component resolved diagnosis (CRD) i.e. IgE-levels to Ana o 1, Ana o 2 and Ana o 3, to finally predict the outcome of the DBPCFC with a model.
3) To address the *clinical consequences of cashew nut allergy* as the clinically relevance of co-sensitisation between cashew nuts, pistachio nut and mango, the effect of DBPCFC test on health-related quality of life in children participating in a study of cashew nut allergy and the rate of cashew nut introduction after a negative challenge test.

The content of the thesis is more comprehensive and contributes also to the knowledge of the cashew nut and cashew nut allergy.

The first paper '*Systematic review on cashew nut allergy'* gives an overview of the relevant literature on the cashew nut and cashew nut allergy.

The second paper *'Multicentre double-blind placebo-controlled food challenge study in children sensitised to cashew nut'* shows the clinical results of the IDEAL- study. This article demonstrates the clinical relevance of cashew nut sensitisation and the clinical reaction patterns during the DBPCFC tests with cashew nut.

The third paper '*Threshold dose distribution and eliciting dose of cashew nut allergy',* shows the threshold distribution curve of the IDEAL-study and the LOAELs in a subgroup of children who reacted to the lowest dose of cashew nut protein (1 mg).

The objectives of the fourth paper *'Measurement and interpretation of skin prick test results'* are to compare different techniques of quantifying SPT results, to determine a cut-off value for a positive SPT for histamine equivalent prick -index (HEP) area, and to study the accuracy of predicting cashew nut reactions in DBPCFC tests with the different SPT methods.

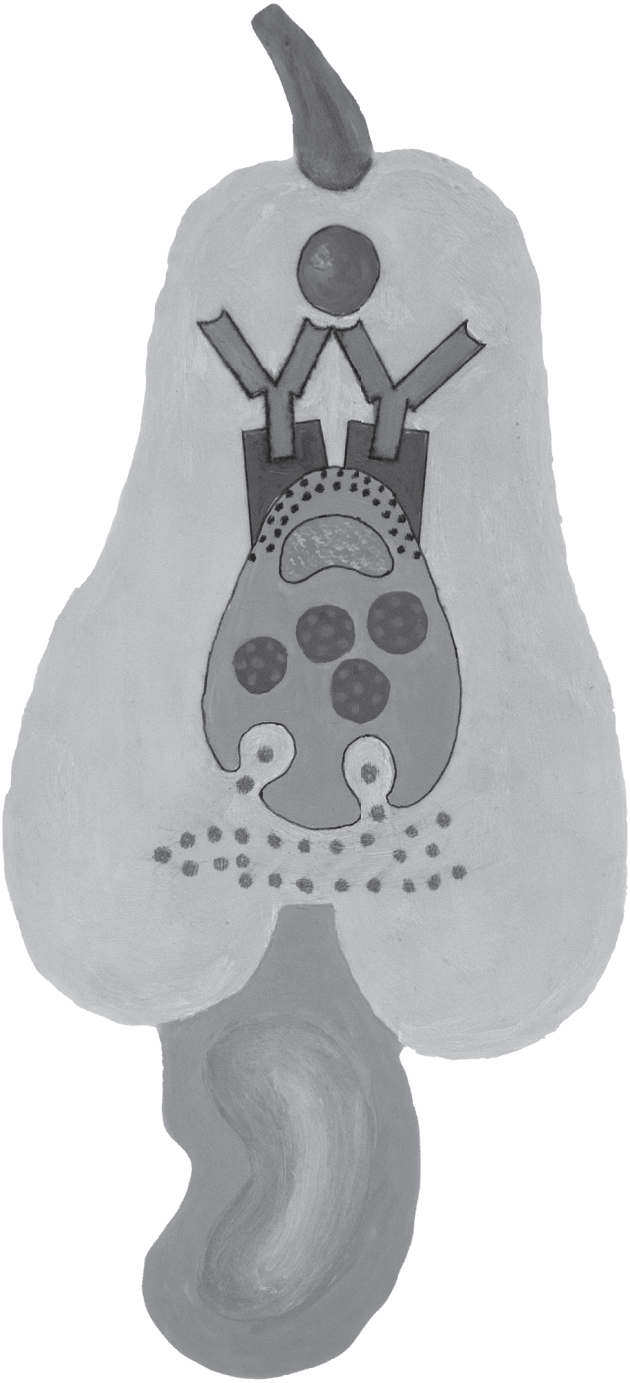
We investigate in the fifth paper *'sIgE Ana o 1, 2 and 3 accurately distinguish tolerant from allergic children sensitised to cashew nuts'* the performance of component sIgE determinations in diagnosing cashew nut allergy.

We describe in the sixth paper *'Prediction of cashew nut allergy in sensitised children'* a multivariate model to predict the outcome of the DBPCFC test with cashew nut.

The seventh paper: *'Low percentage of clinically relevant pistachio nut and mango co-sensitisation in cashew nut sensitised children'* assess the clinical relevance of pistachio nut and mango co-sensitisation in cashew nut sensitised children.

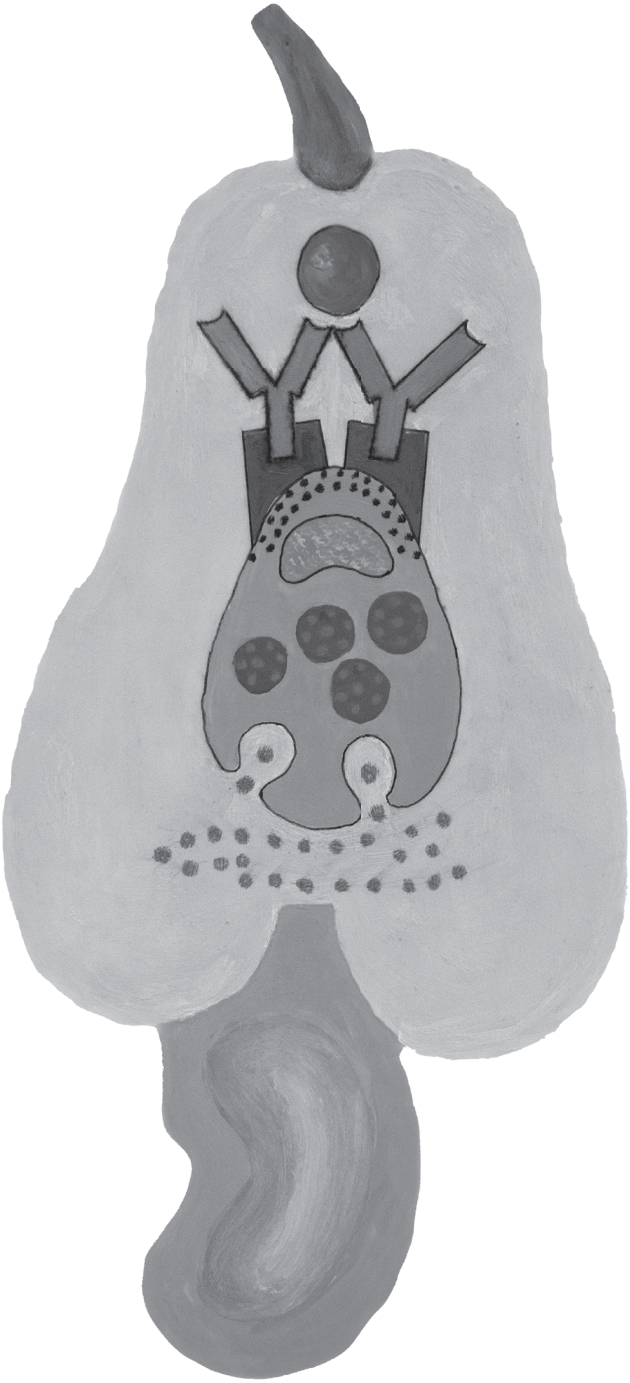
The eight paper '*No difference in health-related quality of life, after a food challenge with cashew nut in children participating in a clinical trial'* demonstrates the effect on quality of life in positive and negative challenged children using the food allergy quality of life questionnaires (FAQLQ's) before and after the DBPCFC test.

The last paper '*Failure of introduction of cashew nuts after a negative food challenge test*' is a letter to the editor on the rate of introduction of cashew nuts after a negative food challenge test.



Chapter 2

Literature overview



Chapter 2.1

Systematic review on cashew nut allergy

This paper gives an overview of the relevant literature on cashew nut and cashew nut allergy. This article is published in Allergy in 2014.

Van der Valk JP, Dubois AE, Gerth van Wijk R, Wichers HJ, de Jong NW. Systematic review on cashew nut allergy. Allergy 2014;69:692-8.

ABSTRACT

Recent studies on cashew nut allergy suggest that the prevalence of cashew nut allergy is increasing. Cashew nut consumption by allergic patients can cause severe reactions, including anaphylaxis. This review summarises current knowledge on cashew nut allergy to facilitate timely clinical recognition and to promote awareness of this emerging food allergy amongst clinicians. The goal of this study is to present a systematic review focused on the clinical aspects of allergy to cashew nut including the characteristics of cashew nut, the prevalence, allergenic components, cross-reactivity, diagnosis and management of cashew nut allergy.

The literature search yielded 255 articles of which 40 met our selection criteria and were considered to be relevant for this review. The 40 articles included one prospective study, six retrospective studies and seven case reports. The remaining 26 papers were not directly related to cashew nut allergy. The literature suggests that the prevalence of cashew nut allergy is increasing, although the level of evidence for this is low. A minimal amount of cashew nut allergen may cause a severe allergic reaction, suggesting high potency comparable with other tree nuts and peanuts. Cashew allergy is clearly an underestimated important healthcare problem, especially in children.

INTRODUCTION

Although peanut allergy has been on the increase for two decades or more, studies indicate that cashew nut is also becoming an important food allergen (1, 2). The rapid increase in consumption of cashew nuts and the change in eating and cooking habits may be responsible for the increasing significance of cashew nut allergy (2). In this paper, we summarise the relevant information available on epidemiology, allergen components, clinical features, diagnosis, clinical and in vitro cross-sensitisation and management of cashew nut allergy.

DATA SOURCES AND LITERATURE SEARCH

In our search, we adhered to the methods and procedures of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines for reporting this systematic review, excluding irrelevant items. Registration number in PROSPERO is CRD42013004047. We used Ovid MEDLINE and EMBASE databases to identify relevant articles using the string: (Anacardi*(tw) OR cashew*(tiab)) AND (Hypersensitiv*(tw) OR hyper sensitiv* (tw) OR allerg*(tw)) for Ovid MEDLINE and (Anacardi* OR cashew*): de, ab, ti AND (Hypersensitiv* OR (hyper NEXT/1 sensitiv*) OR allerg*): de, ab, ti for EMBASE. We also checked references to relevant articles ('snowballing'). We aimed to include studies focused on the clinical aspects of cashew nut allergy. We considered only studies in English. There was no restriction on publication date. Mouse model studies were excluded. Initially, all articles on cashew nut allergy or on cashew nuts were included. Thereafter articles on contact dermatitis, genetics, product labelling, poisoning, detection methods and possible medicinal effects of cashew plants were excluded. Forty of 255 articles found with the literature search (244 articles) and by 'snowballing (11 articles) were considered relevant for the review. Of these 40 articles, one article was a prospective study and six articles were retrospective studies. Five of these seven articles focused on clinical symptoms and constitute a major source for this review. In addition, seven case reports about cashew nut were located. The remaining 26 articles are mainly descriptive and not directly related to cashew nuts or cashew nut allergy. This literature selection procedure is shown schematically in Figure 2.1. Apart from this selection, we added literature to describe the characteristics of the cashew nut, the prevalence, allergenic components, cross-reactivity and diagnosis of cashew nut allergy.

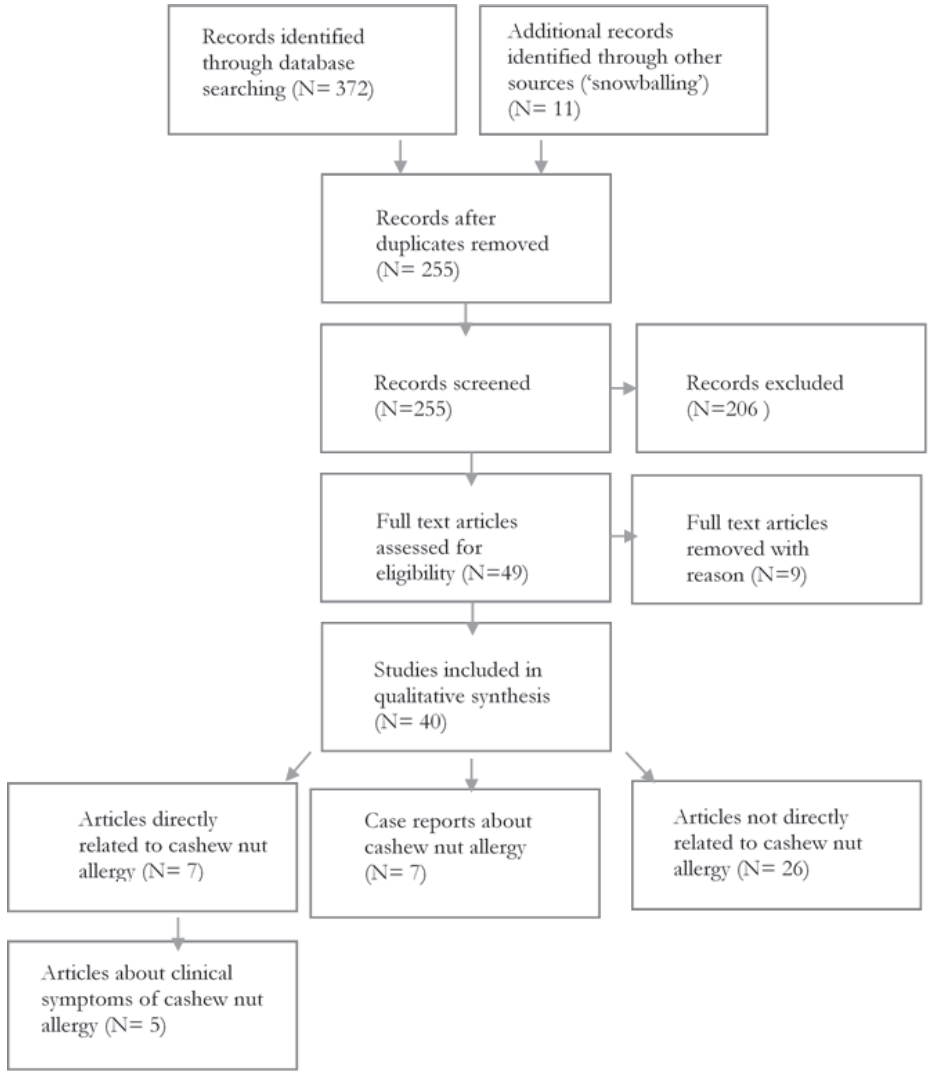


Figure 2.1: Summary of the search and selection.

RELEVANT PAPERS

The five relevant studies on clinical symptoms of cashew nut allergy are presented in Table 2.1. The prospective study by Rance et al. (2) analysed the clinical features and results of skin prick tests (SPT), specific IgE (sIgE) assays and food challenge tests of 42 children with cashew nut allergy without an associated peanut allergy. The study by Davoren et al. (3) described the clinical features, including anaphylactic reactions, to tree nuts and peanuts. Clark et al. performed a retrospective case-matching study in children referred

for either cashew nut (47 children) or peanut (94 children) allergy. The severities of the most severe reactions were compared (4). The paper by Hourihane et al. (1) reported the clinical features of cashew nut allergy in 26 paediatric and three adult subjects, whose history of reaction was supported by positive SPT or raised cashew-specific IgE. Grigg et al. (5) performed a retrospective chart review and phone survey to identify the clinical characteristics of cashew nut allergic patients in comparison with peanut allergic patients. The paper by Corderoy et al. was a retrospective chart review. This study evaluated the mean SPT wheal diameter, cashew sIgE, age at challenge and previous clinical history to determine whether any of these variables predicted the risk of a subsequent reaction during oral food challenges (6). The retrospective study by York et al. (7) investigated the ethnicity of 100 children with a clinical history of cashew nut allergy.

Table 2.1: Relevant studies on clinical symptoms of cashew nut allergy

Author	Year	Type of study	Number of cases	Children/ adults	Symptoms (% and *n* =)
Rance (2)	2003	Prospective study	42	Children	Respiratory 25% (28/112)* Cutaneous 56% (63/112) Gastro-intestinal 17% (19/112)
Davoren (3)	2011	Retrospective chart review	27	Children	Anaphylaxis: 74.1% (20/27)† Respiratory: 15% (3/20) Respiratory, cardiovascular system, skin: 5% (1/20) Respiratory, skin, gastro-intestinal 25% (5/20) Respiratory and skin: 40% (8/20) Respiratory and gastro-intestinal 15% (3/20) Non- anaphylaxis: 25.9% (7/27) Skin 100% (7/7
Hourihane (1)	2000	Retrospective study	29	Children and adults	Wheeze: 48% (14/29) Collapse/feeling faint: 38% (11/29)
Grigg (5)	2009	Retrospective chart review	16	Children	Anaphylaxis: 50% (8/16)‡ Respiratory: 50% (8/16) Cutaneous: 72.4% (11/16) Gastro-intestinal: 18.8% (3/16) Eye symptoms: 18.8% (3/16)
Clark (4)	2007	Case-matching study	47	Children	Cutaneous: 98% (46/47) Gastro-intestinal: 32% (15/47) Rhino-conjunctivitis: 6% (3/47) Wheeze: 40% (19/47) Laryngeal oedema: 9% (4/47) Cardiovascular: 13% (6/47) Lightheaded: 13% (6/47)

*42 cases, 112 events.

†Defined as a rapidly evolving generalized multisystem allergic reaction characterised by cardiovascular involvement and involvement of other systems (skin and/or gastro-intestinal (5).

‡As defined by the Second Symposium on the definition and management of anaphylaxis (36).

CASHEW NUT AND CASHEW NUT ALLERGY

The cashew nut

The Portuguese discovered the cashew nut in northeastern Brazil in the sixteenth century and exported the cashew nut tree to other continents (8). The cashew nut (*Anacardium occidentale*) belongs to the Ancardiaceae family. Botanically the cashew nut is actually a seed and not a nut, but historically it has been referred as to a nut. The cashew nut is kidney-shaped and grows on the bottom of the cashew apple. It is surrounded by a shell as well as a layer of toxic oil. Because of this toxic oil, the cashew nut must be roasted before it is safe to eat. Sixty per cent of cashew nuts are consumed as a snack, and the remaining 40 per cent is processed in products such as butters, pestos, bakery – and confectionary items, sweets, ice creams and chocolates (1, 5, 9). The cashew nut is used especially in the Indian, Thai and Chinese cuisines. In the world production of edible nuts, the cashew nut ranks as third with Vietnam, Nigeria, India and Brazil as the major cashew nut exporters. Cashew nut cultivation is not organized on a plantation scale in most producing countries. The price of the cashew nuts is much higher than that of peanuts and other nuts because of the labour-intensive manner of processing required to turn the raw nut into the edible cashew nut (9). The world production of cashew nuts has experienced a rapid growth. A tenfold increase has been observed during the last 50 years. The world production of cashew nuts was approximately 1.24 million tonnes in 2000 and increased to approximately 3.58 million tonnes in 2010 (9).

EPIDEMIOLOGY

Many published reports deal with the prevalence of tree nut allergy in general. Although cashew nut allergy is reported as a common tree nut allergy, we found only a few studies on the prevalence of cashew nut allergy (10). The search yielded studies suggesting an increase in cashew nut allergy in children and an increased recognition of cashew nut allergy in clinical practice (2, 3, 5). In a study by Tariq et al. (11), 0.08% of children under 4 in the United Kingdom were found to be sensitised to cashew nuts. Moneret-Vautrin et al. (12) reported that 41% of the nut allergic patients in France were sensitised to cashew nut. Hasegawa et al. (13) observed relatively more cashew nut allergy in female adults. The study of York et al. (7) indicates that cashew nut allergy may be more prevalent in the Asian population. Forty-one of 100 patients derived from a multicultural paediatric allergy clinic in Leicester (UK) with a clinical history suggestive of cashew nut allergy were from Asian/ Asian British background compared with only 21% with a history suggestive of allergy to other nuts. A possible explanation for this finding is that Asian children have earlier exposure to cashew nuts because of dietary practices leading to

more cashew nut allergy compared with other populations (7). Despite the impression that sensitisation and clinical allergy to cashew nut are increasing, methodologically rigorous studies documenting this have not yet been performed.

ALLERGENS

The major cashew allergens are Ana o 1, Ana o 2 and Ana o 3. Ana o 1 is a 50 kDa vicilin-like protein resistant to heat and proteolysis. The other two known allergens are Ana o 2, a 33 kDa legume-like protein, and Ana o 3, a 13 kDa 2S albumin (14–16). All three allergens are classified as seed storage proteins. Of patients allergic to cashew nut, 50% (10 of 20 sera) are sensitised to recombinant Ana o 1, 62% (13 of 21 sera) to recombinant Ana o 2 and 81% (21 of 26 sera) to recombinant Ana o 3 determined by Western immunoblotting (14, 15, 17). Allergens from these families of seed storage proteins are known to be allergenic in other tree nuts, legumes and seeds.

CLINICAL CROSS-REACTIVITY, CROSS-REACTIVITY IN VITRO AND CO-SENSITISATION

The cashew nut as well as the pistachio (*Pistacia vera*) nut and the mango (*Mangifera indica*) belong to the *Anacardiaceae* family and are thus botanically related. A high degree of serological cross-reactivity has been established between cashew nut and pistachio (Pis v 1, Pis v 2 and Pis v 3) by sIgE- inhibition tests. This may be explained by the highly conserved primary and three-dimensional structure of these allergen homologue pairs, present in both cashew nut and pistachio (18–22). Clinical cross-reactivity between cashew nut and pistachio was suggested in the study by Noorbakhsh et al. and the study by Willison et al. (20, 23). Garcia et al. and Quercia et al. (19, 24) also reported clinical cross-reactivity between cashew and pistachio, although sIgE inhibition tests were not performed. Cross-reactivity between pistachio nut and mango seed has also been established by sIgE- inhibition tests. Information on the molecular basis of serological or clinical cross-reactivity between cashew and mango fruit, and on which proteins could be involved, is not available (25). Allergens with a high degree of homology with cashew nut in their allergenic proteins include hazelnut, mustard seed, peanut, pistachio, sesame, soybean and walnut (15–18, 23). Co-sensitisation is seen between the cashew nut and almond, hazelnut, orange seed, pistachio, peanut, pectin and walnut (2, 3, 19, 22, 25–27). Sensitisation against cashew nut allergy seems to be a primary sensitisation rather than a cross-reaction between cashew nuts and pollens. An overview of the homology, clinical cross-reactivity, cross-reactivity in vitro and co-sensitisation between cashew nut and other allergens is shown in Table 2.2.

Table 2.2: Overview of allergen homology, co- and cross-sensitisation and co- and cross-reactivity between cashew nut and other food allergens

Allergen	Allergen homology	Co-sensitisation (*n*/total)	Serological cross-reactivity	Clinical dual reactivity (*n*/total)	Probable clinical cross-reactivity
Pistachio	Wilison (23)	Rance (2) (28/42) Garcia (19) (3/3) Sansosti (22) (1/1) Fernandez (2) (4/42)	Willison (23) Noorbakshs (20) Hasegawa (13) Parra (21)	Garcia (19) (1/3) Ferdman (26) (1/1)	Noorbakhsh (20) Willison (23)
Mango	-	-	-	-	-
Walnut	Barre (18) Wang (17) Robotham (15)	Rance (2) (4/42)	-	-	-
Almond	-	Rance (2) (10/42)	-	-	-
Hazelnut	Barre (18)	Rance (2) (6/42)	-	-	-
Peanut	Barre (18) Wang (16)	Clark (3) (13/47)	-	-	-
Soybean	Wang (16) Wang (17)	-	-	-	-
Orange seed	-	O 'Sullivan (27) (35/100)	-	-	-
Pectin	-	Ferdman (26) (1/1)	Rasanen (35)	-	-
Sesame	Wang (17) Robotham (15)	-	-	-	-
Mustard seed	Robotham (15)	-	-	-	-

- Literature not found

CLINICAL FEATURES

The age of onset of cashew nut allergic symptoms varies between 2 months and 27 years with a mean of approximately 3 years (1, 2, 4, 5). Most allergic reactions to cashew nut, such as other food allergies, manifest with skin lesions followed by respiratory and gastro-intestinal symptoms (Table 2.1). The study of Davoren et al. showed cutaneous involvement as the initial symptom in 100% of the non-anaphylactic cases. The initial symptoms in most anaphylactic patients are respiratory, often combined with skin symptoms. On the other hand, 30% of the anaphylactic cases had no cutaneous reaction, which might make it difficult to recognize anaphylaxis (3). Compared with peanut allergy, cashew nut allergy causes more gastro-intestinal symptoms (5). Cashew nut causes severe allergic reactions similar to responses to other tree nuts and peanut (2, 3, 28) and can be lethal in both adults and children (29). However, some studies reported anaphylactic reactions even more frequently to cashew nut than to peanut (50% and 30%, respectively) (5). This was also found in the study by Davoren et al. of 214 children with peanut or tree nut allergy. Thirty per cent of the peanut and 74% of the cashew

nut sensitised patients developed an anaphylactic reaction after ingestion (3). Although being suggestive, this analysis did not correct for other possible factors, which might bias these results, such the eliciting doses or a history of asthma. Clark et al. performed a case-matching study of children with a history of a reaction after cashew nut or peanut ingestion and evidence of sensitisation (positive skin prick test). Children with the most severe reaction to peanut ingestion were matched 2: 1 to children with the most severe reaction to cashew nut ingestion. This study showed no significant differences in clinical features between the cashew nut and peanut group, except asthma (more prevalent in the peanut group). This study reported that allergic reactions to cashew nuts are often more severe than reactions to peanuts, with more frequent bronchoconstriction and cardiovascular symptoms in the cashew group despite the fact that asthma was a more frequent co-morbidity in the peanut group (4). At the Royal Children's Hospital in Melbourne, 117 anaphylactic reactions occurred over a 5-year period, more frequently to cashew nut than to peanut (18% and 13%, respectively). However, it is not described whether this difference is statistically significant and the percentages are not adjusted for other risk factors (28). The study by Davoren et al. showed that five of 27 patients with cashew nut allergy had an allergic reaction after only skin or mucosal contact. One of these five patients developed anaphylaxis (3). This suggests significant reactions even to minimal levels of exposure. Blom et al. determined the eliciting doses in 31 patients with a positive double-blind placebo-controlled food challenge test (DBPCFC) and found that the protein dose, at which 50% of the allergic population was likely to respond (ED50), was 25.4 mg (any type of symptoms) which is comparable to peanut (17.2 mg) and hazelnut (13.5 mg) and clearly lower than that of egg or milk (82.0 and 82.6 mg, respectively) (30). The severity of accidental reactions to cashew reactions could be increased further by the fact that compared with peanut and hazelnut, cashew is more often in particulate form resulting in higher doses. However, further research is necessary. These data collectively suggest that cashew nut allergy may be considered an exceptionally potent allergen that is a relatively frequent cause of anaphylaxis.

DIAGNOSIS

Corderoy et al. showed that patients with positive or negative cashew nut challenge tests do not differ in median cashew nut sIgE. In contrast, however, the SPT was significantly larger in patients with positive challenge tests. Skin prick tests seem to be superior to sIgE in predicting challenge outcome (6). The reliability of SPT depends on several factors such as age, method of skin prick testing and quality of the extract. A cut-off value of ≥ 8 mm (SPT) gave a 95% positive predictive value for a positive challenge test outcome (31). However, the size of the study and the population characteristics limit,

however, the generalizability of the data. There are currently no studies reporting the relative importance of sensitisation to major allergens of cashew in predicting clinical reactivity to cashew nut or the severity of such reactions. A relatively new approach is component-resolved diagnosis, which might be useful to determine sIgE to cashew nut allergens.

ALGORITHM FOR THE DIAGNOSIS OF CASHEW NUT ALLERGY

Cashew nut allergy is often diagnosed by history, combined with measuring sensitisation by skin prick test and in vitro specific IgE tests. As with other foods, the latter tests do not distinguish very well between clinical allergy and asymptomatic sensitisation. For the diagnosis, the gold standard remains DBPCFC, according to international guidelines DBPCFC should not be used in case of a clear-cut history of anaphylaxis after consumption of cashew nuts (32). The fact that this test is time-consuming, labour-intensive, expensive and not entirely without risk has prompted research into the development of models predictive of clinical reactivity based on other parameters. DunnGalvin et al. developed a prediction model for peanut allergy, which might replace DBPCFC. When validated in the same centre, the model showed an AUC of 0.97 to predict peanut allergy (33). However, this prediction model was not able to predict peanut allergy in a Dutch study (34). A prediction model has not yet been developed for cashew nut allergy.

MANAGEMENT

The mainstay of therapy in food allergic patients is avoidance of the allergic food. This is increasingly difficult to achieve in cashew nut allergic patients because of the increase in cashew nuts in many food products. Causal treatment for food allergy in form of oral immunotherapy (OIT) is in development. Oral immunotherapy for food such as egg, milk and peanut seems to be a promising way to induce desensitisation or tolerance despite the difficulties, such as the side effects and doses schedule. Possibly OIT can play a role in the treatment for cashew nut allergy in the future. Furthermore, avoidance of botanically related foods such as pistachio must be advised in case of established cashew nut allergy. More research is needed to better underpin an advice on avoidance of botanically related foods with allergenic homology to cashew nut.

SUMMARY AND CONCLUSION

Recent studies on cashew nut allergy suggest that the prevalence of cashew nut allergy has increased. Whilst this may be a real increase, increased cashew nut consumption may be revealing more cases, and more cases may be noticed because of increased awareness of patients and doctors. The latter seem less likely given the often severe nature of reactions to cashew nut. The major allergenic proteins described in cashew nuts to date are legume-like proteins and 2S albumins. The DBPCFC test is currently the gold standard to establish cashew nut allergy. Cashew nuts allergens are apparently highly potent and can cause relatively severe reactions. They are a relatively common cause of anaphylaxis and can cause death. Avoidance of pistachio nuts must currently be advised in case of a cashew nut allergy, but advice of avoidance of other related allergens needs further investigation. In comparison with literature and research focussed on peanut, cashew allergy is clearly an underestimated but important healthcare problem, especially in children. Further research is urgently needed on this relatively new food allergen, including allergenic content, diagnostic tools and dietary advice for the patients required to prevent severe anaphylactic reactions.

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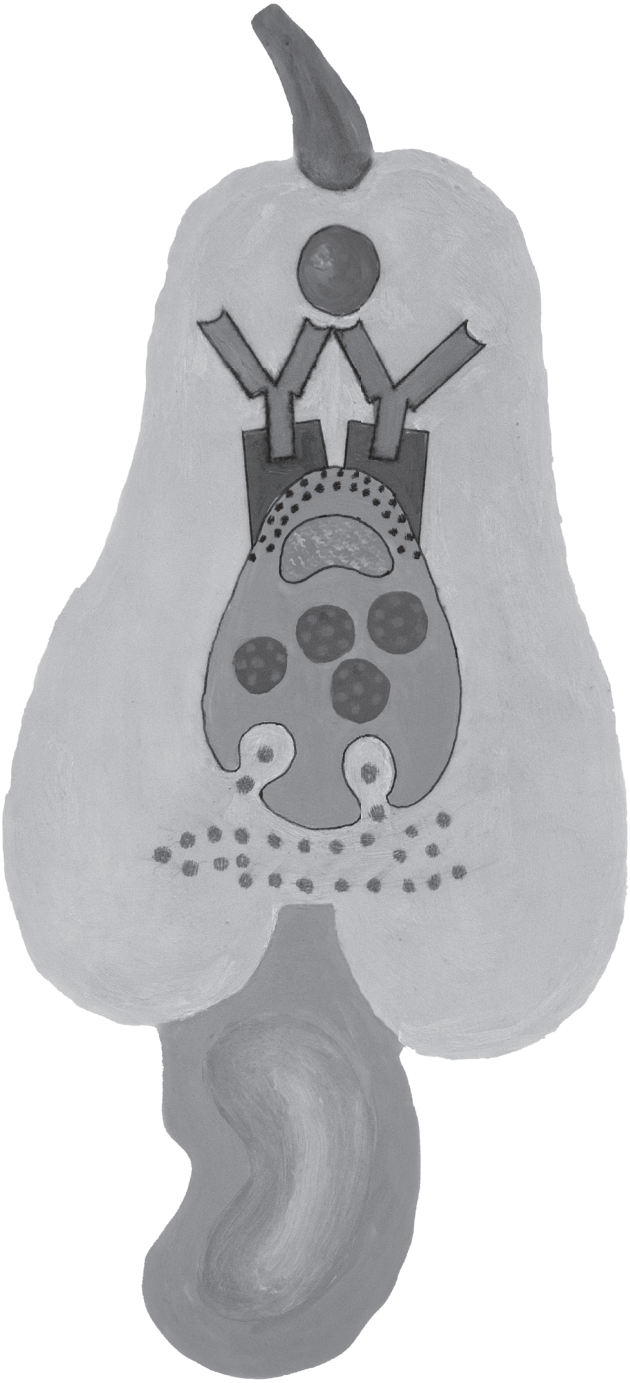
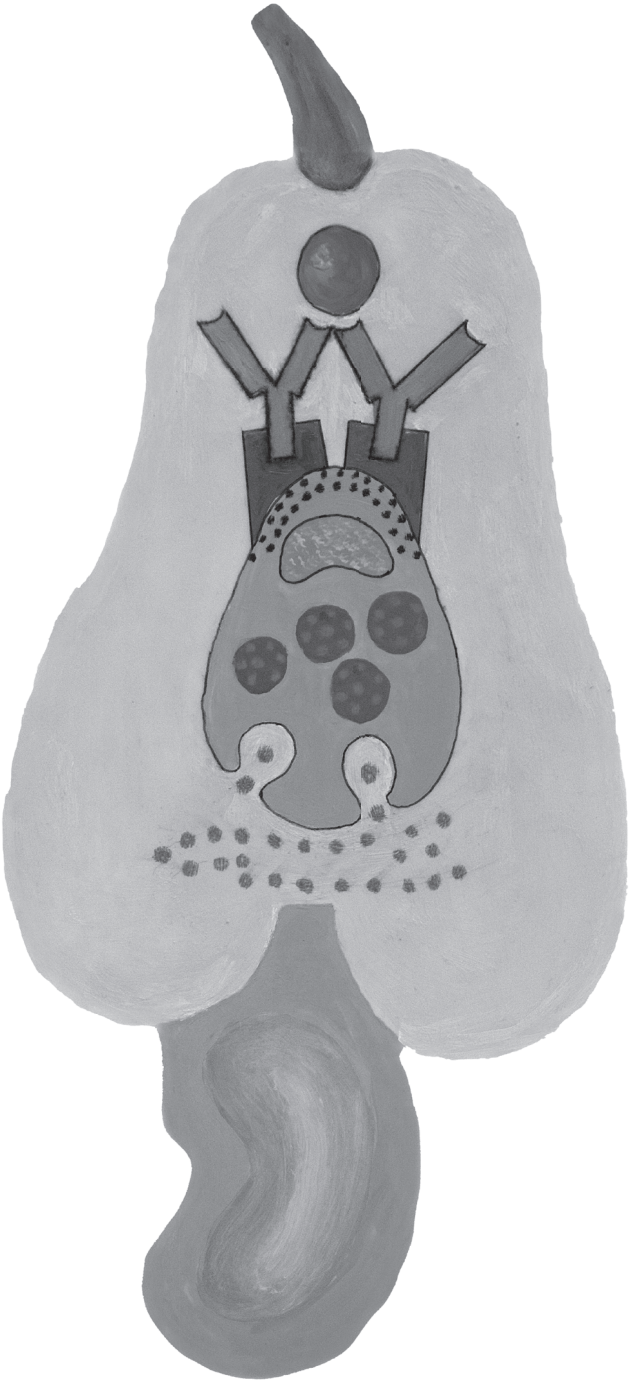


Chapter 3

Clinical results of the IDEAL-study



Chapter 3.1

Multicentre double-blind placebo-controlled food challenge study in children sensitised to cashew nut

This article demonstrates the clinical relevance of cashew nut sensitisation and the clinical reaction patterns during the DBPCFC tests with cashew nut. This article is published in PloS One in 2016.

Van der Valk JP, Gerth van Wijk R, Dubois AE, de Groot H, Reitsma M, Vlieg-Boerstra B, Savelkoul HF, Wichers HJ, de Jong NW. Multicentre double-blind placebo-controlled food challenge study in children sensitised to cashew nut. PLoS One. 2016 Mar 11;11(3).

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.

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.

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 (3).

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 3.1 and a flowchart of the patient inclusion is shown in Figure 3.1.

Table 3.1: Inclusion and exclusion criteria

Inclusion criteria
Age between 2 and 17 years.
Positive skin prick test (mean wheal diameter ≥ 3 mm Ø and HEP-index area ≥ 0.4 and/ or detectible 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.

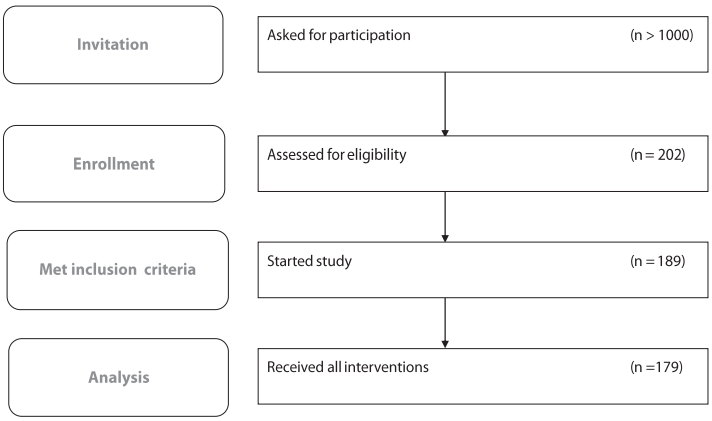


Figure 3.1: Flowchart of patient inclusion

Study procedure

The study program consisted of three clinical visits and a telephone consultation, as shown in Table 3.2. 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.

Table 3.2: 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 Session 1	DBPCFC test Session 2	Result DBPCFC test	Dietary advice
Medical history (2 questionnaires)				
Physical examination				
Blood samples (19 ml)				
Skin prick 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).

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.

METHODS

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 3.3.

Table 3.3: 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.

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.

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 3.4.

Table 3.4: 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%)

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%) (Figure 3.2).

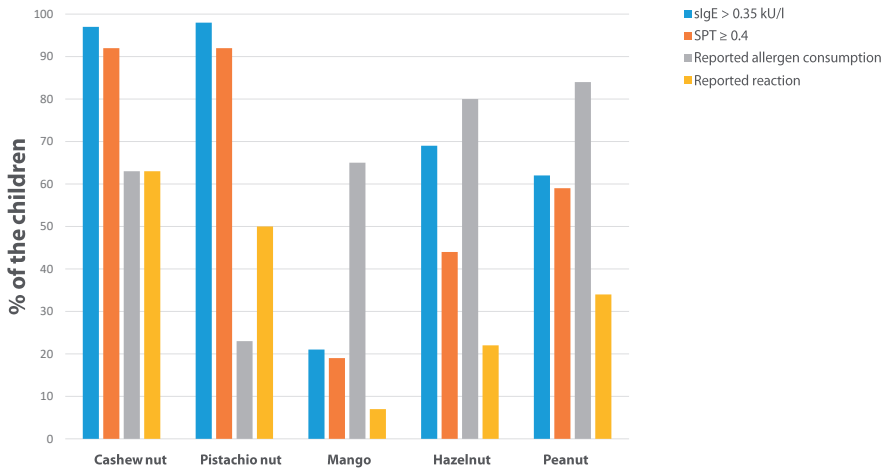


Figure 3.2: History and sensitisation to tested allergens
This figure shows the history and sensitisation to cashew nut and the history and co-sensitisation to pistachio nut, mango, hazelnut and peanut.

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

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 3.5). 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 3.6). A total of 8 children (6%) with a positive reaction

Table 3.5: 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

Table 3.6: Anaphylactic reactions during positive DBPCFC tests

Anaphylaxis	Total N = 49/137 (36%)
S kin 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.

were treated with epinephrine. A single dose (0.15ml < 25 kg and 0.30ml > 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%). Figure 3.3 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.

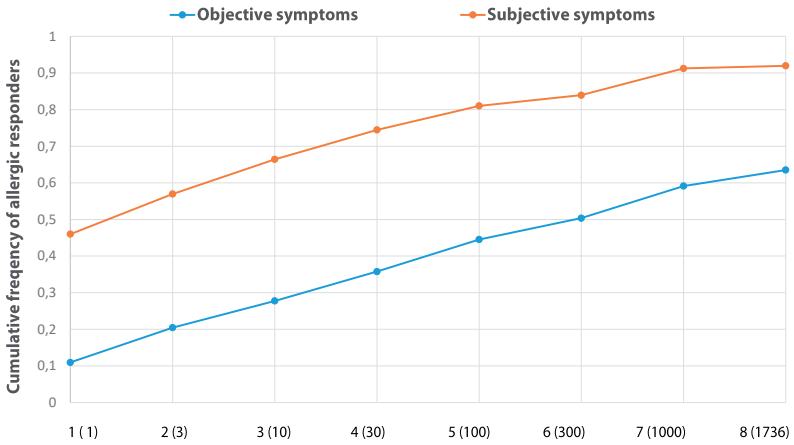


Figure 3.3: Threshold distribution curve for objective and subjective symptoms in cashew nut allergic children

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 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 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 sensitised 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. `

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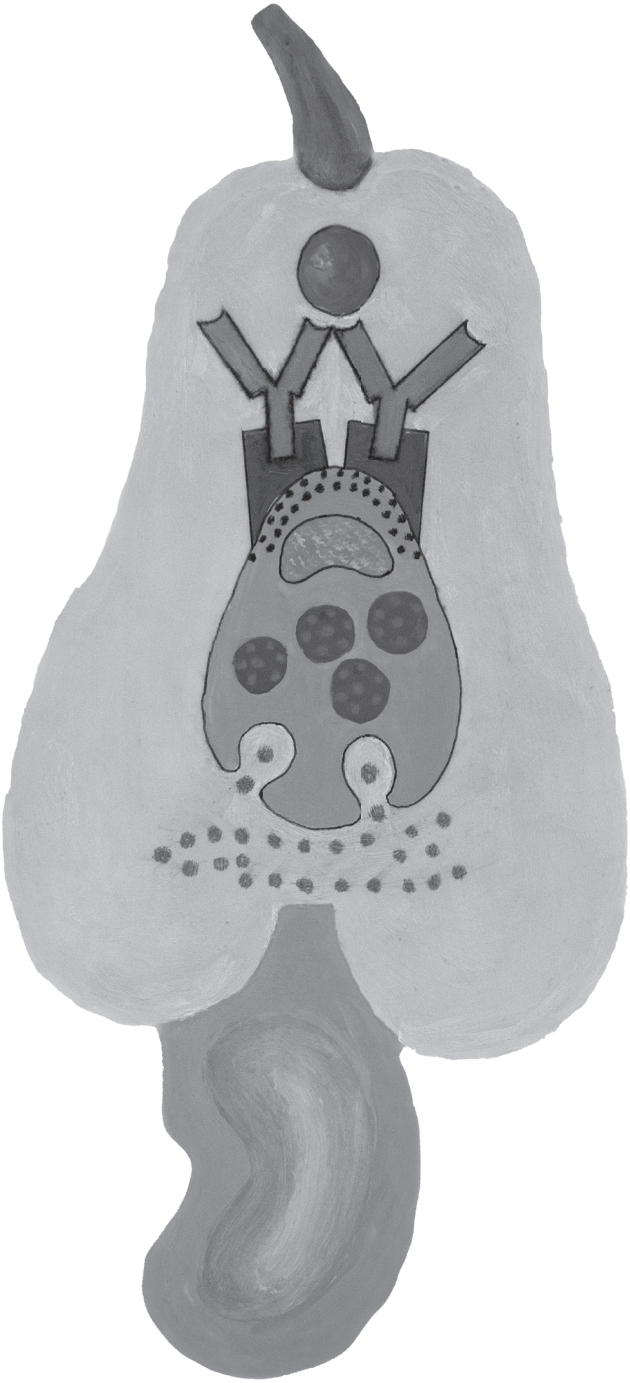


Chapter 3.2

Threshold dose distribution and eliciting dose of cashew nut allergy

This letter shows the threshold distribution curve of the IDEAL-study and the lowest observed adverse effect levels in cashew nut allergic children. This letter is accepted by Annals of Allergy, Asthma & Immunology in 2016.

Van der Valk JP, Gerth van Wijk R, Baumert JL, Nordlee JA, Vlieg-Boerstra B, de Groot H, Dubois AE, de Jong NW. Threshold distribution and lowest observed adverse effect levels in cashew nut allergic children. Ann. of Allergy, Asthma & Immunology. Annals of Allergy, Asthma & Immunology in 2016. Accepted.

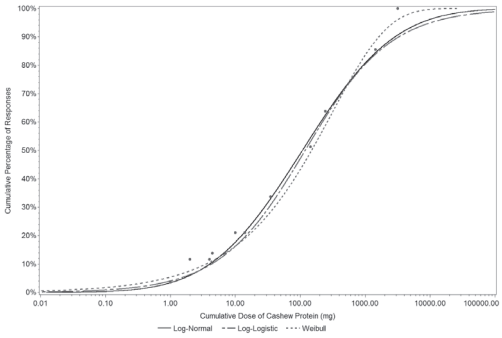
A previous study by our group demonstrated that 137/179 (76.5%) of the cashew nut sensitised children suspected of cashew nut allergy had a positive double-blind, placebo-controlled food challenge (DBPCFC), with 63/137 (46%) children manifesting subjective and/or objective symptoms to the lowest dose (1 mg cashew nut protein) (1). The primary aim of this study was to determine the distribution of threshold doses and the eliciting doses (EDs) in this population. The secondary aim was to investigate whether children that reacted to 1 mg cashew nut (n = 63) could react to even lower doses than 1 mg: the Low-dose follow-up study.

The children participated in the 'Improvement of Diagnostic mEthods for ALlergy assessment' (IDEAL-study, trial number NTR3572). The inclusion and exclusion criteria and detailed study protocol with stop criteria for the DBPCFC were previously described (1). Briefly, we measured sensitisation (specific IgE [sIgE] and skin prick test [SPT]) with cashew nut extract and performed DBPCFC tests with eight-step incremental doses regime (1, 3, 10, 30, 100, 300, 1000, 1736 mg of cashew nut proteins) (2). All children who reacted to 1 mg cashew nut in the IDEAL-study were asked to participate in a Low-dose follow-up study, that consisted in DBPCFC with six-step incremental dose regime, starting with 0.01 mg, followed by increasing doses of 0.03, 0.10, 0.30, 1, and 3 mg cashew protein, performed between 4 and 30 months after the initial IDEAL- challenges. The low-dose challenges were considered positive if objective or subjective symptoms occurred. There were no stop criteria, all patients completed the low-dose DBPCFC test to step 6, unless not medically justified/ unethical or if the patient refused to continue the test. To facilitate the weighing of these small doses, ground cashew nuts were diluted 1:10 with granulated sugar, according to the technical method of Taylor et al. (3).

The Interval-Censoring Survival Analysis (ICSA) approach was utilized to analyse the NOAEL and LOAEL intervals for each allergic individual as described previously (4). The SAS LIFEREG procedure (v.9.2) was used to fit the Log-Normal, Log-Logistic and Weibull parametric distributions based upon cumulative doses for this cashew-allergic population and confidence intervals were calculated. The eliciting doses (EDs) were determined (4).

The patient characteristics and diagnostic results of the 179 participating children of the IDEAL-study were previously described (1). The median age of the children was 9.0 years (range 2–17 years), with 106 boys (59%) and 73 girls (41%). 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) (5). Most children experienced gastro-intestinal symptoms (nausea, vomiting, stomach pain and diarrhea) (72%), followed by oral allergy symptoms (64%), skin symptoms (redness and itchiness) (28%), angioedema (27%) and urticarial symptoms (21%).

The Low- dose follow-up study included 12/63 children (10 girls [83%] median age 13.0 yrs.).



		Cumulative ED_{05} (mg protein)		Cumulative ED_{10} (mg protein)		Cumulative ED_{50} (mg protein)	
Log-Normal	1.6	0.8, 3.3 (95% CI)	4.1	2.2, 7.6 (95% CI)	108.4	64.4, 182.5	(95% CI)
Log-Logistic	1.4	0.6, 3.3 (95% CI)	4.3	2.1, 8.8 (95% CI)	118.8	70.1, 201.3	(95% CI)
Weibull	0.8	0.3, 2.5 (95% CI)	3.5	1.5, 8.4 (95% CI)	149.1	93.9, 236.7	(95% CI)

*Cumulative objective eliciting doses were calculated using Interval Censoring Survival Analysis and were fitted to the Log-Normal, Log-Logistic and Weibull probability distribution models. All doses were calculated in mg of cashew protein.

Figure 3.4: Cumulative distribution (%) of objective threshold in 137 cashew nut allergic children. Distribution curves are based on LOAEL and NOAELs for objective symptoms. Data were fitted with the use of different statistical models (Log- Normal, Log- Logistic and the Weibull).

Cumulative distribution curves for (%) of objective eliciting threshold in the 137 cashew nut allergic children was measured (Figure 3.4). It was not possible to calculate the threshold distribution curve for subjective symptoms, because of the high percentage of children (46%) reacting to dose 1 with subjective symptoms. The doses at which 5, 10 or 50% of the cashew-allergic population (ED_{05}, ED_{10} and ED_{50}, respectively) would be expected to experience objective symptoms ranged from 0.8 to 1.6 mg, 3.5 to 4.3 mg and 108.4 to 149.1 mg of cashew nut protein for the ED_{05}, ED_{10} and ED_{50}, respectively, based upon the Log-Normal, Log-Logistic and Weibull models.

Of the 12 Low-dose challenge tests, 8 were positive, 3 were negative and 1 was undetermined. As only 12 children participated and 51 did not, we compared the groups to exclude selection bias (Fisher's exact test, Mann Whitney u test). There was no significant difference in terms of age ($p = 0.83$), sIgE to cashew ($p = 0.46$), SPT to cashew ($p = 0.21$) and severity of reaction during the DBPCFC with cashew nut ($p = 0.75$). Only gender differed significantly ($p = 0.004$). The lowest dose of cashew nut protein to which subjective symptoms occurred was 0.01 mg, while for transient objective symptoms (red skin), this was 0.30 mg. Placebo reactions during the low-dose challenge test were reported in a higher percentage (4/12 children, 33%) than in the original challenge test (20/179 children, 11%). These 4 placebo reactions during the Low-dose follow-up study, were most likely caused by increased anxiety. One challenge was therefore undetermined, and the other 3 placebo reactions consisted mainly of mild oral allergy symptoms, in contrast to more severe symptoms as stomachache, nausea, tiredness, feeling of swollen

throat and erythema during the verum day. Consequently, there was no doubt about the positive outcome of these challenges.

Three children reacted to a higher dose of cashew nut protein and four patients did not react at all in the low-dose challenge test. We could not find a relation in time interval between the IDEAL-study and the Low-dose follow-up study, (higher or lower doses reactions) as being a cause of the difference in reaction doses in both studies. Previously, Glaumann et al. observed in 29 peanut-allergic patients that only two of these children reacting to the same threshold dose and with the same severity score in two successive food challenge test with peanut (6).

Concerning ED studies, the study by Blom et al. showed a much higher ED_{05} in 31 cashew nut allergic children at 7.4 mg cashew nut protein compared to the ED_{05} in our study (7). The authors indicate in the discussion that this is an unexpectedly high quantity taking into account that cashew nut allergy is considered to be as severe as a peanut allergy. The study by Taylor et al. showed in 286 peanut allergic patients an ED_{05} for objective symptoms of 7.3 mg whole peanut (equivalent to 1.8 mg of peanut protein based on 25% protein in a peanut kernel) (8). Our study demonstrated a lower cashew nut ED_{05} for objective symptoms than $_{EDs}$ for other allergens as reported in the above-mentioned study.

Minimal eliciting doses for different allergenic foods were previously investigated by an expert panel in a study on threshold dose by Taylor et al. (9). This study demonstrated that the eliciting dose on which 1% (ED_{01}) of the population reacted with objective symptoms were 0.1 mg (Log-Logistic) and 0.22 mg (Log-Normal) for peanut. The ED_{01} ranges between 0.02 mg and 0.25 mg protein for hazelnut based upon the Log-Normal, Log-Logistic and Weibull models. The 0.30 mg cashew nut protein as lowest eliciting dose of mild objective symptoms in our Low-dose follow-up study is in the same order of magnitude.

In conclusion, the statistically determined eliciting dose on which 5% of this cashew nut population reacted with objective symptoms was very low (0.8 to 1.6 mg cashew nut protein). Individual patients may react to as little as 0.3 mg and 0.01 mg cashew nut protein with mild objective symptoms and subjective symptoms, respectively. However, the low-dose challenge tests were only performed in 12 children, they were not reproducible, and the children reported mainly subjective symptoms, which makes interpreting the low-dose data with caution.

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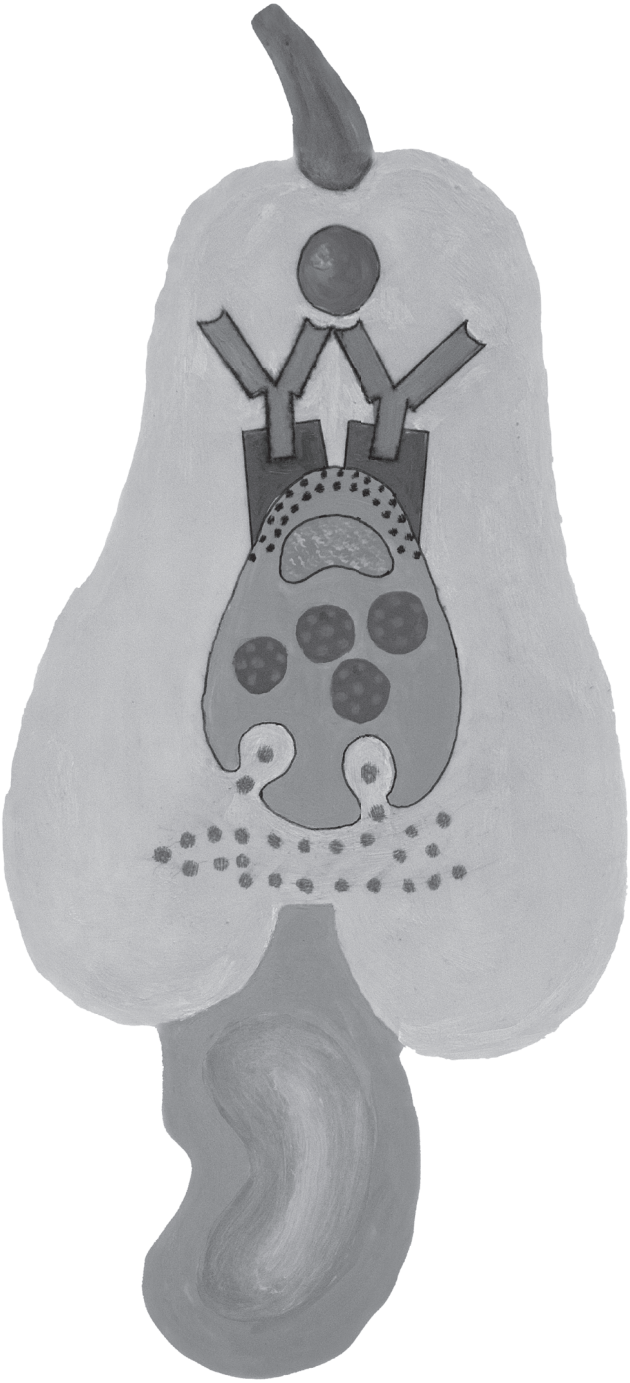
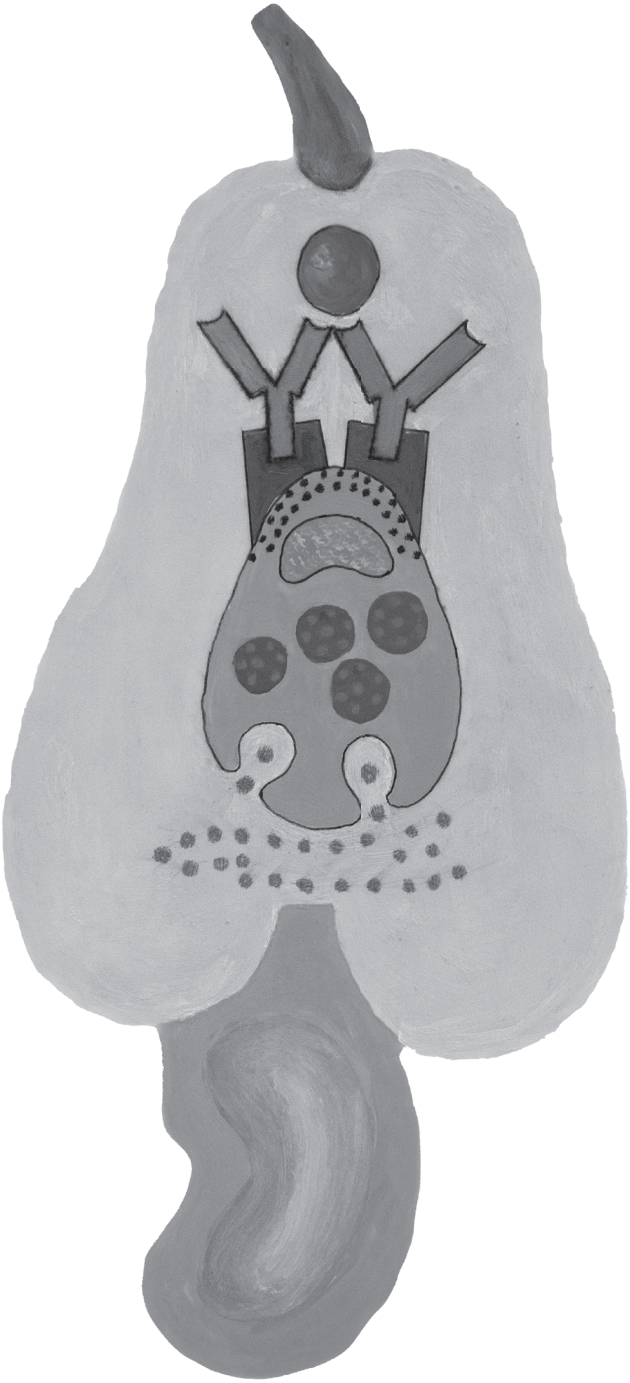


Chapter 4

Improvement of diagnostic methods



Chapter 4.1

Measurement and interpretation of skin prick tests results

In this paper, the different techniques of quantifying SPT results are compared, a cut-off value for a positive SPT for histamine equivalent prick -index (HEP) area is determined, and the accuracy of predicting cashew nut reactions in double-blind placebo-controlled food challenge (DBPCFC) tests with the different SPT methods are studied. This article is published in Clinical and Translational Allergy in 2016.

Van der Valk JPM, Gerth van Wijk R, Hoorn E, Groenendijk L, Groenendijk IM, de Jong NW. Measurement and interpretation of skin prick test results. Clin. Transl. Allergy. 2016.6:8.

ABSTRACT

Background

There are several methods to read skin prick test results in type-I allergy testing. A commonly used method is to characterise the wheal size by its 'average diameter'. A more accurate method is to scan the area of the wheal to calculate the actual size. In both methods, skin prick test (SPT) results can be corrected for histamine-sensitivity of the skin by dividing the results of the allergic reaction by the histamine control. The objectives of this study are to compare different techniques of quantifying SPT results, to determine a cut-off value for a positive SPT for histamine equivalent prick -index (HEP) area, and to study the accuracy of predicting cashew nut reactions in double-blind placebo-controlled food challenge (DBPCFC) tests with the different SPT methods.

Methods

Data of 172 children with cashew nut sensitisation were used for the analysis. All patients underwent a DBPCFC with cashew nut. Per patient, the average diameter and scanned area of the wheal size were recorded. In addition, the same data for the histamine-induced wheal were collected for each patient. The accuracy in predicting the outcome of the DBPCFC using four different SPT readings (i.e. average diameter, area, HEP-index diameter, HEP-index area) were compared in a Receiver-Operating Characteristic (ROC) plot.

Results

The 'scanned area method' is more accurate, as expected, in measuring wheal area size than the 'average diameter method'. A wheal average diameter of 3 mm is generally considered as a positive SPT cut-off value and an equivalent HEP-index area cut-off value of 0.4 was calculated. The four SPT methods yielded a comparable Area under the Curve (AUC) of 0.84, 0.85, 0.83 and 0.83, respectively. The four methods showed comparable accuracy in predicting cashew nut reactions in a DBPCFC.

Conclusions

The 'scanned area method' is theoretically more accurate in determining the wheal area than the 'average diameter method' and is recommended in academic research. A HEP-index area of 0.4 is determined as cut-off value for a positive SPT. However, in clinical practice, the 'average diameter method' is also useful, because this method provides similar accuracy in predicting cashew nut allergic reactions in the DBPCFC.

INTRODUCTION

Standard diagnostics for Type-I acute allergic reactions to foods are based on the patient's history combined with sensitisation tests and, optionally, a food challenge test. Tests to measure sensitisation comprise in vitro specific IgE (sIgE) determination and skin prick testing (SPT). The outcome of the SPT can result in a variety of wheal shapes, and there are several methods to measure these outcomes. In clinical practice and in most academic research, it is common to characterise the wheal shape by the 'average diameter' (1). However, with this method, it is implicitly assumed that the wheal may be described reasonably well by an ellipse or circle, which is not always the case in practice and this method is prone to errors. For this reason, a more advanced scanning method for SPT measurement has been applied for more than a decade in the Erasmus Medical Centre in Rotterdam. To even further increase the accuracy of SPT results, the histamine-induced wheal size of the positive control might be considered as well to correct for skin histamine sensitivity. Furthermore, differences in technique of performing SPTs (inter-observer variability) contribute to the variation in wheal size. We divided the area (or diameter) of the allergen-induced wheal by the area (or diameter) of the positive histamine-induced wheal controls to correct for these factors. This ratio is defined as the Histamine Equivalent Prick (HEP)-index area (or diameter) or histamine-equivalent wheal sizes (HEWS)(2). The first objective of this study is to compare different techniques of quantifying SPT results. The second objective is to determine a cut-off value for area, HEP-diameter and HEP-index area equivalent to the standard used average diameter cut-off value of 3 mm, whereby the HEP-index area is considered as the most important, because of the accuracy of this method (area measurement) and the correction for skin sensitivity (HEP-index measurement). The last objective is to study the accuracy of diagnosing cashew nut allergic reactions in the double-blind placebo-controlled (DBPCFC) tests with the 4 SPT methods.

METHODS

Study design

In this parallel study, data of 172 children (2–17 years of age) with a cashew nut sensitisation (sIgE and/or SPT) were used for the analysis (trial number NTR3572). All patients underwent a SPT with cashew nut extract and a DBPCFC test with cashew nut. Medical ethical approval was obtained and all patients signed informed consent.

Skin prick tests

The children underwent a SPT with homemade cashew nut extracts, a positive control (Histamine 10 mg/ml ALK-Abello, Nieuwegein, the Netherlands) in duplicate and a negative control. The SPT was performed by applying a drop of the allergen extract on the skin of the volar aspect of the forearm. 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 (3).

Different techniques quantifying skin prick test results

The outcome of the SPT can result in a variety of wheal shapes, as shown in Figure 4.1. To determine the average diameter, the mean value of the longest and the midpoint orthogonal diameter (mm) of the wheal were measured (Figure 4.2). The area of the wheal was determined by using a flatbed scanner (Hewlett Packard) in combination with software earlier developed by Erasmus MC: Precise Automated Area Measurement of Skin Test (PAAMOST). Mean values of two histamine-induced wheal sizes of the positive control were collected as well. Based on the measured data the HEP-indices were calculated for both the average diameter and area.

Figure 4.1: Typical observed wheal forms in SPT's

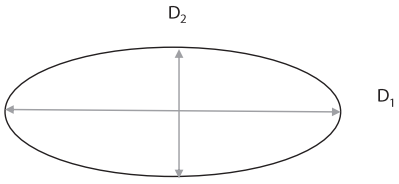


Figure 4.2: Definition of D1 and D2

Consequently the four readings were defined as:

1. Average diameter (allergen-induced average wheal diameter)
2. Area (allergen-induced area measured by scanning device)
3. HEP-index diameter (allergen-induced average diameter divided by histamine-induced average diameter)
4. HEP-index area (allergen-induced area divided by the histamine-induced average area)

Food challenge test

The children underwent a DBPCFC test with cashew nut. The validated and standardised food challenge material used in the DBPCFC was prepared according to the recipe developed by Berber-Vlieg et al (4). The DBPCFC was considered as positive when 1) objective symptoms occurred, 2) when subjective symptoms occurred twice on three successive administration of the challenge material, or 3) when subjective symptoms persisted for more than one hour. In total, 137 children had a positive challenge test.

Analysis

Receiver operating characteristics (ROC) curves and Area under the Curve (AUC) were calculated to evaluate the different SPT methods. An area under the curve of 0.9–1 is considered as excellent, 0.8–0.9 as good and 0.7–0.8 as fair. All analyses were done with SPSS software, 20th edition.

RESULTS

Skin prick test

In total 172 SPT results with cashew, positive (in duplicate) -and negative control were evaluated. Median histamine wheal diameter was 5.38 mm (range 2.75–10.75 mm). All negative controls were negative. Mean variability between the duplicate measurements of histamine was 14% (range 0–100%). Median average diameter, area, HEP-diameter and HEP-index area of the SPT with cashew were 10.50 mm (range 0–26 mm), 71.8 mm^2 (range 0–324.1 mm^2), 1.83 (range 0–5.13) and 2.97 (range 0–15.16), respectively.

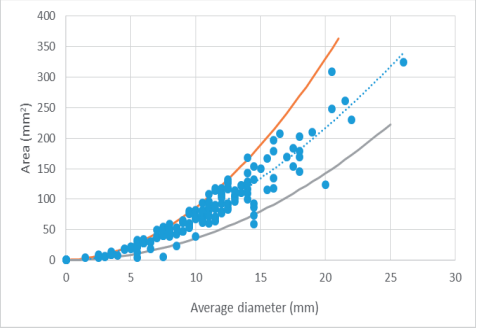


Figure 4.3: Average diameter (method 1) versus scanned area (method 2)

Different techniques of interpreting skin prick test results

As a first step of assessing the different techniques of interpreting the STP results, a comparison is made between the common-used average diameter method ('1') and the scanned area method (PAAMOST) ('2'). These two methods are compared in a scatterplot in Figure 4.3. Every dot represents one patient. The dotted line shows the trend line of the data.

The average diameter on the horizontal axis in Figure 4.3 is defined as the mean value of the longest (D_1) and the midpoint orthogonal diameter (D_2) of the wheal, as shown in Figure 4.3:

$$D = \left(\frac{D_1+D_2}{2}\right) \quad \text{Eq. 1}$$

We introduce the parameter α as the ratio between D_1 and D_2:

$$\alpha = \frac{D_1}{D_2} \quad \text{Eq. 2}$$

In our study population of 172 patients, the parameter α varies between 1.0 and 6.67. Assuming that we can reasonably well estimate the wheal size by an ellipse, the area of the wheal (A) is defined as:

$$A = \frac{\pi}{4} D_1 D_2 \quad \text{Eq. 3}$$

In Eq.3 the wheal area is defined as a function of D_1 and D_2, while the wheal size is commonly characterised by the average diameter, in particular in method '1'. Combining Eqs.1 to 3, the wheal area can be rewritten as a function of the average diameter D and the ratio α:

$$A = \frac{\alpha}{(1+\alpha)^2} \pi D^2 \quad \text{Eq. 4}$$

The lower bound value for α is 1.0 (D_1=D_2). In this case, the wheal shape is circular and Eq. 4 simplifies to the well-known formula describing the area of circle, $A=\pi/4 \cdot D^2$. In Figure 4.3, this lower bound case (area as a circle) is shown by the red line. Based on our set of 172 patients, the upper bound value of α is 6.67. Substituting α = 6.67 into Eq. 4, the upper bound (area as an ellipse) is obtained. This is shown by the grey line in Figure 4.3. Nearly all 172 dots are lying in between these two lines, with only a few exceptions. The reason for these outliers is that an ellipse could not sufficiently well represent the shape of these wheals. From Figure 4.3 it can be concluded that characterizing the wheal size by the average diameter method could be rather inaccurate. For a given average wheal diameter, the actual wheal area could vary between 50% under and 50% above the

trend line, visually in between the red and grey line. For example, if the mean wheal diameter is 15 mm, the real wheal area could lie between 80 mm^2 (α = 6.67) and 176 mm^2 (α = 1.0), which is a rather large variation. Figure 4.3 shows also that the absolute error grows with wheal size. This inaccuracy, of up to 50%, is completely eliminated if one applies the scanning method, i.e. method '2'.

If for practical reasons, one would like to use the average diameter method, the 'best' relationship between the average diameter *D* and the wheal area *A* may be obtained out of dotted trend line in Figure 4.3. This line can be estimated by the following equation:

$$A = \frac{\pi}{6} D^2 \qquad \text{Eq. 5}$$

It is interesting to note that this expression is rather different than the commonly used expression $A=\pi/4 \cdot D^2$, which implicitly assumes a circular wheal shape.

To determine the cut-off value for HEP-index area equivalent to the standard used 3 mm average diameter cut-off value (5), comparison is made between the average diameter method ('1') and the scanned HEP- area method ('4'). These two methods are compared in a scatterplot in Figure 4.4. The dotted line shows the trend line of the data. This trend line can be estimated by the following equation:

$$HEP - index\ Area = 0{,}0096\ D^2 + 0{,}2674\ D - 0{,}5033 \qquad \text{Eq.6}$$

Substituting *D* = 3 mm into Eq. 6, the HEP-index area is obtained and results in 0.4. Therefore, a HEP-index area value of 0.4 is considered as the cut-off value for a positive SPT.

The cut-off values for area and HEP-index diameter were measured on the same method. This results in an area and HEP-index diameter cut-off values of 4.71 mm^2 and 0.6, respectively.

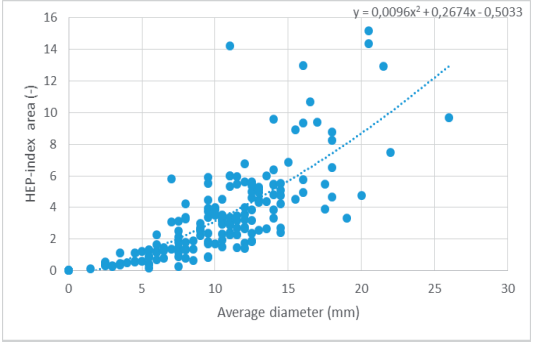


Figure 4.4: Average diameter (method '1') versus HEP-index area (method '4')

Accuracy of diagnosing cashew nut allergy

To study the accuracy of diagnosing cashew nut allergy with the four SPT methods, a ROC plot was generated. The four SPT methods, i.e. the average diameter, area, HEP-index diameter and HEP-index area, yielded a comparable area under the curve of 0.84, 0.85, 0.83 and 0.83, respectively. All four SPT methods were considered as good and equally accurate in diagnosing cashew nut allergy (Figure 4.5).

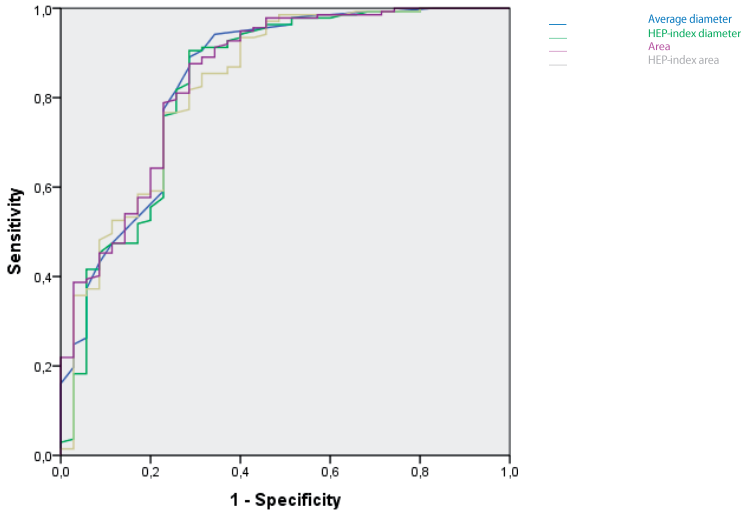


Figure 4.5: Receiver-operating characteristic curves for the 4 SPT methods

DISCUSSION

To determine the outcome of the SPT, it is common to characterise the wheal shape by the 'average diameter'. However, this method is prone to errors, because it is assumed that the wheal size varies between a circle and an ellipse. In fact, the wheals have pseudopodia and interpretation based on two orthogonal diameters is not accurate. This study showed that for a given average wheal diameter, the actual wheal area could vary quite significantly and this inaccuracy grows with wheal size. This inaccuracy is completely eliminated if one applies the scanning method. This more precise method for measuring the wheal size area is previously described by Pijnenborg et. al. (6). The scanning method is also fast, easy in use, has a high reproducibility and is very useful in scientific research (1,3,6,7).

To even further increase the accuracy of SPT results, the HEP-index can be calculated, to rule out differences in skin reactivity. There are several factors that contribute to this difference e.g. poly-sensitised patients and patients with mould sensitisation have

significantly higher skin reactions (8) and the skin response varies in different ethnicities (9). Furthermore, differences in technique of performing SPTs (inter-observer variability) contribute to the variation in wheal size (10). To correct for these factors, the calculation of the HEP-index is useful and also easy to determine with the scanning method.

Notwithstanding all advances of the scanning method inclusive the HEP-index calculation, the 'average diameter' method is as accurate in diagnosing cashew nut allergy as the 'HEP-index area' method. Therefore, the 'average diameter' method can be used if there is no scanning device available. However, the 'best' relationship between the average diameter and the wheal area can be better estimated by the equation $A = \frac{\pi}{6}D^2$ instead of the equation $A = \frac{\pi}{4}\mathrm{D}^2$. Therefore, if one wishes to calculate the area out of the average diameter for e.g. research purposes, the equation $A = \frac{\pi}{6}D^2$ should be used to approximate the area most accurate.

CONCLUSION

This study demonstrates that the scanning method for SPT measurement is more accurate to measure the wheal area in a Type-I allergy than the average diameter. The average wheal diameter gives an overestimation or underestimation of the actual area up to maximal 50%. It is possible to correct for skin sensitivity and inter-observer variability by using the 'HEP-index area' method. The HEP-index area value 0.4 can be considered as an equal cut-off value of 3 mm wheal average diameter. However, in clinical practice, the 'average diameter method' is also useful, because this method is equally accurate in predicting cashew nut allergic reactions in the DBPCFC tests.

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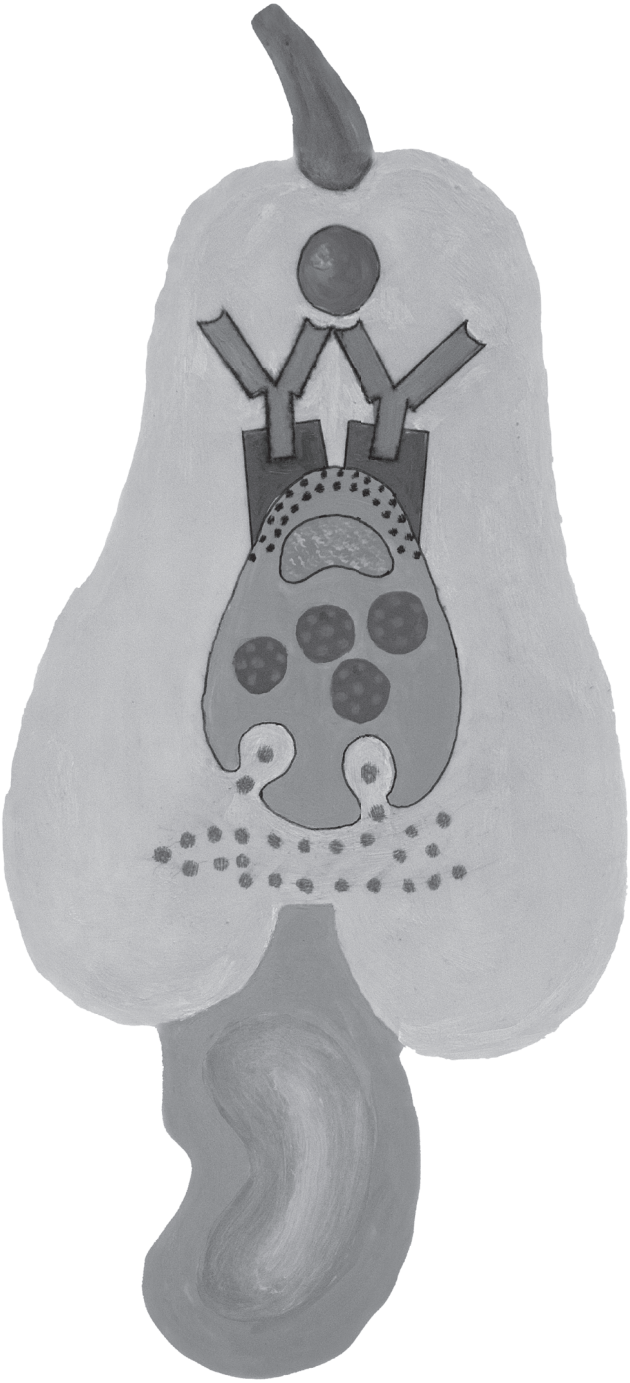

Chapter 4.2

sIgE Ana o 1, 2 and 3 accurately distinguish tolerant from allergic children sensitised to cashew nuts

In this paper, we describe the performance of component sIgE determinations in diagnosing cashew nut allergy. This article is published in Clinical and Experimental Allergy in 2016.

Van der Valk JP, Gerth van Wijk R, Vergouwe Y, Steyerberg EW, Reitsma M, Wichers HJ, Savelkoul HFJ, Vlieg-Boerstra B, de Groot H, Dubois AEJ, de Jong NW. sIgE Ana o 1, 2 and 3 accurately distinguish tolerant from allergic children sensitised to cashew nuts. Clin Exp Allergy. Aug 11 2016.

ABSTRACT

Background

The double-blind, placebo-controlled food challenge test (DBPCFC) is the gold standard in cashew nut allergy. This test is costly, time-consuming and not without side effects. Analysis of IgE-reactivity to cashew nut components may reduce the need for food challenge tests.

Methods

In a prospective and multicentre study, children with suspected cashew nut allergy underwent a DBPCFC with cashew nut. Specific IgE to cashew nut and to the components Ana o 1, 2 and 3 were determined. A skin prick test (SPT) with cashew nut extract was performed.

The association between the outcome of the food challenge test and specific IgE to Ana o 1, 2 and 3 was assessed with logistic regression analyses, unadjusted and adjusted for other diagnostic variables. Discriminative ability was quantified with a concordance index (c-index).

Results

173 children (103 boys, 60%) with a median age of 9 years were included. 79% had a positive challenge test outcome. A steep rise in the risk of a positive challenge was observed for specific IgE to each individual component Ana o 1, 2 and 3 with estimated risks up to approximately 100%. Median values of Ana o 1, 2, 3 were 1.29 kU/l (range 0–100 kU/l), 4.77 kU/l (range 0–100 kU/l) and 8.33 kU/l (range 0–100 kU/l) respectively and varied significantly ($p < 0.001$). Specific IgE to Ana o 1, 2 and 3 better distinguished between cashew-allergic and tolerant children (c-index = 0.87, 0.85 and 0.89 respectively), than specific IgE to cashew nut or SPT (c-index = 0.76 and 0.83 respectively).

Conclusion

The major cashew nut allergens Ana o 1, 2 and 3 are each individually predictive for the outcome of food challenge tests in cashew-allergic children.

INTRODUCTION

Clinicians are seeing a growing number of cashew nut sensitised patients (1, 2). The clinical relevance of cashew nut sensitisation is high as consumption of this nut can cause severe allergic reactions (3). The double- blind, placebo-controlled food challenge (DBPCFC) test is the gold standard to determine the clinical relevance of sensitisation measured by skin prick test (SPT) or specific IgE (sIgE). However, the DBPCFC test is costly and time-consuming for the patients, which warrants the search for cheaper and simpler alternatives. The performance of *in vitro* diagnosis of food allergy has recently improved with the use of component resolved diagnosis (CRD) (4). CRD measures sIgE against individual allergens utilising purified or recombinant allergens.

The major allergens of the cashew nut (*Anacardium occidentale*) are Ana o 1, Ana o 2 and Ana o 3. Ana o 1 is a vicilin-like protein, resistant to heat and proteolysis (5). The other two allergens are Ana o 2, a legume-like protein (6, 7) and Ana o 3, a 2S albumin (8). All three allergens are classified as seed storage proteins.

A case-control study by Savvatianos et al. suggested that sensitisation to Ana o 3 is highly predictive of clinical reactivity in cashew nut sensitised patients (9). To our knowledge, association between the food challenge test with cashew nut and the two other components (Ana o 1 and Ana o 2) has never been investigated.

The primary objective of this multicentre study is to investigate the added value of component analysis (Ana o 1, 2 and 3) to the standard diagnostics (history, gender, sIgE to cashew nut and the SPT) for predicting the outcome of the DBPCFC test with cashew nut.

METHODS

Study design and standard diagnostics

This study was designed as a diagnostic study and registered in the Dutch Trial Register as 'Improvement of Diagnostic mEthods for ALlergy assessment (IDEAL study)' with cashew nut allergy in children as a showcase (trial number NTR3572). 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. 179 children were included from three tertiary care centres in the Netherlands (Erasmus MC Rotterdam, University MC Groningen and Reinier de Graaf Groep Delft) in the period May 2012 to March 2015. A flowchart of the patient inclusion is shown in Figure 4.6. Medical history was obtained and blood samples were drawn to determine sIgE to cashew nut and to Ana o 1, 2 and 3 using the Siemens IMMULITE 2000 XPi Immunoassay System (Med. Imm. Laboratory; Reinier de Graaf Groep (RdGG). SPT

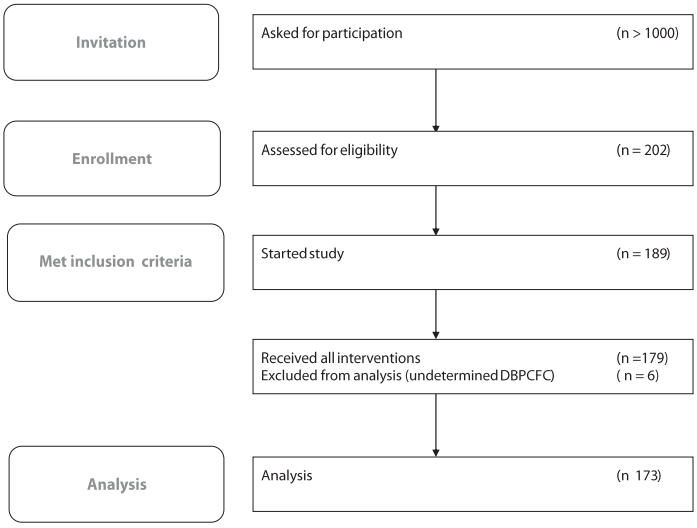

Figure 4.6: Flowchart of patient inclusion

with cashew nut extract was performed and all patients underwent a DBPCFC test with cashew nut.

Participating children underwent a SPT with cashew nut extract, a positive control (histamine 10 mg/ml ALK-Abelló, Nieuwegein, the Netherlands) in duplicate and a negative control. For the cashew nut extract, cashew nuts (blanched, unsalted; Intersnack Doetinchem, the Netherlands) were homogenised mechanically, ground with a mortar and pestle, defatted by ether extraction, and subsequently air-dried. A 10% w/v extract in phosphate-buffered saline (PBS) with this material was made. The SPT was performed by applying a drop of the allergen extract on the skin of the volar aspect of the forearm. Subsequently the dermis was punctured with a 1 mm sharp tip standard sterile lancet with horizontal shoulders (ALK-Abelló, Nieuwegein, the Netherlands) Twenty minutes after application, the contours of the wheal were encircled with a fine-tip pen and transferred to a record sheet with translucent tape (3). A scanner device (Hewlett Packard 2400c) and software (Precise Automated Area Measurement of Skin Test (PAAMOST)) was used to determine the area of the wheals [10]. The area of the allergen-induced wheal was divided by the mean area of the two positive histamine-induced wheal controls (Histamine Equivalent Prick (HEP)-index area). A HEP-index area ≥ 0.4 (corresponding with a 3 mm wheal diameter) and an average wheal diameter ≥ 3 mm was considered positive (10). The HEP-index area method is used because scanning methods are more accurate in measuring the wheal area than estimating the wheal area by the mean diameter. Furthermore, the HEP-index area method corrects for skin-reactivity by considering the histamine-induced wheal size of the positive controls as well (10).

The DBPCFC test consisted of an eight-step incremental dose regime of validated and standardised challenge material (11). The cumulative dose was 3180 mg cashew nut protein (approximately 22 cashew nuts based on 144 mg cashew nut protein per cashew nut with an average weight of 700 milligram; (Intersnack, the Netherlands B.V.)) when all 8-dose steps were consumed. The DBPCFC was considered positive if, on the active challenge (verum) day: 1) objective symptoms occurred, or 2) subjective symptoms recurred twice on three successive administrations of the challenge material, or 3) subjective symptoms persisted for more than one hour (12). Anaphylaxis was defined as described in the EAACI Guidelines for Food Allergy and Anaphylaxis (13). The inclusion and exclusion criteria and detailed study protocols were previously described (3). Medical ethical approval was obtained in April 2012. Parents of children (2–12 years old) and parents and children (≥ 12 years old) signed an informed consent form.

Cashew nut allergens Ana o 1, 2 and 3

Cashew nut allergens Ana o 1, 2 and 3 are not commercially available, so these were purified specifically for this study (14). In short, a total protein extract (in 0.1M ammonium bicarbonate, 0.5M NaCl) was obtained from defatted cashew nut. Stepwise precipitation of this total protein extract using 15%, 30%, 45% and finally 52.5% w/v ammonium sulphate yielded a supernatant containing only Ana o 1 (50kDa) and Ana o 3 (12.6kDa). The two allergens from this supernatant were separated by ultrafiltration ultracentrifugation using a 30kDa Amicon centrifugal filter, collecting Ana o 1 in the retentate and Ana o 3 in the filtrate. Purified Ana o 2 was obtained by gel filtration chromatography (Superdex 200) of a non-defatted cashew protein extract. The purified allergens were conjugated to biotin at a pre-determined protein to biotin ratio to achieve approximately 4–6 biotin groups per antibody molecule, generally requiring a 20 molar excess of biotin to antibody. The quality of the biotinylated cashew allergens was analysed by inhibition testing with unlabelled allergens to verify that the biotinylation procedure had not altered IgE epitopes. Subsequently, the biotinylated allergen concentration required to saturate cashew-specific IgE binding in the Immulite assay was determined by titration experiments using a pooled serum from patients with a history of cashew nut allergy. Quality control for specificity was assessed using in-house control samples with known cashew nut specific IgE values (Siemens IMMULITE 2000Xpi). The IgE values in kU/L for the individual cashew allergens reported here are thus based on the IgE readings obtained with the cashew extract in the commercial IMMULITE assay. The cashew allergen specific IgE assay on the IMMULITE 2000 Xpi system was performed according to standardized procedures (Siemens IMMULITE® 2000/2500 Operators Manual, 2007). Serum samples were used to measure sIgE levels to Ana o 1, 2 and 3 using the Siemens IMMULITE 2000 XPi Immunoassay System (Med. Imm. Laboratory; Reinier de Graaf Groep (RdGG).

Statistical analysis

The patient characteristics were reported in median, ranges and proportions. sIgE values above the upper detection limit of 100 kU/l were set to 100 kU/l (15). The association between the outcome of the DBPCFC with cashew nut (allergic yes/no) and the diagnostic variables were analysed with logistic regression analyses. The form of the association between the outcome of the DBPCFC and the continuous variables sIgE to Ana o 1, 2 and 3, sIgE to cashew nut and the SPT were assessed with restricted cubic spline functions with 2 degrees of freedom. Restricted cubic spline functions are flexible in modelling non-linear associations, but use few extra regression coefficients. The strength of the association was evaluated with the concordance index (c-index). The c-index is equal to the Area Under the Receiver Operating Characteristic Curve (AUC) for a dichotomous outcome. The difference in -2 log likelihood (likelihood ratio, LR) was assessed for nested models including and excluding the variable of interest. The analyses were done with SPSS software, 20th edition and R programming language(16).

RESULTS

Patient characteristics and diagnostic results

Six patients had an uncertain DBPCFC test outcome with cashew nut and were considered as undetermined (e.g. children who did not completed the test). These children were excluded from the analysis. A total of 173 patients were included in the analysis (Table 4.1). The study included 137 (79%) patients with a positive DBPCFC test and 36 (21%) patients with a negative DBPCFC test. In the group of children with a positive test, 64% (88/137) experienced a mild reaction and 36% (49/137) had an anaphylactic reaction during the DBPCFC test (3). The most commonly reported or observed symptoms during the DBPCFC in the non-anaphylactic group, were oral allergy symptoms (68%), followed by gastro-intestinal symptoms such as nausea, stomach pain, vomiting and diarrhea (59%) and redness of the skin (17%). The children with an anaphylactic reaction most commonly experienced a combination of skin and gastro-intestinal symptoms (40 children) followed by respiratory and gastro-intestinal symptoms (6 children) and skin and respiratory symptoms (3 children). All of the patient characteristics and diagnostic test results are summarised in Table 4.1.

Diagnostic variables in patients with no, mild and severe DBPCFC test reactions

Median values of Ana o 1, 2, 3 were 1.29 kU/l (range 0–100 kU/l), 4.77 kU/l (range 0–100 kU/l) and 8.33 kU/l (range 0–100 kU/l) respectively and varied significantly (Wilcoxon signed rank test, $p < 0.001$). The median sIgE values to Ana o 1, 2 and 3 were significantly lower in the patient group with a negative DBPCFC test compared to the patients with a

Table 4.1: Patient characteristics and diagnostic results of the 173 children sensitised to cashew nut with a positive and negative DBPCFC test

	Positive DBPCFC test N (%) 137 (79)	**Negative DBPCFC test N (%) 36 (21)**
Male participants	77 (56)	26 (72)
Age, years*	9.0 (2 -17)	9.5 (2 -17)
Atopic disease symptoms		
Asthma	43 (31)	9 (25)
Eczema	53 (39)	13 (36)
Hay fever	71 (52)	18 (50)
Total IgE, kIU/L*	509 (23-15431)	751 (31-7709)
sIgE to Ana o 1, kU/l*	2.0 (0- ≥ 100)	0.2 (0- 6.7)
sIgE to Ana o 2, kU/l*	6.3 (0- ≥ 100)	1.2 (0- 8.4)
sIgE to Ana o 3, kU/l*	13.0 (0- ≥ 100)	0.6 (0- 30.9)
sIgE to cashew nut, kU/l*	5.8 (0- ≥ 100)	1.2 (0- ≥ 17.1)
SPT with cashew nut extract, HEP index area*	3.4 (0.4-15.2)	0.5 (0-14.3)
Severity of reaction during DBPCFC		
Mild reaction	88 (64)	NA
Anaphylaxis	49 (36)	

* median (range: min- max)

positive DBPCFC test (Figure 4.7, (Mann-Whitney U test, $p < 0.001$)). However, the median sIgE values to Ana o 1, 2 and 3 were similar in the anaphylaxis group compared to the patient group with mild reactions ($p = 0.831$, 0.840 and 0.916, respectively).

The same applies for sIgE to cashew nut and the SPT. The correlation between Ana o 1, 2 and 3 was strong (Pearson correlation coefficients between 0.80 and 0.90), with the highest correlation between Ana o 2 and 3.

Only 6 children had sIgE to cashew nut ≥ 100 kU/l, 1 child had sIgE to Ana o 1 ≥ 100 kU/l, 5 children had sIgE to Ana o 2 ≥ 100 kU/l and 7 children sIgE to Ana o 3 ≥ 100 kU/l. We read the values above 100 kU/L from an extended Master curve, and the comparison between the anaphylaxis group and the group with mild reactions gave similar results.

Performance of the diagnostic variables

Specificity of each cashew allergen was verified through competitive inhibition testing using a cashew specific IgE positive patient serum pool, the cashew allergens and the raw extract. The inhibition plots demonstrated that the allergens tested were nearly completely inhibited by the relevant inhibitor extract in a concentration dependent manner. Additional inhibition studies were conducted to show that the specific allergens

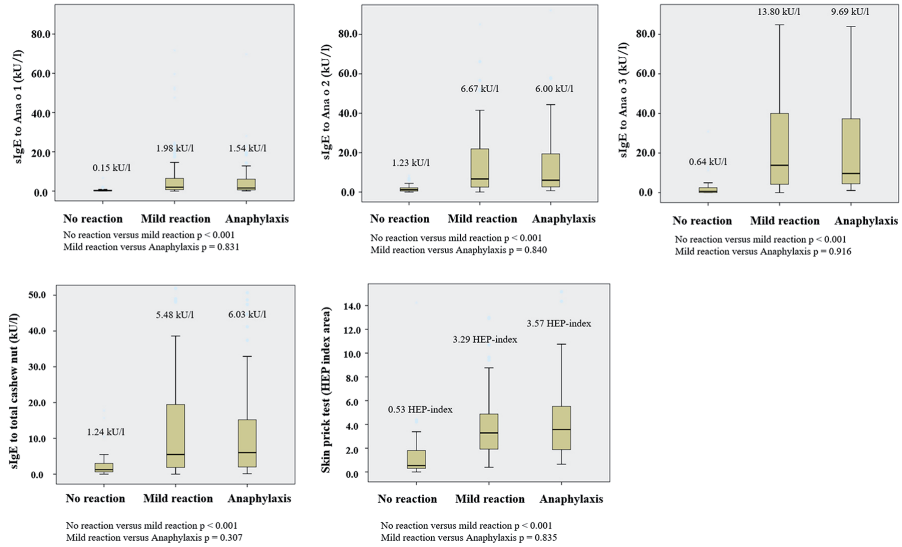

Figure 4.7: Boxplots of Ana o 1, 2 and 3, sIgE and SPT to cashew nut; compared are groups of patients without a reaction during the DBPCFC test with cashew nut, to those with a mild reaction and to those with anaphylaxis

The median values of sIgE to Ana o 1, Ana o 2, Ana o 3, cashew nut and HEP-index areas are given above the respective boxplots, divided in 3 groups of patients: without a reaction, with a mild reaction and those with an anaphylactic reaction during the DBPCFC test with cashew nut.

were not cross-reacting to the unrelated allergens. Testing was performed using one positive sample with several unrelated allergen extracts. A negative sample was used to measure the background response. The analytical sensitivity of the assay was increased by using the appropriate biotin-labelled allergen concentration to allow detection of all specific IgE antibodies present in the sera with the highest cashew specific IgE titers.

Restricted cubic spline functions were used to visualize the form of association between the diagnostic parameters (sIgE to Ana o 1, 2 and 3, sIgE to cashew nut and the SPT) and the risk of a positive DBPCFC test (Figure 4.8). The curves of sIgE to Ana o 1, 2 and 3 with the risk of a positive DBPCFC test increase sharply incline and reach a risk of approximately 100% of cashew nut allergy at the highest levels of sIgE to all three Ana o components. The associations were strong with c-indices of 0.87 (95% CI 0.80–0.93), 0.85 (95% CI 0.79–0.91) and 0.89 (CI 0.83–0.95) for sIgE to Ana o 1, 2 and 3, respectively (Table 4.2). The standard diagnosis by means of sIgE to cashew nut showed a weaker association (c-index = 0.76, 95% CI 0.67–0.84). The association of the SPT and DBPCFC outcome was nearly as strong as the associations of sIgE to the Ana o allergens (c-index = 0.83, 95% CI 0.74–0.91).

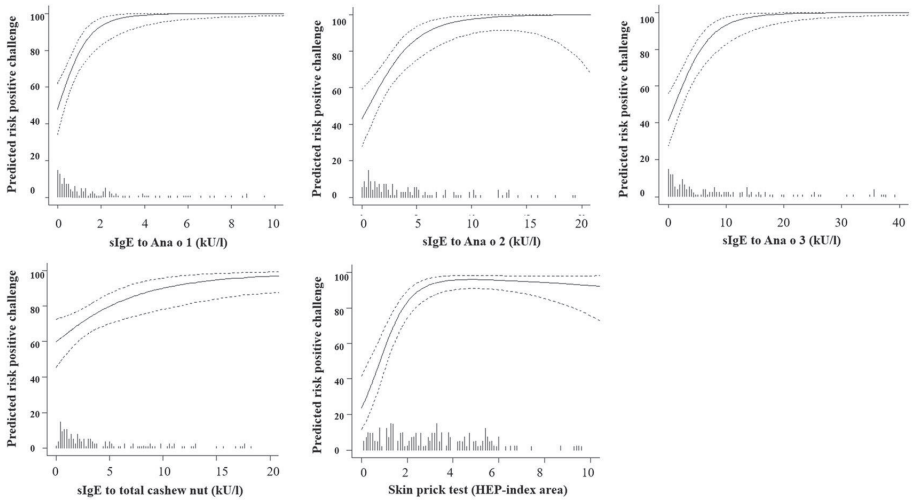

Figure 4.8: Association between the levels of sIgE Ana o 1, 2 and 3 (A) and the levels of sIgE to cashew nut and SPT with cashew extract (B) with the risk of a positive DBPCFC. Curves are modelled with restricted cubic spline functions with 2 degrees of freedom. Spikes at the bottom indicate the distribution of the diagnostic variable.
The line represents the percentage of allergic responders with different sIgE titres to Ana o 1, 2 and 3 and different SPT HEP-index areas. Predictive values of cashew nut allergy of approximately 100% can be obtained at the highest levels of sIgE to all three Ana o components.

Table 4.2: Strength of association between diagnostic variable and outcome of the DBPCFC test

	C- index	LR_0
sIgE Ana o 1	0.866	45.6
sIgE Ana o 2	0.847	47.4
sIgE Ana o 3	0.890	56.0
sIgE cashew nut	0.755	24.8
SPT cashew nut	0.828	49.2

Variables are modeled with a restricted cubic spline function with 2 degrees of freedom.
sIgE= specific IgE, SPT= skin prick test, C-index= concordance index, LR_0=likelihood ratio with the null model

Added value of components Ana o 1, 2 and 3 in multivariable models

We assessed the added diagnostic value of Ana o 1, 2 and 3 to that of easily available patient characteristics and the diagnostic variables. A model with only gender and history of cashew nut allergy showed a c-index of 0.67. With inclusion of Ana o the c index increased to 0.89 maximally for Ana o 3 (Table 4.3). When sIgE to cashew nut was additionally included, Ana o 1, 2 and 3 showed increases up to 0.92, and 0.93 when the

SPT was included. Concordance with sIgE Ana o 2 was slightly better than with sIgE Ana o 1 and 3. The estimates of the LRs showed similar results (Table 4.4).

Table 4.3: Concordance index for multivariable models including Ana o 1, 2 or 3

	History of cashew nut allergy + gender C-index (delta) **(A)**	**A** + sIgE to cashew nut C-index (delta) **(B)**	**A** + **B** + SPT C-index (delta) **(C)**
	0.665	0.819	0.880
sIgE Ana o 1	0.880 (0.215)	0.884 (0.065)	0.905 (0.025)
sIgE Ana o 2	0.881 (0.216)	0.915 (0.096)	0.928 (0.048)
sIgE Ana o 3	0.894 (0.229)	0.899 (0.080)	0.914 (0.034)

sIgE= specific IgE, C-index= concordance index, delta: difference in c-index compared to the model without Ana o.

Table 4.4: Likelihood ratio for the added value of the five diagnostic variables

	History of cashew nut allergy + gender **(A)**	**A** + sIgE to cashew nut **(B)**	**A** + **B** + SPT **(C)**
Diagnostic variable	LR_{added} LR_0	LR_{added} LR_0	LR_{added} LR_0
	10	38	67
sIgE Ana o 1	43 53	18 56	7.3 74
sIgE Ana o 2	48 58	39 77	18 85
sIgE Ana o 3	51 61	29 67	12 79
SPT cashew nut	44 54	29 67	- -
sIgE cashew nut	27 37	- -	- -

SPT= skin prick test, sIgE= specific IgE, LR_0= likelihood ratio of the model compared with a null model, LR_{added} = likelihood ratio of nested models with and without the diagnostic variable
The diagnostic variables are modelled with a restricted cubic spline function with 2 degrees of freedom.

DISCUSSION

We investigated the predictive value of CRD in cashew nut allergy in a multicentre study of 173 children. This study not only examines the diagnostic capacity of CRD with Ana o 3, but also with Ana o 1 and 2.

The measured levels of sIgE to Ana o 1, 2 and 3 varied significantly, however the associations with the DBPCFC were comparable and strong for all components. This is in agreement with the results from studies with other allergens (17), where median values of Ara h 1, 2, 3, and 8, ranged widely from 0.9 kU/l, to 6.5 kU/l in children with a positive

peanut challenge. Consequently, these differences have to be taken into account when one of the cashew nut components is being used in clinical practice or research.

A markedly greater risk of cashew nut allergy as ascertained by DBPCFC was observed for higher values of sIgE to Ana o 1, 2 and 3. At higher levels of sIgE to Ana o 1, 2 and 3 total risks of approximately 100% were observed. The associations were strong with c-indices of 0.87, 0.85 and 0.89 for sIgE to Ana o 1, 2 and 3, respectively. Added value of sIgE to Ana o 1, 2 and 3 remained after considering standard diagnostic variables (gender, history of cashew nut allergy, sIgE to cashew nut and the SPT). However, the cashew nut component analysis could not distinguish between a mild and a severe cashew nut allergy.

In some serum samples, higher sIgE binding was seen to Ana o 1, 2, and 3 than to whole cashew nut extract. This phenomenon was also described in peanut by Aalberse et al. [18]. They found that IgE reactivity to peanut specific components was higher than to the crude peanut extract in 11 plasma samples, and speculated that conventional extracts may have lower concentrations of certain specific allergens in comparison with purified specific allergen components

The association between sIgE to Ana o 3 and a positive challenge test, was previously demonstrated by Savvatianos et al (9). This research group investigated sensitisation to Ana o 3 in 63 children in whom clinical reactivity to cashew nut was documented. Their study demonstrated a near-optimal AUC of 0.97 in the Receiver Operator Characteristics (ROC) curve. However, the clinical diagnosis of cashew nut allergy was based on clinical history in 95% of the cases and only in 5% of the cases on food challenge tests, therefore, the association observed with Ana o 3 levels may have been with sensitisation rather than with clinical allergy to cashew nut with as consequence a stronger association. However, the gold standard is the DBPCFC test that all children underwent in our study.

For other allergies such as that to peanut and hazelnut, the added value of CDR in the diagnosis of clinical allergy to these foods has been proved. Peanut allergen Ara h 2 and hazelnut allergen Cor a 14, which are both 2S Albumins (as is Ana o 3), are of great importance to estimate the risk of a positive challenge test outcome with peanut and hazelnut, respectively (15,19,20). 2S Albumins are resistant to proteolysis, heat denaturation and pH changes and are therefore considered to be clinically relevant allergens (21).

Our data show that sIgE to cashew nut and, to an even greater extent, SPT with cashew nut extract, are associated with a positive DBPCFC test (c-index of 0.76 and 0.83, respectively). The associations with SPT and sIgE were previously shown for other food allergens, e.g. egg, milk, and peanut, and it has been suggested that SPT is superior to sIgE in predicting clinical allergy (22–26). A previous retrospective study on 983 children who underwent food challenge test with egg, milk and peanut also demonstrated that SPT results were more strongly associated with a positive food challenge test than

sIgE (LR 1.23 and 1.04) (27). The study by DunnGalvin et al., which aimed to predict the challenge outcome for 429 patients with suspected peanut, milk and egg allergy also showed a greater predictive capacity of SPT than sIgE (28). Here we show that this is obviously the case for cashew nut allergy. One may speculate that a positive SPT indicates not only the presence of sIgE in the sensitised individual, but also reflects the biological activity of that sIgE.

This is the first study using all currently known cashew nut components to investigate the value of CRD in cashew nut sensitised children. Moreover, we address the predictive value of the SPT with cashew nut extract and sIgE, which has - to our knowledge - not been reported previously. We emphasize that this study comprises a relatively large number of children and that all of them underwent DBPCFC. However, there might have been a selection bias in this study. Approximately 7% of the children or parents of children refused to participate in this study because of fear of severe allergic reactions during the DBPCFC test. As many children reported an unknown history of cashew nut ingestion (66/173 children) and as we observed many anaphylactic reactions (49/173) during the cashew nut DBPCFCs, this form of selection bias is probably limited.

Our sample included only 36 children without cashew nut allergy. Nevertheless, the strong associations of Ana o 1, 2 and 3 are statistically significant. The 95% confidence intervals for the c-indices were wide, which made it not possible to determine which of the three had the strongest association with cashew nut allergy.

In conclusion, sIgE-levels to Ana o 1, 2 and 3 are each individually predictive for the outcome of food challenge tests in cashew-allergic children. The SPT is the second best alternative. Therefore, component analysis in combination with other diagnostic tools needs to be considered in the diagnostic work-up of cashew nut allergy. The diagnostic values of sIgE of the three Ana o components were very similar, as was to be expected because of the high correlation between these components. Therefore, measurement of one of the components Ana o 1, 2 or 3 is probably sufficient.

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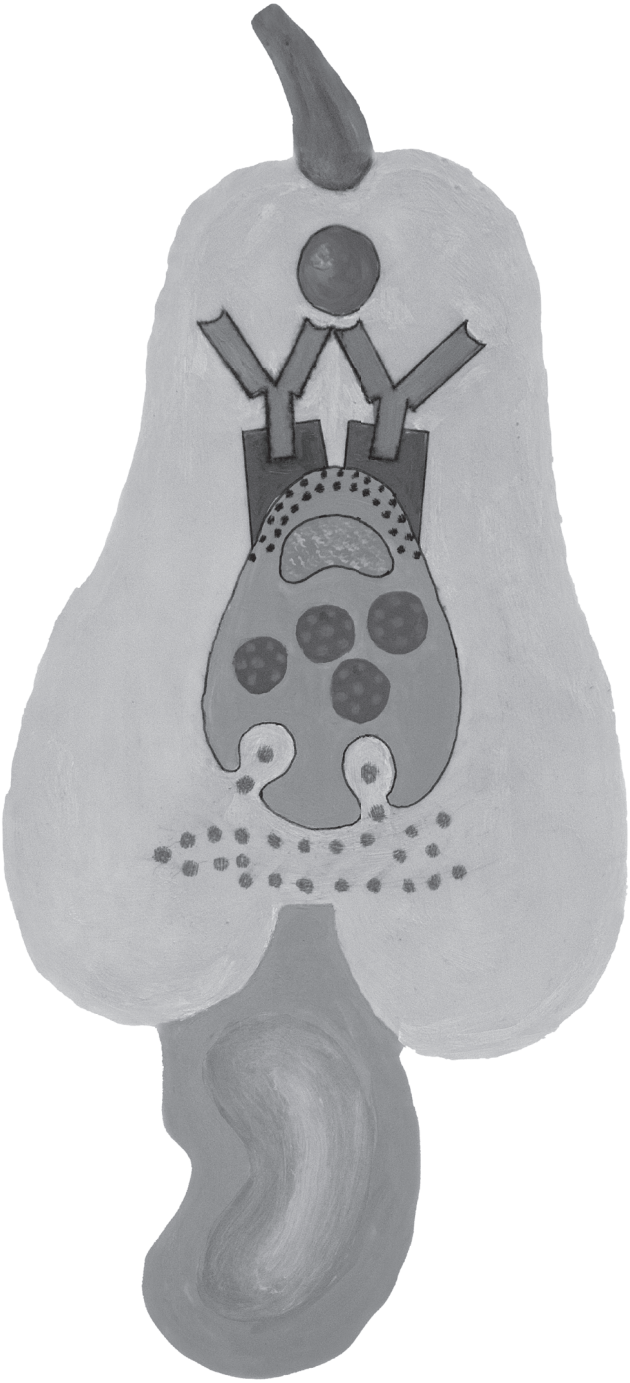

Chapter 4.3

Prediction of cashew nut allergy in sensitised children

We describe in this clinical communication a multivariate model to predict the outcome of the DBPCFC test with cashew nut. Submitted.

Van der Valk JP, Gerth van Wijk R, Vergouwe Y, Steyerberg EW, Reitsma M, Wichers HJ, Savelkoul HF, Vlieg-Boerstra B, de Groot H, Dubois AE, de Jong NW. Prediction of cashew nut allergy in sensitised children. Submitted.

To the Editor,

As an alternative to the costly, time-consuming and possibly stressful double-blind, placebo-controlled challenge (DBPCFC) test, a model to predict the risk of cashew nut allergy was studied incorporating patient characteristics, standard diagnostic parameters (specific IgE (sIgE) and Skin Prick Test (SPT)) as well as component resolved diagnosis (CRD) . We previously demonstrated that sIgE to the components Ana o 1, 2 and 3 discriminated better between cashew nut- allergic and tolerant children sensitised to cashew nut than the current testing methods (SPT and sIgE to cashew nut) (1). The aim of this study was to develop a prediction model for cashew nut allergy.

Results of children who participated in the IDEAL-study (trial number NTR3572) were analysed. The study protocol and inclusion criteria were previously described (2). Briefly, sIgE to cashew nut and to Ana o 1, 2 and 3 was measured, a SPT with cashew nut extract was performed and all patients underwent a DBPCFC test. For the SPT, a scanner device (Hewlett Packard 2400c) and software (Precise Automated Area Measurement of Skin Test (PAAMOST)) was used to determine the area of the wheals. The area of the allergen-induced wheal was divided by the mean area of the two positive histamine-induced wheal controls (Histamine Equivalent Prick (HEP)-index area). A HEP-index area ≥ 0.4 (corresponding with a 3 mm wheal diameter) was considered positive (3). Cashew nut allergens Ana o 1, 2 and 3 were purified specifically for this study (4), and sIgE to these purified allergens was measured by the standardized Siemens IMMULITE procedure.

The DBPCFC test consisted of an eight-step incremental dose regime of validated and standardised food challenge material (5). The cumulative dose was 3180 mg cashew nut protein (approximately 22 cashew nuts) when all 8-dose steps were consumed.

Univariate and multivariable logistic regression analysis was used to assess the contribution of potential predictors to cashew allergy. The odds ratios (ORs) for continuous variables were scaled in a way that they corresponded to a change in one standard deviation of the predictor distribution. The model building process followed the usual order in a diagnostic work-up. We used a relatively high p-value ($p < 0.5$) in the backward selection procedure, because of the limited number of non-events (6). We also applied the 'sign OK' rule (7). Discriminative ability of the models was assessed with the concordance index (c-index). Internal validity was assessed with bootstrapping (8). The regression coefficients in the final model were multiplied with a shrinkage factor. Without shrinkage, predictions are generally too extreme. The prediction models were transformed into score charts for use the use in clinical practice.

The study included 137 (79%) patients with a positive and 36 (21%) patients with a negative DBPCFC test. The predictors: gender (girl), history of cashew nut allergy and atopic features were associated with a positive challenge test with the highest OR for history of cashew nut allergy (OR 2.9, 95% CI 1.4–6.0). sIgE to the cashew nut and the

components Ana o 1, 2 and 3 were strongly associated with a positive challenge test (ORs of 8.6 (95% CI 5.4–13.8), 5.4 (95% CI 3.6–8.1) and 8.6 (95% CI 5.8–12.7), respectively) (Table 4.5).

Table 4.5: Univariate logistic regression of patient characteristics for a positive challenge test with cashew nut, n (%) unless stated otherwise.

Variables	**Positive DBPCFC**		**Negative DBPCFC**			
Anamnestic	**N = 137 (79%)**		**N = 36 (21%)**		**OR (95% CI)**	
Gender (girl)	60	(86%)	10	(14%)	2.0	(0.9-4.5)
Age, years *	9.0	(range 2-17)	9.5	(range 2-17)	1.0	(0.9-1.0)
History of cashew nut allergy	92	(86%)	15	(14%)	2.9	(1.4-6.0)
Atopic features**	102	(81%)	24	(19%)	1.5	(0.7-3.2)
Standard diagnostics						
sIgE to total cashew nut, kU/l*	5.8	(range 0- ≥ 100)	1.2	(range 0-17.1)	2.9	(2.1-4.0)
SPT with cashew nut extract (HEP-index area)	3.4	(range 0.4-15.2)	0.5	(range 0-14.3)	4.9	(2.9-8.5)
Components						
Median sIgE to Ana o 1, kU/l*	2.0	(range 0- ≥ 100)	0.2	(range 0-6.7)	8.6	(5.4-13.8)
Median sIgE to Ana o 2, kU/l*	6.3	(range 0- ≥ 100)	1.2	(range 0-8.4)	5.4	(3.6-8.1)
Median sIgE to Ana o 3, kU/l*	13.0	(range 0- ≥ 100)	0.6	(range 0-30.9)	8.6	(5.8-12.7)

OR= odds ratio, SPT= skin prick test, sIgE= specific IgE
*For continous variables, OR is given for a change in standard deviation of the predictor distribution
**Symptoms reported of hay fever, eczema or asthma

The discriminative ability of the model including gender, history of cashew nut allergy and atopic features was relatively low after correction for optimism (c-index = 0.66). Adding sIgE to cashew nut increased the c-index to 0.80 and further increased when SPT was also included (c-index = 0.86). When CRD was included, only gender and SPT remained in the models after backward selection. Using the CRD in the work-up resulted in the highest discriminative ability with a c-index of 0.89 for Ana o 3 plus gender and a c-index of 0.90, when SPT is also considered (Table 4.6). As a result of the liberal p-value, 95% confidence limits for OR's can include the value 1. Internal validity was satisfactory with shrinkage factors of 0.82 0.88, 0.88, 0.89 and 0.89 for the 5 models.

An easy to use format of the prediction model is based on gender, sIgE to Ana o 3 and the SPT (Figure 4.9) and facilitates calculation of the predictive risk of a positive challenge test in cashew nut sensitised children. Based on this score chart, 58 of the 173 (34%) children in our study had a score of ≥ 8 corresponding to a ≥ 95% chance of a positive challenge test outcome. In 57 of these 58 (98%) children, the cashew nut allergy was established with the DBPCFC test. Of the 115 children with a probability score of

Table 4.6: Multivariate models with demographics and history, standard diagnostics (sIgE to cashew nut and SPT) and component Ana o 3

	Demographics and History Model A		+ Standard diagnostics Model B				+ Component Ana o 3 Model C			
			Without SPT		With SPT		Without SPT		With SPT	
Variables	**OR**	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**
Gender (girl)	1.8	(0.8-4.2)	2.4	(1.0-6.0)	2.9	(1.0-8.6)	2.1	(0.7-6.3)	2.6	(0.8-8.2)
History of cashew nut allergy	2.9	(1.4-6.4)	3.5	(1.5-8.1)	1.8	(0.6-4.9)				
Atopic features*	1.8	(0.8-4.1)	1.6	(0.6-3.9)	2.0	(0.7-5.8)				
sIgE to total cashew nut(kU/l)**			3.2	(2.3-4.4)	2.2	(1.5-3.1)				
SPT with cashew nut extract(HEP-index area)**					4.1	(2.2-7.7)			2.4	(1.3-4.6)
sIgE to Ana o 3 (kU/l)**							8.7	(5.9-12.8)	5.1	(3.4-7.7)
C-index (optimism corrected)	**0.66**		**0.80**		**0.86**		**0.89**		**0.90**	

OR= odds ratio, SPT= skin prick test, sIgE= specific IgE

*Symptoms reported of hay fever, eczema, or asthma

**For continous variables, OR is given for a change in standard deviation of the predictor distribution

< 8 corresponding to a < 95% chance of a positive challenge test outcome, 80 children (70%) had a positive and 35 (30%) had a negative DBPCFC test outcome.

We developed and internally validated a diagnostic model for cashew nut allergy in sensitised children. In situations where there is limited availability of double-blind testing, the use of the model and scoring system presented here may be useful for identifying children who have ≥ 95% chance of having a positive challenge test result and in whom such testing is thus less likely to influence management. In our present series, this pertains to a substantial number of patients (34%).

The specificity of the scoring system may be negatively influenced by several factors, including cross-reacting allergens. Currently there is no data on allergens cross-reacting with cashew nut e.g. PR-10 allergens. More research in this area is needed.

Gender was included in the model, with a higher risk of a positive challenge test for girls. Why sensitisation to cashew nut is more often clinically relevant in girls than boys is currently unknown.

Not all medical settings have the opportunity to perform the SPT (8). Therefore, we developed a model with and without the SPT. If there is no scanner device available to measure the HEP-index area, this can be calculated from the diameter of the wheal with

Predictor	Value	Score
Gender (girl)		1
Ana O 3 (kU/l)	0 - 0.2	0
	0.21 - 1	1
	1.01 - 3	2
	3.01 - 7	3
	7.01 - 20	4
	20.01 - 60	5
	60.01 - 100	6
SPT (HEP-index area)	0 - 0.5	0
	0.51 - 0.75	1
	0.75 - 2.5	2
	2.51 - 10	3
	10+	4
Total sum score		…….

Total	0	1	2	3	4	5	6	7	8	9	10	11
%	0	13	29	44	59	72	83	91	95	98	99	100

Figure 4.9: Score chart for the predictive risk of a positive DBPCFC with cashew nut including SPT for cashew nut sensitised patients

The score chart facilitates calculation of the predictive risk of a positive challenge outcome and is developed for clinical practice. The score chart is based on the variables gender, sIgE to Ana o 3 and the SPT. The continuous scales of sIgE to Ana o 3 and SPT are divided in small steps. The scores are derived from the prediction model an updated intercept:

Lp = -0.487 + 0.847 * Girl + 0.792 * log(AnaO3 + 0.1) + 0.784 * log(SPT + 0.1)

Hypothetical example: a sensitised girl (1 point) with a sIgE to Ana o 3 value of 21 kU/l (5 points) and a SPT of 2.9 (3 points) scores 9 points and has 98% risk on a positive challenge test.

the following equation: HEP-index area = 0.0096D2+ 0.2674D – 0.5033 (D= diameter) (3). If sIgE results to Ana o components are not available, the SPT is second best in the diagnostic model for cashew nut allergy (likelihood ratio test statistic $\chi 2$: Ana o 13.75 and SPT 6).

A prediction model for cashew nut allergy has never been developed previously and the final model in this article has higher discriminability (c-index of 0.90) than the individual Ana o 1, 2 and 3 components in our previous report (c-index of 0.87, 0.85 and 0.89, respectively)(10). Our prediction model is very useful in clinical practice, however, the generalizability of this method needs to be established through external validation.

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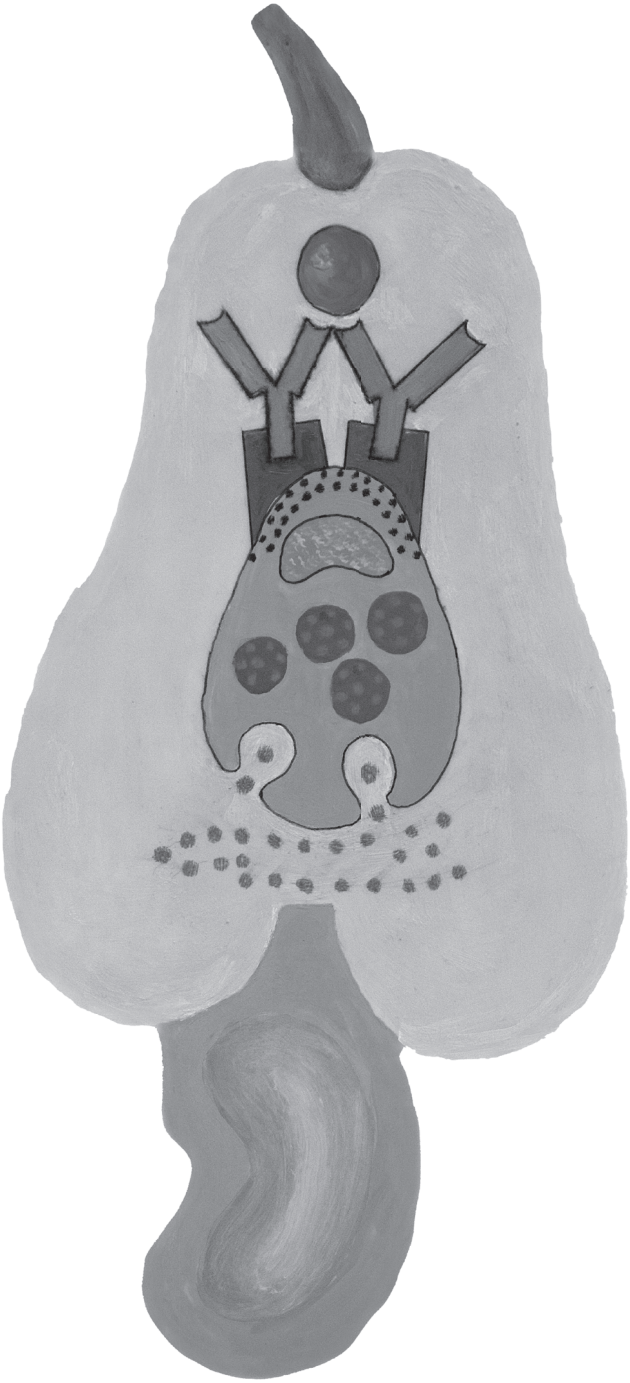
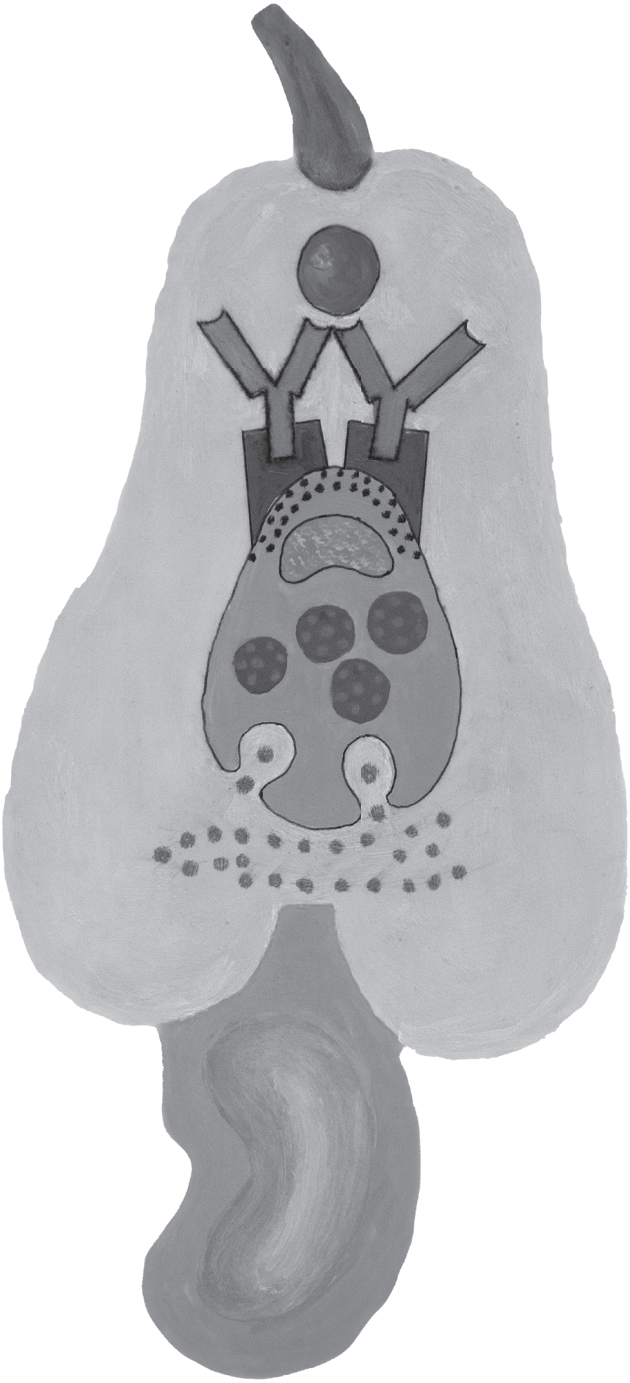


Chapter 5

Clinical consequences of cashew nut allergy



Chapter 5.1

Low percentage of clinically relevant pistachio nut and mango co-sensitisation in cashew nut sensitised children

This short communication assess the clinical relevance of pistachio nut and mango co-sensitisation in cashew nut sensitised children. Submitted

Van der Valk JP, el Bouch R, Gerth van Wijk R, de Groot H, Wichers HJ, Dubois AE, de Jong NW. Low percentage of clinically relevant pistachio nut and mango co-sensitisation in cashew nut sensitised children Submitted.

ABSTRACT

Cashew nut, pistachio nut and mango belong to the *Anacardiaceae* family and are botanically related. The aim of this study is to assess the clinical relevance of co-sensitisation to pistachio nut and mango in cashew nut sensitised children. Children were recruited from the study: 'Improvement of Diagnostic mEthods for ALlergy assessment (IDEAL) (trial number NTR3572). The IDEAL-study showed that children sensitised to cashew nut, were co-sensitised to pistachio nut in 98% of cases and to mango in 21% of cases. All children had undergone a double-blind placebo-controlled food challenge (DBPCFC) with cashew nut in the IDEAL-study, and in this follow-up study a DBPCFC with pistachio nut and an open food challenge with mango was performed. Twenty-nine children (mean age of 11.6 years, 62% male) were included. Pistachio nut sensitisation was clinically relevant in only 34% of cashew sensitised children and only 31% of cashew challenge positive children. None of the children was challenge positive to mango. An oral food challenge is recommended in children co-sensitised to cashew nut and pistachio nut.

INTRODUCTION

Cashew nut, pistachio nut and mango belong to the *Anacardiaceae* family and are botanically related. Cashew nut allergic children are frequently advised to eliminate not only cashew nuts, but also pistachio nuts from their diet. This advice is based on the sensitisation pattern and possible cross-reactivity (1, 2). Cross-sensitisation between cashew nut and pistachio nut has been previously established by specific IgE (sIgE) inhibition tests (3–6). However, studies that confirm clinical cross-reactivity by performing a challenge are rare (7).

The aim of this follow-up study of the 'Improvement of Diagnostic mEthods for ALlergy assessment (IDEAL) study' (trial number NTR3572) is to assess the clinical relevance of pistachio nut -and mango sensitisation in cashew nut sensitised children. The IDEAL-study showed that children sensitised to cashew nut were co-sensitised to pistachio nut in 98% (169/173) and to mango in 21% (37/173).

METHODS

Patient selection and study design

Children were recruited from the multi-centre IDEAL-study and were included between January 2015 and June 2015. The inclusion/exclusion criteria and detailed study protocol of the IDEAL-study were previously described (8). For practical reasons only two of the three centres were invited to participate in this pistachio nut and mango follow-up study. Medical history, sensitisation results (sIgE and SPT) and results from the double-blind placebo-controlled food challenge (DBPCFC) with cashew nut were obtained from the IDEAL-study. The children underwent a DBPCFC with pistachio nut and an open food challenge with mango in this follow-up study. Medical ethical approval was obtained in January 2015. Parents of children (2–12 years old) and parents and children (≥ 12 years old) signed the informed consent.

Skin prick test

All children underwent a SPT with cashew nut and pistachio nut extract and mango juice, a positive control (histamine 10 mg/ml ALK-Abello, Nieuwegein, the Netherlands) in duplicate and a negative control. Cashew nuts (roasted, unsalted) and pistachio nuts (fresh, not roasted, unsalted nuts) were homogenized mechanically, ground with a mortar and pestle, defatted by ether extraction, and subsequently air-dried. A 10% w/v extract in phosphate-buffered saline with the pre-treated material was made. Mango juice was prepared from pieces of ripe mango fruit pulp, without skin or kernel (8). The SPT was performed by applying a drop of the allergen extract on the skin of the volar

aspect of the forearm; subsequently the epidermis was punctured with a standardised 1 mm sharp tip sterile lancet.

We used a precise scanning method to ascertain the SPT results. We divided the area of the allergen-induced wheal by the mean area of two positive histamine-induced wheals. This ratio is defined as HEP-index area. A HEP-index area of ≥ 0.4 corresponding with a wheal diameter of ≥ 3 mm was considered positive (9).

Specific IgE

Serum samples were analysed for sIgE to cashew nut, pistachio nut and mango 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.

Challenge test with pistachio nut

All children underwent a DBPCFC with pistachio nut. The food challenge consisted of an eight-step incremental dose regime. The time interval between each step was 30 minutes. The challenge recipe and dosages used for the DBPCFC with pistachio nut were based on validated and standardised cashew nut recipes (10). Roasted unsalted pistachio nuts were provided by Intersnack, Doetinchem, the Netherlands. The food matrix (muffin dough) mainly consisted of wheat, sugar, gingerbread spice mix and coconut. The challenge dose schedule is shown in Table 5.1 Children under the age of 4 years were not required to complete step 8.

Table 5.1: Challenge doses DBPCFC with pistachio nut and mango

	Pistachio protein (mg)	Pistachio protein cum (mg)	Average amount of Pistachio nut	Mango protein (mg)	Mango protein cum (mg)	Mango volume (gr)
Dose 1	1	1	0.007*	3	3	0.3**
Dose 2	3	4	0.02	10	13	1
Dose 3	10	14	0.07	30	43	3
Dose 4	30	44	0.20	100	143	10
Dose 5	100	144	0.67	300	443	30
Dose 6	300	444	2.08	1000	1443	100
Dose 7	1000	1444	6.94	————	————	————
Dose 8	1736	3180	12.06	————	————	————

*The average weight of one pistachio nut was approximately 700 mg, with 23.8 g pistachio protein per 100 g pistachio nuts. (Intersnack the Netherlands B.V.)

** 100 gram mango (with kernel and peel) contains approximately 1.0 gram mango protein

Challenge test with mango

Children with no history of symptoms after the consumption of mango were considered non-allergic. The remaining children underwent the open challenge (OFC) with mango. The food challenge with mango (pieces of fruit without skin or kernel) consisted of a six-step incremental dose regime. The challenge dose schedule is shown in Table 5.1.

Statistical analysis

Statistical analyses were done with the Fisher exact test, Mann Whitney U test and chi square test. All analyses were performed with IBM SPSS Statistics, version 21.

RESULTS

Patient selection

Eighty-five children sensitised to cashew nut and pistachio nut, from two of the three participating centres of the IDEAL-study were asked to participate in the pistachio nut and mango follow-up study. A total of 29 children (34%) participated, 38 (45%) did not respond to the invitation, 16 (19%) refused to participate citing reasons such as that the food challenge was time-consuming and burdensome for the child or there was fear for a reaction during the challenge. Two children were ultimately excluded because they did not receive all interventions.

Patient characteristics and diagnostic results

Twenty-nine children, 18 boys (62%), mean age of 11.6 years (range 4–20 years) were included. Symptoms, consistent with eczema, asthma or hay fever were reported by 15/29 (52%), 7/29 (24%) and 14/29 (48%) of the children, respectively.

The median cashew nut sIgE was 5.28 kU/l (range 0.4–100 kU/l) and the median pistachio nut sIgE was 7.25 kU/l (range 0.6–82 kU/l). The median SPT HEP-index area of cashew nut and pistachio nut was 2.50 (range 0–8.8) and 2.02 (range 0–9.4), respectively. Twelve of the 29 children were co-sensitised to mango with a median sIgE to mango of 0.71 kU/l (range 0–3.76) and a median SPT HEP-index area of 0.46 (range 0–1.45).

In order to exclude selection bias, we compared the patient characteristics and diagnostic results of the participating children (N = 29) with the non-participating children. There was no significant difference in gender ($p = 0.80$), age ($p = 0.08$), asthma ($p = 0.10$), eczema ($p = 0.52$), hay fever ($p = 0.57$), sIgE to cashew nut ($p = 0.85$), sIgE to pistachio nut ($p = 0.71$), SPT cashew nut ($p = 0.50$) and SPT pistachio nut ($p = 0.13$).

Food challenge with cashew nut versus pistachio nut

The pistachio nut DBPCFC was positive in 10/29 (34%) and negative in 19/29 (66%) children (Table 5.2). Most children with a positive DBPCFC with pistachio nut experienced 'oral allergy' symptoms followed by gastro-intestinal symptoms, skin symptoms and upper airway symptoms. Eight of ten children experienced both objective and subjective symptoms and an anaphylactic reaction according to the EAACI Guidelines for Food Allergy and Anaphylaxis (11) occurred in one child.

Of the 29 children, 22 children (76%) had a positive challenge with cashew nut and 7 children (24%) had a negative challenge with cashew nut. Only 9 of the 29 children (31%) had positive challenge with cashew nut as well as a positive challenge with pistachio nut (Table 5.2). In 6 of the 22 (27%) children, an anaphylactic reaction occurred during the cashew nut challenge. These 6 children had in all cases a negative pistachio nut food challenge outcome.

Table: 5.2: Outcome of the DBPCFC with cashew nut, pistachio nut and OFC with/or home consumption of mango

		Outcome DBPCFC pistachio nut			Outcome OFC or home consumption mango	
		Positive	Negative	**Total**	Positive	Negative or home consumption
Outcome DBPCFC cashew	Positive	9/29 (31%)	13/29 (45%)	**22/29 (76%)**	0	10/11 (91%)
	Negative	1/29 (3%)	6/29 (21%)	**7/29 (24%)**	0	1/11 (9%)
Total		**10/29 (34%)**	**19/29 (66%)**	**29 (100%)**		**11/29 (38%)**

Food challenge with mango

Seventeen children already consumed mango without problems, therefore a challenge was not indicated. One declined to undergo the OFC because it was time-consuming. In total, 11 children participated in the OFC with mango and in all cases the challenge was negative.

Food challenge results versus sIgE levels

In patients with a positive challenge to both, cashew nut and pistachio nut (n = 9), the median sIgE amounted 10.90 kU/l and 17.10 kU/l, respectively. Significantly lower median sIgE values were found in children reacting to either cashew nut (2.48 Ku/L) or pistachio nut (3.77 kU/l), with p-values of 0.014 and 0.024, respectively.

DISCUSSION

In this study we demonstrated that approximately one third of the children with cashew nut sensitisation and co-sensitisation to pistachio nut reacted to pistachio nut. Co-sensitisation to mango was not clinically relevant in any children. Previous studies reported high percentages of co-sensitisation and cross-sensitisation between cashew nut and pistachio nut (1, 3–6, 12–14). The clinically relevance of these co-sensitisations has only been reported in two studies (4, 5). Children with an allergy to cashew nut and co-sensitisation to pistachio nut are often advised to eliminate both nuts from the diet. Based on our results this might not be the best approach. Although higher levels of (co-) sensitisation to cashew nut and pistachio nut are associated with a higher risk of clinical allergy to these nuts, a DBPCFC is clearly indispensable for making a definitive diagnosis in individual patients.

Apart from the need for a well-established diagnosis, unnecessary avoidance of pistachio nuts or mango may have other disadvantages. Unnecessary lengthy elimination of allergenic foods in sensitised patients can increase the risk of a severe allergic reaction after accidental intake of the allergen (15).

In conclusion, this is the first multi-centre study in patients sensitised and clinically allergic to cashew nut investigating the clinical relevance of co-sensitisation to pistachio nut and mango with food challenges. The percentage of clinical relevant co-sensitisation was low (34%) for pistachio nut and absent for mango in this study population. Oral food challenges are recommended in order to avoid unnecessary avoidance of pistachio nut in cashew nut allergic patients.

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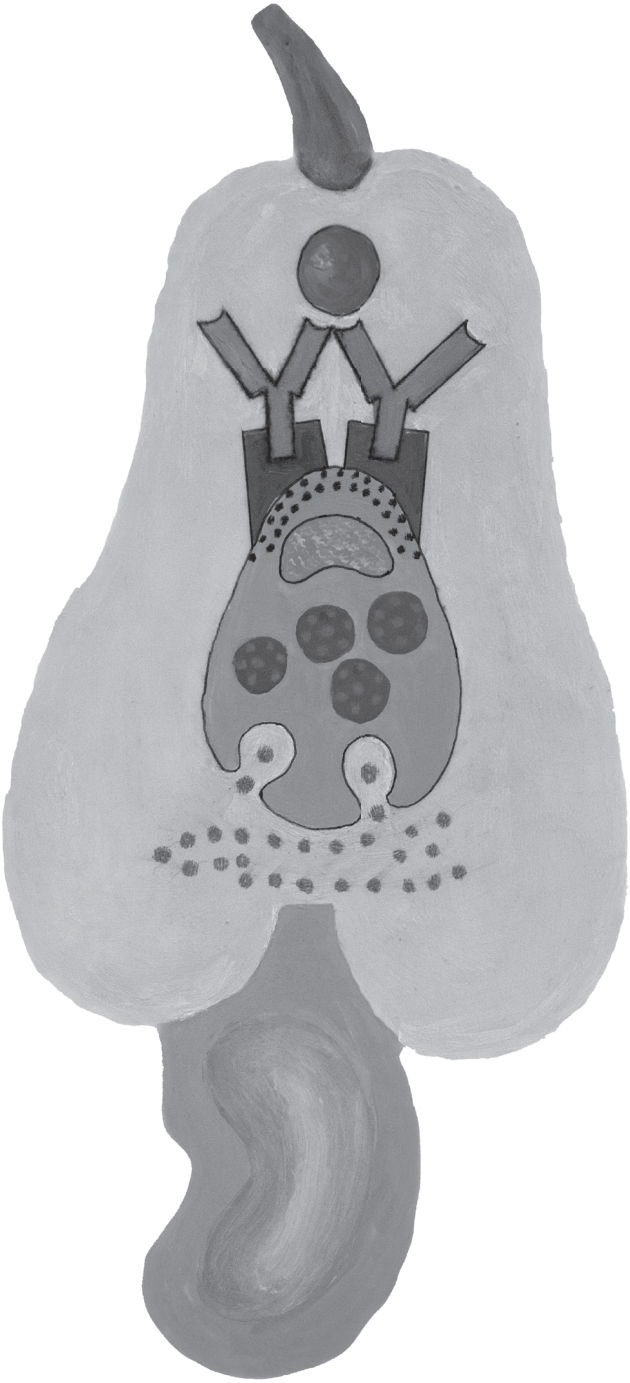

Chapter 5.2

No difference in health-related quality of life, after a food challenge with cashew nut in children participating in a clinical trial.

This paper demonstrates the effect on quality of life in positive and negative challenged children using the food allergy quality of life questionnaires (FAQLQ's) before and after the DBPCFC test. This article is published in Pediatric Allergy and Immunology in 2016.

Van der Valk JP, Gerth van Wijk R, Flokstra-de Blok BM, van der Velde JL, de Groot H, Wichers HJ, Dubois AE, de Jong NW. No difference in health-related quality of life, after a food challenge with cashew nut in children participating in a clinical trial. Pediatr Allergy Immunology. 2016 Aug 6.

ABSTRACT

Background

Previous studies showed that Health-Related Quality of Life (HRQL) significantly improved after the food challenge, with greater improvements in HRQL after a negative outcome than a positive outcome. It is currently unknown whether this also occurs in patients undergoing DBPCFCs with cashew nut in the context of a clinical trial.

Methods

Quality of life was studied in children enrolled in a cashew nut study using food allergy quality of life questionnaires (FAQLQs). Children, teenagers and parents of the children completed the questionnaires before the challenge test and 6 months after the DBPCFC with cashew nut. The difference in the change in HRQL between the children with a positive and negative DBPCFC outcome was studied by Mann-Whitney U test.

Results

In total 112 children (67 boys, median age 9 yrs.) were included. The children, teenagers and parents of the children completed in total 143 sets of questionnaires. There were no significant differences in baseline total and domain scores compared to the follow-up scores in the FAQLQ-CF, -TF and -PF. In children, the delta FAIM score in the negative DBPCFC tested group was significantly better than the delta FAIM score in the positive challenged group ($p = 0.026$). There were no significant differences in the changes in the scores of the FAQLQ-CF and FAQLQ-PF in the children with a positive challenge outcome, compared to the children with a negative challenge result. However, there was a significant difference in the change in score between the latter groups in the domain 'accidental exposure' of the FAQLQ-TF ($p = 0.049$).

Conclusion

This study showed no difference in change of HRQL scores after a DBPCFC with cashew nut in children participating in a clinical trial. The utility of HRQL as an outcome for clinical trials in food allergy may be limited if participant baseline HRQL is relatively unimpaired.

INTRODUCTION

Food allergy is a growing problem in the Western world, especially in children (1). A food allergy may have an impact on the quality of life in children and their parents (2). The fear of severe reactions and the need of strict avoidance of allergenic foods may contribute to this impact on quality of life. Previous studies, with different allergenic foods, demonstrated that Health-Related Quality of Life (HRQL) for patients with a suspected food allergy significantly improved after a double-blind placebo-controlled food challenge (DBPCFC). Greater improvements in HRQL scores were demonstrated in children with a suspected allergy for peanut, nut, milk, egg, wheat, soy and sesame after a negative DBPCFC test outcome (i.e. allergy refuted/passed test) than a positive DBPCFC test outcome (i.e. allergy confirmed/failed test) (3). This study included only patients who had sought medical care for their food allergy. In another study, HRQL of patients seeking medical care was compared to food allergic individuals from the general population (4). HRQL was found to be significantly more impaired in patients seeking medical attention for their food allergy than food allergic individuals in the general population, because this last patient group is probably less afraid of an allergic reaction and have fewer problems with dietary restrictions. Currently, there is little information on the utility of HRQL as an outcome in the setting of diagnostic clinical trials on specific food allergies. The primary aim of this study was to assess the difference in the effect on HRQL before and 6 months after a DBPCFC in children participating in a clinical trial on cashew nut allergy.

METHODS

Participants and procedure

Children (2–17 years of age) participating in the study '*Improvement of Diagnostic mEthods for ALlergy assessment*' with cashew nut allergy in children as a show case (trial number NTR3572) were asked to participate in the evaluation of quality of life (5). Consecutive patients were invited. All patients underwent DBPCFC with cashew nut with an eight-step incremental dose regime of validated and standardised food challenge materials (6). The DBPCFC test was discontinued and considered positive when 1) objective symptoms occurred, or 2) when subjective symptoms re-occurred twice after the same dose of challenge material had been administered, three times consecutively (7), or 3) when subjective symptoms persisted for more than one hour. If the child developed the same symptoms with the same severity on the placebo as on the verum day, the DBPCFC test was considered as undetermined and the child was not included in the analysis.

The children were divided into two groups, patients with a positive and patients with a negative DBPCFC outcome. Anaphylaxis was defined according to the EAACI Guidelines for Food Allergy and Anaphylaxis (8).

The validated Food Allergy Quality of Life Questionnaires (FAQLQ) and Food Allergy Independent Measure (FAIM) questionnaire were used and completed by the patient (8–17 years) and/or parents (0–12 years) before the challenge test and 6 months after the DBPCFC [9]. The FAIM questionnaire was used in this study to measure the longitudinal and cross-sectional validity of the FAQLQs. Only patients who completed both questionnaires in the study period from April 2012 to August 2015 and filled out more than 80% of the questions were included.

Medical ethical approval for this study was obtained (ethics committee Erasmus MC, Rotterdam, 2012–125). All participants signed informed consent (parents of children (2–12 years old) and parents and children (≥ 12 years old). The study is registered in the Dutch Trial register (trial number NTR3572).

Food allergy health related quality of life questionnaires (FAQLQ)

The FAQLQ used in this study is a disease specific instrument that evaluates the impact of food allergy on patients' HRQL. For this study, the FAQLQ child form (FAQLQ-CF) for children between 8 and 12 years of age, the teenager form (FAQLQ-TF) for children between 13–17 years of age and the parent form (FAQLQ-PF) for parents of children between 0 and 12 years of age were used. The FAQLQ-CF contains 24 items and 4 domains (allergen avoidance, risk of accidental exposure, emotional impact and dietary restrictions). The FAQLQ-TF contains 23 items and 3 domains (allergen avoidance and dietary restrictions, risk of accidental exposure and emotional impact). The FAQLQ-PF contains 14 items for children 0–3 years of age, 26 items for children 4–6 years of age and 30 items for children 7–12 years of age; and for all-ages, 3 domains (emotional impact, food anxiety and social and dietary limitations)(2,9,10). The questionnaire items are scored on a seven point scale ranging from 1 to 7. The answer options for children (8–12 years of age) are illustrated with smileys and are simple to use. The FAIM questionnaire is a disease specific instrument that measures self-perceived severity of food allergy (11). The FAIM consists of 4 expectations of outcome questions on the chance of accidental exposure, severe reactions, dying due to the food allergy and effectiveness of self-treatment and 2 questions on extent of disease. The FAIM questionnaire items are also scored on a seven-point scale ranging from 1 to 7.

Statistical analysis

The total FAQLQ score was calculated by adding up the scores, and consequently dividing this score by the number of answered questions at each time point. The domain scores were calculated in the same manner. The FAQLQ mean total score and mean domain scores

were measured at baseline (before DBPCFC) and follow-up (6 months after DBPCFC). The FAIM mean total score was also measured at baseline and follow-up. The statistical significance between the two measurement points (before and after DBPCFC) was calculated by using the paired t-test with 95% confidence intervals and p-values (> 0.05). The changes in FAQLQ scores (follow-up score after the DBPCFC test minus baseline score before DBPCFC test) were calculated for the relevant FAQLQ (all domains) and for the FAIM. The statistical significance between the two groups (patients with a positive and a negative DBPCFC outcome) was calculated by using the Mann-Whitney U test. Multiple linear regressions, correcting for the possibly influencing factors on the change of FAQLQ overtime (dependent variable), was also used to study possible improvement in HRQL. Possible influencing factors (independent variables) used in the multiple linear regression were: age, centre of inclusion (Erasmus Medical Centre Rotterdam, Reinier de Graaf Hospital, University Medical Centre Groningen), atopic features as hay fever, asthma and eczema, history of cashew nut allergy, multiple reported food allergies, anaphylaxis during DBPCFC, baseline score and outcome of the DBPCFC with cashew nut (positive (allergy confirmed)/ negative (allergy refuted) and the dose eliciting a reaction during the challenge with cashew nut. Pearson correlation coefficients were calculated for measuring validity between the baseline, follow-up and change in FAQLQ (CF, TF and PF) scores and FAIM scores. All analyses were done with SPSS software, 20th edition.

RESULTS

Patients and clinical characteristics

In total 112 patients (67 boys, 60%) with a median age of 9.0 years (range 2–17 years) were included. 6 children with an undetermined challenge test were excluded from the analysis. The questionnaires were completed before the challenge test and 6 months after the DBPCFC with cashew nut, and in total 143 pairs of questionnaires were completed from April 2012 to August 2015. Of these 143 pairs of questionnaires, 84 parents' forms 33 child forms and 26 teenager forms were obtained. Of all 112 children, 74 children (66%) had a history of cashew nut allergy and 39 children (34%) had never eaten cashew nut before the challenge test. Positive challenge tests were observed in 85 children (76%), negative tests in 27 children (24%). Most positively tested children had gastro-intestinal symptoms (nausea, vomiting, abdominal pain and diarrhoea) followed by oral allergy symptoms, skin symptoms (redness and itch), angioedema and urticaria. In total 34% (29/85) of the children with a positive DBPCFC to cashew nut had an anaphylactic reaction (8). The children with an anaphylactic reaction most commonly experienced a combination of skin and gastro-intestinal symptoms (n = 22) followed by respiratory, gastro-intestinal and skin symptoms (N = 5) and respiratory and skin

symptoms only (N = 2). Concerning atopic features, 34 (30%) had asthma, 44 (39%) had eczema and 55 (49%) had hay fever. All children were sensitised in either SPT (median 2.97 [0–15.16] HEP-index area) or specific IgE (sIgE) (median 3.42 [0-≥ 100] kU/L).

In order to exclude selection bias, we compared the patient characteristics and diagnostic results between the participating children (n = 112) and the non-participating children from the IDEAL-study (n = 61) (Mann Whitney U test and chi square test). There was no significant difference in gender (p = 0.92), age (p = 0.74) symptoms according to asthma (p = 0.91), eczema (p = 0.68), and hay fever (p = 0.65), history of cashew nut allergy (p = 0.12), sIgE to cashew nut (p = 0.29), SPT cashew nut (p = 0.63) and DBPCFC outcome (p = 0.15).

Initial Health- Related Quality of Life

The mean baseline scores for the FAQLQ-CF, FAQLQ-TF and FAQLQ-PF of 3.32, 3.50 and 2.37 respectively, were measured.

Change in Health-Related Quality of Life after a challenge test with cashew nut

The mean baseline scores for the FAQLQ-CF, FAQLQ-TF and FAQLQ-PF were 3.32, 3.50 and 2.37, respectively. The mean baseline score and the mean follow-up score were compared for the FAQLQ-CF, FAQLQ-TF and FAQLQ-PF and its separate domains. There were no significant differences in baseline total and domain scores compared to the follow-up scores in the FAQLQ-CF and FAQLQ-TF and FAQLQ-PF. However, there was a small but, significant increase measured in FAIM score compared with the follow-up score for children as well as a significant decrease in FAIM score compared with the follow-up score for teenagers (p = 0.025 and p = 0.006 respectively) (Table 5.3).

Change in Health-Related Quality of Life after a challenge test with cashew nut in children with a positive and negative challenge outcome (delta scores)

The mean changes in scores (delta scores) were measured for the FAQLQ-CF, FAQLQ-TF and FAQLQ-PF and the separate domains and the group of children with a positive challenge outcome were compared with the group of children with a negative challenge outcome. There were no significant differences in the changes in the scores of the FAQLQ-CF and FAQLQ-PF in the children with a positive challenge outcome compared to the children with a negative challenge result. However, there was a significant difference in the change in score in the domain 'accidental exposure' of the FAQLQ-TF (p = 0.049). The change in scores for the FAQLQ-TF in total and the other two domains showed no difference between the groups with a positive vs. a negative outcome. The same applies for the change in the FAIM scores of parents and teenagers. In children, the delta FAIM score in the negative DBPCFC tested group was significantly better than the delta score in the group with positive challenge outcomes (p = 0.026)(Table 5.4).

Table 5.3: Food quality of life before and after the DBPCFC test

FAQLQ Form		Base-line Score (mean)	Follow-up score (mean)	Delta Score (mean)	Statistical Significance (P- value)
Child Form	**FAQLQ**				
N = 33	Domain 1	3.06	3.57	0.51	0.102
	Domain 2	3.50	3.79	0.29	0.340
	Domain 3	3.93	3.75	-0.18	0.437
	Domain 4	3.44	3.43	-0.01	0.970
	Total	3.32	3.49	0.17	0.491
	FAIM	2.86	3.27	0.41	**0.025**
Teenager Form	**FAQLQ**				
N = 26	Domain 1	3.45	3.24	-0.21	0.392
	Domain 2	3.31	3.14	-0.17	0.591
	Domain 3	3.73	3.26	-0.47	0.086
	Total	3.50	3.22	0.28	0.286
	FAIM	3.26	2.89	-0.37	**0.006**
Parents Form	**FAQLQ**				
N = 84	Domain 1	2.15	2.24	0.09	0.302
	Domain 2	2.79	2.80	0.01	0.961
	Domain 3	2.28	2.35	0.07	0.532
	Total	2.37	2.43	0.06	0.538
	FAIM	3.17	3.01	-0.16	0.113

FAQLQ= Food Allergy Quality of Life Questionnaire, FAIM= Food Allergy Independent Measure

Possibly influencing factors on Health Related Quality of Life changes

The association of factors that could have influenced the lack of improvement in HRQL were studied using multiple regression analysis in an associative model. Age, centre of recruitment, atopic features as hay fever, asthma and eczema, history of cashew nut allergy, number of reported food allergies, anaphylaxis during DBPCFC, outcome of the DBPCFC with cashew nut, the dose eliciting a reaction during the challenge, were not associated with impairment in HRQL. A higher FAQLQ baseline score (lower HRQL) significantly influenced improvement of HRQL for the child and the teenager group ($p = 0.003$ and $p = 0.015$, respectively) and a higher FAQLQ baseline score for parents of the children, was almost significantly associated with improvement of HRQL ($p = 0.056$).

The possible influence of symptoms caused by (accidental) cashew intake after the challenge in the 6 months before the FAQLQ's were administered, were also evaluated. In the group of children with a positive challenge, there were no reported symptoms due to accidental exposure during this period of 6 months. In the group of children with a negative challenge, we measured introduction rate and reactions during introduction

Table 5.4: Food quality of life before and after DBPCFC test with cashew nut in children with a positive and negative challenge outcome

FAQLQ Form		Delta score * Positive DBPCFC tested group	Delta score* Negative DBPCFC tested group	Statistical significance (P-value)**
Child Form		**N = 24**	**N = 9**	
	FAQLQ			
	Domain 1	0.53	0.44	0.777
	Domain 2	0.33	0.18	0.935
	Domain 3	0.14	-0.41	0.777
	Domain 4	-0.27	0.56	0.292
	Total	0.19	0.10	0.840
	FAIM	0.68	-0.32	**0.026**
Teenager Form		**N = 21**	**N = 5**	
	FAQLQ			
	Domain 1	-0.43	-0.90	0.215
	Domain 2	0.15	-1.49	**0.049**
	Domain 3	-0.26	-1.31	0.090
	Total	-0.06	-1.18	0.085
	FAIM	-0.32	-0.60	0.252
Parents Form		**N = 62**	**N = 22**	
	FAQLQ			
	Domain 1	0.16	-0.11	0.196
	Domain 2	0.08	-0.19	0.113
	Domain 3	0.08	0.03	0.541
	Total	0.11	-0.10	0.176
	FAIM	-0.07	-0.41	0.066

FAQLQ= Food Allergy Quality of Life Questionnaire, FAIM= Food Allergy Independent Measure, DBPCFC= Double-Blind Placebo-Controlled Food Challenge

*** Follow-up score minus baseline score**

**** Statistical difference between the delta score of the positive and negative tested group**

Child form: 1 domain: allergen avoidance, domain 2: risk accidental exposure, domain 3: emotional impact, domain 4: dietary restriction

Teenager form: domain 1: allergen avoidance, domain 2: risk accidental exposure, domain 3: emotional impact

Parents form: domain 1: emotional impact, domain 2: food anxiety, domain 3: social and dietary limitations

(12). Only 3 children experienced mild symptoms during introduction, which did not allow analysis as a possible influencing factor on FAQLQ score.

Longitudinal and cross-sectional validity of Food Allergy of Life Questionnaires

We calculated a significant cross-sectional correlation between the baseline FAQLQ children, teenagers and parents of the children scores and the baseline FAIM scores. The

Pearson correlation coefficients thus obtained were 0.48 (p = 0.005) for the FAQLQ-CF, 0.73 (p = 0.000) for the FAQLQ-TF, and 0.67 (p = 0.000) for the FAQLQ-PF. The follow-up score (after 6 months) also showed a significant correlation for the FAQLQ-CF, -TF and PF of 0.58 (p = 0.000), 0.74 (p = 0.000) and 0.73 (p = 0.000), respectively. The correlation between the change in the FAQLQ score (follow-up score minus baseline score) and the change in the FAIM score were correlated for the FAQLQ-TF (0.43, p = 0.025) and FAQLQ-PF (0.54, p = 0.000), but not for the FAQLQ-CF (0.27, p = 0.124).

DISCUSSION

Previous studies with other allergenic foods showed that HRQL significantly improved after the food challenge, with greater improvements in HRQL after a negative outcome than a positive outcome (3, 13). Our study participants apparently did not experience substantial improvement of food allergy-related quality of life after a cashew nut challenge. However, there was a significant difference in delta score in the FAIM between the children in the group with a positive challenge outcome and those with a negative challenge outcome (p = 0.026). We could not find this difference in the teenagers or the parent group. Apparently, a negative challenge outcome improves the self-perceived severity of food allergy in young children. Perhaps young children are more focused on the tested foods than teens and adults, who may be more broadly concerned about their food allergies. This could result in a greater impact of a negative test outcome in younger children. This is also suggested by the observation that introduction of foods into the diet after a negative food challenge, is more successful in younger children than older children or adults (14). This may be cause and/or consequence of greater improvement in perceived severity of food allergy in these patients seen in our results.

A possible cause for the lack of improvement in HRQL in this study is the relatively low baseline scores, which corresponded to the relatively benign perception of participants of the severity of their food allergy as measured with the FAIM. Although these were not as low as in the general population, baseline scores in the present study were lower than in several other HRQL studies where patients and not researchers initiated participant contact.

In a study also performed in Dutch children, van der Velde et al. found higher mean baseline scores for the child forms (3.80) and teenager forms (3.89) compared to our study with 3.32 for the child forms and 3.50 for the teenager forms (3). Thus there was less room for improvement of HRQL in our study compared to the above mentioned study This is also reflected in the fact that FAQLQ and FAIM scores changed very little in both the challenge positive and even in the challenge negative participants.

Impairment of HRQL may also depend on the food causing the allergy. For example, it is demonstrated that parents of children with milk or egg allergy have a significantly poorer quality of life than parents of children with peanut or tree nut allergy (15). An explanation for this may be the ease to avoid cashew nut from the diet compared to avoidance of other allergens. The risk of accidental exposure is relatively low as it is seldom a hidden allergen. It is known that the numbers of allergic reactions caused by hidden allergens differ extremely per allergen (16). Consequently, the dietary impact of cashew nut allergy on the HRQL might be lower than that of, for example, peanut allergy.

Moreover, a previous study by our group demonstrated a low introduction rate of cashew nuts after a negative DBPCFC of 43.3%, probably due to a low perceived need on the part of parents to do so. If the outcome of the DBPCFC does not change the diet of the children, and/or fear for accidental exposure does not drive the need for accurate diagnosis, then a part of the beneficial effect of the challenge test might be lacking (12).

Possibly, the long-time (6 months) between the challenge with cashew nut and administration of the second questionnaire in our study might also be a reason for the unchanged HRQL in our children. A study by Soller et al. demonstrated an improvement of HRQL after a food challenge test for both allergic and non-allergic patients, however, this effect appeared to wane between 2 and 6 months after the challenge test (17).

The FAIM questionnaire was used to measure the validity of the FAQLQs. The Pearson correlations were significant for the baseline and follow-up scores and established the cross-sectional validity of the FAQLQs for children, teenagers and the parents of the children. The change in FAQLQ scores (follow-up minus baseline score) was significantly correlated with the change in FAIM scores for teenagers and parents of children, but not for children. The latter finding is in contrast to the previous study by Van der Velde et al. (3). However, their study showed a significant change (improvement) in HRQL after a DBPCFC. Therefore, it is likely that longitudinal validity could not be demonstrated because there was insufficient change in FAIM or FAQLQ scores in this study.

CONCLUSION

This study in participants of cashew nut allergy showed no difference in the change of HRQL after a positive vs. a negative DBPCFC test outcome. There was no significant change in HRQL after challenge testing, probably because participants did not experience a significant impairment (low baseline score) of their HRQL. Aside from the relative ease with which cashew nut may be avoided, this study was investigator initiated and not patient initiated. This may have contributed to the relatively good quality of life seen in the study subjects at the start of the study. Researchers in food allergy should thus be aware that participants recruited to a study may have relatively good initial HRQL and

that this may jeopardize efforts to demonstrate improvements in HRQL due to the study intervention.

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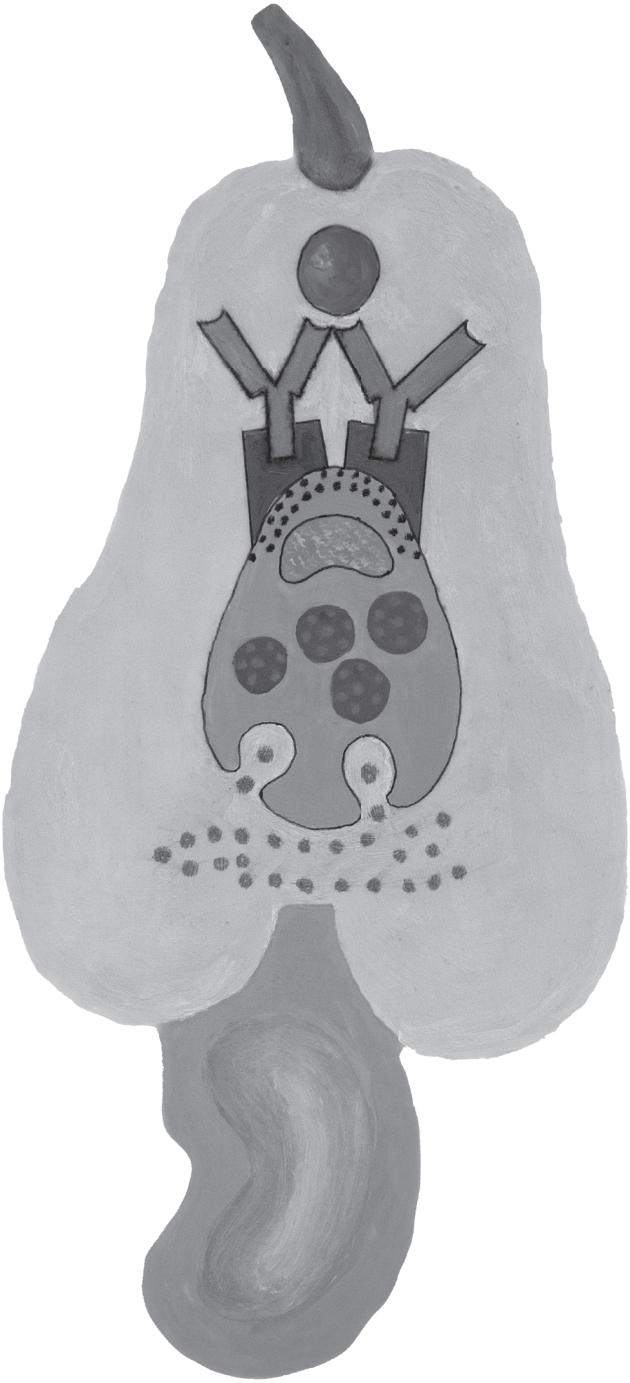

Chapter 5.3

Failure of introduction of cashew nuts after a negative food challenge test

In this letter, we describe the rate of introduction of cashew nuts after a negative food challenge test. This letter is published in Pediatric Allergy and Immunology in 2016.

Van der Valk JP, Gerth van Wijk R, Dubois AE, de Groot H, de Jong NW. Failure of introduction of cashew nut after a negative oral food challenge test in children. Pediatr Allergy Immunol. Sep;27(6):654–8 2016.

To the editor,

Food challenge tests are helpful in assessing the clinical relevance of sensitisation, as determined by skin prick tests and/or specific IgE measurements. After a negative challenge, the patient is advised to introduce the negatively tested food into the diet. Previous studies with commonly challenged allergens demonstrated, however, that in spite of this advice the rate of introduction after a negative challenge is low (1–3).

We studied the introduction rate after a negative challenge in cashew nut sensitised children, ranging in age from 2 to 17 years. The patients were recruited from the study 'Improvement of Diagnostic mEthods for ALlergy assessment' (IDEAL) with cashew nut allergy in children as a show case (trial number NTR3572). The inclusion and exclusion criteria and detailed study protocol were previously described (4). Briefly, all patients underwent a blood draw for sIgE determination, a skin prick test with cashew nut extract and a double-blind placebo-controlled food challenge (DBPCFC) test with cashew nut. The DBPCFC test consisted of an eight-step incremental dose regime of validated and standardised food challenge materials (5). The children consumed 3180 mg cashew nut protein (22 cashew nuts) if all 8 dose-steps were passed. The challenge step was repeated if the child experienced subjective symptoms that disappeared in an hour (6). The DBPCFC test was discontinued and considered positive when 1) objective symptoms occurred, or when 2) subjective symptoms re-occurred twice after the same dose of challenge material had been administered, three times consecutively, or when 3) subjective symptoms persisted for more than one hour (7). Repeating these portions was needed to find out whether the subjective symptoms reported by the child were reproducible and caused by the challenged food or coincidental symptoms due to other reasons.

The patient was contacted by telephone one week after the challenge test. The parent and/or the child was informed about the negative result of the challenge test (no cashew nut allergy). Then the physician explained that the cashew nut should be introduced into the diet and the importance of the introduction was explained. Furthermore, the patient was asked whether they thought they would be able to introduce the cashew into the diet, starting with small amounts of cashew nut, and if the response was affirmative, no further action was taken. When the patient and/or the family was not sure whether introduction would be successful, the physician explained the introduction method in more detail and an introduction schedule was recommended. In some cases the parents still felt that the introduction would give problems, then they were referred to a dietician, who again explained the introduction schedule (8, 9). The introduction schedules comprise instructions and photographs with information on the required amounts of specific food for home introduction.

The patients were asked to complete a questionnaire 6 months after the finish of the IDEAL- study (inclusion May 2012-March 2015), with questions concerning the introduction of cashew nut in the diet of the child. The questionnaire was based on the questionnaire used in a study on the introduction rate of hens' egg, cows' milk, hazelnut and peanut, and slightly adjusted for the IDEAL study (2). The questionnaire contained 14 questions on the successful or failed introduction, symptoms during introduction, the advice received and the understanding of the test result.

Children were divided in 3 groups: those with a successful introduction (starting as well as continuing eating cashew nut on a regular basis) those with a partly successful

Table 5.5: Possible factors associated with a failed introduction after the DBPCFC test with cashew nut

	Successful introduction N (%)	**Partial* introduction N (%)**	**Failed introduction N (%)**
Total	13 (43.3)	6 (20.0)	11 (36.7)
Gender			
Girl	2 (22.2)	2 (22.2)	5 (55.6)
Boy	11 (52.4)	4 (19.0)	6 (28.6)
Age			
0-4	3 (75.0)	0	1 (25.0)
4-8	5 (50.0)	3 (30.0)	2 (20.0)
≥ 9	5 (31.2)	3 (18.8)	8 (50.0)
Symptoms during FC according to the parents			
No	13 (46.4)	5 (17.9)	10 (35.7)
Yes	0	1 (50.0)	1 (50.0)
Advice			
Yes	11 (50.0)	5 (22.7)	6 (27.3)
No	2 (28.6)	1 (14.3)	4 (57.1)
Time to start eating the food			
Never started	0	0	7 (100.0)
A week	5 (83.3)	1 (16.7)	0
A month	5 (62.5)	2 (25.0)	1 (12.5)
A year	1 (50.0)	0	1 (50.0)
Forgotten by patient	2 (33.3)	3 (50.0)	1 (16.7)
Symptoms during introduction			
Yes	0	0	3 (100.0)
No	13 (61.9)	6 (28.6)	2 (9.5)
Never started	0	0	6 (100.0)

* Consumption of only traces or processed products

introduction (consumption of only traces or processed products) and those with a failed introduction (never consumed or only once and never thereafter).

Possible factors influencing successful introduction, which were based on a previous study (i.e. gender, age, symptoms during the DBPCFC test according to the patient, time between the test result and the start of the introduction procedure advice and symptoms during introduction) were recorded (2).

A total of 179 children were included and underwent the DBPCFC of which 36 (20.1%) tests were considered negative. Of these children (or their parents), 30 completed the questionnaire. The mean age of the children was 8.8 years (range 2–17 years), 21 boys (70%) and 9 girls (30%).

Successful introduction was reported in 13 children (43.3%), partly successful introduction in 6 children (20%) and failed introduction in 11 children (36.7%). Possible factors influencing the introduction rate are shown in Table 5.5. Statistical analysis could not be performed because of the small numbers. Other patients' characteristics and diagnostic test results in the groups of children with a failed, partly- or successful cashew nut introduction are shown in Table 5.6. The results do not show obvious differences between the groups nor is there a trend visible.

The main reason for a failed introduction was difficulty in changing the habitual avoidance of this food, followed by fear of the child and aversion to cashew nuts. Parents of one child said they were misinformed. Hence, this child did not introduce the cashew nut in the diet (Figure 5.1).

Table 5.6: Patients' characteristics and diagnostic test results in the groups of children with a failed, partly - or successful cashew nut introduction.

	Failed introduction **N = 11 (%)**	**Partly introduction** **N = 6 (%)**	**Successful introduction** **N = 13 (%)**
Eczema	3 (27)	3 (50)	7 (54)
Asthma	3 (27)	2 (33)	1 (23)
Hay fever	4 (36)	4 (67)	7 (54)
Other nut allergies			
Yes	5 (46)	4 (67)	8 (62)
No	4 (36)	2 (33)	3 (23)
Unknown	2 (18)		2 (15)
History of cashew nut allergy	6 (55)	2 (33)	7 (54)
SPT cashew nut (HEP-index area)	1.24 (range 0.39- 4.22)	0.30 (0- 1.82)	0.96 (0- 14.25)
sIgE cashew nut (kU/l)	2.16 (range 0- 17.80)	0.77 (range 0.43 – 15.70)	1.30 (range 0- 5.48)

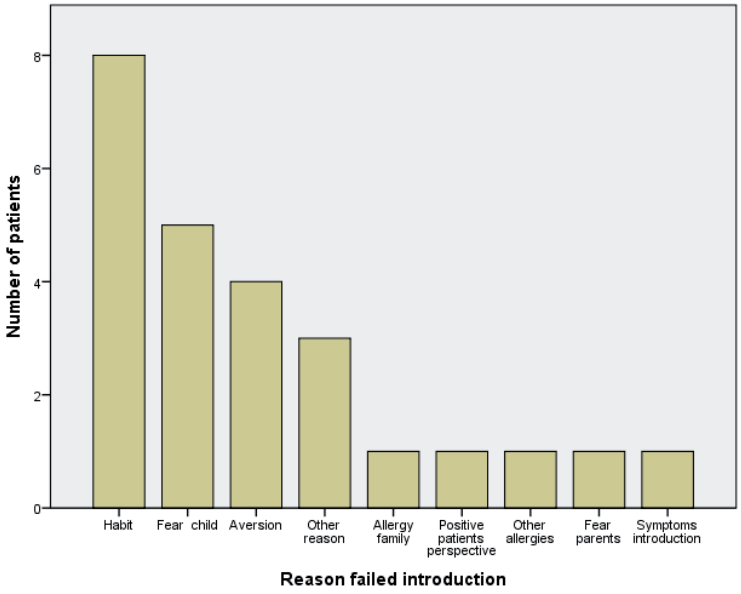

Figure 5.1: Main reason for a failed introduction according to the questionnaires

More boys (52.4%) than girls (22.2%) had a successful introduction. Younger children had higher rates of successful introduction than older children. These results are comparable with an earlier study (2).

Symptoms from the perspective of the parents during the DBPCFC were rarely reported. One child had skin symptoms and one child had respiratory symptoms.

Although all parents and children received advice to introduce the cashew nut into the diet after the negative DBPCFC, only 22/30 parents of the children (73%) reported to have received the advice. Clearly, from the perspective of the patient, the advice as it was given was in some cases insufficient to result in retention by the patient and/or family.

A higher percentage of parents and/or children, who reported to have received advice to introduce cashew nut into their diet after the food challenge (FC) test, had a successful introduction (11/22 children), compared to the parents and/ or children that did not recall receiving such advice (2/7 children). Ten children introduced cashew nuts between one week and one month.

Three of the 30 children who completed the questionnaire, reported symptoms during the introduction of cashew nut at home. Two of them reported 'oral allergy' symptoms and one child had respiratory symptoms in combination with gastrointestinal symptoms.

Our study showed a high failed introduction rate of 36.7% after a DBPCFC test with cashew nut. For the purposes of discussion we might even add this to the 20% of patients who had a partly successful introduction to yield an extremely high percentage of 56.7%. Previous studies have reported rates of failed introduction with different allergenic foods in 25%, 28% and 32% of the patients, respectively (1–3). Studies performed by Flammarion et al., Dambacher et al. and Miceli Sopo et al. showed higher rates of successful introduction of 83%, 81% and 71%, respectively (10–12). The first of these studies (10) included only children with cow's milk allergy and in the second two studies the number of patients with a cow's milk allergy and hen's egg allergy was overrepresented (11,12). A previous study by our group demonstrated that hazelnut and peanut FC test were followed by higher rates of failed introduction (40.6% and 39.5%, respectively) than by hen's egg and cow's milk (13.2% and 9.8%, respectively)(2). The rate of successful introduction for cashew nut in our study was comparable with the rates of successful introduction for peanut and hazelnut. Awareness in patients and the fear of life-threatening reactions as may be caused by nut or peanut allergy, as compared allergenic foods that elicit severe reactions less often, may be the reason for the differences between successful introduction rates of the different allergenic foods (13). Nuts and peanuts are not essential food products and a diet without nuts and peanuts will not lead to missing essential nutrients. It is easier to avoid nuts, in particular cashew nut, because these allergens are less often hidden in food products than hen's egg and cow's milk, although one should realise that cashew nuts are becoming increasingly common in processed food products (14). Recently, the LEAP study demonstrated that early introduction of peanut significantly decreased the development of peanut allergy in high risk children, which lead to new consensus guidelines regarding potential benefits of early, rather than delayed, peanut introduction into the diet of this specific group of children (15,16). Introduction of cashew nut into the diet of the sensitised children in the present study might be of great importance. Moreover, the chance of developing an acute allergic reaction after long time elimination is demonstrated by Flinterman et al. (17).

A shortcoming in the study is that we did not define 'continuing eating the cashew nut on a regular basis'. Currently, there are no published protocols with amount and frequency of exposure to the allergenic food, which may be considered adequate to prevent the occurrence of allergy. Often daily consumption is recommended, but more research is required to adequately define the minimum exposure parameters, which will maintain immunologic tolerance to the food in question.

A previous study by our group demonstrated that age, gender, symptoms during the challenge test, dietary advice and symptoms during introduction significantly influenced introduction rates (2). This study confirms that boys and younger children have a higher successful introduction rate. Concerning advice and introduction rate, in our

study 73% of the children received advice after the negative FC test according to the questionnaire compared to 52% in the abovementioned study (2). Despite the higher rate of children who received advice to introduce the cashew nut in the diet, rates of successful introduction remain low. We speculate that the intention to introduce cashew nut into the diet after a negative challenge may be less than with other allergenic foods. The most frequently reported reason of the parents to consent to the DBPCFC with cashew nut was to determine the clinical relevance of sensitisation and not to be able to introduce the cashew nut in the event of a negative challenge test.

In our study, only 3 children experienced symptoms at home during the introduction of cashew nuts. This is lower than that found in a previous study by our group, where 28% of the children reported symptoms during home introduction of hen's egg, cow's milk, hazelnut and peanut (2). We used challenge material recipes with higher final dosages than in the previous studies. A child consuming all doses would have eaten 3180 mg cashew nut proteins compared to 350 mg of proteins in the recipes used in the previous studies. This higher final challenge dose may be more reassuring.

In this study, more than half of the children did not successfully introduce the cashew nut into their diet after a negative challenge. This emphasizes the need for introduction protocols and better coaching methods after a negative challenge. It also highlights the need for follow up and attention to this somewhat neglected group in comparison to children with a positive challenge test.

In conclusion, firstly, successful introduction is dependent on the type of the tested allergenic food, age, and gender. Secondly, clear and structured advisory protocols are indispensable, to provide the patient with clear-cut information and a follow-up protocol to measure insure successful introduction of the allergenic food. Finally, we advise physicians to discuss the goals of the DBPCFC with patients prior to the test, to perform the test at the youngest age possible, and to use adequate amounts of protein to reduce the chance of false negative testing which may consequently cause symptoms at home during introduction.

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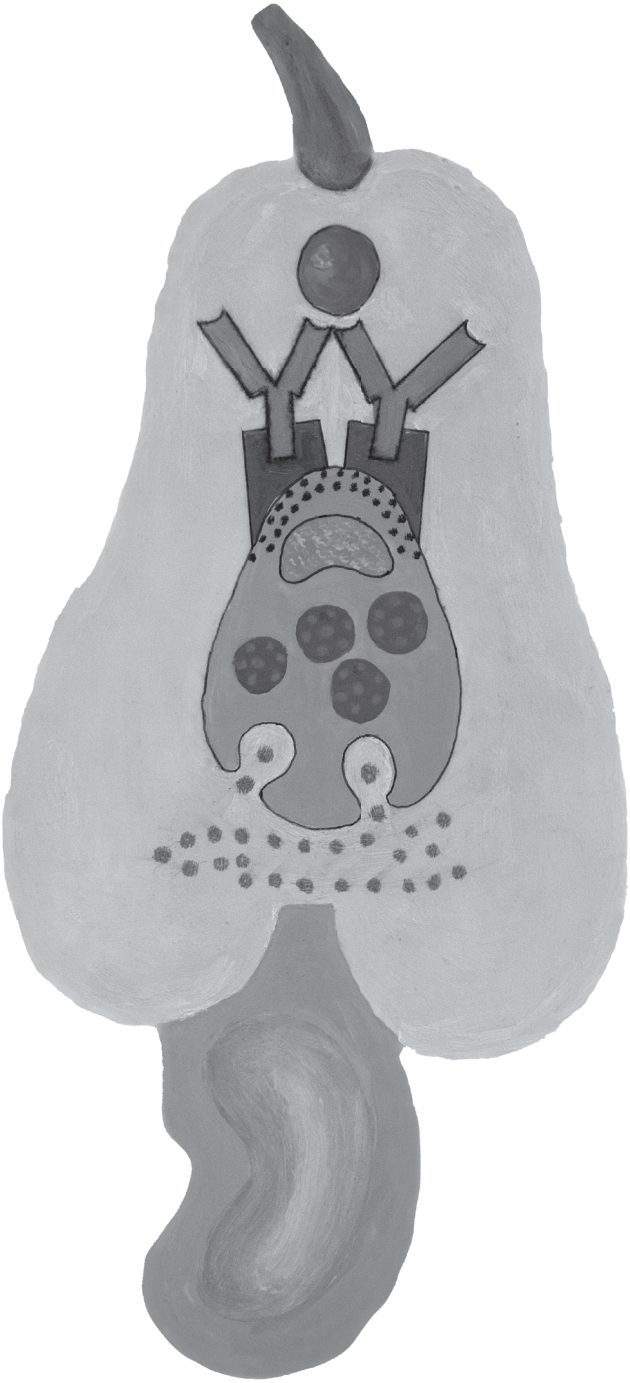
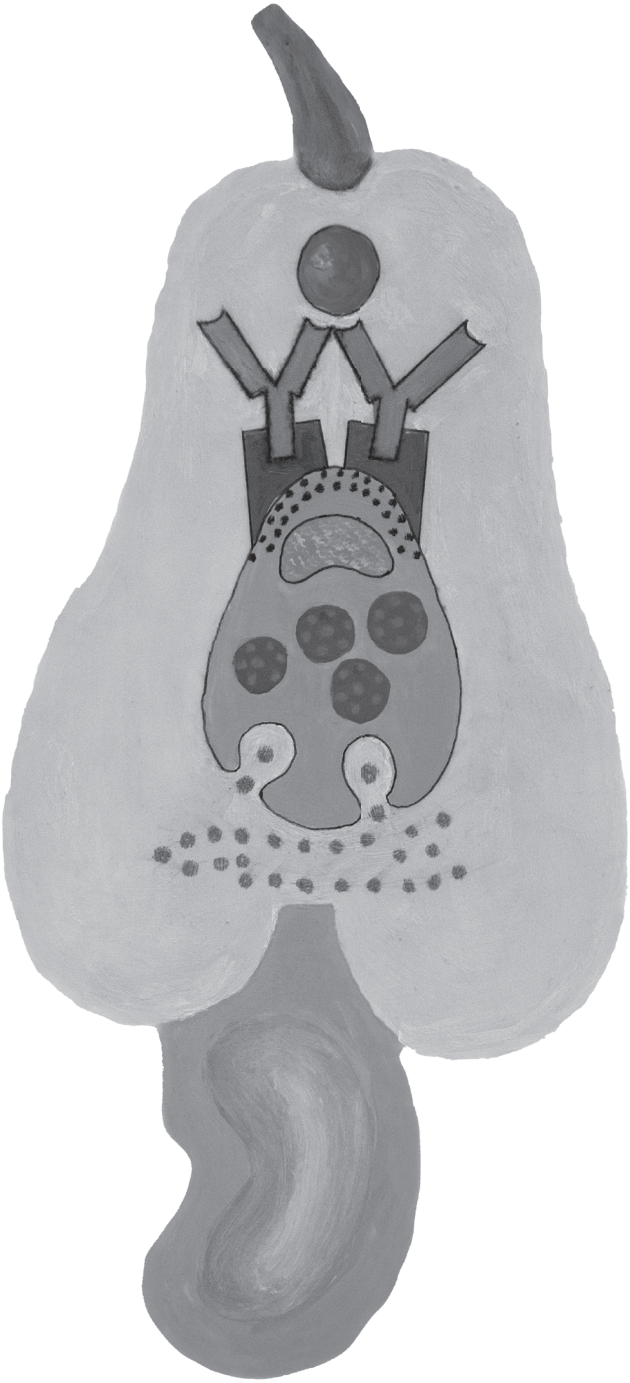


Chapter 6

General discussion and summary



Chapter 6.1

Discussion

Clinical presentation of cashew nut allergy

The IDEAL-study is developed to improve the diagnostics for food allergy with cashew nut allergy as a show-case. We performed a prospective multi-centre study with a relatively large number (n = 179) of cashew nut sensitised children (*chapter 3.1*). More than 75% of these children showed a clinical response in the DBPCFC test. This percentage is much higher than that observed in a previous other cashew nut study (1). Only in 8/42 (19%) children a cashew nut allergy could be confirmed with a positive challenge test in this study. 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 (2). 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 (1). 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 before the challenge test. A negative oral food challenge test after positive testing and/or positive history is reported between 9% and 38% for peanut allergy (3–7). 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.

We showed that cashew nuts allergens are highly potent and can cause relatively severe reactions. With the first dosage of only 1 mg of cashew protein, 46% of the participating, sensitised children experienced subjective and 11% objective symptoms. In our study we demonstrated that the ED_{05}, ED_{10} and ED_{50} for objective signs ranged from 0.8 to 1.6 mg, 3.5 to 4.3 mg and 108.4 to 149.1 mg of cashew nut protein based upon the Log-Normal, Log-Logistic and Weibull models (*chapter 3.2*). The study by Blom et al. showed a much higher ED_{05} in 31 cashew nut allergic children at 7.4 mg cashew nut protein compared to the ED_{05} in our study (8). The authors indicate in the discussion that this is an unexpected high quantity, taking into account that cashew nut allergy is considered to be as severe as a peanut allergy. The study by Eller et al. demonstrated in 780 challenge tests with egg, hazelnut, peanut and milk an ED_{05} causing objective symptoms of 2.08, 8.7, 18.9 and 59.3 mg protein, respectively (9). In contrast to our study, most (89%) of all 449 reactions during the positive challenge test were defined as mild in this study. The study by Taylor et al. showed in 286 peanut allergic patients an ED_{05} of objective symptoms of 7.3 mg whole peanut (equivalent to 1.8 mg of peanut

protein based on 25% protein in a peanut kernel). In this study, almost all the symptoms during the peanut challenge test were assessed as mild (10). Our study demonstrated a low cashew nut ED_{05} for objective symptoms compared to the above mentioned studies on EDs for other allergens. However, comparing the potency of allergens by comparing the EDs expressed as milligram proteins in the allergenic food is debatable, because the allergic reaction occurs in practice to quantities of the allergenic food as a whole. With different percentages of allergenic proteins in these different foods, a comparison of EDs as milligrams of the allergenic food, rather than just the protein it contains, may be more correct. The ED_{05} in our LOAEL- study is based on one study population and to make predictions on the whole at-risk cashew allergic population, the individual data has to be representative of the entire population. Therefore, more studies are needed on cashew nut thresholds to make predictions at a population level. However, this study demonstrates the potency of cashew allergens.

Our Low-dose follow-up study was performed to determine threshold levels lower than 1 mg cashew nut protein (*chapter 3.2*). Therefore, a subgroup of children (N = 12) from the IDEAL-study who reacted to the lowest dose of cashew nut protein (1 mg) were asked to participate in this follow-up study with low dose challenge tests. The LOAELs for objective and subjective symptoms were 0.30 and 0.01 mg cashew nut protein, respectively. Minimal eliciting doses for different allergenic foods were previously investigated by an expert panel in a study on threshold dose by Taylor et al. (11). This study demonstrated that the eliciting dose on which 1% (ED_{01}) of the population reacted with objective symptoms were 0.1 mg (Log-Logistic) and 0.22 mg (Log-Normal) for peanut and 0.08 mg (Log-Logistic) and 0.21 (Log-Normal) for milk. The ED_{01} ranges between 0.05 and 0.21 mg protein for egg and 0.02 mg and 0.25 mg protein for hazelnut based upon the Log-Normal, Log-Logistic and Weibull models. The 0.30 mg cashew nut protein as lowest eliciting dose of mild objective symptoms in our LOAEL follow-up study has the same order of magnitude.

The individual thresholds for LOAELs in our study were not reproducible in all children. Three children reacted to a higher dose of cashew nut protein and four patients did not react anymore in the low dose challenge test, suggesting that their thresholds had increased beyond 3 mg cashew nut protein. Previously, Glaumann et al. observed in 29 peanut-allergic patients that only two of these children reacting to the same threshold dose and with the same severity score in two successive food challenge test with peanut (12).

Furthermore, approximately 40% of the children in our IDEAL- 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 dose 1 (1 mg cashew nut protein). This demonstrated that a minimal amount of cashew nut can cause (severe) allergic reactions.

OPTIMISATION OF THE DIAGNOSTIC PROCEDURE FOR FOOD ALLERGY

History

The first step in the diagnostic procedure of food allergy is taking the history. The history is important to determine if the reaction type is according to an immediate Type-I allergic reaction. Allergy testing starts with demonstrating sensitisation by either sIgE or a SPT.

Specific IgE

sIgE is regularly used to establish sensitisation in patients suspected for a food allergy (13). The sensitivity and specificity of sIgE tests are dependent on the tested allergen, age of the patients, population, geographic region, test method and chosen cut-off values of sIgE.

We demonstrated that sIgE to total cashew nut was associated with a positive DBPCFC test with a concordance-index (c-index) of 0.76 (*chapter 4.2*). The c-index is equal to the Area Under the Receiver Operating Characteristic Curve (AUC) for a dichotomous outcome. This association has to our knowledge never been previously determined. Maloney et al. acquired sIgE levels with 99% PPV for walnut (cut-off level of 19 kU/l) and Buyuktiryaki et al. acquired sIgE levels with 95% PPV for hazelnut (10 kU/l) (14–15).

For the major allergenic foods as hen's egg, cow's milk and peanut, sIgE sensitivity and specificity were determined in several studies with patients suspected for a food allergy (15–20). These studies included children and adults from different continents. The study groups vary between 100 and 324 patients. A PPV of > 95% was determined in this studies for hen's egg (sIgE cut-off value varying between 6 and 13 kU/l), for cow's milk (sIgE cut-off value varying between 13 and 32 kU/l), peanut (sIgE cut-off values varying between 15 and 24 kU/l). The sIgE cut-off values are very high and only a few patients have these values. Furthermore, these data cannot be extrapolated to each patient, because this PPV are dependent on age of the patients, population and geographic region.

Skin prick test

The SPT is an easy to perform and cheap test, recommended in the diagnosis of food allergy (13). The same as for the sIgE applies for the SPT, the sensitivity and specificity of the SPT are dependent on the tested allergen, the age of the patients, population, geographic region, test method and the chosen cut-off values of the SPT.

Our data showed that the SPT with cashew nut extract was strongly associated with a positive DBPCFC test with a c-index of 0.83 (*chapter 4.2*). The study by Ho et al. demonstrated in 86 cashew nut challenge tests in children and teenagers that a SPT weal diameters of ≥ 8 mm predicted a positive food challenge with 95% accuracy (21). The same applies for other nuts as hazelnut and walnut. For almond, pistachio, pecan, and

Brazil nut it was not possible to determine the 95% positive predictive value (PPV) in this study.

The SPT sensitivity, specificity, PPV at different cut-off values for the major allergenic foods as hen's egg, peanut and hazelnut were determined in several studies with patients suspected for a food allergy (22–27). All these studies were performed in children from different continents with study populations varying between 47 and 820 children. A PPV of 95% was determined in these studies for hen's egg (SPT cut-off values varying between ≥ 5 and ≥ 10 mm wheal diameter), for peanut (SPT cut-off values varying between ≥ 4 and ≥ 13 mm wheal diameter) and for hazelnut (SPT cut-off value varying between 8 ≥ and ≥ 12 mm wheal diameter).

Our data (*chapter 4.2*) show that sIgE to total cashew nut and, to an even greater extent SPT with cashew nut extract, are associated with a positive DBPCFC test (c-index of 0.76 versus 0.83, respectively). The stronger associations with SPT than with sIgE were previously shown for other food allergens in predicting clinical allergy (16, 23–25). A previous retrospective study on 983 children who underwent food challenge test with egg, milk and peanut also demonstrated that SPT results were more strongly associated with a positive food challenge test than sIgE (LR 1.23 and 1.04) (28). The study by DunnGalvin et al., who aimed to predict the challenge outcome for 429 patients with suspected peanut, milk and egg allergy also showed a greater predictive capacity of SPT than sIgE (29). We showed that this is obviously the case for cashew nut allergy. One may speculate that a positive SPT indicates not only the presence of sIgE in the sensitised individual, but also reflects the biological activity of sIgE.

Optimisation of the measurement and interpretation of the skin prick test

In clinical practice and in most academic research, it is common to characterise the wheal shape by the 'average diameter'. To determine the average diameter, the mean value of the longest and the midpoint orthogonal diameter (mm) of the wheal were measured. However, we demonstrated in the paper '*Measurement and interpretation of skin prick test results*' *(chapter 4.1)* that this method is prone to errors, because it is assumed that the wheal size varies between a circle and an ellipse. In fact, the wheals have pseudopodia and interpretation based on two orthogonal diameters is not accurate. Our study showed that for a given average wheal diameter, the actual wheal area could vary quite significantly and this inaccuracy grows with wheal size. This inaccuracy is completely eliminated if one applies the scanning method. In the scanning method, the area of the wheal was determined by using a flatbed scanner (Hewlett Packard) in combination with software earlier developed by Erasmus MC: Precise Automated Area Measurement of Skin Test (PAAMOST). This more precise method for measuring the wheal size area is previously described by Pijnenborg et al. (30). The scanning method

is also fast, easy in use, has a high reproducibility and is very useful in scientific research (30–33). To even further increase the accuracy of SPT results, the HEP-index area can be calculated, to rule out differences in skin reactivity. The HEP-index can be calculated by dividing the allergen area by the mean area of two histamine-induced wheal sizes of the positive controls. There are several factors that contribute to this difference e.g. poly-sensitised patients and patients with mould sensitisation have significantly higher skin reactions (34) and the skin response varies in different ethnicities (35). Furthermore, differences in technique of performing SPTs (inter-observer variability) contribute to the variation in wheal size (36). To correct for these factors, the calculation of the HEP-index area is useful and also easy to determine with the scanning method. Notwithstanding all advances of the scanning method inclusive the HEP-index calculation, the 'average diameter' method is as accurate in diagnosing cashew nut allergy as the 'HEP-index area' method. Therefore, the 'average diameter' method can be used if there is no scanning device available.

Component resolved diagnosis

CRD is an advanced method to measure sIgE to specific components of the allergens. We investigated the predictive value of CRD in cashew nut allergy (*chapter 4.2*). A markedly greater risk of cashew nut allergy as ascertained by DBPCFC was observed for higher values of sIgE to Ana o 1, 2 and 3. At higher levels of sIgE to Ana o 1, 2 and 3 total risks of approximately 100% were observed. The associations were extremely strong with c-indices of 0.87, 0.85 and 0.89 for sIgE to Ana o 1, 2 and 3, respectively. This is never previously demonstrated for all three cashew nut components.

The cashew nut component analysis could not distinguish between a mild and a severe cashew nut allergy during the DBPCFC test with cashew nut. Savvatianos et al. previously demonstrated an association between sIgE to Ana o 3 and a positive challenge test (37). This research group investigated sensitisation to Ana o 3 in 63 children in whom clinical reactivity to cashew nut was documented. Their study demonstrated a near-optimal AUC of 0.97 in the Receiver Operator Characteristics (ROC) curve. However, the clinical diagnosis of cashew nut allergy was based on clinical history in 95% of the cases and only in 5% of the cases on food challenge tests. Therefore, the strong association observed with Ana o 3 levels may have been with sensitisation rather than with clinical allergy to cashew nut resulting in a stronger association. However, the gold standard is the DBPCFC test that all children underwent in our study. For other allergies such as that to peanut and hazelnut, the added value of CDR in the diagnosis of clinical allergy to these foods has been proved. Peanut allergen Ara h 2 and hazelnut allergen Cor a 14, which are both 2S Albumins (as is Ana o 3), are of great importance to estimate the risk of a positive challenge test outcome with peanut and hazelnut, respectively

(38–40). 2S Albumins are resistant to proteolysis, heat denaturation and pH changes and are therefore considered to be clinically relevant allergens (41).

CRD for hen's egg, cow's milk and soya had no added value in predicting the challenge outcome (42–50). Several studies demonstrated that the diagnostic sensitivity improves by CRD for wheat, kiwi, cherry, celery, carrot and shrimp (51–58).

Prediction models

CRD can also be used in models to predict the outcome of the food challenge tests. We developed a prediction model for the outcome of the DBPCFC with cashew nut (*chapter 4.3*). The prediction model based on gender and sIgE to Ana o 3 with and without SPT gives the strongest association with an excellent c-index of 0.90 and 0.89 (corrected for optimism), respectively. Gender had a significantly added value in the model with higher risk on a positive challenge for girls. Why sensitisation to cashew nut is more often clinically relevant in girls than boys is currently unknown. The SPT is recommended in diagnosing food allergy, however, not all medical settings have the opportunity to perform the SPT (37). Therefore, we developed a model with and without the SPT. The SPT had minimal added value in the model with gender and sIgE to Ana o 3, however, if Ana o components are not available, the SPT is the second best in the diagnostic method for cashew nut allergy (likelihood ratio test statistic $\chi 2$: Ana o 13.75 and SPT 6). sIgE to total cashew nut had no added value in predicting the outcome of the DBPCFC if sIgE to the Ana o components was determined. Zomer-Kooijker et al. previously developed a prediction model for food allergy (59). This study demonstrated that the outcome of 129 challenge test with different allergens can be predicted accurately on the index food, time gab between ingestion and the development of symptoms and the sIgE levels with and AUC of 0.90 in the ROC curve. The study by DunnGalvin et al. also showed the importance of taking multiple predictors into account as gender, age, history of allergy, total IgE minus sIgE and SPT to develop very strong prediction models for peanut-, egg- and milk, respectively (29). However, only 90% of the diagnoses were established with food challenge test what the correlation may overestimate. Klemans et al. validated the above-mentioned model and conclude that the validation was good, however, the calibration poor, probably caused by overfitting of the data (40). The model predictions support the selection of children requiring a challenge test to diagnose cashew nut allergy and this can saves cost, time and burdensome for the patient. In situations where there is limited availability of double blind testing for suspected cashew allergy, the use of the model and scoring system may be useful for identifying children who have ≥ 95% chance of having a positive challenge test result and in whom such testing is less likely to influence management. In our present series, this pertains to a substantial number of patients (58/173 children, 34%).

Although, our prediction model is very useful in clinical practice and the number of food challenge tests can be decreased, beside internal validation, external validation is needed to extrapolate our model to other populations. For clinical practice, we developed an easy-use score chart based on the models. The score chart was developed to calculate the chance on a positive DBPCFC test outcome for each cashew nut sensitised patient individually.

Food challenge test

Notwithstanding, CRD and predictive models are incorporated into clinical use; the DBPCFC test remains the gold standard in diagnosing food allergy.

However, the DBPCFC test is a costly and time-consuming test for the physician and patient. Further disadvantages of the test are e.g. that test is not appropriate for each patient, intrinsic and extrinsic factors are absent in the artificial test setting, certain medications should be stopped and not all medical care centres have the opportunity to perform the DBPCFC test (60). Placebo reactions during the challenge test (61–62) and reactions during the introduction of the allergen after a negative food challenge test (63) are not only described in exceptional cases.

In the IDEAL-study, placebo reactions were observed in 11% (20/179) of the children and consisted of reported subjective reactions in 70% (14/20) and both, subjective and objective in 35% (6/20). Placebo reactions were in 5 children the reason for of an undecided challenge outcome. We observed placebo reactions in 4 of the 12 patients (33%) in the Low-dose follow-up study (*chapter 3.2*). This is a relatively high percentage for DBPCFC as compared to placebo reactions in other studies (61–62). Because of all these disadvantages, the search for better, cheaper and simpler alternatives as CRD and predictive models is needed. We demonstrated that prediction models may reduce the need for food challenge test. Therefore, this should be developed for all (major) allergens.

CONSEQUENCES OF CASHEW NUT ALLERGY

Co-sensitisation

We studied the clinical relevance of co-sensitisation between cashew nut and pistachio nut and mango in the pistachio nut part of the IDEAL-study (N = 29) (*chapter 5.1*). Cashew nut, pistachio nut and mango belong to the *Anacardiaceae* family and are botanically related. In line with previous reports, we showed a high rate of co-sensitisation between cashew nuts and pistachio nuts in SPT and sIgE (respectively 92% and 98%). 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. We demonstrated that the pistachio nut sensitisation was only clinically relevant in 34%. In ad-

dition, co-sensitisation to mango did not result in clinical reactivity to mango. Previous studies reported high percentages of co-sensitisation and cross-sensitisation between cashew nut and pistachio nut (64–70). The clinical relevance of these sensitisations has only been reported in two studies (66–67).

Because of the high amount of positive food challenges with cashew nut compared to the pistachio nut, it is most likely that the primary sensitiser is the cashew nut. However, confirmation with inhibition tests is needed.

Health related quality of life

With the advent of clinical trials for the treatment of food allergy, Health-Related Quality of Life (HRQL) is becoming an increasingly important outcome measure to document benefit of treatment. Previous studies with other allergenic foods showed that HRQL significantly improved after the food challenge, with greater improvements in HRQL after a negative outcome than a positive outcome (71–72). In contrast, we showed a significantly different change in FAQLQ-TF only for the domain 'risk accidental exposure' in teenagers with a negative and a positive challenge outcome ($p = 0.049$). All the other FAQLQ scores and the FAIM score showed no significant differences in patients with positive vs negative test outcomes (*chapter 5.2*). A possible cause for this lack of improvement is the relatively low baseline scores, which corresponded to the relatively benign perception of participants of the severity of their food allergy as measured with the FAIM.

Impairment of HRQL may depend on the food causing the allergy. For example, it is demonstrated that parents of children with milk or egg allergy have a significantly poorer quality of life than parents of children with peanut or tree nut allergy (73). An explanation for this may be the ease to avoid cashew nut from the diet compared to avoidance of other allergens. Aside from the relative ease with which cashew nut may be avoided and the fact that many participants had not had previous exposure to cashew nut, this study was investigator initiated and not patient initiated. This may have contributed to the relatively good quality of life seen in the study subjects at the start of the study.

Failure of introduction of cashew nut after a negative challenge test

One of the main aims of the DBPCFC test in case of a negative test outcome is to introduce the tested allergen into the diet. Our follow-up study '*Failure of introduction of cashew nuts after a negative food challenge test*' (N = 30) (*chapter 5.3*) showed a high failed introduction rate of 36.7% after a negative DBPCFC test with cashew nut. Previous studies have reported rates of failed introduction with different allergenic foods in 25%, 28% and 32% of the patients, respectively (63, 74–75). Studies performed by Flammarion et al., Dambacher et al. and Miceli Sopo et al. showed higher rates of successful introduction of 83%, 81% and 71%, respectively (76–78). The first of these studies (77) included only

children with cow's milk allergy and in the second two studies; the number of patients with a cow's milk allergy and hen's egg allergy was overrepresented (77–78). A previous study by our group demonstrated that hazelnut and peanut FC test were followed by higher rates of failed introduction (40.6% and 39.5%, respectively) than by hen's egg and cow's milk (13.2% and 9.8%, respectively)(2). The rate of successful introduction for cashew nut in our study was comparable with the rates of successful introduction for peanut and hazelnut.

Successful introduction is dependent on the type of the tested allergenic food, age, advice and gender. Clear and structured advisory protocols are indispensable, to provide the patient with clear-cut information and a follow-up protocol to measure insure successful introduction of the allergenic food. We advise physicians to discuss the goals of the DBPCFC with patients prior to the test, to perform the test at the youngest age possible, and to use adequate amounts of protein to reduce the chance of false negative testing which may consequently cause symptoms at home during introduction.

RECOMMENDATIONS FOR FUTURE RESEARCH

With the increase of processing of cashew nuts and change in eating habits and as consequence the increase of exposure to cashew nuts in the population, we expect that the prevalence of cashew nut allergy will rise. Therefore, clinicians will have to deal with cashew nut allergy more often. It is important to diagnose cashew nut allergy in an efficient and save way. We improved the diagnostic process for cashew nut allergy with predictive models including gender and Ana o (with and without SPT). These models facilitate the selection of children who have ≥ 95% chance of having a positive challenge test result and in whom such testing is less necessary. This may save time and costs for a selected group of patients. However, more research is needed to investigate if we can extrapolate this model to other populations. Furthermore, it is important to keep this predictive model for cashew nut allergy up to date for our own population. The prevalence of cashew nut sensitisation and cashew nut allergy increases over time, which raises the a priori probability of a cashew nut allergy. This will require adjustments to our model to ensure the usability of the model for our own population in the future.

In this study, we used purified Ana o 1, 2 and 3. The strong association between sIgE to Ana o components with a positive challenge test asks for commercially available Ana o components.

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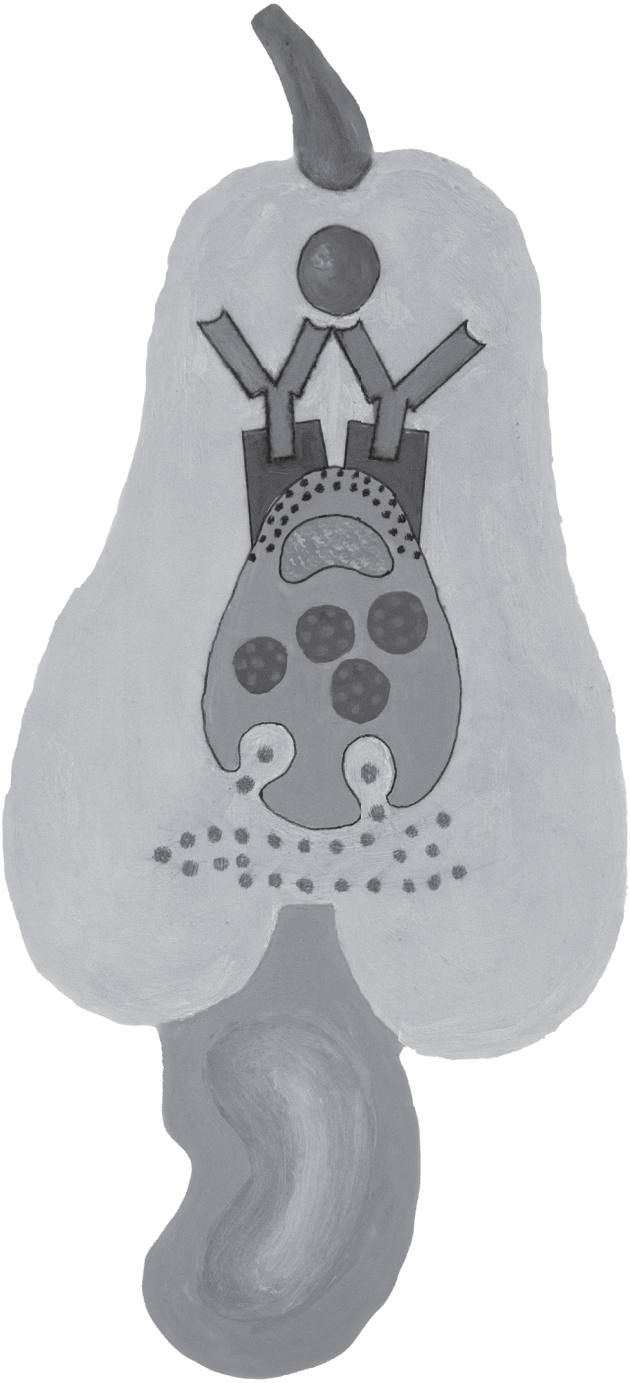


Chapter 6.2

Summary

The content of the thesis contributes to the knowledge of the cashew nut and cashew nut allergy. Cashew nut allergy is an important healthcare problem, especially in children. We performed a prospective multicentre study (IDEAL-study) with a 179 cashew nut sensitised children.

Chapter 1. General introduction

The cashew nut (*Anacardium occidentale*) belongs to the *Ancardiaceae* family and is botanically related to pistachio (*Pistacia vera*) nut and the mango (*Mangifera indica*). The major allergen components of the cashew nut are Ana o 1, Ana o 2 and Ana o 3.

Cashew nut consumption has increased and post aut propter sensitisation rates has also increased, especially in children. Accurate interpretation of sensitisation is mandatory.

Chapter 2. Literature overview

The paper '*Systematic review on cashew nut allergy*' summarises the current knowledge on cashew nut allergy. The literature search yielded 255 articles of which 40 met our selection criteria and were considered to be relevant for this review.

In comparison with literature and research focussed on peanut, cashew allergy is clearly an underestimated but important healthcare problem, especially in children. Further research is urgently needed on this relatively new food allergen.

Chapter 3. Clinical results of the IDEAL-study

The first paper in this chapter '*Multicentre double-blind placebo-controlled food challenge study in children sensitised to cashew nut' shows* the clinical results of the IDEAL-study.

In total 179 children with cashew nut sensitisation and a clinical history of reactions to cashew nuts or unknown exposure (median age 9.0 years; range 2–17 years) participated in the study. Of these sensitised children, 137 (76.5%) had a positive DBPCFC test. 46% manifesting subjective symptoms to the lowest dose of 1 mg cashew nut protein and 11% 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.

The second paper in this chapter '*Threshold distribution and lowest observed adverse effect levels in cashew nut allergic children',* shows the threshold distribution curve of the IDEAL-study and the lowest observed effect level. The eliciting dose on which 5%, 10% and 50% of the population reacted with objective symptoms ranges between 0.8 and 1.6 mg (ED_{05}), 3.5 and 4.3 mg (ED_{10}) and 108 and 149.1 mg (ED_{50}) of cashew nut protein based upon the Log-Normal, Log-Logistic and Weibull models. This ED_{05} for cashew nut

(objective symptoms) is low compared to the ED_{05} of peanut, hazelnut, milk and egg in other studies. The lowest dose on which mild objective and subjective symptoms occurred was 0.30 mg and 0.01 mg cashew nut protein, respectively.

Chapter 4. Improvement of diagnostic methods

The first paper in this chapter *'Measurement and interpretation of skin prick test results'* shows that the scanning method for SPT measurement is more accurate to measure the wheal area in a Type-I allergy than the average diameter. It is possible to correct for skin-sensitivity and inter-observer variability by using the 'HEP-index area' method. Therefore, we divided the area of the allergen-induced wheal by the area of the positive histamine-induced wheal controls. This ratio is defined as the (HEP)-index area. The HEP-index area value 0.4 can be considered as an equal cut-off value of 3 mm wheal average diameter. However, in clinical practice, the 'average diameter method' is also useful, because this method is equally accurate in predicting cashew nut allergic reactions in the DBPCFC tests.

The second paper in this chapter, *'sIgE Ana o 1,2 and 3 accurately distinguish tolerant from allergic children sensitised to cashew nuts'* demonstrates a steep rise in the risk of a positive challenge for sIgE to each individual component Ana o 1, 2 and 3 with estimated risks up to approximately 100%. sIgE to Ana o 1, 2 and 3 better distinguishes between cashew-allergic and tolerant children (c-index = 0.87, 0.85 and 0.89 respectively), than sIgE to cashew nut or SPT with a c-index of 0.76 and 0.83 respectively (c-index is equal to the Area Under the Receiver Operating Characteristic Curve for a dichotomous outcome).

We describe in third paper *'Predictive model for cashew nut allergy in children'* a multivariate model to predict the outcome of the DBPCFC test with cashew nut and showed easy to -use format of the models to calculate the risk of cashew nut allergy in sensitised children. Gender and Ana o components (with and without SPT) showed the highest discriminative ability (c-index = 0.90 with SPT and 0.89 without SPT). The models facilitate the selection of children requiring a challenge test to diagnose cashew nut allergy and may save time and cost for a selected group of patients. In situations where there is limited availability of double blind testing for suspected cashew allergy, the use of the model and scoring system may be useful for identifying children who have ≥ 95% chance of having a positive challenge test result and in whom such testing is less likely to influence management. In our present series, this pertains to a substantial number of patients (58/173 children, 34%).

Chapter 5. The clinical consequence of cashew nut allergy

In the first paper in this chapter '*Low percentage of clinically relevant pistachio nut and mango co-sensitisation in cashew nut sensitised children*' we assessed the clinical relevance of pistachio nut and mango co-sensitisation in cashew nut sensitised children. All children sensitised to cashew and pistachio nut and/or mango underwent a DBPCFC with cashew nut, with pistachio nut and an open food challenge with mango.

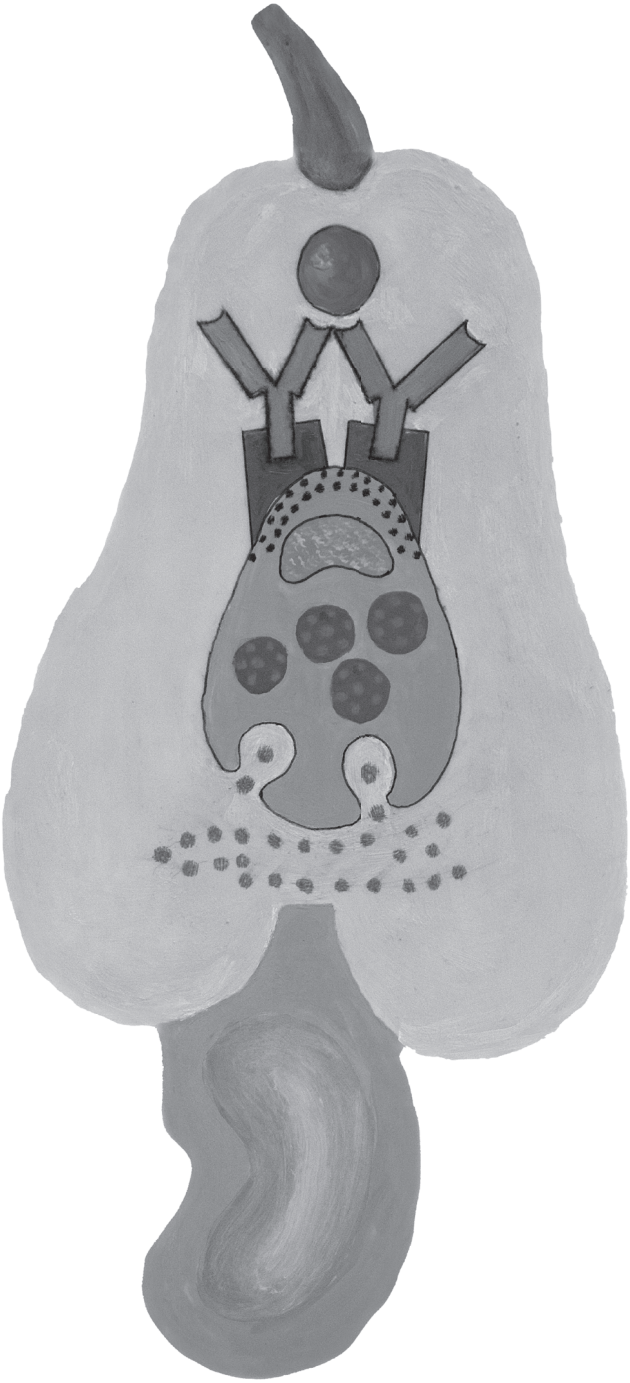
Although co-sensitisation in skin test between cashew nut and pistachio nut was observed in 98%, the pistachio nut sensitisation was only clinically relevant in 34.5% of the children. Therefore, in case of a co-sensitisation between cashew nut and pistachio nut, a DBPCFC is recommended. None of the children was mango-allergic.

The second paper in this chapter *'No difference in health-related quality of life, after a food challenge with cashew nut in children participating in a clinical trial'* demonstrates the effect on quality of life in positive and negative challenged children using FAQLQ's before and after the DBPCFC test. The cashew nut allergic patients, both with positive and negative challenge outcome, experienced no significant change of HRQL in this study.

Aside from the relative ease with which cashew nut may be avoided and the fact that many participants had not had previous exposure to cashew nut, this study was investigator initiated and not patient initiated. This may have contributed to the relatively good quality of life seen in the study subjects at the start of the study.

We describe in last paper in this chapter '*Failure of introduction of cashew nuts after a negative food challenge test'* the introduction rate of cashew nut after a negative challenge test.

After a negative challenge, the patient is advised to introduce the negatively tested food into the diet. Previous studies with commonly challenged allergens demonstrated, however, that in spite of this advice the rate of introduction after a negative challenge is low. In this study, successful introduction (starting as well as continuing eating cashew nut on a regular basis) was reported in 13 children (43.3%), partly successful introduction (consumption of only traces or processed products) in 6 children (20%) and failed introduction (never consumed or only once and never thereafter) in 11 children (36.7%). Successful introduction was dependent on the type of the tested allergenic food, age, and gender. Secondly, clear and structured advisory protocols are indispensable to provide the patient with clear-cut information and a follow-up protocol to measure insure successful introduction of the allergenic food.



Chapter 6.2

Samenvatting

De inhoud van dit proefschrift draagt bij aan de kennis van de cashewnoot en cashewnootallergie. Cashewnootallergie is een belangrijk gezondheidsprobleem, voornamelijk bij kinderen. We hebben een prospectieve multicenter studie (IDEAL-studie) uitgevoerd met 179 cashewnoot gesensibiliseerde kinderen.

Hoofstuk 1. Algemene introductie

De cashewnoot (*Anacardium occidentale*) behoort tot de *Ancardiaceae* familie en is botanisch verwant aan de pistachenoot (*Pistacia vera*) en mango (*Mangifera indica*). De belangrijkste allergenen van de cashewnoot zijn Ana o 1, Ana o 2 en Ana o 3.

De consumptie van cashewnoten neemt toe en daardoor ook het aantal cashewnoot gesensibiliseerde mensen, waaronder voornamelijk kinderen.

Hoofdstuk 2. Literatuur overzicht

Het artikel '*Systematic review on cashew nut allergy*' geeft een samenvatting van de literatuur over cashewnootallergie. Het literatuuronderzoek leverde 255 artikelen op, waarvan 40 voldeden aan onze selectiecriteria en relevant waren voor het review.

In vergelijking met literatuur over pinda-allergie is cashewnootallergie een onderschat, maar belangrijk gezondheidsprobleem, voornamelijk bij kinderen. Meer onderzoek is dringend noodzakelijk naar dit relatief nieuwe voedselallergeen.

Hoofdstuk 3. Klinische resultaten van de IDEAL-studie

Het eerste artikel in dit hoofdstuk '*Multicentre double-blind placebo-controlled food challenge study in children sensitised to cashew nut'* toont de klinische resultaten van de IDEAL-studie.

In totaal deden er 179 kinderen mee aan de studie (mediane leeftijd 9.0 jaar; range 2–17 jaar) met een cashewnootsensibilisatie en een allergische reactie in de voorgeschiedenis na de consumptie van cashewnoten of nooit blootgesteld aan cashewnoten. Van deze gesensibiliseerde kinderen hadden 137 (76.%) een positieve DBPGVP test. 46% van de kinderen rapporteerde subjectieve klachten na inname van de laagste provocatiedosis van 1 mg cashewnooteiwit en 11% ontwikkelde objectieve klachten na deze dosis. De kinderen hadden het meest frequent maag-darmklachten, gevolgd door 'oral allergy' en huidklachten. Totaal had 36% (49/137) van de kinderen een anafylactische reactie en 6% (8/137) van deze kinderen werd behandeld met adrenaline.

Het tweede artikel in dit hoofdstuk '*Threshold distribution and lowest observed adverse effect levels in cashew nut allergic children',* toont de drempelwaarde distributiecurve van de IDEAL-studie en de laagste hoeveelheid allergeen waarop een reactie plaats vond.

De uitlokkende dosering waarop 5%, 10% en 50% van de populatie reageerde met objectieve symptomen varieert tussen de 0.8 en 1.6 mg (ED_{05}), 3.5 en 4.3 mg (ED_{10}) en

108 en 149.1 mg (ED_{50}) gebaseerd op de Log-Normal, Log-Logistic en Weibull modellen. Deze ED_{05} voor cashewnoot (objectieve symptomen) is laag vergeleken met de ED_{05} voor pinda, hazelnoot, melk en ei in andere studies. De laagste doses waarop milde objectieve en subjectieve klachten ontstonden was 0.30 mg en 0.01 mg cashewnooteiwit, respectievelijk.

Hoofstuk 4. Verbetering van de diagnostische methoden

In het eerste artikel van dit hoofdstuk *'Measurement and interpretation of skin prick test results'* tonen we aan dat de scanmethode voor het meten van de huidpriktestresultaten nauwkeuriger is in het schatten van de 'wheal'-oppervlakte door gebruik te maken van de gemiddelde diameter. Het is mogelijk om te corrigeren voor de huidgevoeligheid en de observatievariabiliteit tussen de personen die de huidtesten aflezen, door gebruik te maken van de HEP-index oppervlakte-methode. Daarvoor deelden we de oppervlakte van het allergeen-geïnduceerde 'wheal'- oppervlakte door het gemiddelde 'wheal'-oppervlakte van de positieve histamine-geïnduceerde controles. De ratio is gedefinieerd als de HEP-index oppervlakte. De HEP-index oppervlakte waarde van 0.4 kan gelijk beschouwd worden aan een grenswaarde van 3 mm van de gemiddelde 'wheal'- diameter. Echter, in de klinische praktijk is de gemiddelde diametermethode bruikbaar, omdat het even nauwkeurig de uitkomst kan voorspellen van de DBPGVP test.

Het tweede artikel in dit hoofdstuk *'sIgE Ana o 1,2 and 3 accurately distinguish tolerant from allergic children sensitised to cashew nuts'* laat bij de kinderen die deelnamen aan de IDEAL-studie, een snelle risico toename zien op een positieve provocatie bij stijgende sIgE- waarden tegen elke individuele component Ana o 1, Ana o 2 en Ana o 3 met risico's op een positieve provocatie tot 100%. sIgE tegen Ana o 1, 2 en 3 kan beter onderscheid maken tussen cashewnoot allergische en tolerante patiënten (c-index = 0.87, 0.85 en 0.89 respectievelijk) dan sIgE tegen cashewnoot en de huidpriktest (c-index 0.76 en 0.83, respectievelijk) (De c-index is gelijk aan de 'Area Under the Receiver Operating Characteristic Curve' voor dichotome uitkomsten).

In het derde artikel in dit hoofdstuk *'Predictive model for cashew nut allergy in children'* beschrijven we een model om de uitkomst van de cashewnootprovocatie te voorspellen en tonen we een eenvoudig bruikbaar format van de modellen om het risico op een cashewnootallergie te berekenen voor gesensibiliseerde kinderen. De hoogste discriminerende vermogens werden gevonden met geslacht en Ana o componenten (met en zonder huidpriktest) (c-index 0.90 met huidpriktest en 0.89 zonder huidpriktest). De modellen maken een selectie mogelijk van kinderen die een voedselprovocatietest nodig hebben om de diagnose cashewnootallergie te stellen en zouden tijd en kosten kunnen besparen in de geselecteerde groep van patiënten. In situaties waar er gelimiteerde

mogelijkheden zijn in het dubbelblindtesten van een cashewnootallergie kunnen de modellen en scoringssysteem bruikbaar zijn om kinderen te identificeren met een kans op een positieve provocatie uitkomst van ≥ 95% en waarbij de provocatietest minder waarschijnlijk het beleid veranderd. In onze studie betrof dat een substantieel aantal kinderen (58/173 kinderen, 34%).

Hoofdstuk 5. De klinische consequenties van een cashewnootallergie

Het eerste artikel in dit hoofdstuk '*Low percentage of clinically relevant pistachio nut and mango co-sensitisation in cashew nut sensitised children*' beoordeelt de klinische relevantie van de cashewnoot en mango co-sensibilisatie in cashewnoot gesensibiliseerde kinderen.

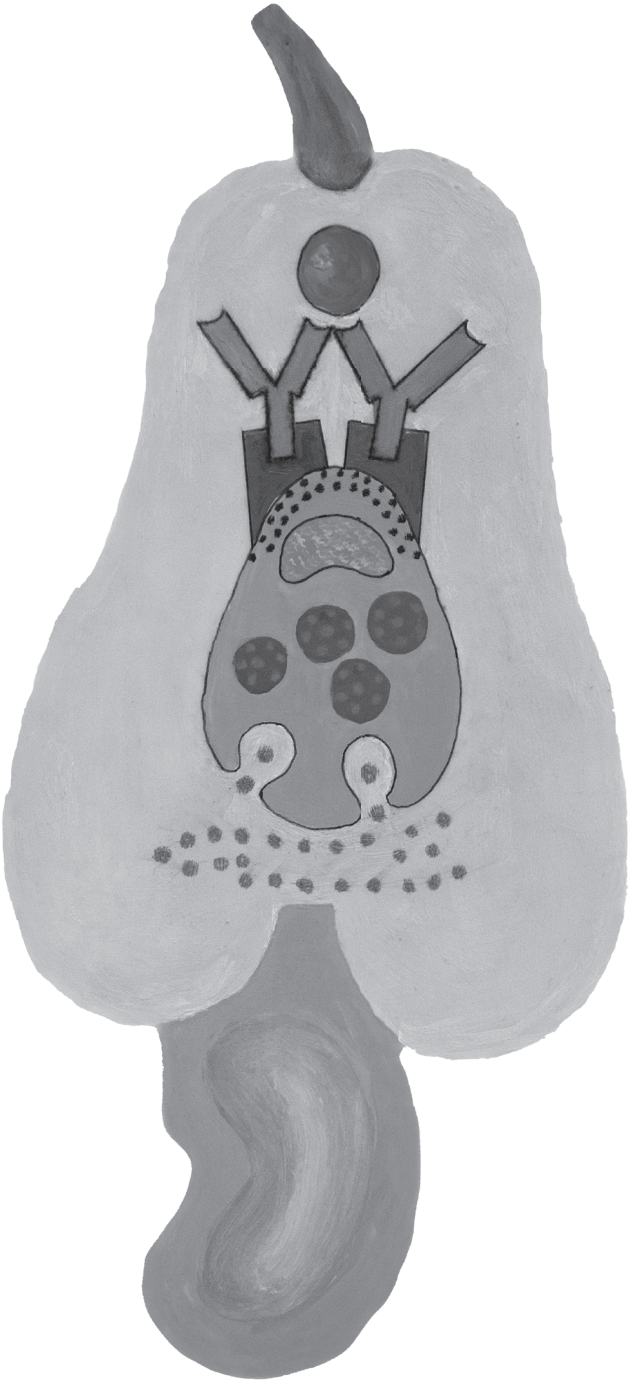
Alle kinderen, gesensibiliseerd voor cashewnoot en pistachenoot en/of mango ondergingen een DBPGVP test met cashewnoot en pistachenoot en een open voedselprovocatie met mango. Ondanks dat we een co-sensibilisatie in de huidpriktest zagen van 98% tussen cashewnoot en pistachenoot, is de pistachenootsensibilisatie maar klinisch relevant in 34.5% Daarvoor adviseren een DBPGVP test in geval van een co-sensibilisatie tussen cashewnoot en pistachenoot. Geen van de kinderen had een mango-allergie.

Het tweede artikel in dit hoofdstuk *'No difference in health-related quality of life, after a food challenge with cashew nut in children participating in a clinical trial*" toont het effect op kwaliteit van leven in de positief en negatief geteste kinderen voor en na de voedselprovocatie.

De cashewnoot-allergische patiënten, zowel met een positieve als een negatieve provocatie-uitslag ervaarden geen significante verbetering in kwaliteit van leven in deze studie. Naast dat het relatief makkelijker is om cashewnoten te vermijden en dat een groot aantal kinderen nog nooit blootgesteld is aan de cashewnoot, is deze studie ook geïnitieerd door onderzoekers en niet door de patiënt zelf. Mogelijk heeft dit bijgedragen aan de relatieve goede kwaliteit van leven van de kinderen voor de start van de studie.

We beschrijven in het laatste artikel in dit hoofdstuk '*Failure of introduction of cashew nuts after a negative food challenge test'* de introductie graad van cashewnoten na een negatieve provocatietest. Na een negatieve provocatietest worden de kinderen geadviseerd om het negatief geteste voedingsmiddel te introduceren in het dieet. Eerdere studies toonden aan dat de graad van introductie laag is van algemeen geteste allergenen, ondanks het advies om te introduceren. In deze studie, werd succesvolle introductie (starten en blijven eten van cashewnoot op regelmatige basis) gerapporteerd door 13 kinderen (43.3%), gedeeltelijke introductie (het eten van sporen cashewnoot of cashewnoot verwerkt in andere voedingsmiddelen) in 6 kinderen (20%) en gefaalde introductie

(nooit cashewnoten gegeten of eenmalig en daarna nooit meer geprobeerd) door 11 kinderen (36.7%). Succesvolle introductie is afhankelijk van het type geteste allergeen, de leeftijd en geslacht. Verder zijn een duidelijke en gestructureerde protocollen noodzakelijk om de patienten van informatie te voorzien en is een vervolg protocol noodzakelijk om te meten of het voedingsmiddel daadwerkelijk geïntroduceerd is.



Chapter 6.3

Dankwoord

In het jaar 2012 ben ik vol enthousiasme aan mijn promotieonderzoek begonnen. Vanaf de eerste dag was het hard werken om dit onderzoek succesvol af te ronden in 4 jaar. En het is gelukt! Echter, zonder de goede samenwerking met ons onderzoeksteam en de steun van mijn familie en vrienden was dit boekje niet tot stand gekomen.

Nicolette de Jong, co-promotor, ik wil je hartelijk danken voor je eindeloze enthousiasme en doorzettingsvermogen voor dit project. Ik waardeer jouw doelmatigheid en de grenzeloze communicatie mogelijkheden ontzettend in onze samenwerking. Zelfs midden in de nacht kon ik je 'appen' voor mijn prangende vragen of kon ik je bellen in je vakantie om je op de hoogte te brengen van onze publicatiesuccessen en even te zeggen: 'zie je wel, we zijn een top-team'! Mede door jouw inzet en organisatie talent is mijn boekje geworden wat het is.

Roy Gerth van Wijk, promotor, 4 jaar geleden heeft u mij overtuigd om te kiezen voor het specialisme Allergologie. Ik kreeg de mogelijkheid om te werken en promoveren op de afdeling. Ik denk dat dit een ontzettend goede keuze is geweest voor een begin van een leuke en wetenschappelijke carrière en ik ben u daar heel dankbaar voor.

De leescommissie, Patrick Bindels, Suzanne Pasmans en Edward Knol dank ik voor het beoordelen van mijn proefschrift.

Ewoud Dubois, mijn dank voor uw inzet om de artikelen tot een succesvolle publicatie in correct Engels te brengen is enorm. Ook de inhoudelijke uitleg hierbij is mij heel veel waard geweest. Ik hoop dat ik in de toekomst nog mag blijven vragen om goede raad, dit zou mijn carrière zeker ten goede komen.

Nicolette Arends, zonder jou had ik alle voedselprovocaties niet aangedurfd. Je hebt me enorm goed begeleid op de Medium Care in het Sophia kinderziekenhuis. Naast dat ik daar veel geleerd heb, was onze samenwerking ook ontzettend leuk.

Het multicenterkarakter van deze studie maakt dat ik ook Hans de Groot en o.a. de verpleegkundige Anne-Marie Oomkes, Gerda Bal en Arzu Koca-Algur dankbaar ben voor de inzet om patiënten te includeren in Delft en Groningen. Zonder jullie had ik niet zo'n mooie studie kunnen doen.

Yvonne Vergouwe en Ewout Steyerberg, door jullie heb ik heel veel geleerd op het gebied van de statistiek. Hier zal ik mijn hele carrière baat van hebben. Hartelijk dank hiervoor.

Harry Wichers, Marit Reitsma, Shanna Bastiaan-Net en Huub Savelkoul, de samenwerking tussen Wageningen Universiteit en het Erasmus Medisch Centrum is de succesformule van dit onderzoek geweest. Ik wil jullie daarvoor hartelijk danken.

Berber Vlieg-Boertra, Bertine Flokstra-de Blok, Tina van der Velde, Harold Overkamp en Cees van Egeraat, dank ik voor alle goede adviezen tijdens de studie.

Loes en Ilse, wat ben ik blij geweest met jullie hulp bij het controleren en invoeren van alle data. Dit gaf me een goed vertrouwen in onze database. Maar bovenal bedankt voor

de gezelligheid, ik keek er altijd naar uit om jullie op de onderzoekskamer te zien. Dan wist je zeker, 'dit wordt lachen'.

Dit onderzoek is financieel ondersteund door Stichting voor de Technische Wetenschappen (STW). Ik waardeer uiteraard de financiële ondersteuning, maar ook de grote betrokkenheid tijdens onze halfjaarlijkse STW-vergaderingen.

Guido, lieverd, mijn dank aan jou is onbeschrijfelijk. Tijdens mijn promotie ben je altijd geïnteresseerd geweest en was je telkens weer bereid om mee te denken als ik vast liep. Je wilde het niet oppervlakkig begrijpen, maar vroeg me uit tot in detail. Ik durf te beweren dat jij als ingenieur meer weet van de allergologie dan menig dokter. Onze avondjes dat we weer samen aan het 'neurden' waren hebben gezorgd dat mijn promotie op tijd en succesvol is afgerond. Bedankt voor je grenzeloze steun.

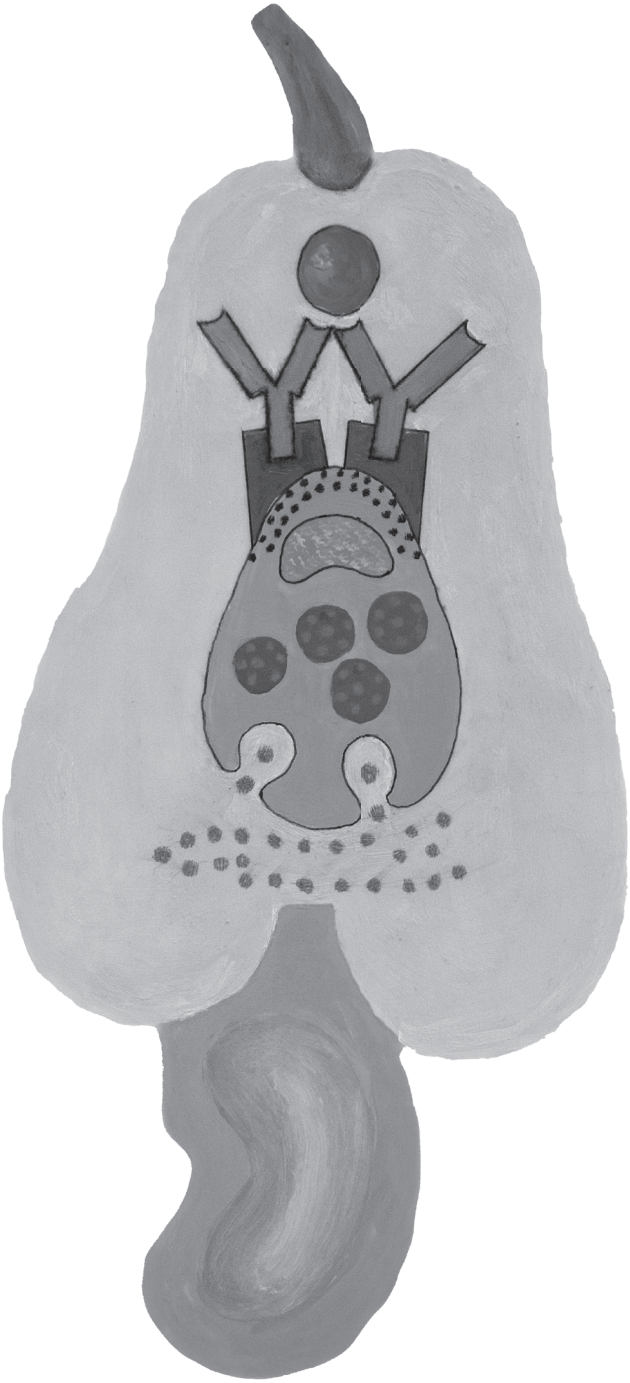
Mijn kinderen Saar, Loes en Lars, aan jullie heb ik mijn stelling 11 te danken: 'Een kind maakt een promotie onderzoek vruchtbaar'. De stelling is niet wetenschappelijk te onderbouwen, maar wel persoonlijk. Jullie drie geboortes in deze vier jaar promotie hebben mij euforisch gemaakt en gaven me ontzettend veel levensgeluk en energie. Schatjes, ik ben jullie intens dankbaar.

Mijn ouders, Joke en Pieter, heel erg bedankt voor jullie steun en aanmoediging in deze promotietijd. Pieter, de keren dat ik je 'face-timede' voor vragen en discussies zijn ontelbaar. Je hebt me leren nadenken en leren schrijven. Je bent en blijft mijn grote voorbeeld. Joke, jouw eindeloze betrokkenheid bij mij en ons gezin is goud waard. De prachtige voorkant van mijn boekje is aan jou te danken.

Mijn schoonouders, jullie interesse in mijn promotie heb ik erg gewaardeerd. Ton, er is bijna geen artikel ongelezen gebleven door jou en vele tips heb ik mogen ontvangen.

Ook mijn broers Thijs, Joost en Dirk, schoonfamilie en vrienden bedank ik zeer voor jullie steun.

Hanna Kuiper-van der Valk



Chapter 6.4

PhD Portfolio

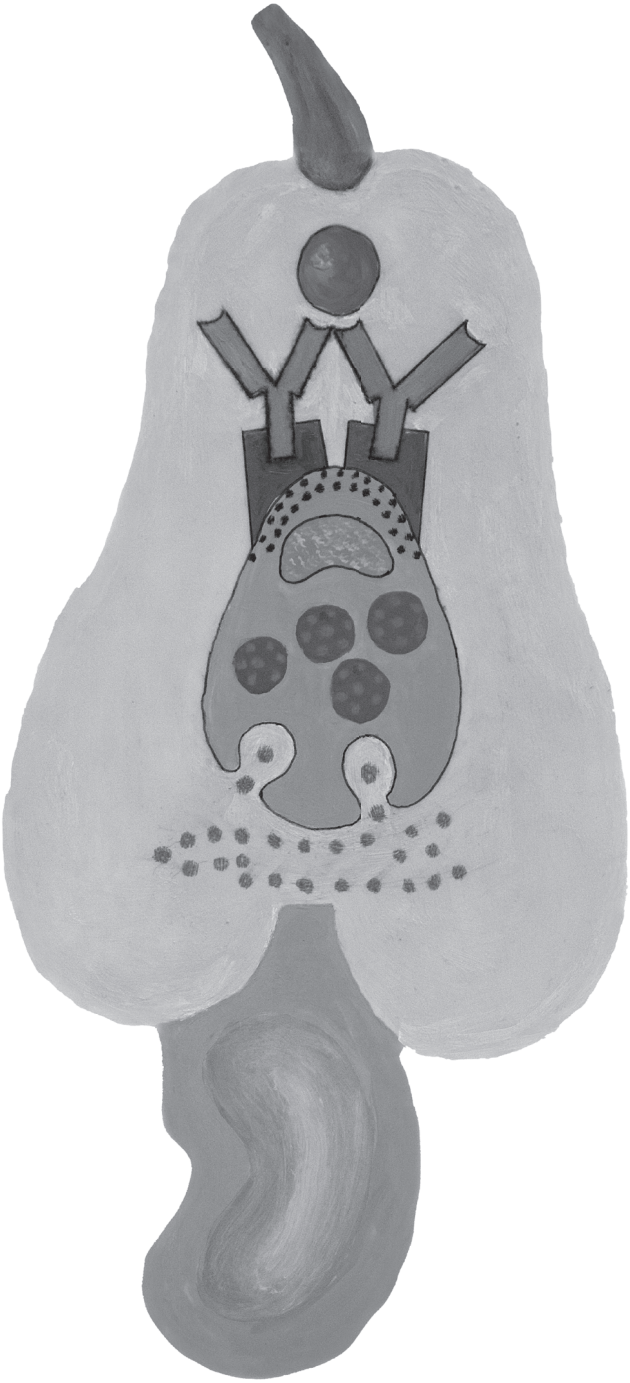
Name PhD student: J.P.M. Kuiper- van der Valk
Erasmus MC Department: Allergology
Research School: Erasmus Medical centre

PhD period: 01-04 2012 to 22-11-2016
Promotor(s): Prof. R. Gerth van Wijk
Supervisor: Dr. N.W. de Jong

1. PhD training

	Year	Workload (hours)
General courses		
Endnote	2012	2
Pubmed	2012	2.5
Other databases	2012	2
Specific courses		
BROK	2013	20
Introduction to Clinical Research (EWP01)	2013	51
Biostatistical Methods I: Basic Principles Part A (CC02a)	2013	56
Biostatistics for Clinicians (EWP22)	2014	20
Regression Analysis for Clinicians (EWP23)	2014	39
Seminars and workshops		
General practioner education	2012	2
Immunology meeting	2012	2
EAACI Exam meeting	2012	3
Immunology meeting	2012	2
EAACI Exam meeting	2012	3
Presentations		
STW (8)	2012- 2016	4
Clinical immunology	2012	1
Guidelines food allergy/food challenge test	2012	0.5
RADAR	2012	0.5
Food Allergy foundation	2013	0.5
Science days Internal Medicine	2013	0.5
EAACI Copenhagen	2014	1
EAACI Barcelona	2015	0.5
(Inter)national conferences		
- EAACI Genève	2012	
- PAAM Athene	2013	
- Science days Internal Medicine Antwerp	2014	40
- EAACI Copenhagen	2014	80
- EAACI Barcelona	2015	40
Guideline Food allergy/ challenge test		
- Meetings (3)	2012	15
- Work	2012-2015	240
2. Teaching		
Lecturing		

- Student education Urticaria (Minor)	2012	2
- Student education Food allergy (Minor)	2012	2
- Student education Urticaria (Minor)	2013	2
- Student education Food allergy (Minor)	2013	2
Prices		
Poster price, Science days Antwerp	2014	
Publications next to thesis:		
1. *Van der Valk JPM, Gerth van Wijk R, Vergouwe Y, de Jong NW. Failure of introduction of food allergens after negative oral food challenge tests in children. Eur J Pediatr. 2015 Aug;174(8).*		160
2. *Van der Valk JPM, de Jong NW, Gerth van Wijk R. Review on immunotherapy in airway allergen sensitised patients. Neth J Med. 2015 Jul;73(6):263-9.*		160
3. *Reitsma M, Bastiaan-Net S, Sforza S, van der Valk JP, van Gerth van Wijk R, Savelkoul HF, de Jong NW, Wichers HJ. Purification and Characterization of Anacardium occidentale (Cashew) Allergens Ana o 1, Ana o 2, and Ana o 3. J Agric Food Chem. 2016 Feb 10;64(5):1191-201.*		160
4. *Van der Valk JPM, Gerth van Wijk R, de Jong NW. Meerwaarde van componentenanalyse bij patiënten met latex- en fruitsensibilisatie. Ned Tijdschr Allergie & Astma 2016;16:3-10.*		160
5. *Van der Valk JPM, Schreurs MWJ, el Bouch R, Arends NJT, de Jong NW. Mono-sensitisation to peanut component Ara h 6: a case series of 5 children and literature review. Accepted Eur J Pediatr. 2016 May 20.*		160
Total (hours)		1432
Total (ECTS)		48



Chapter 6.5

Curriculum vitae

Hanna Kuiper- van der Valk was born in Groningen, the Netherlands, on 22 January 1985. After graduating at the Stedelijk Gymnasium Nijmegen in 2003, she attended the Faculty of Medicine in Nijmegen. She received her MD-degree in 2009. After one year at the department of internal medicine at the Vlietland Ziekenhuis, she worked for one year at the outpatient department of Allergology Erasmus Medical Centre. Thereafter, she started a PhD-project on food allergy at this department. She received a poster price on the Internal Medicine science days in Antwerpen in 2014 for her contribution to the knowledge of food allergy/cashew nut allergy.

She was a co-author of the National Guidelines of Food Allergy/Food Challenge Tests implemented in 2016. She published several articles on different aspects in allergology. She moved to Norway in 2014 and was able to finish her PhD project there. During her stay in Norway, she worked at the department of Allergy Centre Stavanger. She was there to meet the Norwegian practice of allergology. She will start with her training for allergologist/immunologist in January, 2017

She is married to Guido Kuiper and is the happy mother of three wonderful children.

LIST OF PUBLICATIONS 2012-2016

Thesis:

1. Van der Valk JP, Dubois AE, Gerth van Wijk R, Wichers HJ, de Jong NW. Systematic review on cashew nut allergy. Allergy. 2014 Jun;69(6):692–8.
2. Van der Valk JP, Gerth van Wijk R, Hoorn E, Groenendijk L, Groenendijk IM, de Jong NW. Measurement and interpretation of skin prick test results. Clin Transl Allergy.2016.6:8.
3. Van der Valk JP, Gerth van Wijk R, Dubois AEJ, de Groot H, Reitsma M, Vlieg-Boerstra B, Savelkoul HFJ, Wichers HJ, de Jong NW. Multicentre double-blind placebo-controlled food challenge study in children sensitised to cashew nut. PLoS One. 2016 Mar 11;11(3).
4. Van der Valk JP, Gerth van Wijk R, Dubois AE, de Groot H, de Jong NW. Failure of introduction of cashew nut after a negative oral food challenge test in children. Pediatr Allergy Immunol. 2016: Sep;27(6):654–8.
5. Van der Valk JP, Gerth van Wijk R, Vergouwe Y, Steyerberg EW, Reitsma M, Wichers HJ, Savelkoul HF Vlieg-Boerstra B, de Groot H, Dubois AE. de Jong NW. 1sIgE Ana o 1, 2 and 3 accurately distinguish tolerant from allergic children sensitised to cashew nuts. Clin Exp Allergy. 2016 Aug 11.
6. Van der Valk JP, Gerth van Wijk R , Flokstra-de Blok BMJ, van der Velde JL, de Groot H, Wichers HJ, Dubois AEJ, de Jong NW. No difference in health-related quality of life, after a food challenge with cashew nut in children participating in a clinical trial. Pediatr Allergy Immunolol. 2016 Aug 6.
7. Van der Valk JP, Gerth van Wijk R, Baumert JL, Nordlee JA, Vlieg-Boerstra B, de Groot H, Dubois AE, de Jong NW. Threshold distribution and lowest observed adverse effect levels in cashew nut allergic children. Ann. of Allergy, Asthma & Immunology. Accepted.

Other:

8. Van der Valk JP, Gerth van Wijk R, Vergouwe Y, de Jong NW. Failure of introduction of food allergens after negative oral food challenge tests in children. Eur J Pediatr. 2015 Aug;174(8).
9. Van der Valk JP, de Jong NW, Gerth van Wijk R. Review on immunotherapy in airway allergen sensitised patients. Neth J Med. 2015 Jul;73(6):263–9.
10. Reitsma M, Bastiaan-Net S, Sforza S, van der Valk JP, van Gerth van Wijk R, Savelkoul HF, de Jong NW, Wichers HJ. Purification and Characterization of Anacardium occidentale (Cashew) Allergens Ana o 1, Ana o 2, and Ana o 3. J Agric Food Chem. 2016 Feb 10;64(5):1191–201.

11. Van der Valk JP, Gerth van Wijk R, de Jong NW. Meerwaarde van componentenanalyse bij patiënten met latex- en fruitsensibilisatie. Ned Tijdschr Allergie & Astma 2016;16:3–10.

12. Van der Valk JP, Schreurs MW, el Bouch R, Arends NJ, de Jong NW. Mono-sensitisation to peanut component Ara h 6: a case series of 5 children and literature review. Eur J Pediatr. 2016 May 20.