DIAGNOSING FOOD ALLERGY IN CHILDREN

Peanuts?

Francine van Erp

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COLOFON

Diagnosing food allergy in children, peanuts?
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DIAGNOSING FOOD ALLERGY IN CHILDREN, PEANUTS?

Het diagnosticeren van voedselallergie bij kinderen, peanuts?

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
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door

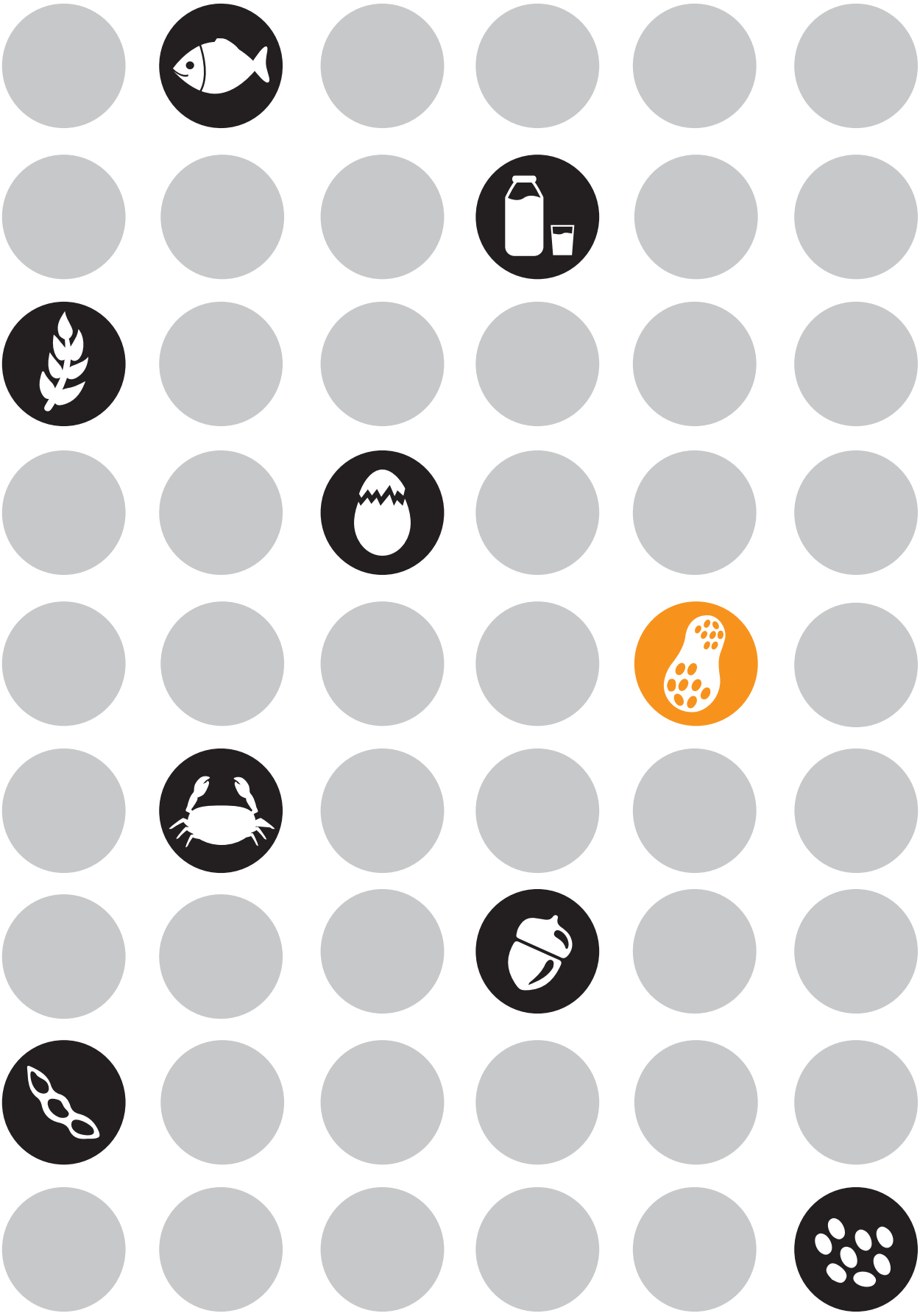
Francine Clemency van Erp

geboren op 14 september 1986 te Huizen

Promotor: Prof.dr. C.K. van der Ent

Copromotor: Dr. A.C. Knulst

TABLE OF CONTENTS



CHAPTER 1

General introduction

GENERAL INTRODUCTION

Allergy: change in reactivity

For more than two thousand years it has been advocated that food can cause disease and concerns for some people. Hippocrates (469–370 b.C) already recorded gastric upset and hives due to cheese and encouraged dietary measures to eliminate milk. However, the term allergy was not introduced until 1906 by Von Pirquet.(1) At that time it was used to describe that when an individual comes into contact with an allergen such as food, a change in reactivity of the immune system occurs. This change could be protective (no symptoms after exposure: immunity) or harmful (signs and symptoms after exposure: hypersensitivity). Throughout the years, the interpretation and usage of the term allergy has changed. Moreover, the word has grown in popularity and is frequently used as a synonym for aversion and rejection.(2) Currently, allergy is defined as a hypersensitivity reaction initiated by specific immunologic mechanisms.(3) Hypersensitivity is used to describe objectively reproducible symptoms or signs initiated by exposure to a defined stimulus at a dose tolerated by normal persons.(3) In this thesis, the term food allergy as a hypersensitivity reaction in which antigen specific IgE molecules are involved (IgE-mediated food allergy) will be used.

Food allergy can cause a variety of symptoms ranging from oral (itch), skin (urticaria), gastro-intestinal (vomiting), upper and lower respiratory (rhinitis, laryngeal oedema, wheezing) to cardiovascular symptoms (drop in blood pressure). A food allergic reaction can be triggered after ingestion, by skin contact or inhalation of food.(4) Symptoms can be aggravated by co-factors like exercise, infections or activity of other allergic diseases like asthma or allergic rhinitis.(5) Food allergic children are often atopic: they have a genetic tendency to produce IgE antibodies in response to allergens.(3) As a consequence, children with food allergy frequently have other burdensome allergic diseases like eczema, asthma and allergic rhinitis.(6) The most prevalent food allergies in children are: cow's milk, egg, wheat, soy, peanut, tree nuts and (shell) fish.(7) In this thesis we primarily focus on children with peanut allergy. Peanut is one of the most common food allergens capable of eliciting severe allergic reactions.(8) Moreover peanut allergy appears early and often persists throughout life.(9) Depending on the geographical region studied and definition of allergy used, peanut allergy is estimated to affect 0.2-3% of the population, with higher estimates in older age groups compared to younger children.(7,10)

The impact of food allergy

Food allergy has a major impact on quality of life of children and their parents.(11) Allergic symptoms like itch, urticaria and gastro-intestinal symptoms are burdensome and respiratory and cardiovascular symptoms can be life-threatening. Although food allergic patients usually do not experience daily symptoms, they are faced with dietary restrictions and the risk for a severe reaction every day. Consequently patients and their families are restricted in daily activities like meal preparation and social activities like

birthday parties and eating out.(12,13) The need for continuous awareness when eating or shopping and coping with changing or incomplete food labels also compromises quality of life.(14) Additionally, the fear of an allergic reaction and the presence of a severe allergy illustrated by the need to carry emergency medication like an epinephrine auto-injector has a negative impact on quality of life of children and their parents.(15,16) When compared to other diseases parents of children with peanut allergy reported more disruption of daily activities and social problems than parents of children with rheumatologic disease.(17) Moreover, they experience more fear than children with insulin-dependent diabetes mellitus.(12)

In current clinical practice large amounts of data on the incidence and severity of symptoms, medication use and impact of disease are gathered during routine patient care. Very little of these data are however available for research purposes because data are not recorded in a structured way. For that reason we developed a nationwide electronic data collection system with validated questionnaires on respiratory and allergic diseases: the Electronic Portal. The Electronic Portal also facilitates the evaluation of the interaction between different atopic diseases. The rational and design of the Electronic Portal is described in **chapter 2** of this thesis. In **chapter 3** we used the Electronic Portal to investigate the impact of atopic comorbidities and food allergy related characteristics on quality of life in children referred for food allergy.

Diagnosing food allergy

A correct diagnosis of food allergy is important to prescribe adequate emergency medication, educate children and their families in avoiding allergens and other triggers and prevent fatal reactions. Excluding food allergy can be important to improve quality of life and prevent children from using unnecessary elimination diets.(18,19) In clinical practice food allergy is suspected based on a clinical history with previous reactions to the allergen together with a positive skin prick test (SPT) and / or a raised level of allergen specific IgE (sIgE).(20) In recent years the role of sIgE to allergen components in the diagnostic work-up of patients with suspected food allergy has been extensively studied. Instead of relying on crude allergen extracts, component resolved diagnostics (CRD) depends on sensitization to purified or recombinant allergenic proteins within the allergen. In **chapter 4** we describe the current knowledge and advances of the use of CRD in the management of peanut allergic patients.

Food challenges

To ascertain the presence of food allergy to the suspected allergen, objectifying the allergic reaction is necessary.(21) At present, the double blind placebo controlled food challenge (DBPCFC) represents the 'gold standard' for the definitive diagnosis of food allergy.(22) In short, increasing amounts of allergen or placebo are given with close monitoring by a specialized team of physicians and nurses in a hospital setting with emergency equipment. The DBPCFC is terminated and considered positive when

objective or severe persisting subjective symptoms occur after the ingestion of allergen. (22) If a DBPCFC is negative the allergen can be introduced in the diet.(23) Food challenges are expensive due to the time consuming procedure and the need of trained staff and dedicated hospital facilities. Moreover, challenges are not without risk and severe allergic reactions can occur.(24) In **chapter 5** we investigated whether known risk factors for anaphylaxis in daily life (like asthma) could be used as predictors for positive and severe challenge outcomes in children who underwent a peanut challenge. In **chapter 6** we evaluated in a prospective study whether we could predict or exclude peanut allergy using sIgE and a functional assay (the basophil activation test) to peanut components Ara h 2 and h 6.

Peanuts?

There are several pitfalls in performing and interpreting food challenges. Especially in children, symptoms during challenge in particular subjective symptoms (abdominal complaints, oral symptoms or change of behaviour) are sometimes difficult to interpret. Likewise, stress associated with hospitalization and aversion of food required for the challenge can mimic allergic symptoms like abdominal pain. Furthermore, subtle objective symptoms (such as mild bronchoconstriction or slight changes in blood pressure) are easily missed. In **chapter 7** the degree of variability in the interpretation of symptoms during challenge between and within observers is evaluated. In **chapter 8** it is described whether monitoring of vital and respiratory parameters contributes to the accuracy of food challenges.

One of the other limitations of food challenges is the occurrence of false negative tests.(25) This is further demonstrated by a considerable number of children that fails to introduce the allergen in the diet despite negative challenge.(26) In **chapter 9** we determine the added value of open challenges and guided reintroduction after negative peanut challenge. In **chapter 10** we evaluate the success rate of introduction and occurrence of allergic reactions after negative peanut challenges.

AIMS OF THIS THESIS

In this thesis we aimed to: 1) investigate the impact of food allergy in children; 2) predict the presence and severity of peanut challenge outcomes; 3) investigate and improve the diagnostic accuracy of food challenges and 4) evaluate post-food challenge outcomes. The implications and future perspectives of our findings are discussed in **chapter 11**.

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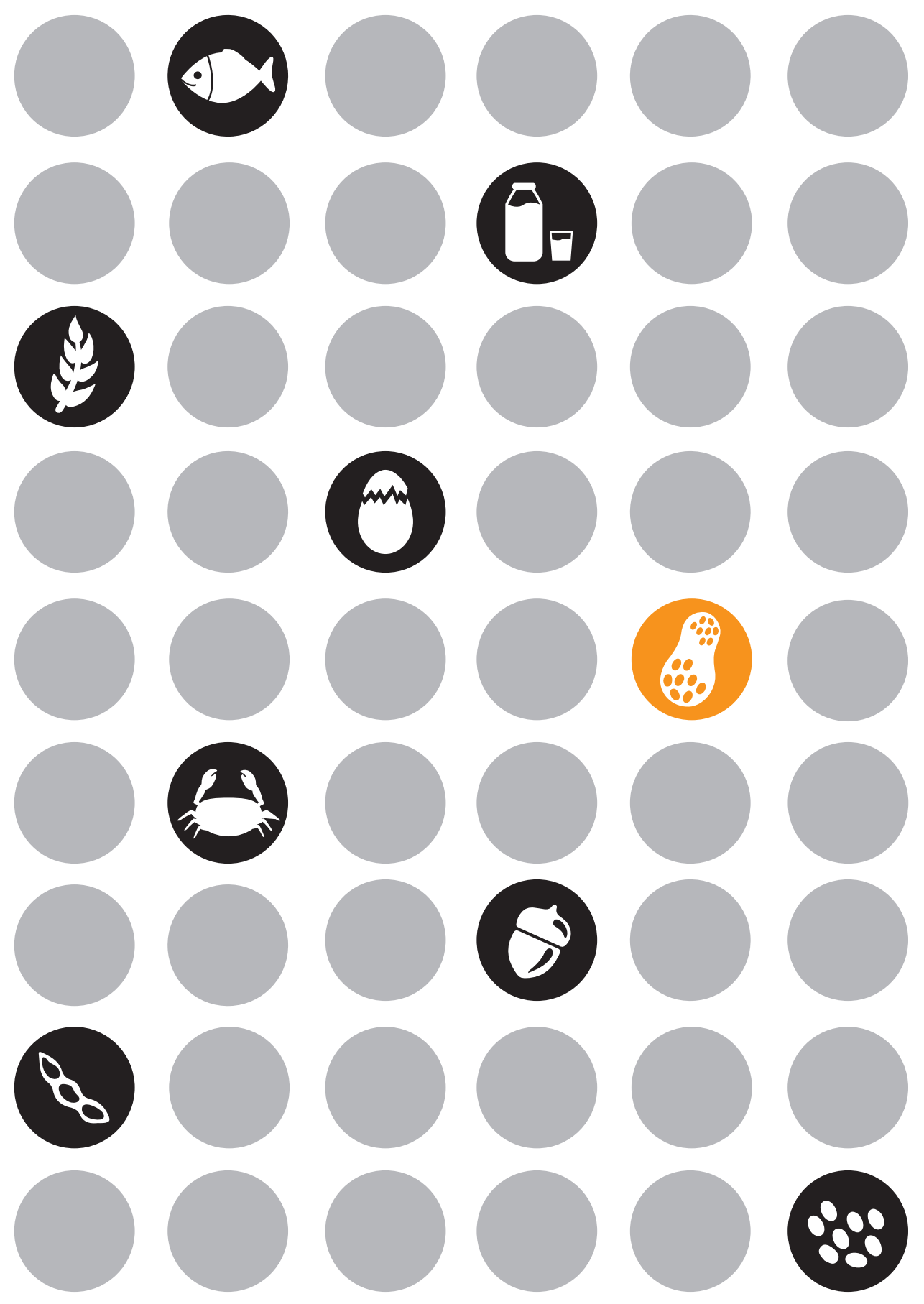
PART

Impact of food allergy



CHAPTER 2

The expert network and electronic portal for children with respiratory and allergic symptoms: rationale and design

BMC Pediatr 2013: 13:9

Kim Zomer-Kooijker
Francine C. van Erp
Walter A.F. Balemans
Bart E. van Ewijk
Cornelis K. van der Ent

on behalf of the Expert Network for children with respiratory and allergic symptoms

ABSTRACT

Data on baseline characteristics of children with asthma to predict individual treatment responses are lacking. We aimed to set up a data-collection system which can easily fill this gap in clinical practise. A web-based application was developed, named 'Portal for children with respiratory and allergic symptoms', hereafter called Electronic Portal (EP). It contains health- and disease-related questionnaires on respiratory- and allergic diseases. All patients, 1-18 years of age, with respiratory- and/or allergic complaints are invited to enter the EP before their first visit. By using the EP large amounts of data, gathered during routine patient care can be used for research purposes. This may help to further investigate the different treatment related asthma phenotypes and will be helpful to monitor risk factors for other atopic diseases and respiratory infections.

INTRODUCTION

Asthma is the most prevalent chronic illness in childhood.(1) The prevalence of asthma is ranging from 4 to 12 percent of school age children.(2) A recent study in The Netherlands showed that in a population of 1614 school age children 5% had physician-diagnosed asthma, while an additional 8% had asthma symptoms without knowing to have asthma.(3) Despite advances in the management of asthma in children, it continues to be a condition that has significant impact on children and their families. In a Dutch study both children with diagnosed and undiagnosed asthma had impaired quality of life scores compared to healthy peers and had higher rates of absence from school.(4) The AIRE (Asthma Insight and Reality) study showed only partial effectiveness of asthma care in daily life.(5) In addition, Fuhlbrigge et al. showed that goals of therapy in asthma, based on the National Asthma Education and Prevention Program guidelines, have not been achieved for the majority of children, although more than 70% had mild intermittent disease.(6) The impact of asthma on daily activities is substantial; avoiding exertion (47%) and staying inside (37%) are common approaches to improve control of asthma symptoms. These data indicate poor control of asthma in school-age children in affluent countries. To improve patient care in clinical practise there is an urgent need for predictors of asthma treatment responses. Scarce data are available on predictors of treatment response. Several studies addressed the predictive capacity of family history, clinical symptoms, or lung function parameters for the effect of different treatment regimens. For example, a parental history of asthma or increased levels of exhaled Nitric Oxide (eNO) might predict a beneficial effect of ICS.(7-10) while in adults LTRAs might be especially beneficial in asthma patients who smoke.(11) In cases where group-wise differences between different therapies are lacking.(12,13) predictive baseline characteristics might be helpful to predict which therapy has the best risk-benefit ratio in the individual child. The evaluation of the predictive capacity of comprehensive clinical and laboratory parameters for treatment responses requires analysis of a large and diverse patient population from different clinical settings.

Recently, we started an extensive nationwide study in The Netherlands to compare different treatment strategies for children with respiratory and allergic symptoms and to evaluate predictors of treatment responses. In a strongly internet-supported network of academic and general pediatricians in The Netherlands (the 'Expert Network´) large numbers of patients are recruited and evaluated using an Electronic Portal. Here we aim to describe the design of both the Expert Network and the Electronic Portal.

METHODS

Study design

The Electronic Portal (EP) is used by the members of the Expert Network (EN) as a clinical tool to prospectively collect data in children with respiratory and allergic symptoms. The EP is used firstly to thoroughly screen patients on the presence of certain symptoms and possible risk factors, before their first outpatient department-visit. Secondly,

patients can be followed-up on a regular basis without intervention of their caregivers. At start uniform information about atopic diseases, respiratory infections, exposure to potentially toxins, and demographic information is collected by the patients. Afterwards data on treatment, disease control and treatment effects are monitored. In this way pre-treatment patient characteristics can be related to treatment and disease outcomes. Recruitment and follow up of children started in June 2011.

The Expert Network

In a nationwide collaborative network of Dutch caregivers at least 3000 children presenting with asthma symptoms will be included from June 2011. The EN is a nationwide collaborative network and consists of caregivers in the primary-, second- and third line health care. The members of the EN are general practitioners, pediatricians and specialized pediatricians in pulmonology, allergology, dermatology, infectiology and otolaryngology. We aim to include at least 15 large pediatric clinics (for current status see figure 1). Members of the EN are be personally instructed how to use the EP. The EN has three-monthly meetings in which data from the EP are analysed and compared between centres. Information about meetings, diagnostic and treatment protocols, and scientific updates on atopic diseases can be found on a supporting website. Children between the ages of 1-18 years, referred to a member of the EN because of respiratory- or atopic complaints are eligible to participate and are asked to participate in the EP. Also known patients are eligible to participate in the EP. Each centre has its own account. With this, access is given to the data of their own patients, and records can be made and printed with results per patients. Patients with congenital pulmonary defects or cystic fibrosis are excluded. Also (parents of) patients who do not understand the Dutch language will be excluded. Informed consent for use of the questionnaires and clinical information is given by an electronic tick, and the medical ethics committee of the University Medical Centre Utrecht has approved the protocol.

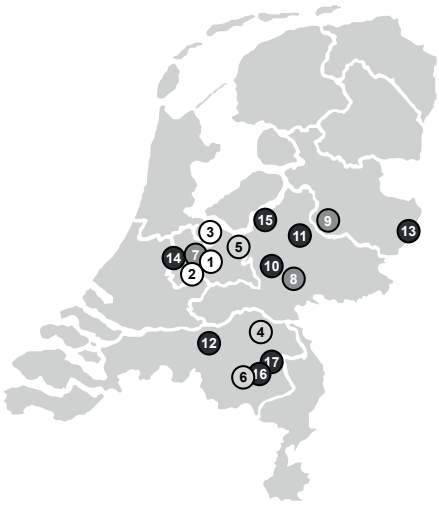


Figure 1 The Dutch Expert Network

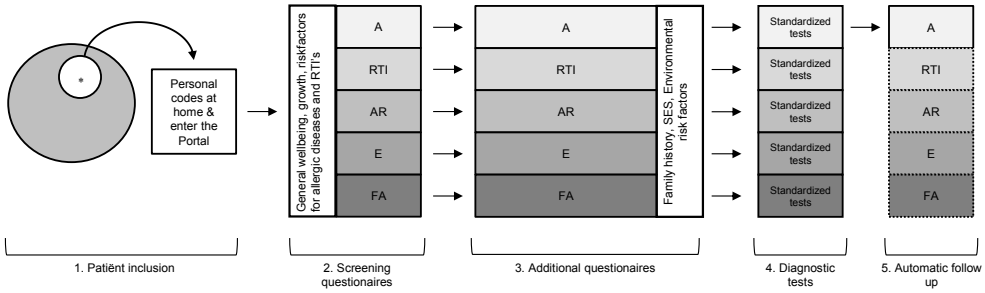
1) Utrecht, 2) Nieuwegein, 3) Blaricum,
4) Oss, 5) Amersfoort, 6) Veldhoven,
7) Utrecht, 8) Arnhem, 9) Deventer, 10) Ede,
11) Apeldoorn, 12) Tilburg, 13) Enschede,
14) Woerden, 15) Harderwijk, 16) Helmond,
17) Eindhoven

○ Centers started in 2011
○ Centers that will start first half of 2012
○ Centers that will start second half of 2012
○ Centers that will follow later on

The Electronic Portal

The Electronic Portal is a web-based application developed by the University Medical Centre Utrecht, in collaboration with Vital Health software. The EP can be approached via the url https://www.luchtwegportaal.com. The supporting website presents information on three levels: for the patient, the parents, and the members of the Expert Network, and contains disease information, information on the EP, and protocols for physicians. From this website the EP can be entered with a unique personal code. The information in the EP consists of personal patient information, validated questionnaires, diagnostic test results, and an automatic follow up function. Individual data in the EP are accessible for both the patient and his caregiver and structured reports can be generated on screen and on paper. The content of the EP is summarized in Table 1. The structure of the EP, and the following order in which the EP is used is shown in figure 2.

Figure 2 Structure and way of usage of the Electronic Portal



*	Patients consulting one of the EN members	E	Eczema
A	Asthma	FA	Food Allergy
RTI	Respiratory Tract Infections	.....	To be developed
AR	Allergic Rhino-conjunctivitis		

Baseline examination

Screening questionnaires

After entering the EP, parents are asked to fill in screening questionnaires which aim to screen on the presence of atopic diseases. Therefore the core questions of the ISAAC questionnaire on asthma, allergic rhino conjunctivitis and eczema are used. In addition, questions about respiratory infections and food allergy are included. Based on the answers in the screening part, additional specific questionnaires on each disease topic are selected or not, to be filled in subsequently. Information about growth parameters, breast feeding and vaccination status are obtained from personal health care files by the parents. This health care file is a document that every child in the Netherlands owns and is used in the primary care setting during the first years of live. The general health status is determined based on the RAND questionnaire (table 2). The screening questionnaires also contain questions about known risk factors for infections (as use of a consoler, day care) and atopic diseases (as smoking, pets, and breastfeeding).

Additional questionnaires

The aim of the additional questionnaires is to extensively explore the complaints of the patient, his medication use and habits, and measure the disease related quality of life. Details of the supplementary questionnaires in the EP, and the meaning of the corresponding scores are given in table 2. Questionnaires about asthma, respiratory tract infections, allergic rhino conjunctivitis, eczema and food allergy are included. In addition to the questionnaires mentioned in table 2, additional questions about asthma and rhino conjunctivitis are included.(14) Besides disease specific questionnaires, information on environmental factors, pet exposure, smoking and social economic status are obtained, partially adopted from the ISAAC questionnaire.(15)

Table 1 Structure and way of usage of the Electronic Portal

1. Screening Part		Includes
Personal data		DOB, weight at birth, development, vaccination status
General Health Status		RAND questionnaire
General medical history questions		Known risk factors for atopic diseases
Screening questions on atopic and infectious diseases		ISAAC core questions and non-validated questions
2. Additional Part		
Asthma	Symptoms	ISAAC additional questions, ACT, medication use
	Treatment compliance	MARS
	Quality of life	PAQoL
Infections	Symptoms	Non-validated questionnaire
	Quality of life	OM-6
Allergic Rhinoconjunctivitis	Symptoms	ISAAC additional questions, ARIA, medication use
	Quality of life	RQLQ
Food allergy	Symptoms	Non-validated questionnaire
	Quality of life	FaQoL
Eczema	Symptoms	SA-EASI
	Quality of life	IDQL or CDLQI
3. Diagnostic test results		
Lung function tests		FEV1, NO, BDR or Methacholine challenge test
Laboratory results		Inhalation screening (sIgE)
Allergy test results (when applicable)		SPT, Food challenge results
4. Follow-up Part		
Treatment		Medication use
Symptom control		ACT
Treatment compliance		MARS

DOB, Date of Birth; FEV1, Forced Expiratory Volume in 1 second; NO, Nitric Oxide; BDR, Bronchodilator response; SPT, Skin Prick Test For abbreviations concerning questionnaires: see table 2

Table 2 Questionnaires in the additional part of the Electronic Portal

	Questionnaire	Description	Score range
General	RAND GHRI (26,27)	7-item general health questionnaire. Developed for use in children 0,5-12 years of age	Range: 7-32 32 = good health
Asthma	C-ACT (28)	7-item questionnaire. Developed to measure asthma control in children 4-11 years of age. 4 questions are for the child, 3 for the parent.	Range: 0-27 > 20 = well controlled
	ACT (29)	5-item questionnaire developed to measure asthma control in children ≥12 years.	Range: 5-25 > 20 = well controlled
	MARS (30)	9-item questionnaire, developed to measure medication adherence.	Range: 0-5 Mean score >4.5 = 'adherent'
	PAQLQ (31)	23-item questionnaire, in 3 domains. Developed to measure asthma-specific health-related QoL in children 6-18 years of age.	Range 0-7 0 = maximal impairment
Infections	Brouilette (32)	3-item questionnaire to assess presence of OSAS	> 3,5: OSAS present - 1 to 3.5: uncertain OSAS < -1: OSAS not present
	OM6 (33)	6-item questionnaire in 6 domains. Developed to measure change in ear-related handicap in children with recurrent acute otitis media and otitis media with effusion	Range: 0-7 (mean) 7= severe
Allergic rhinoconjunctivitis	ARIA (34)	5-item questionnaire, developed to measure presence and severity of rhino-conjunctivitis	Classification into: intermittent or persistent and mild or moderate/severe
	PRQLQ (35)	23-item questionnaire in 5 domains. Developed to measure the functional problems in rhino-conjunctivitis in children 6-12 years of age	Range: 0-6 (mean) 6 = maximal impairment
	AdolRQLQ (36)	25-item questionnaire in 6 domains. Developed to measure the functional problems in rhino-conjunctivitis in children 12-17 years of age	Range: 0-6 (mean) 6 = maximal impairment
Food Allergy	FAQLQ-CF (37)	24-item questionnaire, in 4 domains. Developed to measure food allergy related QoL in children 8-12 years of age	Range: 1-7 (mean score) 7 = maximal impairment
	FAQLQ-TF(38)	23-item questionnaire, in 3 domains. Developed to measure food allergy related QoL in children 13-17 years of age	Range: 1-7 (mean score) 7 = maximal impairment
Eczema	SA-EASI (39,40)	10-item questionnaire. Developed to measure the caregiver's self-assessment of the severity of his/her child's atopic dermatitis	Range: 0-72 (acute score) 72 = very severe
	IDQL (41)	10-item questionnaire, < 4 years of age	Range: 0-30 30 = maximal impairment
	CDLQI (42)	10-item questionnaire, 4-16 years of age	Range: 0-30 30 = maximal impairment

RAND GHRI, RAND General health rating index; C-ACT, Child-Asthma Control Test; ACT, Asthma Control Test; MARS-9, 9-item Medicine Adherence Rating Scale; PAQLQ, Paediatric Asthma Quality of Life Questionnaire; OM6, 6-item Otitis Media questionnaire; ARIA, Allergic Rhinitis and its Impact on Asthma; RQLQ, Rhinitis Quality of Life Questionnaire; FAQoL, Food Allergy Quality of Life Questionnaire; SA-EASI, Self-Administered Eczema Area and Severity Index; IDQL, The Infant's Dermatitis Quality of Life Index questionnaire; CDLQI, Children's Dermatology Life Quality Index. OSAS; Obstructive Sleep Apnoea Syndrome.

Diagnostic tests

Caregivers from the EN can add results of diagnostic tests to the EP. Protocols are written to ascertain uniform performance of different tests.

Respiratory function

In all new patients suspected for asthma, lung function- and allergy tests are performed according to the Dutch national guideline.(16) Maximal flow-volume curves are measured according to the ATS/ERS standards.(17) The highest values of three correct performed manoeuvres are used for analysis. Recorded parameters are FEV1 (forced expiratory volume in one second) and FVC (Forced Vital Capacity). To measure the bronchodilator response 800 microgram of salbutamol is administered via a metered dose inhaler using a volumatic spacer (GSK, Uxbridge, UK). Airway reversibility is defined as an increase of FEV1 of ≥ 12% of the predicted value 10 minutes after administration of salbutamol. Bronchial hyper responsiveness is assessed by a challenge with nebulized methacholine according to the ERS/ATS guidelines.(18) All children will be asked to withhold from taking rescue medication for at least 12 hours beforehand. A child will be defined as having BHR when FEV1 has dropped by ≥20% from baseline during the inhalation challenge. In children with a baseline FEV1 ≤70% no challenge will be performed. In all known patients with asthma spirometry (BDR or on indication a challenge test) is annually performed in patients, according to the national guideline.(16)

Other test results

Depending on the situation of the patient, more diagnostic tests may be performed when this is considered necessary for patient care by the physician. For instance, in a child presenting with recurrent infections initially a culture may be taken and lab tests may be performed, before a lung function test will confirm the diagnosis of asthma. The EP does offer the opportunity to enter those test results in the system in a structured way. Cultures (nasopharyngeal, sputum, ear, and nose) and lab results in case of suspicion of an immune deficiency can be entered when applicable. Also other atopic test results, as an ImmunoCAP for food allergens or inhalation allergens, food challenge results or skin prick test results can be entered. Test results can be filled in on predefined schedules. Also the doctors-diagnosis will be entered in the EP, and other diagnoses can be entered over time.

Follow up and study endpoints

By activating the follow up function in the EP, each season patients are informed by email that a short questionnaire is ready to be filled in by parents and/or patient in the EP (for details, see table 1 section follow-up). In order to obtain a validated measure of asthma control, the EP uses the validated C-ACT, or ACT, depending on the age of the child. Adherence to treatment is assessed by using the Medication Adherence Report Scale (MARS) comprising questions on medication use behaviour and adherence.(19) Medication use is registered by parents.

Privacy

The handling of personal data complies with the Dutch Personal Data Protection Act. All data are stored in a large database, which is maintained by Vital Health Software. Storage and protection of the data is done according to the NEN 7510 guideline. Privacy is protected by encrypted storage of personal information in the database. Exchange of data is protected by a security protocol to prevent damage, loss, unauthorized access or abuse of data. The EP can only be accessed with personal access codes.
The EP offers different user levels. Each level has its own function and privileges, such as a professional (to give access to the EP to patients, and to view their own recruited data), an application manager (to give access to the EP to professionals; access to all processes and modules, including the databases), and patient (access to their own data). Each participating centre has its own access codes, and data from other centres cannot be seen or modified.

RESULTS

Recruitment

At the time of writing 1500 children have been invited to participate, of whom the baseline questionnaire has been completed in 740 (49%) patients. 478 patients were selected to be followed up based on a diagnosis of a recent asthma diagnosis or new symptoms that were assigned to asthma by the pediatrician. Recruitment has been underway for 1 year in 3 centers (Figure 2), for 5 months in 2 centers and 2 months in one center. Two other centers have confirmed participation in the study, and will start at the end of 2012 with inclusion.

DISCUSSION

In current clinical practice large amounts of data are gathered during routine patient care. Very little of this data are available for research purposes because data are not recorded in a structured way. Here we describe an EP which facilitates the EN to collect data in a structured way with minimal effort of the caregivers themselves. This EP offers several opportunities.

Since the start of inclusion, in June 2011, 1485 patients were invited to participate. Two third has started filling in the questionnaire and at present 661 patients (66%) have completed the baseline questionnaire. In 95% of the cases informed consent was given to use EP-data for research purposes. 498 patients have been selected for follow-up until date. These patients have been recruited in the past 9 months in 1 centre (figure 2), for 6 months in 2 centres and 3 other centres have confirmed participation in the study, and are currently starting up. This shows the EN is able to gather a large number of patients within a relative short period. As a result a large database will be available within a relatively short time.

Large population based observational studies, mainly birth cohort studies, have been published, that mainly studied determinants of asthma.(20-22) These data are not suitable to study treatment related asthma phenotypes of asthma in children; firstly because of the small number of patients with asthma in most of these studies. Although birth cohorts may be large, asthma may be present in about 5% of the children above the age of five. The number of patients using asthma medication on regular basis, which is only a sample of this 5%, does not allow comparing therapy response within the different treatment regimens. Especially in a heterogeneous disease, as asthma, large patient numbers are needed to explore those treatment defining phenotypes.

Strict inclusion criteria are used in randomized trials to study the efficacy of treatment trials. The outcomes of those studies are applicable to this selected group, but difficult to generalize in the heterogenic asthmatic population seen in daily practise. The EP enables collection of data gathered during daily practise of an unselected population with asthma (and other atopic diseases), for research purposes. By including large samples of patients, the outcomes will be usable in daily practice. Data from the EP will be used to study the effectiveness instead of the efficacy, which makes the outcomes more applicable in daily practice.

Currently, the automatic follow up function is enabled for asthmatic patients. However, in the future, this function can be built for the other disease topics included in the EP: allergic rhino conjunctivitis, eczema, food allergy and (upper and lower-) respiratory tract infections. Apart from the research relevance, the patients participating in the EP will be followed up in time, which means that their complaints will be monitored actively by the EP without extra effort from the doctor. In regular asthma care, the frequency of visits is often once per year in stable periods. During this visit it may be difficult for parents and patient to recall how the last 12 months have been. The EP makes it possible to have a whole year through-overview of the treatment response and medication use for the doctor, as well for the patient himself. Transparency in hospital care is also increased by access to their test results in the electronic EP by each individual patient, which may increase the involvement of the patient in his treatment.(23)

The EP supports a more structured way of working within the collaborative network. This may support the use and implementation of national guidelines on atopic diseases. Each participating hospital creates its own patient database. With this database the performance of each centre can be monitored and compared to other centres. Furthermore, working strategies or other knowledge can be exchanged to improve daily practice within the centres. Due to the use of a web based application, there will be a selection in the population that is included in the EP. Currently in the Netherlands, 1% of all persons between 11-45 years of age do not have access to internet at home.(24) The main reason for not having internet-access is 'no interest'. Because financial reasons seem to play a much smaller role, this will probably not lead to a selection in our patient

group (in social economic state). However, also a good understanding of, and ability to read the Dutch language is an inclusion criterion. This will lead to a selection of patients, because the 1.5 million functional analphabetic persons in the Netherlands will mainly evolve within the lower social economic class. One third of those persons are foreigners. (25) How large this selection is will be analysed.

CONCLUSION

We conclude that the use of current web-based services like the described EP can be helpful to support extensive data collection in Expert Networks.

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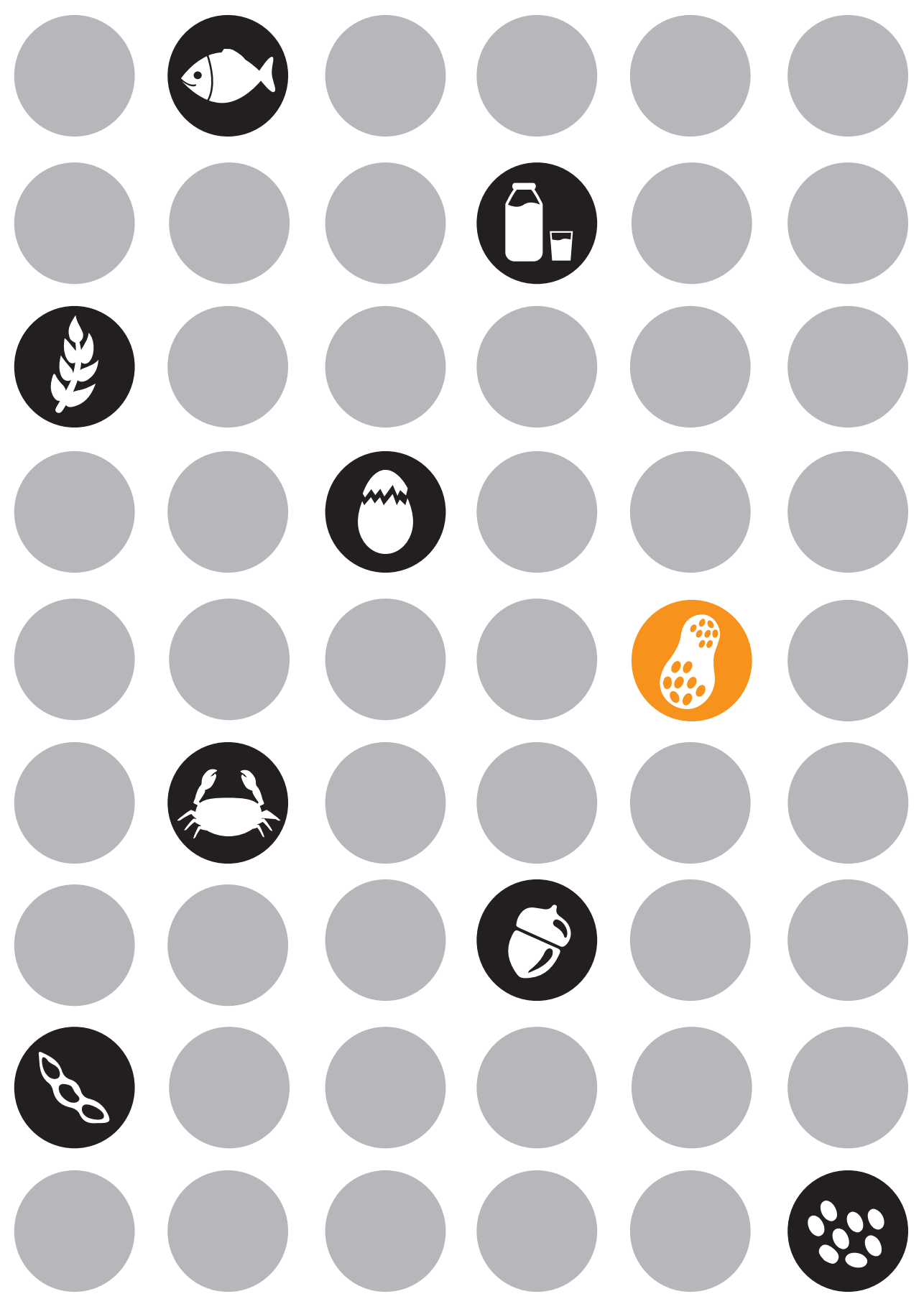

CHAPTER 3

Generic and disease specific quality of life in children referred for food allergy

Submitted

Francine C. van Erp
Kim Zomer-Kooijker
Walter A.F. Balemans
Bart E. van Ewijk
Maartje F. van Velzen
Gordon Slabbers
Diana M.W. Gorissen
Esther de Vries
André C. Knulst
Yolanda Meijer
Cornelis K. van der Ent

ABSTRACT

Background

Health related quality of life (HRQL) is a measure of the perceived severity of disease in food allergic children. Information about determinants of HRQL can be used to guide diagnostic procedures and management of patients at outpatient clinics. With this study we aimed to investigate generic and food allergy specific HRQL in children referred for food allergy and compare their generic HRQL to other referred children and controls.

Methods

A cross-sectional study with data collected in secondary and tertiary care including children referred for food allergy (n = 565), asthma (n = 620), infections (n = 351) and controls (n = 269) was performed. Uni- and multivariable linear regression analysis was used to investigate (determinants of) food allergy specific and generic HRQL.

Results

In children referred for food allergy generic HRQL was impaired but not to the same extent as in children referred for asthma or infections. Younger age, an extensive elimination diet, family history of food allergy, respiratory symptoms, eczema and recurrent respiratory tract infections had significant negative impact on generic HRQL. A severe allergic reaction in history and extensive elimination diets had significant negative impact on food allergy specific HRQL.

Conclusions

Children referred with food allergy have an impaired generic and specific HRQL. Generic HRQL is most affected by respiratory and atopic comorbidities and food allergy specific HRQL is impaired by a severe allergic reaction in history and an extensive elimination diet.

INTRODUCTION

Food allergy prevalence is high (1 to 4%) and even higher for self-reported food allergy (up to 25%).(1) Currently no preventive treatment for the occurrence of acute food allergic reactions or cure is available. Management of food allergy is therefore limited to elimination diets and emergency treatment prescription.(2) As a result, children with food allergy and their families are faced with dietary but also with social restrictions.(3) Furthermore, food allergic children and their parents show high levels of anxiety about the risk of potentially severe reactions.(4,5)

It is difficult to measure disease severity in food allergy as mortality is relatively low and physical symptoms occur only occasionally and are generally absent. In the absence of adequate severity scores, health related quality of life (HRQL) can be used to measure the perceived burden of disease in food allergic patients.(6) Instruments that measure food allergy specific HRQL are suitable to measure food allergy related impairments in quality of life and differences in quality of life over time. Previous studies on food allergy specific HRQL have shown that especially dietary restrictions, fear of allergic reactions and the need to carry an epinephrine auto-injector (EAI) decrease HRQL.(3) Generic measures of HRQL do not focus on disease specific symptoms and make direct comparison to the general population and patients with other conditions possible.(7) Studies investigating generic HRQL have shown that (parents of) children with food allergy have lower general health perception than healthy controls and children with diabetes mellitus, but better generic HRQL than patients with asthma, irritable bowel syndrome and rheumatoid arthritis.(8) Information about the influence of patient- and food allergy related characteristics on generic and specific HRQL is important for clinicians to guide diagnostic procedures and management of patients at outpatient clinics. However, only limited data on determinants of HRQL in children with suspected food allergy is available. Furthermore, children presenting with food allergy often have other burdensome atopic conditions like asthma, allergic rhinitis and eczema. The relative importance of those comorbidities with respect to impairment of HRQL is largely unknown.(9) Moreover, it is unknown how generic HRQL of children referred for food allergy compares to children referred for other allergic and respiratory conditions.(10) In this study we investigated determinants of both generic and food allergy specific quality of life in children referred for food allergy. We also compare their generic HRQL with children referred for asthma and recurrent infections.

METHODS

Design and setting

A cross-sectional study with data collected within a nationwide collaborative network of Dutch caregivers in secondary and tertiary care from June 2011 – March 2015 was performed.(11) Children between 0 – 18 years referred for food allergy to one of the participating centres were included. For comparison of HRQL between referral groups

we also included children referred for asthma or recurrent infections. Furthermore, a control group of un-referred children between 6 – 12 years was recruited from a prospective birth cohort, the Wheezing Illnesses Study Leidsche Rijn (WHISTLER).(12) Informed consent was obtained from parents and children before enrolment and the study was reviewed and approved by the ethical committee of the University Medical Centre Utrecht.

Data collection

Data were collected with electronic questionnaires for children and their parents on a personal page in the Electronic Portal (EP). The EP is web-based system and contains health- and disease related questions on respiratory- and allergic diseases, as well as questionnaires about exposures and demographic information.(11) Questionnaires were filed in before an outpatient visit in referred children. Children of the control group received an invitation for the EP by e-mail as part of the follow-up of the WHISTLER study. A doctor's diagnosis of food allergy was defined as yes to the question 'Has your child ever been diagnosed with food allergy by a general practitioner, paediatrician/ dermatologist or allergologist?'. For the number food allergens avoiding, reported allergens were categorized into: cow's milk, chicken egg, peanut, nuts, wheat and other. Food related symptoms were defined as the worst symptoms ever occurred after ingestion of any food allergen and classified according to organ system. Information about asthma and allergic rhinitis related symptoms were adopted from the ISAAC questionnaire.(13) Eczema was defined as yes to the question 'Has your child had an itchy rash (which was coming and going)?'. Recurrent lower respiratory tract infections were defined as three or more episodes of bronchitis (= coughing, dyspnoea and fever). Recurrent upper respiratory tract infections were defined as four or more episodes of otitis (= earache, otorroea, fever), 12 or more episodes of colds (= nasal congestion, rhinoroe and coughing) or three or more episodes of croup (= barking cough, stridor, hoarseness).

Quality of life questionnaires

Generic HRQL was measured in parents of all referred children and controls. Food allergy specific HRQL was assessed in children referred for food allergy older than 8 years (n = 167). The 7-item RAND general health-rating index (RAND) was used to asses generic HRQL.(14) The RAND is translated and validated for Dutch parents and assesses perceptions of general health and susceptibility to illness of caregivers regarding their child.(15) The total score has a minimum of 7 and a maximum of 32 with higher scores indicating better general health. Food allergy specific quality of life was measured in children with the validated Dutch version of the FAQLQ Child Form (CF) for children aged 8 – 12 and the FAQLQ Teenage Form (TF) for adolescents aged 13 – 17 years (16,17). The FAQLQ contains 23 (CF) or 24 (TF) questions and is divided in different domains: allergen avoidance, dietary restrictions, risk of accidental exposure and emotional impact. The total FAQLQ score is the mean of all items and ranges from 1 (minimal impairment) to

7 (maximal impairment) with lower scores indicating better food allergy specific quality of life. A difference in total FAQLQ scores of ≥0.5 points was considered to be clinically relevant.(16)

Statistical Methods

Descriptive statistics including means, standard deviations (SD) and proportions were used to describe the study population. To avoid bias that might result from a complete case analysis, missing data were imputed by using multiple imputations. The RAND and FAQLQ scores were expressed as continuous variables with a normal distribution. Differences in the RAND-score between referral groups and determinants of the RAND-score in children referred for food allergy were investigated using uni- and multivariable linear regression analysis. Atopic comorbidities and allergic or respiratory symptoms in the last 12 months and referral reason were included as independent variables in the multivariable model. Age, gender, educational level, family history of atopy, season of questionnaire, referral to secondary or tertiary care were included as confounders. To investigate determinants of the FAQLQ, all relevant food allergy related variables which were associated with the FAQLQ after univariable analysis ($p < 0.200$) including the type of FAQLQ (TF or CF) were included in a multivariable linear regression model. Floor and ceiling effects (percentages of patients with minimal and maximum scores) of the quality of life questionnaires were calculated to verify responsiveness. These effects were considered present if >20% of the sample achieved the highest or lowest possible scores. The correlation between generic and specific quality life questionnaires were compared using Pearson's correlation coefficients. Moreover we investigated whether the 10% of children with the worst or best generic HRQL did correspond to the 10% of children with the worst or best food allergy specific HRQL with the Mc-Nemar test for paired proportions. Statistical analysis was performed using IBM SPSS statistics version 21 (Armonk, New York, USA), p values < 0.05 were considered significant.

RESULTS

Study population

By March 2015 data of 1805 referred subjects with complete questionnaires were available in the EP for data analysis. The available data included 565 children referred for food allergy, 620 children referred for asthma, 351 children referred for recurrent infections. Furthermore we could include 269 controls from the WHISTLER study. Baseline and food allergy related characteristics of children referred for food allergy are shown in table 1a and 1b. Characteristics of other children are shown in supplementary table E1. Children referred for food allergy had a mean (SD) age of 6.1 (4.2) years. At the time of referral a parent reported doctor's diagnosis of food allergy was present in 420 (80%) children. Most parents presented with elimination diets for more than two foods (50%). One third (36%) of parents reported to carry an EAI and 84 (14%) children reported anaphylaxis in history. Allergic comorbidities and associated symptoms were often reported: current eczema (60%), asthma (26%), allergic rhinitis (35%) and recurrent infections (11%).

Table 1a Characteristics of children referred for food allergy, n = 565

Patient characteristics	**n (%)**
Male sex	359 (64)
Mean age (SD)	6.14 (4.18)
Dutch ethnicity mother*	517 (92)
High educated mother*	330 (59)
Family history of	
allergic rhinitis*	396 (70)
eczema*	380 (67)
asthma	237 (42)
food allergy*	158 (45)
Referred to tertiary care	348 (62)
Referred by general practitioner	263 (47)
paediatrician	212 (38)
other	90 (16)
Season of questionnaire (winter)	162 (29)
Comorbidities	
Asthma diagnosis*	140 (26)
Wheeze in the last 12 months	226 (40)
Cough at night in the last 12 months*	246 (44)
Allergic rhinitis diagnosis	197 (35)
Nasal and eye symptoms in 12 months*	237 (42)
Eczema diagnosis	456 (81)
Active eczema in the last 12 months	334 (60)
Recurrent upper respiratory tract infections	29 (5)
Recurrent lower respiratory tract infections*	59 (11)
Quality of life	
Mean RAND-score (parents n = 565)	21.97 (4.93)
Mean FAQLQ-CF score (children n = 118)	3.8 (1.5)
Mean FAQLQ-TF score (children n = 49)	3.9 (1.4)

* Variables with missing values that were imputed
EAI, epinephrine auto-injector; SD, standard deviation

Table 1b Food allergy related characteristics, n = 565

Characteristic	**n (%)**
Food allergy diagnosis	420 (74)
Number of food allergens avoiding	
0-1	19 (3)
2	184 (33)
>2	338 (60)
Type of food allergen with symptoms	
Cow's milk	187 (33)
Hen's egg	149 (26)
Peanut	276 (49)
Nuts	247 (44)
Wheat	78 (14)
Other	287 (51)
Severity of symptoms	
Rhinitis	33 (5)
Skin	72 (12)
Gastro-intestinal	108 (19)
Respiratory	221 (39)
Anaphylaxis	84 (14)
Other	47 (8)
Carrying an EAI	127 (23)
Growth problem	101 (18)
Eating problem	71 (12)

EAI, epinephrine auto-injector

Differences in generic HRQL

In children referred for food allergy the mean RAND-score was 21.97 (SD 4.9) which was significant lower compared to controls (adjusted mean difference 3.67, p <0.001) However, their generic HRQL was significant better compared to children referred for asthma or recurrent infections (adjusted mean difference: -0.70, p = 0.037 and -3.51, p < 0.001). Mean differences in generic HRQL are presented in table 2.

Determinants of generic HRQL

A doctor's diagnosis of food allergy or a history of a severe food allergic reaction did not have a significant impact on the RAND-score in children referred for food allergy. Younger age, an extensive elimination diet, family history of food allergy, respiratory symptoms (cough and wheeze), active eczema and recurrent respiratory tract infections significantly impaired generic HRQL. Regression coefficients of the variables independently related to the RAND in children referred for food allergy are shown in table 3.

Table 2 Mean difference in RAND-score between referral groups and controls

Referral reason (n)	**Mean** (SD)	**Mean difference** (95%CI)	**p-value**	**Adjusted Mean difference** (95%CI)[#]	**p-value**
Food allergy (565)	21.97 (4.93)	*Ref*		*Ref*	
Asthma (620)	20.62 (4.55)	-1.35 (-1.86;-0.83)	< 0.001	-0.70 (-1.36;-0.04)	0.037
Infections (351)	17.23 (4.83)	-4.74 (-5.34;-4.13)	< 0.001	-3.51 (-4.26;-2.76)	< 0.001
Controls (269)	28.22 (2.99)	6.26 (5.6;6.92)	< 0.001	3.67 (2.84;4.49)	< 0.001

SD, standard deviation; Ref, reference category [#]Adjusted for: age, gender, educational level, season of questionnaire, family history of atopy, tertiary or secondary care, respiratory and allergic symptoms and diagnosis

Table 3 Determinants of generic HRQL in children referred for food allergy, n = 565

Determinant	**Multivariable B** (95% CI) [#]	**p-value**
Age in yrs.	0.14 (0.03;0.25)	0.010
Season of questionnaire (winter)	-0.87 (-1.67;-0.06)	0.034
Avoiding > 2 food allergens	-1.84 (-2.62;-1.07)	<0.001
Family history of food allergy	-1.46 (-2.28;-0.64)	<0.001
Cough at night in the last 12 months	-1.5 (-2.28;-0.72)	<0.001
Wheeze in the last 12 months	-1.05 (-1.87;-0.23)	0.012
Active eczema in the last 12 months	-1.27 (-2.07;-0.48)	0.002
Recurrent upper respiratory tract infections	-2.53 (-4.2;-0.85)	0.003
Recurrent lower respiratory tract infections	-2.64 (-3.89;-1.38)	<0.001

B, Unstandardized regression coefficient of the predictor in the linear regression model; CI. Confidence Interval
Negative B-values indicate a negative impact on the RAND-scores (poorer general health). The explained variance of the multivariable model was 24%. R^2 = 0.244.
[#]Adjusted for all other variables in the model (age, gender, educational level , season of questionnaire , family history of atopy, tertiary or secondary care, respiratory and allergic symptoms and diagnosis)

Determinants of food allergy specific HRQL

The total FAQLQ scores (mean, SD) were 3.8 (1.5) for children and 3.9 (1.4) for adolescents. Univariable analyses showed that a family history of food allergy, the number of eliminated food allergens, the type of food allergen (hen's egg, cow's milk and peanut) and the severity of symptoms (respiratory or anaphylaxis) were related to poorer quality of life. After multivariable analysis, an elimination diet of more than 2 foods (Regression coefficient (B) 0.89, p = 0.040), respiratory (B 0.75, p = 0.047) and anaphylactic (B 0.97, p = 0.043) symptoms to food had an independent and clinically relevant negative impact on the FAQLQ. Carrying an epinephrine auto-injector (EAI) had no significant impact on the FAQLQ score (B 0.36, p = 0.192). An EAI also had no effect on different subdomains of the FAQLQ score (data not shown). Results of the multivariable analysis are shown in table 4.

Table 4 Determinants of food allergy specific HRQL in children and adolescents referred for food allergy, n = 167

Determinant	**Multivariable B** (95% CI)[#]	**p-value**
Type of food allergen		
Other	*Ref*	
Cow's milk	0.23 (-0.34;0.81)	0.430
Chicken egg	0.32 (-0.30;0.94)	0.310
Peanut	-0.098 (-0.61;0.41)	0.705
Number of food allergens avoiding		
0-1	*Ref*	
2	0.75 (-0.12;1.62)	0.082
>2	0.86 (0.02;1.7)	**0.046**
Type of symptoms		
All other symptoms	*Ref*	
Respiratory	0.62 (0.12;1.12)	**0.015**
Anaphylaxis	0.79 (0.003;1.57)	**0.049**

B, Unstandardized regression coefficient of the predictor in the linear regression model; CI. Confidence Interval; EAI. Epinephrine auto-injector. Positive B-values indicate a negative impact on the FAQLQ-scores (more impairment of quality of life). The explained variance of the multivariable model was 16%, $R^2 = 0.156$

[#] Adjusted for other variables associated with the FAQLQ in the univariable analysis (p < 0.200): Age category (8-12 years or > 12 years), family history of food allergy, doctors' diagnosis of food allergy, carrying an EAI.

Bold p-values indicate a significant effect of the determinant on the FAQLQ score

Comparison of generic and food allergy specific HRQL

No floor and ceiling effects were seen: proportion of children with lowest and highest score for the RAND 0.2% and 1.8% and for the FAQLQ 0.6% and 0%. This is confirming the responsiveness of the questionnaires. No significant correlation was found between the generic and food allergy specific quality of life scores (R -0.131, p = 0.093). Moreover, the 10% of children with the worst or best generic HRQL differed from the 10% of children with the worst or best food allergy specific HRQL (agreement worst scores: 3% p = 0.049 and agreement on best scores: 0%, p = 0.114).

DISCUSSION

This is the first study that evaluates both generic and food allergy specific HRQL in children referred for food allergy. Our results show that children referred for food allergy have an impaired generic HRQL which is for an important part explained by respiratory and atopic comorbidities. Moreover, we demonstrate that especially children with reported severe allergic reactions and extensive elimination diets are at risk for a lower food allergy specific HRQL.

We compared generic HRQL between different referral groups and controls. Our study shows that parents of children referred for food allergy have an impaired HRQL but a better HRQL compared to parents of children referred for asthma or respiratory infections. These results are not explained by differences in patient characteristics as age, gender or other comorbidities between referral groups or controls because we included those confounders in the multivariable model. The frequency and burden of symptoms associated with asthma and infections may have a stronger impact on general health compared to a more chronic condition like food allergy. Another explanation could be that the generic questionnaire used (RAND) was not able to capture all food allergy specific impairments like the burden of allergen avoidance and the risk of severe accidental reactions.(8) This may have led to an underestimation of HRQL in children referred for food allergy. The poor correlation between the generic and food allergy specific instrument underlines that different aspects of HRQL are captured in both instruments.

Our findings underline the impact of food allergy on generic HRQL but also highlight the importance of comorbidities in these children. It is known that food allergy is associated with an increased risk of asthma and eczema.(18,19) The high proportion of children with asthmatic symptoms (44%) and active eczema (60%) and the impact of those symptoms on generic HRQL we found in this study indicate that part of the children with food allergy could be suffering from uncontrolled comorbidities. Thorough examination and adequate management of those comorbidities in children referred for food allergy is warranted, especially because (uncontrolled) respiratory and atopic diseases could be related to more severe food allergic reactions.(20,21)

Our results further indicate that in children older than 8 years referred for food allergy, impairment of food allergy specific HRQL seems not to be allergen dependent. This is in contrast to previous studies that did found a relation between the type of allergen and impairment of HRQL.(22–24) This difference could be explained by the fact that in our study children were referred for suspected food allergy and the exact food allergen was not yet confirmed. In contrast to a study in venom immunotherapy and a previous study in children with food allergy, carrying an epinephrine auto-injector had no significant impact on food allergy specific HRQL of children in our study.(25,26) A possible explanation for this is that we investigated child reported HRQL and that

in (younger) children especially parents suffer from the responsibility to carry an EAI. As was suggested in a recent study, the previous proposed relationship between EAI prescription and HRQL may be confounded by self-perceived disease severity.(22) As an alternative for this perceived severity we included the type of food allergic symptoms in the model as we expected that these are also closely related to both the prescription of an EAI and HRQL. After inclusion of disease severity EAI prescription did no longer had a significant effect on HRQL.

A limitation of this study is that we investigated generic HRQL in parents and food allergy specific HRQL in children only. Although perceived severity of food allergy is comparable between children and parents, the latter tend to report better quality of life.(27) Therefor the comparison between the two instruments has to be interpreted carefully. Furthermore we are not able to conclude whether differences in generic HRQL are clinically relevant as no data on the smallest change in RAND score that is considered to be clinically important by patients is available. Moreover, our data are based on referred Dutch children with reported food allergy and no data regarding sensitization tests or oral challenges could be taken into account. This decreases the generalizability of the results to children with confirmed food allergy and other populations. However, in line with a recently published study our study is able to demonstrate that also in children with undiagnosed food allergy generic HRQL is impaired.(10)

CONCLUSION

In summary, this study demonstrates an impaired generic HRQL in children referred for food allergy. The results of this study can be used to guide patient oriented management early in the diagnostic process of children referred with food allergy. A multidisciplinary approach in those children is important as respiratory and other allergic comorbidities are important determinants of generic HRQL. Clinicians should also be aware that especially children with reported severe allergic reactions and extensive elimination diets are at risk for a lower food allergy specific HRQL.

Table E1 Baseline characteristics of children referred for asthma, infections and controls

Patient characteristics (%)	**Asthma** (n = 620)	**Infections** (n = 351)	**Controls** (n = 269)
Male sex	387 (62.3)	186 (53)	120 (44.6)
Mean age (SD)	8.94 (4.03)	4.33 (3.55)	9.11 (2.18)
Dutch ethnicity mother*	554 (89.2)	316 (90)	244 (90.7)
High educated mother*	311 (50.1)	172 (49)	192 (71.6)
Family history of			
allergic rhinitis*	429 (69.2)	196 (55.8)	153 (56.9)
eczema*	398 (64.2)	171 (48.7)	134 (49.8)
asthma	302 (48.7)	127 (36.2)	66 (24.5)
food allergy*	147 (32)	29 (29.3)	46 (17.1)
Referred to tertiary care	205 (33)	313 (89.2)	.
Referred by a general practitioner	390 (62.8)	188 (53.6)	.
Referred by a general paediatrician	135 (21.7)	118 (33.6)	.
Referred by other	96 (15.5)	45 (12.8)	.
Season of questionnaire (winter)	147 (24)	131 (37)	10 (4)
Respiratory symptoms and diagnosis (%)			
Food allergy diagnosis	111 (17.9)	23 (6.6)	7 (2.6)
Avoiding > 2 food allergens	82 (13.2)	25 (7.1)	2 (0.7)
Severe reaction to food	95 (15.3)	9 (2.6)	3 (1.1)
Asthma diagnosis*	313 (51.7)	31 (9.1)	9 (3.4)
Wheeze in the last 12 months	410 (66.6)	163 (46.6)	9 (3.4)
Cough at night in the last 12 months*	408 (65.8)	175 (51)	29 (10.8)
Allergic rhinitis diagnosis	221 (35.8)	20 (5.7)	29 (10.8)
Nasal and eye symptoms in 12 months*	266 (43)	51 (14.53)	31 (11.5)
Eczema diagnosis	374 (60.5)	139 (39.6)	95 (35.3)
Active eczema in the last 12 months	221 (35.7)	81 (23.1)	44 (16.4)
Recurrent upper respiratory tract infections	55 (8.9)	134 (38.2)	5 (1.9)
Recurrent lower respiratory tract infections*	231 (38.2)	135 (38.6)	0 (0)

* Variables with missing values that are imputed

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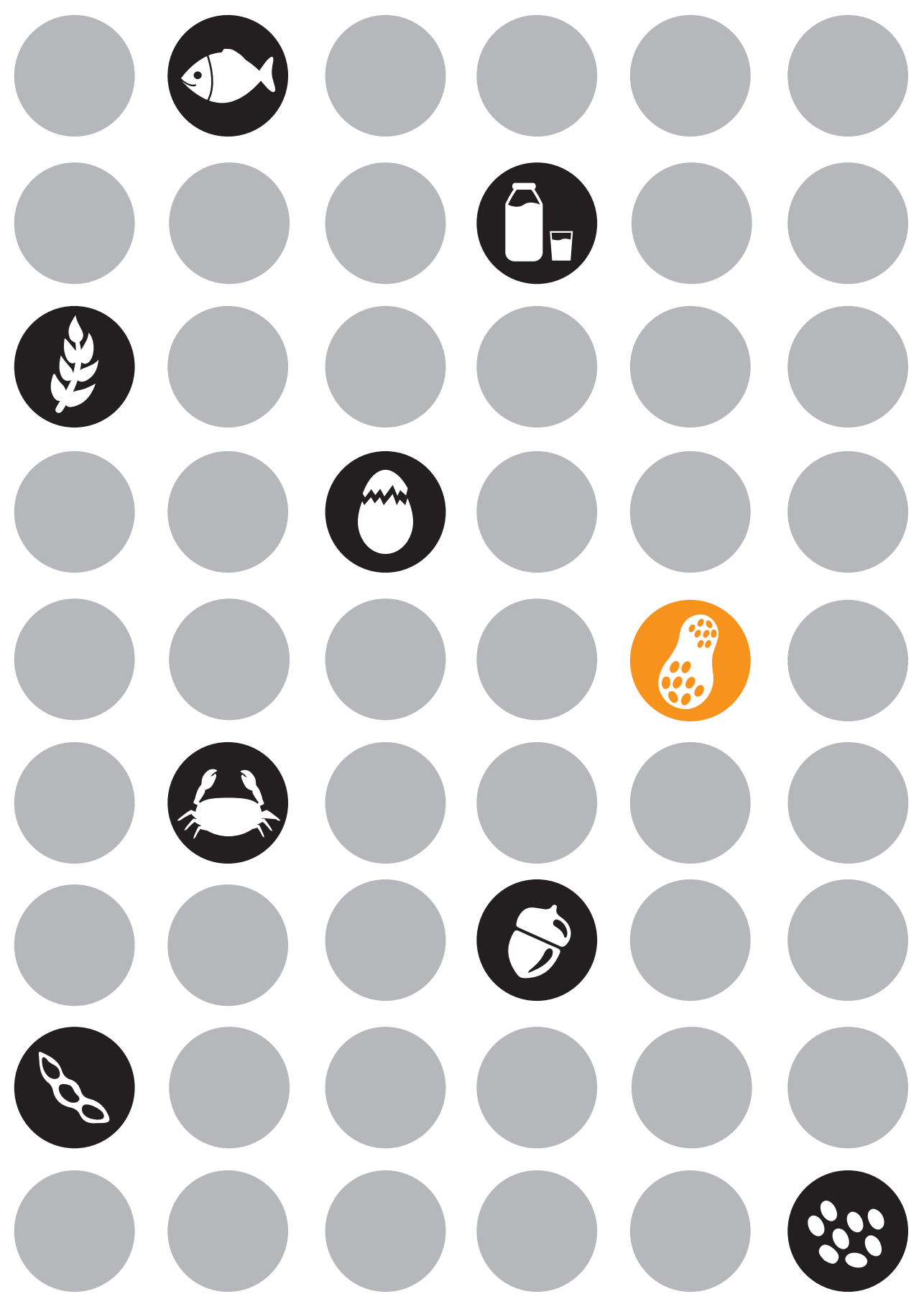
PART II

Predicting peanut challenge outcomes



CHAPTER 4

Using component resolved diagnostics in the management of peanut allergic patients

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Francine C. van Erp
Rob J. B. Klemans
Yolanda Meijer
Cornelis K. van der Ent
André C. Knulst

ABSTRACT

Instead of relying on crude peanut extract, component-resolved diagnostics (CRD) uses sensitization to allergenic proteins within peanut. In this review we describe the recent advances and future perspectives of the use of CRD in the management of peanut allergic patients. There is strong evidence that sensitization to Ara h 2 is the best predictor for clinically relevant peanut allergy in children and adults. Isolated sensitization to other peanut components is only rarely present in patients with systemic reactions to peanut. It is however important to remark that cut-off points of sIgE to Ara h 2 that predict tolerance or allergy vary between different study populations, different age groups and geographical regions, and validation studies performed in different settings are necessary to implement cut-offs in daily practice. Future studies should focus on the role of CRD in risk-assessment early in life, predicting long term tolerance and monitoring treatment responses following immunotherapy.

INTRODUCTION

Peanut is one of the most common allergens capable of eliciting severe allergic reactions.(1) Moreover, peanut allergy can already appear during early childhood and often persists throughout life.(2) Depending on the geographical region studied and definition of allergy used, peanut allergy is estimated to affect 0.2-3% of the population.(3,4) Peanut allergy is suspected when immediate allergic symptoms occur after peanut ingestion together with positive sensitization. Sensitization to peanut can be detected by a raised level of specific IgE (sIgE) or positive skin prick test (SPT). Sensitization is not always accompanied with clinical reactivity. The gold standard to diagnose peanut allergy is a double blind placebo controlled food challenge (DBPCFC).(5) However, the DBPCFC is a burdensome, expensive, and potentially dangerous procedure and therefore alternative ways to predict peanut allergy are strongly required.(6) In addition, previous work indicated that (double blind) food challenges can be false-negative and are subject to observer variability, especially when objective symptoms are absent.(7,8)

In recent years the role of sIgE to peanut components in the diagnostic work-up of patients with suspected peanut allergy has been extensively studied. Instead of relying on crude peanut extract, component-resolved diagnostics (CRD) uses sensitization to purified or recombinant allergenic proteins within peanut. CRD has proven to strongly increase the diagnostic accuracy to test for peanut allergy. Moreover it is able to identify cross-reactivity and has the potential to classify patients at higher risk for systemic reactions.(9) Moreover, reactivity to individual peanut allergens might be able to predict resolution of peanut allergy and be a target for immunotherapy.(10,11) In this review we describe the recent advances and future perspectives of the use of CRD in the management of peanut allergic patients.

RESULTS

Peanut extract and components

Peanut (Arachis hypogaea) belongs to the botanical family Fabaceae which is also known as Leguminosae and commonly known as the bean or pea family. The protein content of peanut lies between 24-29% and is mostly made of seed or storage proteins.(12) Currently, 17 allergens (components) of peanut (Ara h 1 – 17) have been identified in the official allergen nomenclature database.(13) Only the first nine of those allergens have been studied in relation to peanut allergy in humans and will therefore be part of this review. These allergens belong to the cupin (Ara h 1, 3), conglutin or prolamin (Ara h 2, 6, 7), profilin (Ara h 5), Bet v 1-like or Pathogenesis Related (PR-10) proteins of class 10 (Ara h 8) or lipid-transfer protein (Ara h 9) family. The allergens can also be divided in more functional and clinically relevant categories: storage proteins, pollen-associated proteins and plant-pan allergens. The characteristics of peanut allergens are shown in table 1.

Storage proteins

Seed storage proteins Ara h 1, 2, 3, 6 and 7 have a high degree of thermal and digestive

stability.(14) As a result, they are the major peanut allergens. Ara h 1 is a 7S globulin and is recognized in 26-92% of peanut allergic patients.(15–22) Between 20-80% of peanut-allergic patients are sensitized to the 11S globulin Ara h 3.(15–22) Ara h 3 and Ara h 4 are isoforms of each other and considered to be the same allergen.(23) Ara h 2, h 6 and h 7 belong to the 2S albumin protein family and have a high amount of amino acid sequence identity.(24) Ara h 2 and Ara h 6 are considered as the most potent allergens and are recognized by the majority (60-100%) of peanut allergic patients in western Europe and the US.(16,17,19,21,25) However, only up to 60% of the Mediterranean peanut allergic patients show raised levels of sIgE to Ara h 2.(20,26)

Bet v 1 homologous proteins

Ara h 8 is an allergen of the PR-10 family and has low stability to roasting and digestion. PR-10 allergens are common pan-allergens in pollens and also present in vegetables and fruits. Due to cross-reactivity with the birch pollen allergen Bet v 1, sensitization to Ara h 8 is common especially in North-West Europe.(27,28) Furthermore, Ara h 8 is also cross-reactive with Gly m 4 from soy and potentially with white lupine.(29,30)

Lipid Transfer Proteins

Ara h 9 has been identified as an important Lipid Transfer Protein (LTP) allergen in peanut, especially in the Mediterranean area.(28,31) LTPs are very stable and LTP-sensitized patients can experience systemic allergic reactions in addition to oral allergy. A strong association between sensitization to the LTPs in peach (Pru p 3) and peanut in Spain has been described.(32) Besides Pru p 3 it has also been suggested that LTP form plane tree (Pla a 3) or mug wort (Art v 3) can act as primary sensitizers.(33,34)

How to use CRD: in the diagnosis of peanut allergy

There is strong evidence that sIgE to Ara h 2 is the best predictor for peanut allergy in children and adults.(38) Depending on the population studied and definitions used sensitivity ranges from 60-100% and specificity from 60-96% when using a cut-off of 0.35 kUA/L.(15–19,21,39–41) The best combination of positive and negative likelihood ratio was also found when using sIgE to Ara h 2. Although it has been suggested that the prevalence and relative importance of sIgE to Ara h 2 is lower in Mediterranean countries, sIgE to Ara h 2 also emerged as the best predictor in studies from Southern France and Spain.(20,26)

In daily practice, sIgE to Ara h 2 and peanut extract are both suitable to exclude peanut allergy.(38) However, sIgE to Ara h 2 is more specific and sensitive to diagnose peanut allergy. A 80-95% NPV was reached when using sIgE to Ara h 2 levels of < 0.35 kUA/L and a 100% NPV when a cut-off level of < 0.1 kUA/L was used.(25,42) Moreover a 95-100% PPV was reached when using sIgE to Ara h 2 levels > 5 kUA/L to diagnose peanut allergy.(25,40) By using optimal cut-off points for sIgE to Ara h 2 (i.e. with the highest NPV and PPV) peanut allergy could be diagnosed without a food challenge in the majority of

Table 1 Characteristics of currently characterized relevant peanut allergens

Name	Protein family	Test availability	Stability	% of total protein	Type	Cross-reactivity	Related proteins
Ara h 1	Cupin 7S globulin	ISAC/ CAP	+++	12-16%	Storage	+	Gly m 8 Cor a 11
Ara h 3/4*	Cupin 11S globulin	ISAC/ CAP	+++	38-76%	Storage	+	Cor a 9 Gly m 9
Ara h 2	Conglutin 2S albumin	ISAC/ CAP	+++	5.9-9.3%	Storage	+	Cor a 14 Gly m 8
Ara h 5	Profilin	-	-	No data	Pollen-associated	+++	Bet v 2 Gly m 3
Ara h 6	Conglutin 2S albumin	ISAC	+++	4-14%	Storage	+	Cor a 14 Gly m 8
Ara h 7	Conglutin 2S albumin	-	No data	0.5%	Storage	No data	Cor a 14 Gly m 8
Ara h 8	PR-10	ISAC/ CAP	-	< 0.1%	Pollen-associated	+++	Bet v 1 Mal d 1
Ara h 9	Lipid Transfer Protein	ISAC/ CAP	++	*No data*	Plant -pan allergen	+++	Pru p 3 Art v 3

Bet, birch pollen; Gly, soy; Pru, peach; Cor, hazelnut; Art, mug wort; Ses, sesame
* Ara h 4 shares 91.3% nucleotide sequence homology with Ara h 3 (35)
References used for this table (9,13,36,37)

subjects suspected of peanut allergy. However, it should be noticed that current available cut-off points were estimated in a selected group of referred patients and therefore cannot be generalized to other centres without validation studies. Furthermore, data in adults and young children (< 4 years) are currently lacking.

It should also be kept in mind that in some cases of peanut allergy other peanut components are relevant.(43,44) Allergic patients without sIgE to Ara h 2 but with Ara h 1 or Ara h 3 sensitization have been reported occasionally.(28,42) The sensitivity of sIgE to Ara h 1 and Ara h 3 is generally low but varies extensively between studies (26-92% and 21-84%), mainly depending on geographical region.(38) Two studies in children and adults describe that the diagnostic accuracy of sIgE to Ara h 6 is comparable to sIgE to Ara h 2.(20,39) This can be explained by the homology and cross-reactivity between these two 2S albumins.(24) In adults it was advocated that Ara h 6 could have additional value to Ara h 2 in individual cases with a strongly suspected peanut allergy in which sIgE to Ara h 2 was absent or very low.(22,42) The diagnostic value of sIgE to Ara h 8 is low with a sensitivity ranging from 16–42% and specificity from 31-100%. Isolated Ara h 8 sensitization is often related to Bet v 1 sensitization and associated with tolerance or mild local symptoms.(27,45) In a Mediterranean region Ara h 9 can detect LTP related peanut sensitization, however the added value of sIgE to Ara h 9 is questionable as cases

of peanut allergy with isolated Ara h 9 sensitization are rare.(22,42)
In summary, sIgE to Ara h 2 is the best diagnostic test to diagnose or exclude a possible peanut allergy. In case of a suspected peanut allergy and absence of sIgE to Ara h 2, additional peanut components can be determined to detect relevant other sensitization. In older children and adults with a suspected Bet v 1 related peanut allergy sIgE to Ara h 8 can be useful. In adults and children with highly suspected primary peanut allergy sensitization to other storage proteins (Ara h 1, h 3 and h 6) can be relevant.

How to use CRD: in the prediction of severe peanut allergy

We concluded that sIgE to Ara h 2 could reduce the number of food challenges. However, next to diagnostic purposes, food challenges are used to provide useful information regarding the severity of peanut allergy and subjective and objective eliciting doses.
The severity of allergic symptoms during challenge correlated with higher levels of Ara h 2 in several studies.(16,22,46) Furthermore, higher levels of sIgE to Ara h 2 were associated with lower thresholds during food challenges in children and adults.(16,47) However, contrasting results and large individual variation in the relation between Ara h 2 and severity of peanut allergy exist.(16,48) There are several explanations for the absence of a strong and consistent association between sIgE to Ara h 2 and severity of peanut allergy. Firstly, challenges can underestimate severity of peanut allergy because they are usually stopped when objective and not necessarily severe symptoms occur. Secondly, in contrast to daily life, patients are in a relative stable situation during challenge (absence of co-factors like active allergic disease, infections or exercise).(48,49) In addition, it was suggested that the correlation between sIgE to Ara h 2 and thresholds only applies to higher dose therefore to selected patient populations only.(16)

sIgE to the storage components Ara h 1, Ara h 3 and Ara h 6 have also been related to severity but the best correlation was found for Ara h 2.(16,22) As is mentioned before, isolated sIgE to Ara h 8 is often related to mild symptoms. It has been reported that allergic reactions can occur in rare cases if a large amount of peanut is eaten over a short period of time.(44) Furthermore, Ara h 8 is able to activate basophils in mono sensitized children and a recent report shows that natural Ara h 8 from roasted peanuts has a reasonable degree of proteolytic and thermal stability.(50,51) In summary, severe peanut allergy is unlikely without IgE to any of the seed storage proteins Ara h 1, 2, 3 or 6. Although Ara h 2 is correlated to severity we cannot use the level of sIgE to Ara h 2 or other components to classify individual patients at higher risk for severe allergic symptoms during challenge or in daily life.

Other aspects of CRD

Despite the promising results of CRD in diagnosing and excluding peanut allergy, there are several important aspects of CRD that have to be considered when using and interpreting CRD in daily practice.

Singleplex versus multiplex

Besides determination of sIgE to individual components (singleplex) with the ImmunoCAP method (Thermo Fisher, Uppsala, Sweden) it is also possible to simultaneously determine sIgE to a large number of components by the use of biochip technology (multiplex) like the ImmunoCAP ISAC (Thermo Fisher, Uppsala, Sweden). The multiplex assay requires less blood and allergen and facilitates the identification of (cross-reactive) sensitization patterns.(52) Several studies compared the singleplex and multiplex method for peanut allergens and showed high correlation between the two methods.(53,54) However, it has to be considered that the multiplex method has potentially lower analytical sensitivity. (20) Furthermore, the ISAC requires manual procedures and results are semi-quantitative (expressed in standardized units). In general, multiplex CRD should therefore be used to investigate complex cases like patients with multiple food allergies, idiopathic anaphylaxis or severe allergic symptoms without sensitization to Ara h 2 and not as primary diagnostic test.(55)

Availability of allergens

One of the limitations of the use of whole peanut extract in sIgE testing is that the conventional extracts vary in composition and are deficient in some IgE-components. (56) However, relevant sensitization can still be missed when using CRD because some peanut allergens related to (severe) allergy might not have been identified. Moreover, some allergens that were characterized are not yet commercially available or investigated in clinical studies (Ara h 5, Ara h 7) or are only available in multiplex tests (Ara h 6), see also table 1.

Variability in CRD pattern within and between patients groups

The diagnostic value of different components is affected by several patient related factors. Age dependency of sensitization patterns was described. Several studies demonstrate that older peanut allergic patients were more often sensitized to Ara h 8 in contrast to children with early onset allergy that recognize predominately Ara h 2 and to a lesser extent Ara h 1 and h 3.(28,57,58) Additionally, geographical variation in sensitization patterns presumably due to differences in exposure to other plant allergens, dietary habits and genetics.(28,31) Although sIgE to Ara h 2 seems accurate in diagnosing and excluding peanut allergy in different parts of the world, cut-off points may vary between countries. As mentioned before additional components may play a role in certain regions (like Ara h 9 in Mediterranean countries).

Validation studies

Prospective validation studies that include follow-up to detect false negative (e.g. allergic reactions in patients with undetectable sIgE to Ara h 2) or false positive tests (negative challenges despite high levels of sIgE to Ara h 2) are needed. Those studies are necessary to further confirm the added value of CRD in daily practice and determine

cost-effectiveness. Furthermore validation of CRD in other settings (e.g. secondary care), young children and in different regions of the world is necessary as the diagnostic accuracy and therefore cut-off points of a test vary with the pre-test probability of disease.

Future perspectives of CRD

Risk-assessment early in life

The Learning About Peanut Allergy study showed that early oral introduction of peanuts was able to successfully prevent allergy in high-risk infants.(59) Peanut avoidance was associated with an increase in peanut wheal size and a higher proportion of patients with high levels of sIgE to peanut. At this moment it is unknown whether CRD in those very young children can be used to predict an increased risk for (severe) peanut allergy and is useful in deciding which children should introduce peanut early in life.

Predicting development of tolerance

Results from the population-based Health Nuts Study showed that in 22% of children with positive challenge in their first year of life outgrew their peanut allergy at year four.(2) Like in several other studies an increased SPT and sIgE response to peanut indicated persistent peanut allergy.(60–62) However, sIgE to Ara h 2 at year one was not predictive for persistence of peanut allergy.(2) Further studies are required to investigate whether the course of sIgE to Ara h 2 over time is related to resolution or persistence of peanut allergy.

Selection of patients for food challenges

The DBPCFC is the current gold standard to diagnose peanut allergy but burdensome and expensive, partly because the test takes two or even three days (open challenge). Based on validated cut-off points, sIgE to Ara h 2 can be used to reduce the amount of food challenges or food challenge days. For example, patients with sIgE to Ara h 2 above the cut-off point with a 100% PPV (5 kUA/L) are considered to have peanut allergy in our centre. If objectification of symptoms and more information about the threshold and severity is necessary it could be speculated that in these patients the DBPCFC can be replaced by a single day verum challenge. Furthermore, in patients with a low suspicion of peanut allergy and absence of sIgE to Ara h 2, clinical reintroduction (i.e. an open challenge with peanut butter) or even introduction at home can be advocated. It should be investigated whether certain diagnostic strategies are safe and associated with reduced health care costs and improved quality of life.

Usefulness of components in other diagnostic tools

Peanut components Ara h 1, Ara h 2, Ara h 3 and Ara h 8 have been investigated in the basophil activation test (BAT).(50,63,64) These results indicated that the basophil response to peanut components was related to clinical relevant peanut allergy and

might have a higher PPV compared to sIgE to peanut components. However, more data is necessary and several practical limitations (absence of a standardized protocol, fresh whole blood samples are necessary, high costs) prevent current implementation of the BAT in daily practice.(65) Furthermore in our recent study we could not confirm the added value of the BAT in predicting severe peanut allergy (manuscript submitted). Skin prick tests (SPT) with peanut components were performed in the past and indicated the relative importance of Ara h 2 and Ara h 6 compared to Ara h 1 and Ara h 3.(66) Furthermore a correlation between the number of components detected in the skin prick test correlated with severity of peanut allergy was found on group level.(67) Although results are promising, the future of the SPT as a diagnostic test is questionable as the SPT is prone to observer (measurement and interpretation of wheal size), device and extract variability. Furthermore tight regulations have made the production of extracts problematic.(68)

Relation to treatment response

Patients responsive to oral and sublingual immunotherapy had lower sIgE levels to peanut components Ara h 1, Ara h 2 and h 3 at baseline and at the end of studies while

Table 2 The role of CRD in the management of peanut allergic patients

CRD as diagnostic tool	○ Sensitization to Ara h 2 is the best predictor for clinically relevant peanut allergy in children and adults ○ Cut-off points of sIgE to Ara h 2 that predict tolerance or allergy vary with age, geographical region and study populations and validation studies performed in different settings would be necessary to implement cut-offs in daily practice ○ In absence of sensitization to Ara h 2, other peanut components should be considered in select patient groups: ○ Isolated Ara h 1, h 3 and h 6 sensitization ○ Isolated Ara h 8 sensitization is not rare and often related to Bet v 1 sensitization and mostly associated with tolerance or mild local symptoms in older children and adults ○ Especially in Mediterranean patients relevant isolated sensitization to Ara h 9 occurs in rare cases ○ Singleplex assays are preferred in patients presenting with suspected peanut allergy
CRD in relation to severity	○ Levels of sIgE to Ara h 2 or other components are correlated to severity but cannot be used to classify individual patients at higher risk for severe allergic symptoms
Future perspectives of CRD	○ At this moment the role of CRD in risk-assessment early in life, predicting long term tolerance or treatment responses is unclear

IgG4 binding increased at the same epitopes.(10,69,70) In those studies cut-off points of baseline Ara h 2 with 65-70% sensitivity and 90% specificity have been published that could predict (long term) responsiveness to immunotherapy. However, the added value of sIgE to Ara h 2 was debatable as sIgE to peanut extract had comparable discriminative capacity. At this moment further studies are required to determine whether CRD is able to select patients for immunotherapy and predict the long term outcome of treatment strategies.

CONCLUSION

The highlights of this review are presented in table 2. In summary, we can conclude that CRD plays an essential role in the diagnostic evaluation of a patient with suspected peanut allergy. sIgE to Ara h 2 is the best predictor for peanut allergy and is preferred as first diagnostic step. Clinicians should be aware that cases of (severe) peanut allergy, sIgE to other peanut components (Ara h 1, Ara h 3, Ara h 6, Ara h 8 and Ara h 9) may be relevant. At this moment CRD cannot be used to predict the risk of a severe allergic reaction in individual patients. The role of CRD in predicting long term tolerance early in life and treatment response deserves further investigation.

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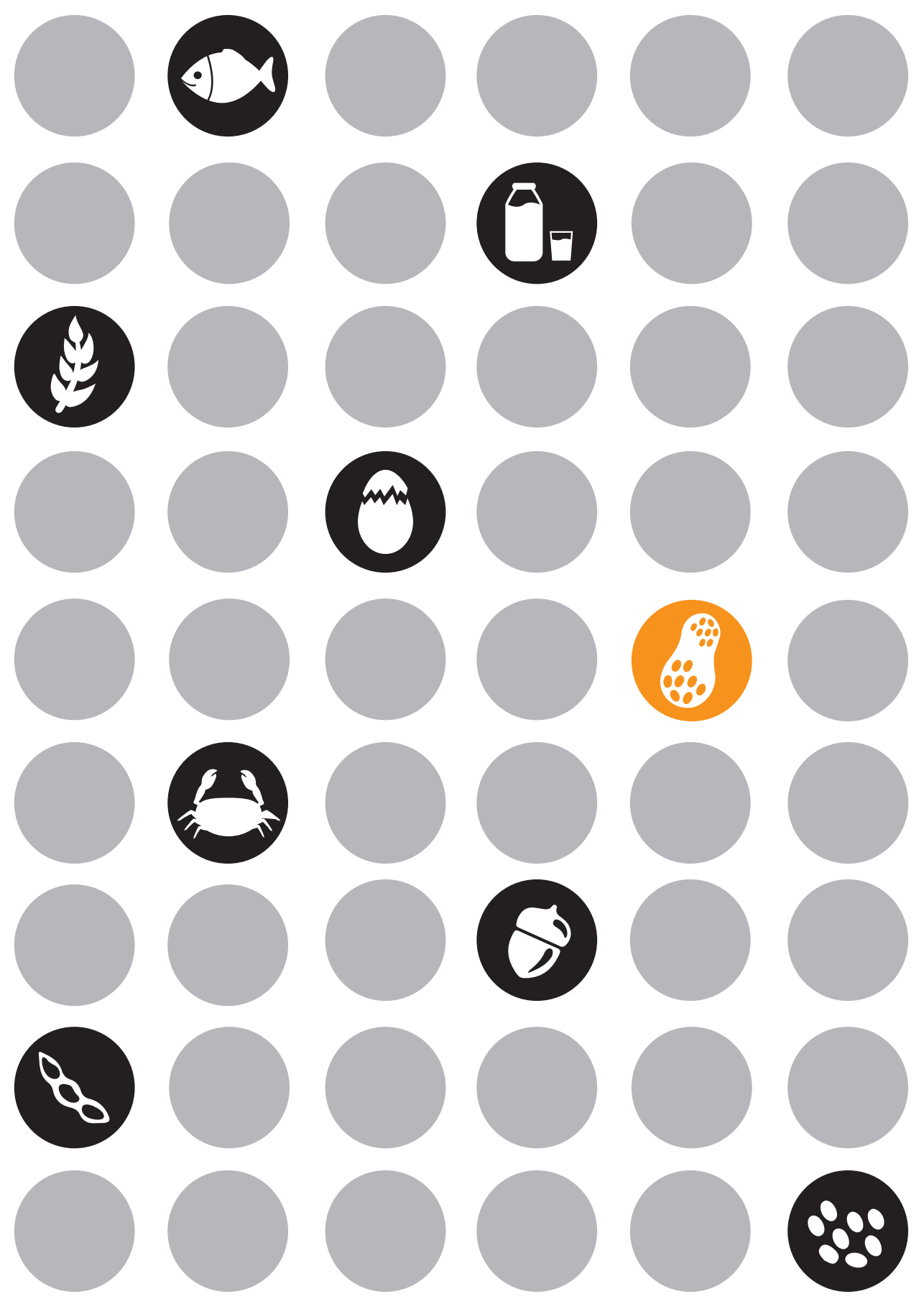

CHAPTER 5

Can we predict severe reactions during peanut challenges in children?

Pediatr Allergy Immunol 2013: 24:596–602

Francine C. van Erp
André C. Knulst
Petra A. Kentie
Suzanne G.M. Pasmans
Cornelis K. van der Ent
Yolanda Meijer

ABSTRACT

Background

Limited and contrasting data are available about risk factors for severe reactions during double blind placebo controlled food challenge (DBPCFC). Knowing these risk factors would help to improve safety precautions and choosing the best setting for challenge. We assessed whether we could determine predictors for positive and severe food challenge outcome (FCO) with regular available patient data in children suspected for peanut allergy.

Methods

A retrospective study in children referred for DBPCFC with peanut was performed during a three-year period. Reactions during challenge were classified as mild/moderate (Sampson grade 1-3) and severe (Sampson grade 4-5). We performed uni- and multivariable logistic regression to determine predictors for positive and severe FCO.

Results

A group of 225 children with a median age of 6.7 (IQR 5.0-9.5) years was studied. In 109 (48%) children food challenge outcome was positive and 24 (11%) children developed a severe reaction. The level of sIgE for peanut OR 1.14 (1.08-1.20), male gender OR 0.40 (0.20-0.81), having another food allergy OR 0.43 (0.20-0.88), were independently related to positive FCO. No significant differences were found between children with severe and non-severe FCO with respect to age, gender, asthma, sIgE or previous reaction to peanut.

Conclusion

Although predictors of positive FCO could be identified, none of the studied risk factors could predict a severe reaction during peanut challenge. When challenging a child sensitized to peanut, clinicians should be prepared and equipped to handle any reaction in all cases.

INTRODUCTION

Allergic reactions to food can vary from mild localized to severe generalized reactions with involvement of the respiratory and/or cardiovascular system.(1) A Double Blind Placebo Controlled Food Challenge (DBPCFC) is considered to be the gold standard for diagnosing food allergy.(2) Severe reactions can occur during DBPCFC, and are reported in 10-12% of patients challenged for peanut in previous studies.(3–5) In common practice a variety of patient related factors, like previous severe reactions and age is used to estimate the risk of a DBPCFC and to determine the precautions and the setting of the challenge.(6–9) Additionally, retrospective studies of community reactions showed that children with a fatal or severe reaction to food often had asthma.(6,7,10,11) However, the situation in daily life is different from that during DBPCFC, where the influence of other possible co-factors (e.g. active allergic disease, infections, alcohol or exercise) is minimized.(12) Moreover, when symptoms occur during DBPCFC, challenges are discontinued and treatment is given in an early stage. The evidence for using risk factors known from severe reactions in daily life to predict severity of food challenge outcome seems therefore limited and contradictory.(3,4,13) Because peanut is one of the most common foods known for its capacity to elicit a severe life threatening reaction,(7) we investigated whether we could identify known risk factors for anaphylaxis in daily life as predictors for positive and severe FCO in children who underwent a DBPCFC for peanut.

METHODS

Study design

Data were collected retrospectively from electronic patient records of oral food challenges, performed in the Wilhelmina Children's Hospital during a three-year period. All children from urban and rural area with suspected peanut allergy who required DBPCFC were referred to this tertiary hospital. Children eligible for DBPCFC were more than 3 years old and suspect for having a peanut allergy by a previous allergic reaction to peanut and/or a raised sIgE and/or positive SPT. All children fulfilling these criteria were accepted for DBPCFC and included for this study, unless a previous reaction to peanut required hospitalization at an intensive care unit was reported. Data were obtained as part of regular patient care and were used in strictly anonymous form, according to the code of conduct for medical research approved by the hospital's Medical Ethical Committee.

Patient characteristics

Measurement of peanut specific IgE (sIgE) was performed in all children within 1 year prior to DBPCFC, using Immuno CAP-technique (Phadia, Uppsala, Sweden), IgE levels of ≥ 0.35 kUA/L were considered positive. In a subset of children with available stored blood samples sIgE for peanut component Ara h 2 was determined. Skin Prick Testing (SPT) was performed within 2 years prior to DBPCFC using a commercial peanut extract (ALK-Abelló, Nieuwegein, The Netherlands). For positive and negative control histamine dihydrochloride 10 mg/mL and glycerol diluent were used, respectively. Mean diameter

of peanut wheal size in millimetres was calculated from the average of the largest wheal diameter plus largest wheal diameter perpendicular to this. SPT was considered positive when the wheal was 3 mm greater in diameter than the negative control.(14) Previous reactions to peanut were reported by the parents of the child and classified according to the Sampson classification described below based on the most severe reaction observed. The presence of asthma, atopic dermatitis, allergic rhinitis and other food allergies was determined in out-patient clinic consultations before DBPCFC. Asthma was defined as having a doctor's diagnose of asthma, together with the prescription of inhalant medication or inhaled corticosteroids (ATC code R03BA). Atopic dermatitis and allergic rhinitis were defined by doctor's diagnosis. Other food allergies were confirmed by a previous reported reaction combined with positive sensitization and/or a positive DBPCFC.

DBPCFC for peanut

Challenges were performed in a clinical setting equipped for resuscitation and monitoring of vital signs according to protocol. Only clinically stable children without recent infection, uncontrolled atopic disease or recent allergic reactions were eligible to start the challenge procedure. The DBPCFC protocol for peanut was described earlier by Flinterman et al.(15) The challenge was considered positive and terminated when objective symptoms occurred or when consistent subjective symptoms occurred on at least 3 subsequent doses. The eliciting dose (ED) was determined as the lowest dose of peanut protein in mg eliciting objective allergic reaction. The cumulative dose (CD) was determined by the cumulative portion of peanut protein administered to the child in mg. Symptoms during challenge were graded after physical examination by one of the clinical experts and classified based on the most severe reaction observed in an organ system, according to the Sampson classification.(1) Severe food challenge outcome (FCO) was defined as a positive FCO with a severe respiratory and/or cardiovascular reaction of Sampson grade 4 or 5 (severe stridor and/or dyspnoea and/or wheezing and/or hypotension). Severe respiratory reactions were further classified into respiratory reactions with involvement of the upper airways (severe inspiratory stridor) and reactions with involvement of the lower airways (expiratory stridor or wheezing). Children with mild/moderate reactions were defined as having a reaction according Sampson grade 1-3.

Statistical methods

Analyses were performed using SPSS for Windows (version 15.0 SPSS Inc., Chicago, IL, USA). A previous reaction to peanut in history was defined as a categorical variable with no previous ingestion / no previous reaction as reference category. Age was categorized in three groups (< 6, 6-12 and > 12 years). SPT and sIgE were used as continuous variables, sIgE values >100 kUA/L were deemed to be 101 kUA/L. To avoid bias that might result from a complete case analysis, we imputated missing SPT data using multiple imputation.(16) Univariable associations between candidate predictor variables and

positive FCO and subsequently severe FCO were investigated using univariate logistic regression. Predictors that were univariable associated with the outcome (with a p-value < 0.20) and or clinically relevant were included in stepwise backward fashion selection in a multivariable logistic regression model to evaluate their independent value (p-value < 0.05) in the prediction of positive FCO. Reliability (of goodness of fit) of the final model was estimated using the Hosmer-Lemeshow test. Prognostic capacity to differentiate between patients with and without positive FCO was estimated using the area under the receiver operating characteristics curve (AUC). Food challenge characteristics in children with mild/moderate reactions and severe reactions were compared using Pearson's Chi-Square test for categorical data and Mann-Whitney U tests for non-parametric continuous data.

RESULTS

A total of 225 DBPCFC's for peanut were performed and included for this study. Our study population consisted of predominantly boys (66%) and children had a median age of 6.7 (IQR 5.0-9.5) years. All children were sensitized for peanut by raised sIgE for peanut or positive SPT. In 88 (39%) children stored blood samples were available to determine sIgE for peanut component Ara h 2. In 109 (48%) children there was no known previous reaction to peanut in history. In 96 (43%) children no previous known ingestion was reported and the other 13 (6%) children did consume peanut without developing symptoms in history but were set on a prolonged elimination diet for other reasons (e.g. eczema). Patient characteristics of the included study population are shown in table 1.

Food challenge outcome

During challenge 116 (52%) children appeared to be tolerant for peanut and 109 (48%) children developed symptoms of Sampson grade 1 – 4 and had a positive FCO. No delayed reactions (after more than 4 hours observation) occurred. ED, CD, symptoms and treatment during challenge in children with positive FCO are shown in table 2.

Because asthma is thought to be an important risk factor for developing respiratory symptoms we further compared asthmatic children with non-asthmatic children. In children with positive FCO, asthmatics did not report a severe respiratory reaction in history more often (29% compared to 22%, p=0.50) and did not had a reaction with involvement of the lower airways during challenge more often (12% compared to 15%, p=0.60) than non-asthmatics (data not shown).

Univariate analyses showed that six patient related factors were significantly associated with FCO: male gender, having another food allergy, sIgE for peanut and sIgE for Ara h 2, wheal size of SPT and a severe previous reaction in history. Based on clinical relevance and available data male gender, asthma, presence of another food allergy, sIgE for peanut, SPT and the reaction to peanut in history were included in a multivariable model. The results show that the level of sIgE for peanut was positively associated with positive

Table 1 Characteristics of included children with suspected peanut allergy and univariate relation with positive and severe FCO

Characteristic	**All children** n = 225	**Positive FCO** n = 109	**Severe FCO** n = 24	**OR for positive FCO** (95% CI)	p-value	**OR for severe FCO** (95% CI)	p-value
Median age (IQR. in yrs.)	6.7 (5.0-9.5)	6.6 (4.8-9.0)	6.6 (4.8-7.7)	0.96 (0.89-1.04)	0.964	0.96 (0.84-1.09)	0.961
Age groups							
< 6 years	121 (54)	63 (58)	14 (58)	Reference	-	Reference	-
6 – 12 years	74 (33)	32 (29)	7 (29)	0.70 (0.39-1.26)	0.701	0.80 (0.31-2.08)	0.799
> 12 years	30 (13)	14 (13)	3 (13)	0.81 (0.36-1.80)	0.806	0.85 (0.23-3.17)	0.849
Male gender (%)	148 (66)	63 (58)	15 (63)	**0.50 (0.29-0.87)**	0.015*	0.85 (0.36-2.05)	0.720
Current other atopic diseases (%)							
Allergic rhinitis	96 (43)	47 (43)	9 (38)	1.04 (0.61-1.76)	0.894	1.03 (0.43-2.47)	0.444
Atopic dermatitis	184 (82)	88 (81)	10 (42)	0.87 (0.44-1.72)	0.873	0.85 (0.36-2.00)	0.835
Asthma (using ICS)	83 (37)	43 (39)	9 (38)	1.24 (0.72-2.13)	0.441	1.13 (0.36-3.50)	0.703
Asthma (using inhalants)	102 (45)	50 (46)	12 (50)	**1.04 (0.62-1.76)**	0.875	1.40 (0.60-3.25)	0.948
Any of above	217 (96)	103 (95)	24 (100)	0.66 (0.26-1.71)	0.392	1.02 (0.22-4.70)	0.983
Other food allergy	151 (67)	63 (58)	14 (58)	**0.44 (0.25-0.77)**	0.004*	0.65 (0.28-1.55)	0.335
SPT wheal (IQR. in mm)	8.50 (3.85-13.90)	11.60 (6.40-16.00)	10.10 (6.33-14.58)	**1.14 (1.08-1.78)**	<0.001*	1.05 (0.95-1.15)	0.303
sIgE for peanut (IQR. in kU_A/L)	2.60 (0.60-19.20)	17.80 (4.35-86.0)	14.5 (2.7-39.0)	**1.13 (1.08-1.19)**	<0.001*	1.00 (1.00-1.02)	0.123
sIgE for Ara h 2 (IQR. in kU_A/L)	n = 88	n = 43	n = 11	**1.92 (1.27-2.91)**	0.002*	1.02 (0.99-1.04)	0.097
	0.58 (0.07-6.46)	6.48 (0.62-38.30)	7.2 (2.1-41.0)				
Reaction to peanut in history (%)							
No symptoms/ ingestion unknown	109 (48)	45 (41)	11 (46)	Reference	-	Reference	-
Previous non-severe reaction	76 (34)	40 (37)	7 (29)	**1.58 (0.88-2.84)**	0.370	0.90 (0.33-2.45)	0.842
Previous severe reaction	40 (18)	24 (22)	6 (25)	**2.13 (1.02-4.67)**	0.044*	1.57 (0.54-4.58)	0.407

FCO. Food challenge Outcome; ICS. Inhaled corticosteroids; IQR. Inter Quartile Range; OR. Odds Ratio; SPT. Skin Prick Test
Bold data were entered in the multivariate logistic regression model
* significantly related to the outcome. $p < 0.05$

FCO (OR 1.14 (1.08-1.20), p<0.001). Male gender (OR 0.40 (0.20-0.81), p=0.01) and the presence of another food allergy (OR 0.43 (0.21-0.88), p=0.02) were both negatively associated with a positive FCO. The reaction to peanut in history and SPT were not independently related to positive FCO after adjustment for the other factors. The final model showed good discrimination of children with positive and negative FCO with an AUC of 0.89 (0.84-0.93). However, calibration was poor which is indicated by a significant Hosmer Lemeshow test statistic of 34.99 (df = 8 and p < 0.001) (table 3).

Table 2 Reaction thresholds, symptoms and treatment during challenge in children with positive, mild/moderate and severe FCO

Characteristic	**Positive FCO** n = 109	**Mild/Mod FCO** n = 85	**Severe FCO** n = 24	p-value
Median ED (IQR, in mg peanut protein)	150 (50-500)	150 (50-500)	150 (50-1500)	0.65
Median CD (IQR, in mg peanut protein)	706 (206-2206)	706 (206-2206)	706 (56-2206)	0.91
Symptoms during challenge (%)*				
Skin	50 (46)	41 (48)	9 (38)	0.35
Grade 1 (loc. pruritus, flushing, urt.)	15 (14)	13 (15)	2 (8)	
Grade 2 (gen. pruritus, flushing, urt.)	35 (32)	28 (33)	7 (29)	
Gastro-intestinal tract	93 (85)	75 (88)	18 (75)	0.12
Grade 1 (OAS)	14 (13)	11 (13)	3 (13)	
Grade 2 (+ nausea / emesis)	71 (65)	58 (68)	13 (54)	
Grade 3 (+ repetitive vomiting)	6 (6)	5 (6)	1 (4)	
Grade 4 (+ diarrhoea)	2 (2)	1 (1)	1 (4)	
Respiratory tract	67 (62)	44 (52)	23 (96)	0.00
Grade 2 (nasal congestion / sneezing)	32 (29)	32 (38)	-	
Grade 3 (rhinorrhoea, throat prur./tight.)	12 (11)	12 (14)	-	
Grade 4	23 (21)	-	23 (96)	
Upper airways (insp. stridor)	8 (7)	-	8 (33)	
Lower airways (wheezing, exp. stridor)	15 (14)	-	15 (63)	
Cardiovascular (grade 4, hypotension)	1 (1)	-	1 (4)	0.22
Neurological	2 (2)	1 (1)	1 (4)	0.34
Grade 2 (change in activity level)	1 (1)	-	1 (4)	
Grade 3 (+ anxiety)	1 (1)	1 (1)	-	
> 2 organ systems involved	88 (81)	66 (78)	22 (92)	0.15
Treatment during challenge (%)				
AH and CS	84 (77)	84 (1)	-	-
+ Inhalation therapy	9 (8)	-	9 (38)	-
+ Adrenaline i.m./i.v.	16 (15)	1 (1)	15 (63)	-

CD, Cumulative Dose; ED, Eliciting Dose; FCO, Food Challenge Outcome; Gen, generalized; IQR, Inter Quartile Range; Loc, localized; Urt, urticaria * Symptoms during challenges were graded according to Sampson et al.(1)

We also performed multivariable analysis in the subgroup of 88 children with available Ara h 2 results. In a model with male gender and the presence of other food allergy, there was a clear and strong association of Ara h 2 with positive FCO (OR 3.52 (1.71-7.29), p <0.001). This model also showed good discrimination of children with positive and negative FCO with an AUC of 0.97 (0.93-1.00) and was well calibrated with a non-significant Hosmer Lemeshow test statistic of 6.30 (df=7, p=0.51) (table 3).

Severe food challenge outcome

Twenty four children (11%) developed a severe reaction during DBPCFC and had a severe FCO. In 92% of these children more than two organ systems were involved in the allergic reaction during challenge. The majority of children with severe FCO had severe respiratory symptoms of Sampson grade 4. Upper respiratory symptoms with severe throat tightness occurred in 8 children and wheezing due to lower airway involvement in 15 children. Only one child developed a severe drop in blood pressure. The occurrence of skin symptoms, gastro-intestinal symptoms and neurological symptoms was comparable between children with mild/moderate FCO and children with severe FCO. In 9 (38%) children with severe FCO treatment with inhalants was indicated as lower respiratory symptoms were the main manifestation of anaphylaxis, moreover 15

Table 3 Multivariable analyses of predictors for children with positive or severe FCO

Predictor	**Adjusted OR positive FCO** (95% CI)	p-value	**Adjusted OR positive FCO** (95% CI)*	p-value	**Adjusted OR severe FCO** (95% CI)	p-value
Male gender (%)	0.40 (0.20-0.81)	0.011	0.03 (0.003-0.19)	<0.001	-	-
Other food allergy	0.43 (0.21-0.88)	0.021	0.25 (0.05-1.17)	0.077	-	-
sIgE for peanut (kU_A/L)	1.14 (1.08-1.20)	<0.001	-	-	1.00 (0.99-1.02)	0.104
sIgE for Ara h 2 (kU_A/L)	-	-	3.52 (1.71-7.29)	0.001	-	-
Reaction to peanut in history (%)						
No reaction / known ing.	-		-		Reference	-
Non-severe reaction					0.80 (0.29-2.2.21)	0.661
Severe reaction					1.49 (0.50-4.42)	0.469
ROC area (95% CI)	0.89 (0.84-0.93)		0.97 (0.93-1.00)			
Hosmer Lemeshow (Chi-square statistic)	34.99 (df = 8)	<0.001	6.30 (df = 7)	0.506		

FCO, Food Challenge Outcome; ing, ingestion; OR, Odds Ratio; ROC, Receiver under the operator curve

* Subgroup of children with known sIgE to Ara h 2, n = 88

(63%) children received adrenaline. The ED (median [IQR]) was not significantly different between children with mild/moderate (150[50-500] mg) and severe (150[50-1500] mg) FCO, p=0.65. Moreover, there was no difference in the CD of peanut ingested (median [IQR]) between children with mild/moderate and severe FCO (706[206-2206] mg and 706[56-2206] mg, p=0.91) (table 2).

Univariate analyses did not show significant associations of any of the candidate predictors with severe FCO (table 1). In the subgroup of 88 children with available Ara h 2 results, sIgE for Ara h 2 was also not significantly related to severe FCO, OR 1.02 (0.99-1.04), p=0.10. Due to the relatively low incidence of severe FCO only the most clinical relevant predictors (asthma, sIgE for peanut and reaction to peanut in history) were entered in a multivariate model. None of the predictors were significantly associated with severe FCO (table 3).

DISCUSSION

The main goal of food challenges is to confirm or exclude the presence of food allergy under safe conditions. However, severe reactions during challenges do occur, in our study 11% of peanut challenged children had severe FCO which is comparable to previous published results.(3–5) Our results confirm that there are patient related factors associated with positive food challenge outcome, but no independent risk factors for severe FCO could be determined.

Improving diagnostics in food allergy is a topic leading to much discussion in literature and daily practice and several prediction rules to predict FCO and reduce the need for oral food challenges have been published.(17,18) In this study the level of sIgE and not SPT reactivity, appeared to be an independent predictor for positive FCO. Earlier studies showed an AUC of 0.79 to 0.86 for SPT and 0.77 to 0.87 for sIgE to peanut respectively.(19–22) However as is stated before (23,24), cross-reactivity with other allergens (e.g. grass-pollen or other foods) can interfere with levels of sIgE and reactivity of SPT which can explain the relatively low predictive capacity of both measures. In the near future, promising results regarding component resolved diagnostics provide opportunities to improve the diagnostic capacity of sIgE and SPT in predicting positive FCO.(25)

We did not expect to find that male gender was related to negative FCO due to the gender specific prevalence of food allergy before puberty which is higher in boys than in girls.(26) However, previous studies in young children with respiratory symptoms showed that there are gender differences in health care utilization by parents and prescription of medication by physicians in favour of boys.(27,28) It is therefore not beyond possibility that clinicians tend to perform DBPCFC's more easily in boys. Although peanut sensitization is thought to be more present in children with other atopic disorders including food allergies,(29) having another food allergy was negatively associated with positive FCO in our study. It could be that there is a higher proportion of children with

unjustified suspected peanut allergy among children with other food allergies compared with children without any other (suspected) food allergy in our study.

In this study important risk factors for anaphylaxis in daily life as having asthma, the level of sIgE and severe reaction in history were not independently associated with severe FCO. Severity of allergic reactions during challenge may depend on the interaction between several host and event related factors.(30) Of the event related factors, ED and the amount of food ingested during challenge can be associated with the severity of reaction during challenge.(31) In our study no differences were found between children with mild/moderate and severe FCO regarding to the CD of peanut given, indicating that children with mild/moderate FCO were not "prevented" from developing a severe reaction. Because all eligible patients with clinically suspected peanut allergy were challenged in our hospital, our study population is most likely reflecting the complete spectrum of peanut allergy.

Our results indicate that previous reactions reported by parents cannot be used to predict severity of food challenge outcome, which is supported by others.(4,13) This could be due by an inaccuracy in the description of allergic symptoms by the parents but can also be due to the different circumstances and absence of co-factors during challenge which can aggravate reactions in daily life.

Our results regarding to the value of sIgE for peanut and SPT in predicting severe FCO are in contrast with two other studies who did found a correlation between the level of sIgE for peanut and anaphylaxis during challenge.(13,32) However, the suggested cut-off values of both studies have not been validated in other less selected populations. Moreover, others confirm our results that levels of sIgE for peanut cannot be used to estimate the risk of anaphylaxis during challenge.(3,19,20,33,34) Studies investigating accidental anaphylaxis to food indicate that children with fatal or severe reactions to food in daily life often have asthma, suggesting this to be an important risk factor for severe reactions.(35–37) As has been shown before (3,13), our results point out that we cannot generalize these findings to the setting of food challenges. Children eligible for challenge have clinical stable asthma without recent exacerbations in contrast with possible uncontrolled circumstances during severe reactions in daily life.(38,39) Our results also indicate that lower respiratory tract symptoms are relatively rare in children with positive FCO (14%) but if they occur this is both in children with and without (stable) asthma.

CONCLUSION

In conclusion this study shows that clinical information gathered before food challenge can be used to predict positive FCO in contrast we were unable to identify children with suspected peanut allergy as high or low risk for severe FCO. To extrapolate these results to daily practice and develop a well calibrated prediction model for positive and severe

FCO, internal and external validation in a larger study population is necessary. Until more extensive and prospective studies are conducted, possible risk factors known from daily life studies cannot guide the eligibility of a patient for food challenges or the setting and safety precautions taken. Clinicians performing peanut challenges in sensitized patients should be prepared and well equipped for treatment of a severe reaction in all cases.

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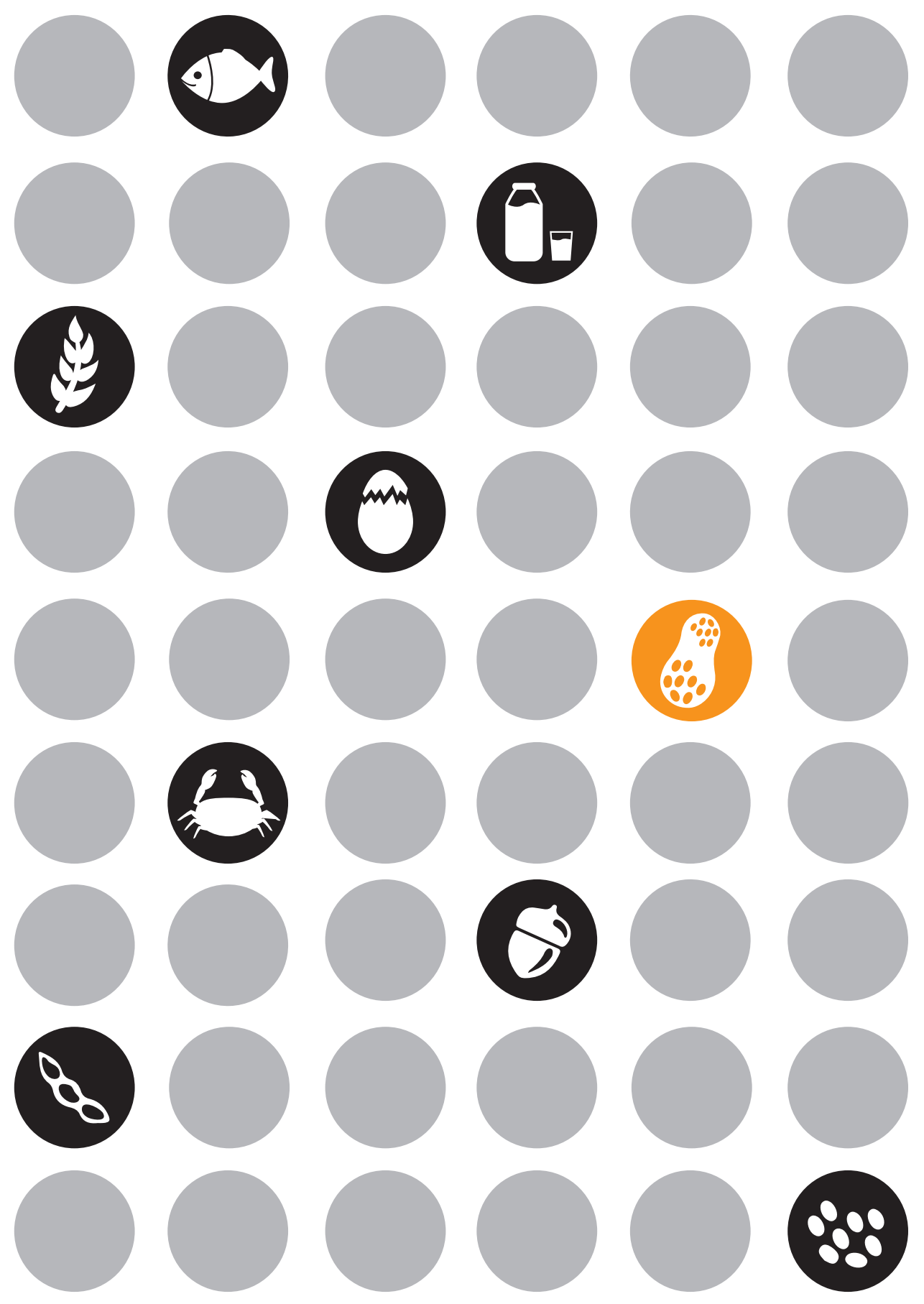


CHAPTER 6

The IgE and basophil response to Ara h 2 and h 6 are strong predictors of peanut allergy in children

Submitted

Francine C. van Erp
Edward F. Knol
Bo Pontoppidan
Yolanda Meijer
Cornelis K. van der Ent
André C. Knulst

ABSTRACT

Background

Double blind placebo-controlled food challenge (DBPCFC) is the gold standard to diagnose peanut allergy. In children sensitized to peanut, the detection of allergen-specific IgE (sIgE) and /or basophil sensitivity to Ara h 2 and Ara h 6 could be an alternative way to predict clinical peanut allergy and thereby avoid burdensome and expensive challenges. We aimed to prospectively evaluate the most accurate diagnostic approach in children with suspected peanut allergy using sensitization tests and the Basophil Activation Test (BAT) to peanut components, with focus on Ara h 2 and Ara h 6.

Methods

A total of 83 children with suspected peanut allergy underwent diagnostic evaluation for peanut allergy including DBPCFC. The diagnostic value of sensitization tests and the BAT in predicting (severe) peanut allergy was evaluated.

Results

Peanut allergy was confirmed in 48 (58%) children, including 15 (18%) with severe allergy. Ara h 2 and h 6 showed high discriminatory capacity in sIgE and the BAT. Ara h 6 had significant higher diagnostic value than Ara h 2 in the BAT. With sIgE to Ara h 2 we could classify 62% of children correctly as tolerant or allergic, when subsequently adding the BAT we could increase this to 80%. The BAT had no added diagnostic value over sIgE testing in predicting severe peanut allergy.

Conclusions

This study shows that Ara h 2 and h 6 are both strong predictors of peanut allergy. A stepwise approach including sIgE to Ara h 2 and subsequently the BAT to Ara h 2 and Ara h 6 is able to predict peanut allergic status in the majority of children.

INTRODUCTION

Peanut is one of the most common foods eliciting life threating allergic reactions.(1) Accurate diagnosis of peanut allergy is important to guide precautionary measures in case of allergy, and prevent unnecessary elimination diets and improve quality of life in case of tolerance. Allergy is suspected when immediate allergic symptoms occur after peanut ingestion together with sensitization.(2) Sensitization can be detected by a raised level of specific IgE (sIgE) or positive skin prick test (SPT). The diagnosis of peanut allergy is confirmed by the reference standard: a positive double blind placebo controlled food challenge (DBPCFC).(3) The DBPCFC provides information on the presence and severity of peanut allergy, as well as the threshold dose of peanut that elicits an allergic reaction. The DBPCFC is however a burdensome, expensive, and potentially dangerous procedure as 10-20% of children develop severe allergic symptoms.(4,5)

Component resolved diagnostics showed promising results in predicting DBPCFC outcome. A discriminative ability of sIgE to the peanut components Ara h 2 and Ara h 6 up to 90% was found in previous retrospective studies with children and adults. (6,7) Although the use of Ara h 2 and Ara h 6 was promising in reducing the need for food challenges, prospectively collected data in children with suspected peanut allergy are lacking. Moreover, sIgE tests are influenced by cross-reactivity with other allergens and do not account for crosslinking between IgE molecules. Additionally, the presence of blocking antibodies, i.e. IgG4 is not taken into account in the determination of sIgE.(8) As a result, a subgroup of patients has detectable sIgE without having peanut allergy.

The Basophil Activation Test (BAT) could be an alternative way to differentiate between sensitization and relevant allergy. The BAT is a functional assay in whole blood samples and based on the flow-cytometric detection of surface protein expression caused by allergen induced activation of the basophil.(9) Previous studies indicated that basophil sensitivity to peanut allergen was significantly increased in children with peanut allergy. (10,11) More recently it has been demonstrated that the BAT could be superior to sensitization tests and discriminates between children with mild and severe peanut allergy.(12)

In this study we aimed to prospectively evaluate the most accurate diagnostic approach in children suspected of peanut allergy using the SPT, sIgE and the BAT using peanut components with focus on Ara h 2 and Ara h 6.

METHODS

Study design and study population

This cross sectional prospective diagnostic study was performed in the Wilhelmina Children's Hospital a tertiary centre for food allergy. All children referred to our hospital for peanut allergy between January 2012 and May 2015 were screened for the study. Children with suspected peanut allergy, between 3 and 18 years who were eligible for DBPCFC and willing to participate to the study were included. Suspected peanut allergy was based on (1) a clinical history of a reaction to peanut with or without peanut

sensitization or (2) peanut sensitization without previous known ingestion to peanut. Peanut sensitization was defined at the outpatient clinic as a peanut sIgE ≥ 0.35 kUA/L or a peanut SPT mean wheal size of ≥ 3 mm. Children with previous ICU admission after a severe peanut allergic reaction in history and children who ingested peanut without symptoms were excluded from this study. All included children subsequently underwent clinical evaluation, determination of sIgE to peanut and peanut components, SPT, BAT and a two day DBPCFC for peanut. This study was reviewed and approved by the ethical committee of the University Medical Centre Utrecht. Written informed consent was obtained from parents and/or children before enrolment in the study.

Clinical history and sensitization tests

In each patient a careful history of peanut allergy and other atopic diseases was obtained. A previous allergic reaction to peanut was defined as the most severe symptoms after peanut ingestion or contact in history and graded according to the Sampson classification of anaphylaxis.(13) Asthma was defined as a doctors diagnosis of asthma together with the use of inhalant medication. Allergic rhinitis, eczema and other food allergies were defined according to a doctors diagnosis. Levels of sIgE to peanut and recombinant components Ara h 1, 2, 3, 6, 8 and 9 using the ImmunoCAP method (Thermo Fisher Scientific, Uppsala, Sweden) and expressed as continuous variables in kUA/L ranging from 0.1 - 100 or higher after sample dilution as required. SPT was performed with peanut extract and a single-headed lancet (ALK-Abello, Nieuwegein, The Netherlands). SPT was expressed as the peanut wheal index which was calculated by dividing the peanut wheal size by the wheal size of the positive control (histamine).

Basophil activation test

Whole heparinized blood samples were collected at the start of DBPCFC and stored at room temperature for a maximum of 24 hours. Basophils were stimulated for 30 minutes at 37˚C with increasing concentrations (0.1, 1, 3, 10, 100 and 1000 µg/ml) of purified peanut proteins Ara h 2 and h 6 (Indoor Biotechnologies, Warminster, United Kingdom) diluted in RPMI (Gibco, Life technologies, New York) supplemented with 1 ng/ml IL3. Polyclonal goat antihuman-IgE (Anti-IgE AI-3040, 1 µg/ml, Vector Laboratories, Burlingame, United States) and formyl-methionyl-leucyl-phenylalanine (fMLP, 1 µM, Sigma F3506-10MG) were used as positive controls and RPMI medium and supplemented with IL3 as negative controls. Leukocytes were stained with a cocktail of CD63-PE (Monosan, MON9000R), CD203C-APC (Biolegend, 324610), CD123-FITC (Biolegend, 306014), CD45-PO (Invitrogen, MHCD4530), CD41-PECY7 (Beckman Coulter, 6607115) and HLA-DRPB (Biolegend, 307624) and analysed by flow cytometry (FACS Canto II) with FACSDiva software (version 8.0.1, BD Biosciences, San Jose, Calif). Basophils were defined as CD45+ CD203+ CD123+ and HLA-Dr- and CD41- in the lymphocyte scatter region. Degranulating basophils were determined as CD63-bright cells and calculated as percentage positive cells. Results of the BAT were expressed as the percentage CD63-positive cells after stimulation with 0.1 - 1000 µg/L allergen. For this study, different

outcomes of the BAT were evaluated: I) the maximal CD63 response after allergen stimulation; II) the ratio between the maximal response to allergen and the response to Anti-IgE; III) the mean CD63 response after 10 and 100 µg allergen; IV) the ratio between the mean CD63 response after 10 and 100 µg allergen and the response to Anti-IgE; V) the inverse of the concentration allergen with 50% of maximum CD63 up-regulation (CD-sens) and VI) the area under the allergen - CD63 response curve. Children with basophils which after anti-IgE stimulation responded with < 5% CD63-positive basophils were regarded as non-releasers. The BAT was performed and interpreted by an investigator who was blinded for patient characteristics and the reference test.

The reference test: diagnosis of peanut allergy

The diagnosis of peanut allergy was made after DBPCFC and if no reaction occurred on both days, a consecutive open challenge. Challenges were performed in a clinical setting equipped for resuscitation with monitoring of vital signs and lung function throughout the whole challenge procedure. Intravenous access was established before the first portion. The challenge protocol consisted of 7 increasing portions of ginger bread containing 8 g defatted peanut flour (50% peanut protein) on the verum day but not on the placebo day.(14) All signs and symptoms during challenge were classified and recorded on a food challenge score sheet adapted from Nowak et al.(15) Challenges were stopped and treated according to protocol when objective symptoms indicating an allergic reaction occurred (i.e. rash, urticaria, angioedema, rhinitis, conjunctivitis, vomiting, diarrhoea, dyspnoea, wheezing, hypotension). Oral allergy syndrome, nausea, abdominal pain, change in behaviour or aversion were referred to as subjective symptoms, but no reason to stop the challenge. Children were observed for two (no symptoms) or four (symptoms) hours after DBPCFC was stopped. If no or inconclusive symptoms occurred during either verum or placebo challenge, a confirmatory open challenge with 10 g of whole peanuts was performed. An expert panel of three independent experienced allergologists, blinded for the sensitization tests and the BAT individually classified children as peanut allergic or tolerant and determined severity of symptoms (by Sampson grade 1 - 5) and eliciting dose based on all available information of DBPCFC, open challenge and subsequent introduction at home. In the absence of consensus in the first instance, a face-to-face panel discussion meeting was held until agreement among all experts was reached. Severe peanut allergy was defined as peanut related symptoms of Sampson grade 4 - 5 and/ or objective symptoms to peanut after ingestion up to 300 mg peanut protein during challenge.

Statistical analysis

Continuous variables were expressed as mean ± SD or as the median and interquartile range in case of a skewed distribution. Comparison of differences in diagnostic test parameters between allergic and tolerant or mild and severe allergic children for continuous variables was done by the Student's T-test or nonparametric Mann-Whitney U test, as appropriate. The significance of difference in proportions was tested

with use of the X_2-statistic. To avoid bias that might result from an incomplete case analysis, missing sensitization data were imputed by using multiple imputations.(16) Non-releasers in the BAT were excluded for the determination of optimal cut-offs but included when evaluating the diagnostic capacity using logistic regression and Receiver under the Operating Characterstic (ROC) analysis. Univariable logistic regression analysis was used to evaluate which of the sensitization tests and which of the basophil activation outcome measures were significantly associated with peanut allergic status (or severe peanut allergy). Multivariable logistic regression analysis was used to quantify which combination of diagnostic tests performed best in predicting the presence of peanut allergy (or severe peanut allergy). Data of diagnostic test variables with a non-normal distribution were log-transformed before entering the regression models. Performance of nested models was compared using the log likelihood ratio test. The ability of each diagnostic test (or combination of tests) to discriminate between peanut allergic and tolerant patients (or non-severely and severely reacting patients) was estimated using the area under the ROC-curve (AUC). Optimal cut-off points and corresponding sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) were determined for diagnostic tests (or combination of tests) with the highest AUC. The added value of the BAT was also evaluated as a second diagnostic step in the diagnostic procedure in the population that could not be predicted using sensitization tests. Based on cut-offs with the highest NPV and PPV, a flow chart was created for children with complete BAT-results (n = 81) showing in how many children a DBPCFC could be avoided using different diagnostic approaches. All statistical analyses were performed using SPSS for Windows (version 21) and a p-value < 0.05 was considered statistically significant.

RESULTS

Study population

A total of 174 children referred for peanut allergy were screened for this study. Children that were excluded did not fulfil in- and exclusion criteria (n = 20), were not eligible for challenge (n = 15) or were not willing to participate (n = 56), supplementary figure E1. Children had a median (IQR) age of 7.8 (5.5-11) years and often had eczema (57%), allergic rhinitis (58%), asthma (48%) or other food allergies (74%). Suspected peanut allergy was based on a previous reaction to peanut in 70% (including 5 children without sensitization to peanut) and no previous known ingestion but with sensitization in 30%. In a subset of children missing sensitization data was imputed because the SPT could not be performed (n = 15) or sIgE to peanut components was missing due to failure of blood collection (n = 5). The BAT was successfully performed in all but two children (due to failure of blood collection at the day of challenge). All children underwent the reference test (DBPCFC) and peanut allergy as determined by the expert panel was confirmed in 58%. There were no statistical differences with respect to patient characteristics between tolerant and allergic children. Baseline characteristics of included children are shown in table 1.

Table 1 Baseline characteristics for tolerant and allergic children n = 83

	Total n = 83	**Tolerant** n = 35	**Allergic** n = 48	**Mild-Mod** n = 33	**Severe** n = 15	**All vs Tol** p-value	**Mild-Mod vs Severe** p-value
Age, median (IQR)	7.8 (5.5-11)	6.9 (5.5-10.6)	7.9 (5.5-11.7)	8.5 (6.2-11.2)	6.7 (4.7-11.7)	0.50	0.09
Gender (male), n (%)	58 (70)	26 (74)	32 (67)	24 (73)	8 (53)	0.46	0.19
Atopy n (%)							
Eczema	47 (57)	20 (57)	27 (56)	16 (49)	11 (73)	0.94	0.11
Food allergy	61 (74)	25 (71)	36 (75)	27 (82)	9 (60)	0.72	0.11
Asthma	40 (48)	13 (37)	27 (56)	18 (55)	9 (60)	0.09	0.72
Hay fever	48 (58)	17 (49)	31 (65)	20 (61)	11 (73)	0.15	0.39
Suspected peanut allergy n (%)							
Sensitization	25 (30)	13 (37)	12 (25)	5 (15)	7 (47)	0.23	**0.02**
Previous reaction	58 (70)	22 (63)	36 (75)	28 (85)	8 (53)	-	-
Previous reaction n (%)*							
Never ingested peanut	25 (30)	13 (37)	12 (25)	5 (15)	7 (47)	0.28	0.20
Grade 1	9 (11)	5 (14)	4 (8)	3 (9)	1 (7)		
Grade 2	12 (15)	2 (6)	10 (21)	6 (18)	4 (27)		
Grade 3	5 (6)	2 (6)	3 (6)	2 (6)	1 (7)		
Grade 4	23 (28)	11 (31)	12 (25)	11 (33)	1 (7)		
Grade 5	2 (2)	1 (3)	1 (20)	1 (3)	- -		
Contact^	7 (8)	1 (3)	6 (13)	5 (15)	1 (7)		

All, Allergic; Mild-Mod, Mild to moderate; Tol, tolerant. ***** Most severe reaction according to the Sampson classification of anaphylaxis. ^ No previous ingestion but a local reaction (redness or urtica) after skin contact with peanut.
Bold faced p-values indicate a significant difference between the outcome group.

The diagnostic value of sensitization tests

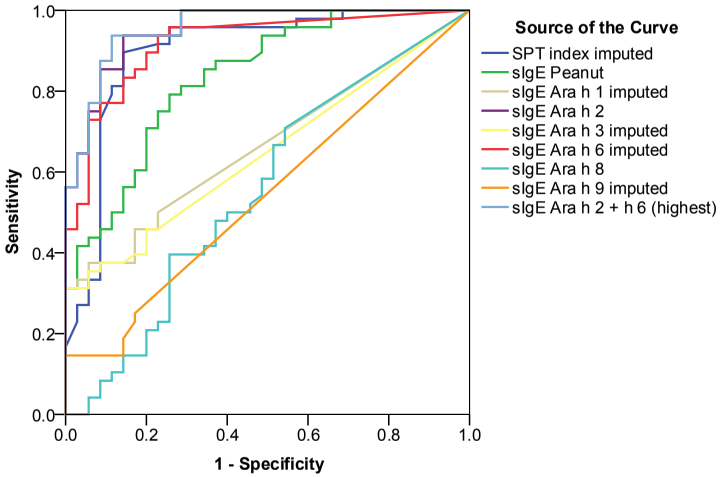
The levels of sIgE to the storage proteins Ara h 1, h 2, h 3 and h 6 were significantly higher in peanut allergic children (table 2). sIgE to Ara h 2 and Ara h 6 were strong predictors of peanut allergy and showed high discriminatory capacity (AUC (95%CI): 0.95 (0.91-0.99) and 0.92 (0.86-0.99)). The levels of sIgE to Ara h 2 and Ara h 6 were highly correlated, r^2 = 0.948. Two tolerant children recognized Ara h 6 (0.12 kUA/L and 0.71 kUA/L) but not Ara h 2 (<0.1 kUA/L) and one allergic child recognized Ara h 2 (0.13 kUA/L) but no Ara h 6. When using the best performing cut-off points of Ara h 2 (highest NPV and PPV) to exclude or confirm peanut allergy (0.1 kUA/L and 5 kUA/L) in the population with complete BAT results (n = 81) we could classify 50/81 (62%) children correctly (including 25/34 (74%) tolerant and 25/47 (53%) allergic children). With the best cut-off points of Ara h 6 (0.1 kUA/L and 6.7 kUA/L) we could predict 44/81 (54%) patients correctly

Table 2 Diagnostic tests under study in tolerant, mild-moderate or severe peanut allergic children, n = 83

Test (median, IQR)	**Tolerant** n = 35	**Mild-Mod** n = 33	**Severe** n = 15	**All vs Tol** p-value	**Mild-mod vs Severe** p-value
Sensitization tests					
Skin prick test peanut (index)*	1 (0-1.67)	9.75 (8-12.5)	9.25 (7.25-12.25)	**<0.001**	0.57
Skin prick test peanut (mean wheal)*	4.5 (0-7.5)	2.75 (2.3-3.5)	2.38 (2.25-3.25)	**<0.001**	0.44
sIgE Peanut (kUA/L)	0.76 (0.11-2.73)	7.74 (2.06-22.79)	25.37 (6.94-79.2)	**<0.001**	0.05
sIgE Ara h 1**	0.1 (0.1-0.1)	0.1 (0.1-7.72)	0.6 (0.1-35.38)	**<0.001**	0.16
sIgE Ara h 2	0.1 (0.1-0.39)	4.8 (1.06-20.18)	11.65 (3.54-50.16)	**<0.001**	0.13
sIgE Ara h 3**	0.1 (0.1-0.14)	0.1 (0.1-0.4)	0.26 (0.1-4.87)	**0.01**	0.07
sIgE Ara h 6**	0.1 (0.1-0.35)	2.47 (1.12-18.06)	16.11 (5.29-24.09)	**<0.001**	**0.03**
sIgE Ara h 8	0.62 (0.1-7.34)	2.1 (0.23-7.14)	0.55 (0.1-7.05)	0.37	0.46
sIgE Ara h 9**	0.1 (0.1-0.1)	0.1 (0.1-0.1)	0.1 (0.1-0.17)	0.34	**0.03**
Basophil Activation Test^					
Ara h 2					
Maximal CD63%	2.3 (1-4.6)	50.05 (21.9-64.9)	41.1 (17.85-63.65)	**<0.001**	0.75
Maximal CD63% / aIgE	7.79 (4-22)	102.48 (62.64-139.97)	109.19 (66.47-167.98)	**<0.001**	0.73
Mean CD63% 10–100µg	1.1 (0.6-1.95)	31.73 (14.43-52.08)	32.2 (10.68-47.33)	**<0.001**	0.89
Mean CD63% / aIgE	4 (1.73-10.39)	79.82 (33.45-105.77)	78.63 (43.73-110.89)	**<0.001**	0.67
CD-sens	0.01 (0.01-0.01)	23.31 (14.51-60.21)	19.81 (2.52-31.02)	**<0.001**	0.19
AUC	5.42 (2.3-7.77)	100.3 (36.42-160.8)	84.69 (32.89-123.8)	**<0.001**	0.47
Ara h 6					
Maximal CD63%	1.9 (1.2-3.5)	48.65 (25.55-65.6)	54 (41.7-58.55)	**<0.001**	0.67
Maximal CD63% / aIgE	6.49 (3.23-21.84)	104.07 (60.18-130.82)	120.56 (90.07-199.07)	**<0.001**	0.17
Mean CD63% 10–100µg	1.15 (0.5-2.25)	36.15 (11.6-58.95)	37.45 (30.93-44.1)	**<0.001**	0.83
Mean CD63% / aIgE	4.18 (1.45-17.24)	85.12 (27.62-109.82)	93.01 (64.8-143.02)	**<0.001**	0.32
CD-sens#	0.01 (0.01-0.01)	16.82 (1.98-21.9)	11.71 (3.89-26.15)	**<0.001**	0.47
AUC	4.52 (2.31-7.07)	91 (30.17-150.95)	97.66 (76.07-107.25)	**<0.001**	0.81

All, Allergic; AUC, Area under the dose response curve; aIgE, anti IgE; sIgE, specific IgE, Tol, Tolerant
* SPT was not performed in n = 15 (19%) due to eczema, daily use of antihistamines or logistic reasons, missing values were imputed. ** sIgE to Ara h 1, 3, 6, 9 was not performed in n = 4 (5%) due to failed blood collection, missing values were imputed. ^ BAT was performed in n = 81, the 8 non-releasers are excluded from this table
# Missing n = 1 concentration in the BAT due to small blood sample. **Bold faced** p-values indicate a significant difference between the outcome groups.

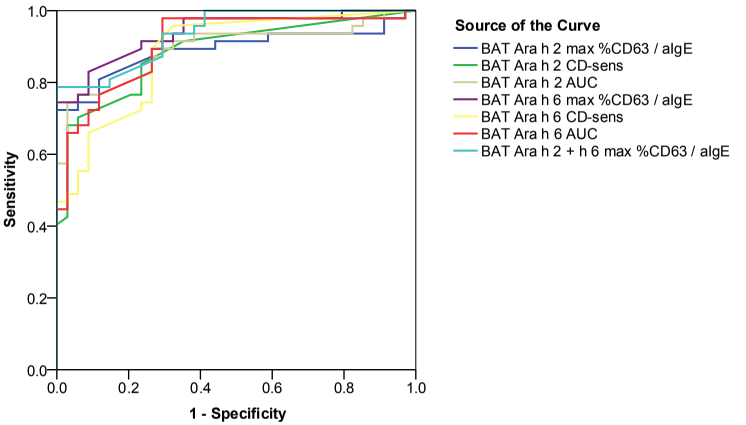
Figure 1A ROC-curves of sensization tests, n = 83



Sensitization test	AUC	95%CI	
SPT index	.899	.823	.974
sIgE peanut	.835	.750	.921
sIgE Ara h 1	.666	.551	.781
sIgE Ara h 2	.954	.913	.994
sIgE Ara h 3	.648	.531	.764
sIgE Ara h 6	.919	.859	.979
sIgE Ara h 8	.557	.428	.685
sIgE Ara h 9	.545	.420	.669
sIgE Ara h 2 + Ara h 6	.957	.917	.996

AUC, Area under the curve; ROC, receiver operating characteristic

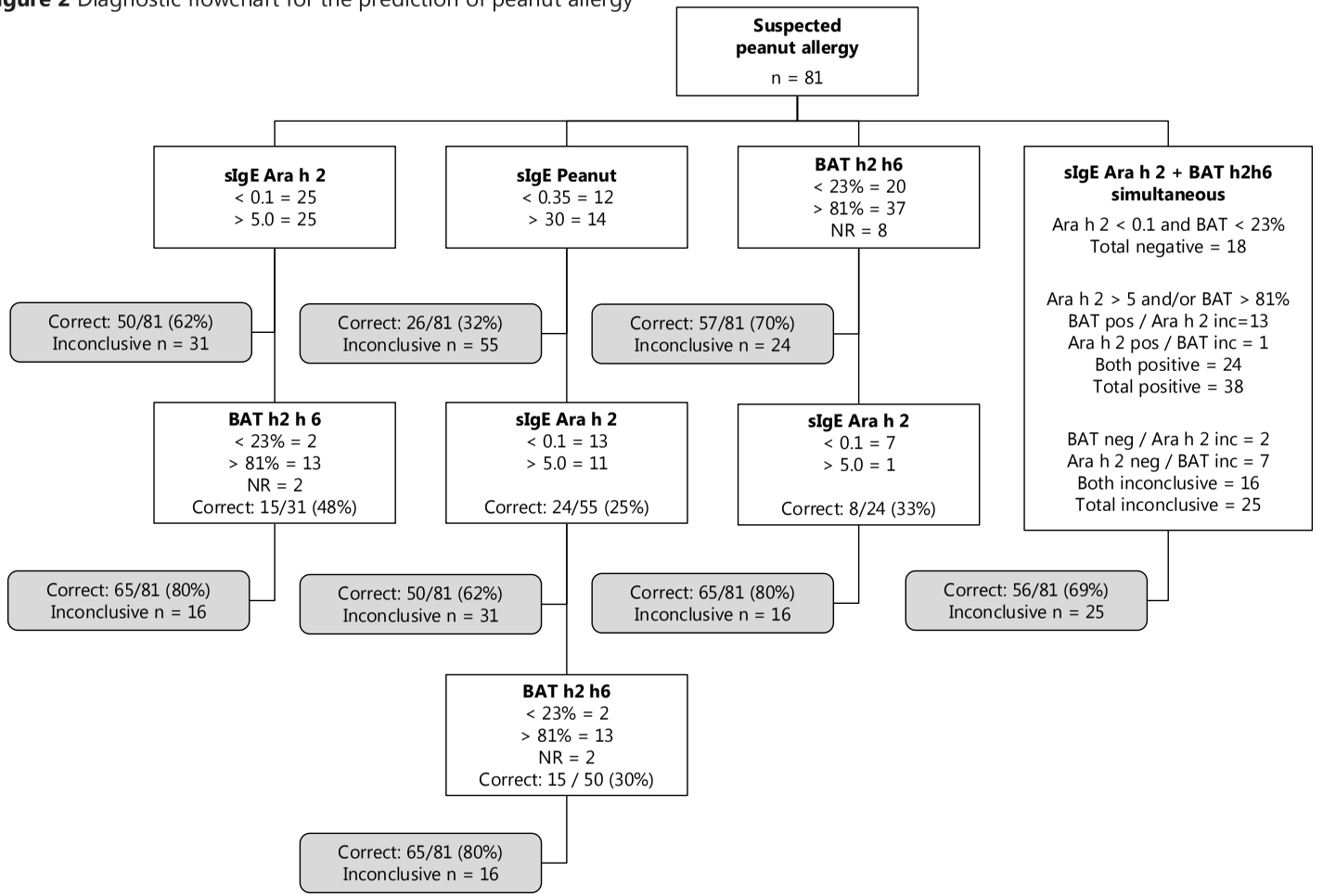
Figure 1B ROC-curves of basophil activation test, n = 81



Outcomes of the BAT	AUC	95%CI	
Ara h 2			
Maximal CD63%	.898	.828	.968
Maximal CD63% / aIgE*	.892	.818	.967
Mean CD63% 10–100µg	.901	.833	.968
Mean CD63% 10–100µg / aIgE	.907	.838	.976
CD-sens*	.886	.814	.958
AUC*	.894	.823	.965
Ara h 6			
Maximal CD63%	.925	.866	.984
Maximal CD63% / aIgE*	.939	.890	.988
Mean CD63% 10–100µg	.920	.861	.979
Mean CD63% 10–100µg / aIgE	.913	.852	.975
CD-sens*	.885	.813	.956
AUC*	.909	.845	.973
Ara h 2 + Ara h 6 (highest)			
Maximal CD63% / aIgE*	.938	.892	.985

* BAT-outcomes displayed in ROC figure. AUC, Area under the curve; ROC, receiver operating characteristic

Figure 2 Diagnostic flowchart for the prediction of peanut allergy



(including 23/34 (68%) tolerant and 21/47 (45%) allergic children. However, two false negative Ara h 6 tests were also found. Ara h 6 did not have significant added value in combination with Ara h 2. The SPT and sIgE to peanut extract had a lower discriminant capacity compared to sIgE to Ara h 2 and Ara h 6. sIgE to peanut extract had no added value when used before sIgE to Ara h 2. The SPT had very limited added value when used before Ara h 2 (three extra patients could be predicted correctly). ROC-curves of sensitization tests are presented in figure 1A, a diagnostic flow-chart is shown in figure 2 and cut-off points with corresponding sensitivity and specificity are presented in table E2.

The diagnostic value of the BAT with Ara h 2 and h 6

All evaluated BAT-outcomes were significantly associated with peanut allergy. The ratio between the maximum response and anti-IgE yielded the highest AUC for Ara h 6: 0.94 (0.89-0.99) and was 0.89 (0.82-0.97) for Ara h 2. Ara h 2 and Ara h 6 were highly correlated $r^2 = 0.842$ but differences were seen at the individual patient level. Five children (including 2 mild allergic and 1 severe allergic) were positive (maximal CD63% > 5%) in the BAT to Ara h 6 but negative to Ara h 2. Those children did respond with sIgE to both Ara h 2 and Ara h 6 although levels were low (< 2 kUA/L) Furthermore, three children (including 1 allergic) only responded to Ara h 2. When using cut-off points with a 100% NPV (23%) and 100% PPV (81%) the BAT using both Ara h 2 and Ara h 6 could be used to classify 20/34 (59%) tolerant and 37/47 (79%) allergic children correctly. Eight children (10%) including three allergic subjects were non-releasers in the BAT. There were no statistical differences between non-releasers and releasers. Allergic children that were non-releasers in the BAT did have a positive SPT with peanut. ROC-curves of the BAT-outcomes are presented in figure 1B and cut-off points with corresponding sensitivity and specificity are presented in table E2.

The added value of the BAT

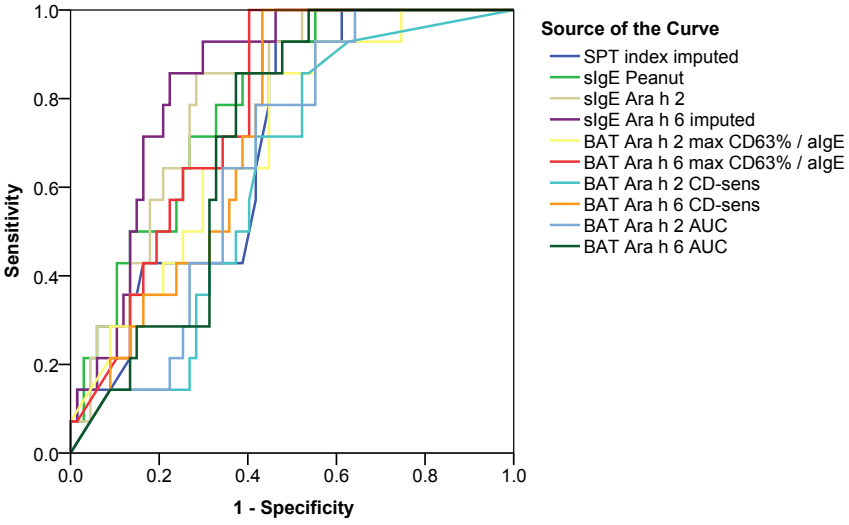
Logistic regression analysis and our flow-chart approach (figure 2) showed no significant added value of the BAT in combination with sIgE to Ara h 2 simultaneously. However, when used as a second or third step in the diagnostic process, the BAT had added value. When the BAT was used in children that could not be classified with Ara h 2 (n = 31) an additional 15 children could be classified correctly. The total amount of correctly classified children was hereby increased from 62 to 80% (figure 2).

Prediction of severe peanut allergy

Fifteen children (18%) had a severe peanut allergy: 11 (13%) with a Sampson grade 4 reaction and 4 (5%) with milder reactions who developed objective symptoms at a challenge dose of < 300 mg peanut protein. Nine children with a severe peanut allergy received epinephrine. Children with severe peanut allergy had more often a lifelong elimination diet for peanut ($p = 0.019$), higher levels of sIgE to Ara h 6 ($p = 0.030$) and Ara h 9 ($p = 0.029$) compared to children with mild-moderate peanut allergy. Logistic

regression analysis on the total population showed a significant association of severe peanut allergy with the SPT, the level of sIgE to Ara h 2 and Ara h 6 and the BAT for Ara h 2 and Ara h 6. sIgE to Ara h 2 and Ara h 6 were the best predictors of severe peanut allergy. At a cut-off of 5 kUA/L the PPV was 38% and 44% and the NPV 91% and 95%. For the BAT, the ratio of the maximum response to Ara h 6 and the response to anti-IgE showed the highest AUC 0.77 (0.67-0.88). None of the BAT-outcomes had significant added diagnostic value for severity compared with sIgE to Ara h 2 or Ara h 6. The use of different definitions of severe peanut allergy (ED < 300 mg only, Sampson grade 4/5, Muller grade 3/4, epinephrine use), did not alter our results. ROC-curves of diagnostic tests related to severe peanut allergy are shown in figure 3.

Figure 3 ROC-curves for severe peanut allergy, n = 81



Diagnostic tests for severe peanut allergy

Test	AUC	95%CI	
SPT index	.703	.576	.829
sIgE peanut	.791	.682	.900
sIgE Ara h 2	.802	.699	.905
sIgE Ara h 6	.840	.751	.930
BAT Ara h 2 max CD63% / aIgE	.713	.581	.846
BAT Ara h 6 max CD63% / aIgE	.773	.668	.878
BAT Ara h 2 CD-sens	.623	.487	.758
BAT Ara h 6 CD-sens	.724	.610	.838
BAT Ara h 2 AUC	.663	.539	.788
BAT Ara h 6 AUC	.711	.597	.826

AUC. Area under the curve; ROC, receiver operating characteristic. * BAT-outcomes displayed in ROC figure

DISCUSSION

As DBPCFCs are burdensome, expensive and limited to expertise centres there is need for less invasive diagnostic tools. This study shows that when using the combination of sensitization tests and the BAT to Ara h 2 and Ara h 6 it is possible to predict peanut allergy in the majority of children.

Our study is the first to investigate sIgE and the BAT using both Ara h 2 and Ara h 6 in a population of children with suspected peanut allergy. Although differences were seen at the individual patient level, Ara h 2 and Ara h 6 performed similar in predicting the presence of peanut allergy which confirms previous research in adults where the diagnostic value of sIgE to Ara h 6 was comparable to that of Ara h 2.(17) This can be explained by the homology and cross-reactivity between these two 2S albumins.(18) We did not find any peanut allergic patient mono-sensitized in sIgE to Ara h 6. However, three patients, including one severely allergic, were strongly positive in the BAT for Ara h 6 but negative for Ara h 2. This might reflect the more functional nature of the BAT which takes in to account the concentration and affinity of sIgE and other (blocking) antibodies. It could also be that differences between sIgE and the BAT are the result of the different nature (purified versus recombinant) and manufacturer between the allergens used in both tests. Nevertheless it should be kept in mind that rare cases of (severe) peanut allergy have been described in absence of reactivity to Ara h 2.(17,19) The SPT had only limited added value compared to Ara h 2. Moreover, the future of the SPT as a diagnostic test is questionable as the SPT is prone to observer (measurement and interpretation of wheal size), device and extract variability.(20)

Using the BAT as a second diagnostic step in the diagnostic process in children that cannot predicted with sIgE to Ara h 2, we could predict the presence or absence of peanut allergy in 80% of children without a DBPCFC. We compared different outcome parameters of the BAT and concluded that the ratio of the maximum response with anti-IgE is the best performing outcome measure. This is most likely due to the donor-dependent releasability after IgE crosslinking of basophils and highlights the importance of incorporating the response to anti-IgE in the interpretation of the basophil response to allergens.(21) A major drawback in BAT is the occurrence of non-releasers as in those subjects the BAT cannot be interpreted. In our population there were 8 (10%) non releasers which is comparable to that in previous studies.(12,22) Interestingly, the peanut allergics that were non-releasers still demonstrated a positive SPT response, indicating that the non-releaser phenotype was limited to basophils and not mast cells. Other drawbacks that need to be taken into account before further implementation are that the BAT needs to be performed on whole blood samples within 24 hours of blood collection and requires a specialized laboratory with expertise in cell activation.(23) There are several previous studies which demonstrate the (added) value of the BAT in children with peanut allergy.(10,12,24–26) However, different cut-off points and other diagnostic accuracies were found. Differences could be explained by the fact that the studies substantially differ in study population, definition of positive DBPCFC, protocol of the BAT and outcome measures of the BAT investigated. Inconsistencies between

study populations also occur because peanut consumption differs between countries as well as the occurrence of cross-reactive allergens, such as Ara h 9 in Spain.(27) In line with previous studies sIgE to Ara h 6 and to a lesser extent Ara h 2 were related to severe peanut allergy.(28,29) Despite the relatively high AUC of both components, the capacity to detect children at risk for a severe reaction before DBPCFC was limited as the PPV was relatively low (40% and 44% at a cut-off of 5 kUA/L). Despite the evaluation of several outcome parameters of the BAT and different definitions of severe allergy the BAT could not be used to predict severe peanut allergy in our study population. This is in contrast to previous studies in which the BAT did discriminate between mild-moderate and severe peanut allergy.(30–33) However, in those studies the performance of the BAT was evaluated in children with proven peanut allergy which is not the study design suitable to evaluate its diagnostic value.

A limitation in this study is that a substantial proportion of parents were not willing to participate in the study as they refused the (two day) DBPCFC. We don't think this has led to a selection of more tolerant or less severe allergic patients as the rate of severe reactions in this study (18%) was comparable to our and other previous retrospective studies in children undergoing peanut challenges.(4,34,35) Furthermore this study was prospective, comprises a realistic representation of referred children and peanut allergy was diagnosed accurately in all patients. Panel diagnosis including the DBPCFC and open challenge was used as reference test as the DBPCFC is susceptible for inter- and intra-observer variability.(36) No incorporation bias occurred as the outcome of DBPCFC was assessed blinded for sensitization tests and the BAT. An other limitation of this study is that we did not perform in- or external validation and therefore did not adjust for over fitting of the diagnostic models. Moreover generalizability to other populations such as secondary care is not clear as the PPV and NPV of a diagnostic test are dependent on the prevalence of peanut allergy in the population investigated. Further research should focus on validation of our proposed diagnostic strategy and its efficiency including costs and quality of life.

CONCLUSION

In conclusion, this prospective study shows that Ara h 2 and Ara h 6 are both strong predictors of peanut allergy. In the majority of children, peanut allergic status can be predicted using a stepwise approach including sIgE to Ara h 2 and subsequently the BAT to Ara h 2 and Ara h 6. For optimal insight in eliciting doses or severity a food challenge remains the test of choice.

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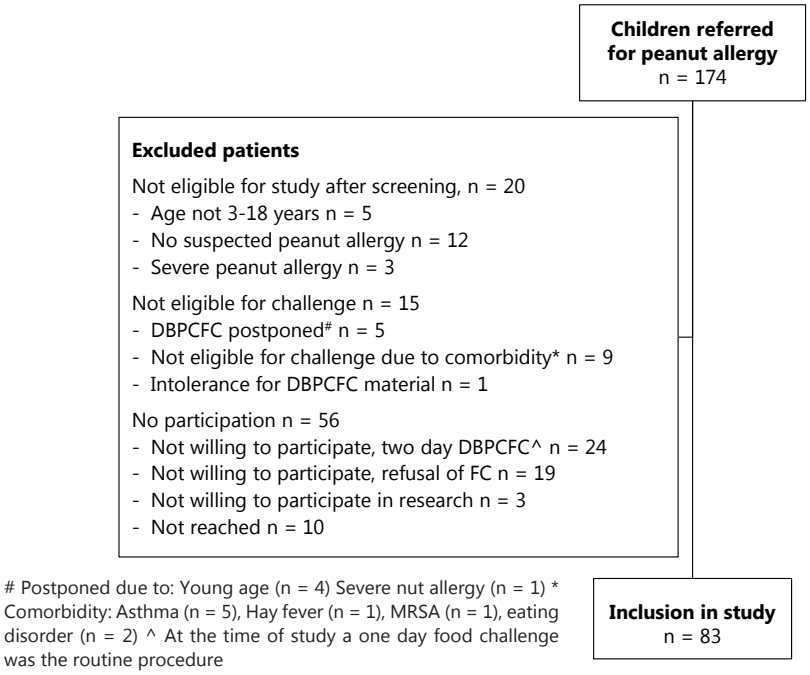
Figure E1 In- and exclusion of patients



Postponed due to: Young age (n = 4) Severe nut allergy (n = 1) * Comorbidity: Asthma (n = 5), Hay fever (n = 1), MRSA (n = 1), eating disorder (n = 2) ^ At the time of study a one day food challenge was the routine procedure

Table E2 Cut-off points of the diagnostic test under study to predict peanut allergy, n = 81

	Correct Classification, n (%)*	Cut-off	Sens	Spec	PPV	NPV
Sensitization tests (n = 81)						
SPT index imputed	19 (23)	**0.84**	100	31	67	100
		3.59	17	100	100	47
sIgE peanut	26 (32)	**0.36**	100	34	59	100
		30.33	31	100	100	51
sIgE Ara h 2	50 (62)	**0.10**	100	71	69	100
		5.00	54	100	100	61
sIgE Ara h 6 imputed	44 (54)**	**0.10**	92	66	67	92
		6.67	48	100	100	59
Basophil Activation Test (n = 81)						
Maximal CD63% to Ara h 2/ aIgE	35 (43)	**0.86**	100	3	8	100
		65.19	72	100	100	72
Maximal CD63% to Ara h 6/ aIgE	42 (52)	**2.76**	100	21	31	100
		80.61	74	100	100	69
Maximal CD63% to Ara h 2 + h 6 / aIgE	57 (70)	**23.22**	100	59	42	100
		81.21	79	100	100	77

* The total number of patients that were correctly classified as tolerant or allergic using the cut-off points with the highest PPV and NPV ** Including n = 2 false negative test results
Sens, sensitivity; Spec, specificity; PPV, positive predictive value; NPV, negative predictive value.

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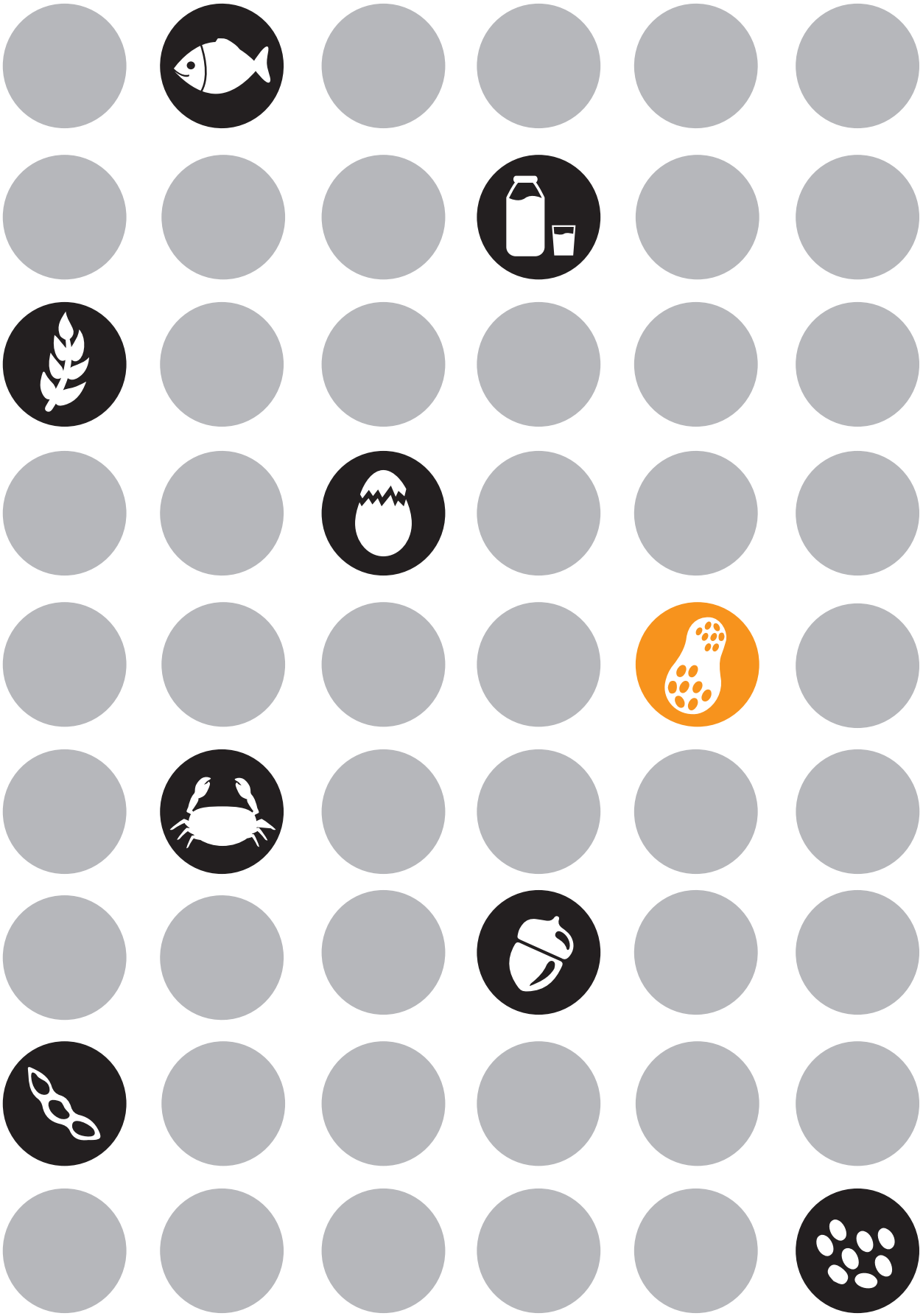
PART III

The diagnostic accuracy of food challenges



CHAPTER 7

Standardized food challenges are subject to variability in interpretation of clinical symptoms

Clin Transl Allergy 2014: 4:43

Francine C. van Erp
André C. Knulst
Yolanda Meijer
Carmelo Gabriele
Cornelis K. van der Ent

ABSTRACT

Background

Food challenge tests are the gold standard in diagnosing food allergy. Guidelines provide scoring systems to classify symptoms during challenge and typically recommend that challenges are considered positive when objective symptoms occur. However, currently no standard criteria for the definition of a positive challenge outcome exist and interpretation of food challenges mainly depends on clinical judgment. This study aims to assess inter- and intra-observer variability in outcomes of routinely performed peanut challenges in children.

Methods

All complete food challenge score sheets of double blind placebo controlled peanut challenges performed in 2008-2010 in an academic hospital were included. Score sheets were reassessed independently by three clinical experts including double reassessment in a subset of score sheets. Inter- and intra-observer variability was evaluated using kappa statistics.

Results

We included 191 food challenge score sheets. Inter-observer agreement on overall challenge outcome was moderate (κ = 0.59-0.65) and was fair (κ = 0.31-0.46) on challenges with symptoms. Intra-observer agreement on overall challenge outcome was good (κ = 0.63-0.77) but was moderate (κ = 0.50-0.60) on challenges with symptoms. Subjective symptoms (oral symptoms, abdominal complaints, food aversion) were significantly associated with disagreement between observers.

Conclusion

We demonstrate that, despite strict adherence to guidelines, there is a considerable amount of variability in reassessment of symptoms recorded on food challenges sheets between and within well trained clinicians, especially when subjective symptoms occur.

INTRODUCTION

Food challenge tests are the gold standard in diagnosing food allergy.(1) Several guidelines and symptom score sheets exist to classify symptoms during challenge. A food challenge is usually considered positive when clear objective symptoms occur on verum and not on placebo.(1–3) Whenever possible, symptoms are supported and objectified by measuring clinical parameters such as blood pressure, oxygen saturation and lung function tests. However, no standard criteria for the definition of a positive challenge outcome exist and the interpretation of food challenges mainly depends on clinical judgment. Especially when clear objective symptoms are absent, determination of food challenge outcome can be difficult. Clinicians may then take other factors (course and reproducibility of symptoms over time, patient characteristics, a "gut feeling" or lessons learned from previous cases) into account to determine challenge outcome. These factors come along with clinical judgment in general and are not easily standardized nor implemented in guidelines. Until now no data on the diagnostic accuracy of the interpretation of symptoms during food challenge are available. In this study we describe inter- and intraobserver variability in reassessment of the outcome of previous performed standardized food challenges by measuring the agreement on the outcome of food challenge score sheets.

METHODS

All complete DBPCFCs (n = 191) for peanut performed in an academic hospital from 2008-2010 were selected for this study. Data were obtained as part of regular patient care and collected retrospectively from electronic patient records in 2012. Food challenge score sheets were used in strictly anonymous form, according to the code of conduct for medical research approved by the hospital's Medical Ethical Committee.

The DBPCFC protocol used in this study was described earlier by Flinterman et al.(4) In short, increasing amounts of defatted peanut flour from 0.01 to 3000 mg, were given with time-intervals of 15-30 minutes with randomly dispersed placebo's. Challenges were performed by a nurse practitioner specialized in food allergy and interpreted under supervision of an allergologist. When symptoms occurred the patient was fully examined and in case of doubt or severe symptoms the allergologist was called to interpret these symptoms. All signs and symptoms observed during DBPCFC were recorded in detail on paper food challenge score sheets including timing and administration of doses by a trained nurse and any abnormalities in vital signs (figure 1). Challenges were discontinued and considered positive in case of persistent objective symptoms or if suggestive subjective symptoms (OAS) occurred at 3 subsequent doses or a severe subjective symptom (abdominal pain / nausea with discomfort) lasted for more than 45 minutes. Symptoms within 15 minutes after a placebo dose were considered as placebo reactions. The three observers were clinical experts in food allergy, regularly interpreted food challenges according to the most recent clinical guidelines (2), had the same criteria

for classifying a challenge was positive and worked in close cooperation with each other within an expert centre of food allergy. Observer 1 (a paediatric allergologist) performed food challenges in children for 10 years and supervised included food challenges (2-4 years ago). Observer 2 (a paediatric allergologist in training) performed challenges for more than 5 years. Observer 3 (dermatologist and immunologist) performed food challenges in adults for more than 10 years. Anonymous food challenges score sheets (blinded for patient characteristics, randomization and challenge outcome) were individually administrated to the observers . The observers received 25% duplicated score sheets randomly dispersed with the other score sheets without their knowledge. They were asked to determine and argue DBPCFC outcome as positive, negative or when information was insufficient or doubtful as inconclusive. Agreement between observers was defined as a concordant classification of all three observers. Disagreement was defined as a discordant classification between two or three observers.

Statistics

The kappa statistic (κ) was used to determine intra-observer and inter-observer variability between different pairs of observers on overall challenge outcomes and on individual symptoms in challenges with symptoms respectively. Interpretation of the Kappa value: <0.20 = poor agreement; 0.21-0.40 = fair agreement; 0.41-0.60 = moderate agreement; 0.61-0.80 = good agreement; 0.81-1.0 = excellent agreement.(5) For univariable analyses of the association between type of symptoms and the agreement between observers, the chi-square statistic or univariable logistic regression analysis was used. A p-value < 0.05 was considered statistically significant.

RESULTS

Initial DBPCFC outcome was positive in 89 (46%) and negative in 103 (54%) included challenges. Reactions ranged from Sampson grade 1 to grade 4, only one child showed significant changes in vital signs (tachycardia). Baseline characteristics of children who underwent DBPCFC are shown in table 1.

Agreement of observers with initial challenge outcome ranged from 79% - 87%. Based on the reassessment of score sheets the observers fully agreed on 132 of 191 (69%) DBPCFCs, whether the challenge outcome was positive or negative. In 47 (25%) challenges one observer disagreed with the other two, in 12 (6%) challenges complete disagreement (negative, positive and inconclusive classification) was present. Inconclusive challenge outcome was recorded by different observers in 58 (10%) reassessments. Reasons reported for inconclusive judgment were insufficient information (50%), nonspecific symptoms (47%) or unknown (3%). Overall 111 (58%) score sheets could be used to assess inter-observer agreement on individual symptoms. On the remaining 80 (42%) food challenge score sheets no symptoms were reported. Results of inter- and intra-observer analysis are shown in table 2.

Table 1 Baseline characteristics of children who underwent DBPCFC, n = 191

Age, mean (range) in yrs.	7.8 (3.4-18.6)
Male sex, n (%)	132 (70)
Peanut sIgE, median (IQR) in kU_A/L	2.60 (0.60-18.80)
Previous reaction to peanut, n (%)	
No ingestion / no reaction	96 (50)
Non severe	63 (33)
Severe	32 (17)
DBPCFC outcome, n (%)*	
Negative	103 (54)
Grade 1	2 (1)
Grade 2	51 (27)
Grade 3	15 (8)
Grade 4	20 (11)

* According to the Sampson classification of anaphylaxis (6)

Table 2 Agreement and variability in classification of DBPCFC outcome

	All DBPCFC (n =191)			**DBPCFC with symptoms** (n =111)		
	1	**2**	**3**	**1**	**2**	**3**
Agreement with initial DBPCFC outcome	79%	82%	87%	65%	69%	78%
	1:2	**1:3**	**2:3**	**1:2**	**1:3**	**2:3**
Inter-observer agreement	78%	76%	76%	76%	61%	60%
κ (95% CI)	0.65 (0.56-0.74)	0.59 (0.50-0.68)	0.59 (0.50-0.68)	0.46 (0.39-0.53)	0.35 (0.22-0.48)	0.31 (0.25-0.38)
Overall agreement		69%			50%	
	1	**2**	**3**	**1**	**2**	**3**
Intra-observer agreement*	77%	83%	88%	67%	70%	81%
κ (95% CI)	0.63 (0.45-0.82)	0.71 (0.54-0.89)	0.77 (0.62-0.92)	0.50 (0.37-0.63)	0.52 (0.39-0.65)	0.60 (0.45-0.60)

DBPCFC, Double Blind Placebo Controlled Food Challenge; κ, Kappa.

* n =48 (All DBPCFC) and n =27 (DBPCFC with symptoms).

Bold numbers express different observers. For example; 1 = observer 1 and 1:2 = observer 1 versus observer 2.

Table 3 Univariate association of symptoms during challenge with observer agreement, n=191

Tract	**Symptoms**, n (%)	**Disagree** (n =59)	**Agree** (n =132)	p-value
Upper airways	Red / itchy eyes	10 (17)	13 (10)	0.168
	Sneezing	10 (17)	10 (8)	0.056
	Nasal congestion / rhinorrhoea	-	10 (8)	0.043*
Lower airways	Cough	5 (9)	6 (5)	0.282
	Hoarseness / difficulty swallowing	-	3 (2)	0.243
	In- and/or expiratory stridor	-	5 (4)	0.130
	Wheezing	-	4 (3)	0.177
	Dyspnoea	3 (5)	2 (2)	0.153
Gastro-intestinal	OAS^	28 (48)	12 (9)	0.000**
	Abdominal complaints^	21 (36)	6 (5)	0.000**
	Vomiting	4 (7)	5 (4)	0.374
	Diarrhoea	-	-	-
Skin	Contact urticaria#	9 (15)	13 (9)	0.283
	Redness	12 (20)	9 (7)	0.008**
	Pruritus	4 (7)	6 (5)	0.525
	Urticaria	-	10 (8)	0.043*
	Angioedema	-	2 (2)	0.342
Neurological	Change in activity level / loss of consciousness	-	-	-
Other subjective signs	Discomfort^	2 (3)	10 (8)	0.283
	Food aversion^	14 (24)	10 (8)	0.003**
Number of different objective symptoms	No objective symptoms	18 (32)	2 (4)	Ref
	1 symptom	22 (39)	23 (40)	0.005**
	2 symptoms	13 (23)	22 (40)	0.001**
	3 symptoms	3 (5)	8 (15)	0.002**

OAS, Oral Allergy Symptoms; Ref, Reference category.
^ Symptoms referred to as subjective symptoms.
# Local urticaria after direct contact between the challenge material and skin.
* Statistical significant association with agreement.
** Statistical significant association with disagreement.

The inter-observer agreement on overall food challenge outcome was moderate with κ = 0.59-0.65. Analysis of agreement in challenges with symptoms (n = 111) showed only fair agreement between observers, κ = 0.31-0.46. To assess intra-observer variability 48 (25%) randomly selected duplicated score sheets including 27 (14%) score sheets with reported symptoms could be used. The intra-observer agreement on overall challenge outcomes in duplicated challenges was, based on the kappa value, relatively good (κ = 0.63-0.77). The agreement within observers in challenges with symptoms (n = 27) was however moderate, κ = 0.37-0.60.

Clear objective symptoms (nasal and severe respiratory symptoms and urticaria) were associated with agreement whereas mild objective symptoms (mild respiratory symptoms, eye symptoms, sneezing and skin symptoms other than urticaria) and subjective signs and symptoms (OAS, abdominal complaints and food aversion) were associated with disagreement between observers (table 3). The more different objective symptoms were present the more agreement between observers was observed (table 3). The occurrence of subjective symptoms (e.g. abdominal complaints and OAS) was associated with disagreement within observers whereas disagreement was never present when respiratory symptoms occurred (data not shown). Four children (2%) experienced symptoms on a placebo portion during challenge, observers disagreed on challenge outcome in two of these children. Exclusion of children with placebo reactions did however not change the results of our study (data not shown).

DISCUSSION

Our results indicate that when presented with the same clinical information about symptoms during food challenges, clinical experts often (in more than 30%) disagree on food challenge outcome. While this fair amount of disagreement could be seen as disappointing, results could have been expected. It is known from previous studies in other disciplines that variability in interpretation of clinical symptoms is often present, despite the use of guidelines or scoring systems. Investigators of the Paediatric Rome II criteria for diagnosing functional gastrointestinal disorders in children showed low inter observer agreement among gastroenterologists (45% agreement, κ = 0.4), even when using a standardized symptom scoring system.(6) A study on the agreement between nurses who triaged patients presenting in the emergency room revealed only 52% agreement (κ = 0.3).(7) Moreover a low level of agreement (κ = 0.3) among pediatric asthma specialists in classifying asthma serverity according to the NIH guidelines was found previously.(8)

The origin of disagreement between and within observers observed in this study can be explained in several ways. Our results indicate that not the number but the origin and severity of symptoms is related to the amount of disagreement between observers. This is in contrast to previous suggestions that there is less room for doubt about challenge outcome when two or more organ systems are involved or when

symptoms are reproducible or persisting (9). Due to the amount of variability in course of symptoms during challenge between patients, we were unfortunately not able to demonstrate whether the timing of symptoms was related to the level of agreement between observers.

Subjective symptoms or mild objective symptoms (1 episode of vomiting or a transient rash) frequently occur in children, usually as the first sign of an allergic reaction during food challenges. However these symptoms can also indicate fear associated with the clinical setting of the challenge or intolerance for the amount of food or the matrix chosen. As mainly subjective symptoms were present in cases on which observers disagreed one could argue that observers have difficulties in the interpretation of food challenge outcome when clear objective symptoms are absent. Moreover, guidelines only provide information on symptoms likely to be associated with positive challenge outcome and can therefore be interpreted and implemented by each observer differently. Reliability of the assessment of food challenges outcome also depends on the information provided. In our study lack of knowledge of the guidelines is unlikely to influence the results as all observers were clinical experts in the field of food allergy and used to perform and interpret food challenges. The same clinical information was administered to all observers excluding the possibility of sampling error. Assessment of challenge outcome was based on paper score sheets eliminating the possibility that the interpretation of observers and results of this study were influenced by other (patient related) factors as level of sensitization, age or previous challenge results.

To our knowledge this is the first study exploring agreement between clinical experts in assessing food challenge outcome. Observers reassessed a large number of challenges in a blinded, standardized and accurate way. Due to the retrospective nature there are some limitations that should be considered when interpreting the results of this study. Placebo reactions can influence challenge outcome in young children.(10) DBPCFCs were performed with randomly interspersed placebo's, but observers had only access to blinded score sheets. Unfortunately we were therefore not able to analyse differences between placebo or verum challenges.

Challenges were reassessed after two years, based on recorded symptoms during challenge, no additional (photographic or real life) patient information was available. The food challenges score sheet was not validated and lack of information could have caused differences between observers. Based on our results we can therefore not conclude that observers would classify challenge outcome of actual patients in the same manner as they did based on paper score sheets. However it is possible that the lack of agreement we found is even an underestimation of variability in assessment of 'real life' challenges since conditions in this study were standardized in contrast to real life reactions where observers are influenced by many other (patient related) factors.

CONCLUSION

Although our study using symptom score sheets might not fully reflect procedures in a real life setting, our observations indicate that different observers may have different opinions about symptoms during food challenge tests. To further investigate whether this variability is also present during real life challenges future prospective studies using an expert panel or for example a scoring system with weightage points for each (type of) symptom to assess food challenge outcomes are needed. To improve standardization of food challenges and diminish variability in interpretation new preferably objective parameters might also be helpful in the future.(11–15)

Until now, clinicians should be aware that although experienced and familiar in working according to international guidelines variability in interpretation of food challenge outcome is present when reassessing score sheets of challenges, especially when objective symptoms are absent.

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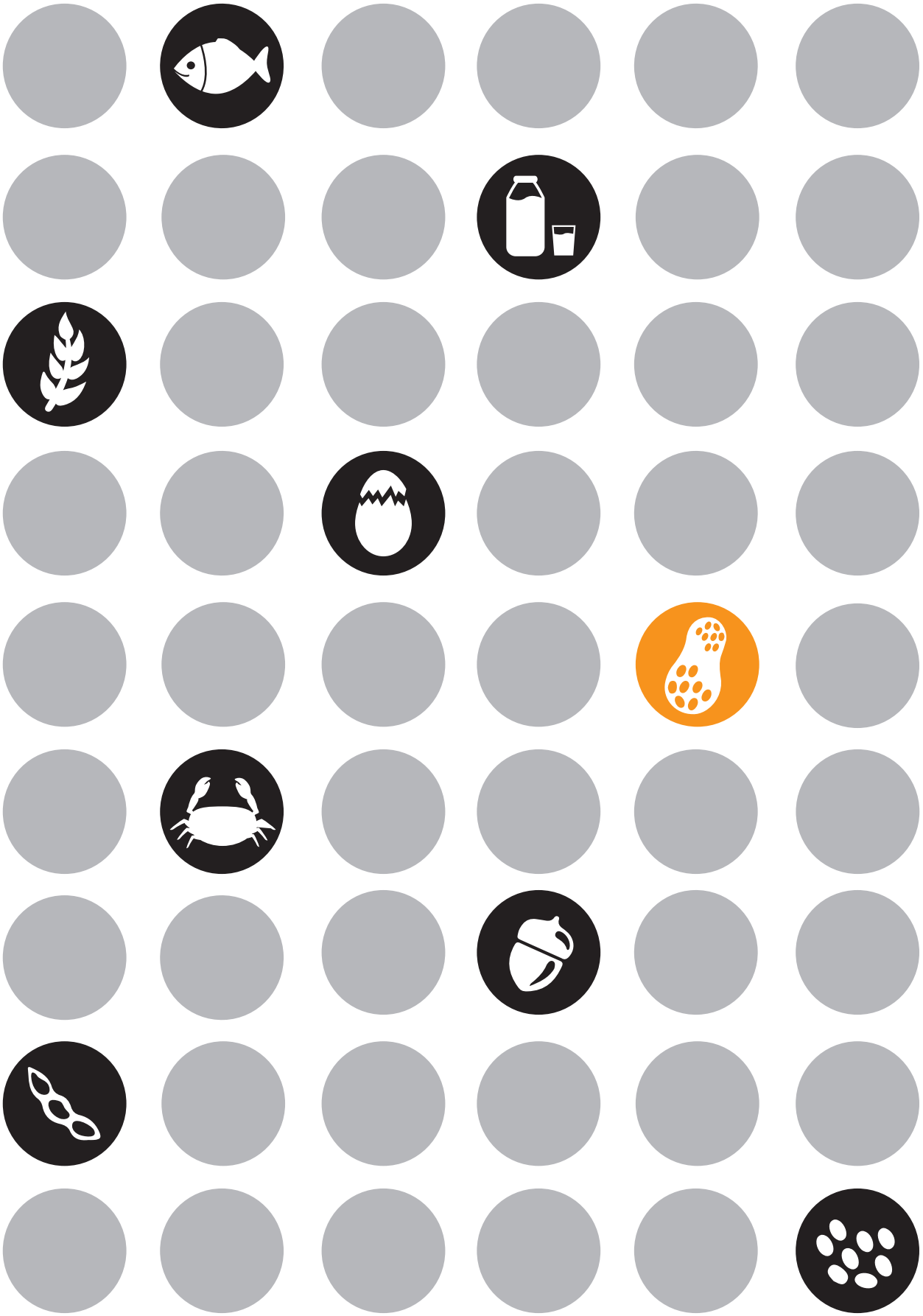


CHAPTER 8

The added value of monitoring respiratory and vital signs during food challenges

Submitted

Francine C. van Erp
Cornelis K. van der Ent
André C. Knulst
Yolanda Meijer

ABSTRACT

Background

A double blind placebo controlled food challenge (DBPCFC) is usually terminated and considered positive when objective symptoms occur. Subjective symptoms are often difficult to interpret. Less obvious objective symptoms (as mild dyspnoea) are easily missed. Our aim was to investigate if monitoring of respiratory and vital signs contributes to the interpretation of DBPCFC.

Methods

A prospective study in 83 children with suspected peanut allergy that underwent a DBPCFC and subsequent open challenge was performed. The following parameters were monitored during challenge: oxygen saturation, heart rate, blood pressure, respiratory function and respiratory sounds. Differences in the course of the parameters over time between peanut and placebo for allergic and tolerant children were investigated using linear mixed effects modelling.

Results

Allergic children had a significant decrease in mean FEV1 (forced expiratory volume in one second) on peanut compared to placebo (B (95%CI) -0.58 (-0.92;-0.25)% predicted per 30 minutes, $p < 0.001$). This decrease was not related to a history of asthma or lower respiratory symptoms during challenge. A persistent decrease in FEV1 > 20% on the peanut day was distinctive for peanut allergy. No significant differences between peanut and placebo for allergic or tolerant children were seen for the course of other respiratory and vital parameters during challenge.

Conclusion

A decrease in FEV1 > 20% is indicative for an allergic reaction during DBPCFC. Other respiratory and vital signs cannot be used to discriminate children with and without peanut allergy.

INTRODUCTION

The gold standard to diagnose food allergy is a double-blind placebo-controlled food challenge (DBPCFC).(1) During challenge increasing amounts of a specified allergen are administered and symptoms are monitored carefully. A DBPCFC is usually terminated and considered positive when objective symptoms or severe and recurrent subjective symptoms occur.(1) However, less obvious objective symptoms as mild dyspnoea or small changes in blood pressure are easily missed.(2) It has been described that patients may develop bronchial obstruction during challenge without clinical symptoms.(3) On the other hand, subjective symptoms like abdominal complaints and change of behaviour are difficult to confirm and are subject to inter- and intra-observer variability.(4) Some children with a negative DBPCFC have symptoms during the introduction of food, suggesting false-negative outcomes of food challenge tests.(5,6) Ideally, symptoms should be confirmed by measuring clinical parameters such as changes in blood pressure and oxygen saturation. Until now studies on monitoring objective parameters (e.g. respiratory function, nasal temperature and blood pressure) during food challenge are scarce.(3,7,8) These studies did focus on one type of monitoring and were not performed in patients with suspected allergy that all underwent double blind placebo controlled challenges. In this study we performed blinded monitoring of multiple objective parameters during both placebo and peanut day of DBPCFC in children with suspected peanut allergy. We investigated if measurement of vital signs, respiratory function and respiratory sounds during food challenges contributes to the interpretation of the DBPCFC.

METHODS

Study population and setting

This study is part of prospective diagnostic study performed in the Wilhelmina Children's Hospital a tertiary centre for food allergy between January 2012 and May 2015. Children with suspected peanut allergy were selected based on (1) a clinical history of a reaction to peanut with or without peanut sensitization or (2) peanut sensitisation without previous known ingestion to peanut. Peanut sensitization was defined as peanut sIgE > 0.35 kUA/L or a peanut skin prick test (SPT) mean wheal size of > 3 mm. This study was reviewed and approved by the ethical committee of the University Medical Centre Utrecht. Written informed consent was obtained from parents and children before enrolment in the study.

Diagnosis of peanut allergy

Food challenges

All children underwent a two day DBPCFC for peanut. Only clinically stable children without recent infection or with controlled asthma (based symptom scores and a FEV1 > 70% predicted) and without active allergic rhinitis were eligible to start the challenge procedure. Challenges were performed by a trained nurse under supervision

of an allergologist in a clinical setting equipped for resuscitation. Intravenous access was established before the first allergen dose. The DBPCFC protocol consisted of a validated recipe with 7 increasing portions of ginger bread containing 8 g defatted peanut flour (50% peanut protein) or placebo in randomized order.(9) Standard signs and symptoms during challenge were classified and recorded on a score sheet adapted from Nowak et al.(10) Challenges were stopped when objective symptoms or severe subjective symptoms that lasted longer than 45 minutes occurred. When symptoms during DBPCFC were absent or inconclusive an open challenge followed with 10 g whole peanuts directly after day two. Children were observed for two (no symptoms) or four (symptoms) hours after the last portion of administration of treatment.

Panel diagnosis

An expert panel of three independent experienced allergologists, blinded for patient characteristics, sensitization tests and measurements during challenge was used to determine food challenge outcome. Using the scoring sheets, the experts individually classified children as peanut allergic, tolerant or inconclusive and determined severity of symptoms (by Sampson grade 1 - 5). A severe reaction was defined as symptoms occured according to Sampson grade 4 or 5. They evaluated the placebo day, peanut day and open challenge independently. A face-to-face panel discussion meeting was held until consensus among all experts about the total challenge outcome (DBPCFC and open challenge) was reached. The challenge outcome was considered inconclusive if the panel agreed that symptoms during DBPCFC and/or open challenge were inconclusive and reintroduction at home was needed to determine definitive food challenge outcome.

Parameters monitored during challenge

All parameters that were monitored during challenge were measured by an independent researcher that was blinded for the challenge material, not involved in the interpretation of symptoms during challenge and not part of the expert panel. Physicians performing food challenges were blinded for the parameters during challenge until an objective allergic reaction occurred.

Respiratory function

Respiratory function was measured in children from age six that were able to produce valid flow-volume curves. Flow volume measurements were performed at baseline and after each dose during DBPCFC with a mobile calibrated spirometer (Flowhandy ZAN100USB of Nspire Health). The guidelines of the American Thoracic Society (ATS) and the European Respiratory Society (ERS) on acceptability and reproducibility of flow-volume curves were applied.(11) The Dutch reference values for paediatric lung function were used.(12) The forced expiratory volume in one second (FEV1), peak expiratory flow (PEF), and the forced vital capacity (FVC) were expressed as percentage predicted.

Vital signs

Oxygen saturation and pulse rate were measured continuously during challenge with a Wrist pulse oximeter MD300W with finger sensor. The mean pulse rate and saturation per challenge step during DBPCFC were calculated. The pulse-rate change over time was expressed as the absolute increase in beats per minute from baseline. Blood pressure and temperature were measured at baseline and 15 minutes after each dose during DBPCFC and observation period. Blood pressure was measured at the same arm in sitting position and expressed as the percentage of change from baseline. We used the age specific low blood pressure definition according to the Anaphylaxis Network (defined as < 70mmHg from 1 month to 1 year, < 70 mm Hg + [2 x age] from 1 to 10 years, and < 90mmHg from 11 to 17 years).(13) Temperature was measured with an in-ear thermometer and expressed in absolute degree Celsius (°C).

Respiratory sounds

Respiratory sounds were recorded and analysed during DBPCFC using an automatic wheezing detection device WHolter (KarmelSonix Pulmotrack, KarmelSonix, Haifa, Israel). For procedures regarding the measurements and definitions of wheeze we refer to a previous publication.(14) The wheeze rate, the ratio between the wheeze time and recorded breathing time at each sensor was expressed as a mean percentage per 30 minutes. The inspiratory and expiratory wheeze rate (IWR and EWR) for both sensors (tracheal (TR) and chest wall (CW)) were used in this study.

Statistical methods

To explore relevant changes of the parameters over time linear mixed effects modelling was used. All measurements performed during DBPCFC and open challenge until 30 minutes after the last administered portion were included. The parameters were analysed as dependent variables and time from start until the last portion (per 30 minutes), challenge material (placebo vs. peanut) and allergic status (allergic vs. tolerant) and their three-way interaction were included as fixed effects to investigate their influence on the pattern of dependent variables. A random slope (for time) and intercept were included in the model with an unstructured covariance structure to account for repeated measurements. The effect of learning (day 1 vs. day 2) and asthma on the course of the parameters was investigated by including those factors as fixed effect in the models. A parameter was considered informative when a significant difference in course over time was seen between peanut and placebo for allergic children and on peanut between allergic and tolerant children. Furthermore clinical relevant cut-off points for informative diagnostic parameters were evaluated in allergic, tolerant and inconclusive children. The proportion of children with a relevant change in parameter was compared between placebo and peanut day with McNemar's test to account for paired data. Analyses were performed with SPSS, version 21. Results with a p-value of less than 0.05 were considered statistically significant.

RESULTS

Baseline characteristics

We included 83 children with a mean (SD) age of 8.4 (3.5) years. The expert panel classified 22 (27%) children tolerant, 45 (54%) allergic and 16 (19%) inconclusive (figure 1). Inconclusive classifications were due to: missing placebo day and open challenge (n = 1), < 3 urticae (n = 1), feeling throat tightness (n = 1), feeling dyspnoea (n = 1), intermittent oral symptoms (n = 5), mild abdominal complaints (n = 4) or late symptoms at home (n = 3). A severe reaction was seen in 10/45 (22%) allergic children. They developed lower respiratory symptoms and were treated with epinephrine and inhalants if necessary. No objective cardiovascular symptoms occurred during challenge. The placebo day of DBPCFC was cancelled due to severe allergic symptoms including administration of epinephrine on the peanut day in 4 allergic children (parents did not agree to perform the placebo challenge) and due to poor asthma control in one child. Baseline characteristics and available measurements for the different diagnostic parameters per food challenge outcome are shown in table 1.

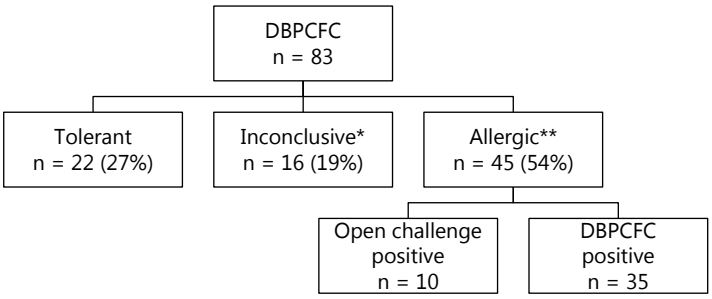
A decrease in FEV1 is associated with peanut allergy

There was no significant difference in baseline lung function parameters between tolerant and allergic children (Table 1). Allergic children had a significant decrease in mean FEV1 over time on the peanut day compared to placebo (B (95%CI) -0.58 (-0.92;-0.25) per 30 minutes, p = 0.001), table 2 and figure 2. Their FEV1 also tended to decrease on verum compared to tolerant children, but this difference was not significant (B (95%CI) -0.48 (-1.01;0.05) per 30 minutes, p = 0.077), table 2 and figure 2. A drop in FEV1 > 20% occurred in 3/32 (20%) allergic children on the peanut day and not on placebo. A drop in FEV1 > 20% was also seen in one child with inconclusive outcome on peanut and in one tolerant child on placebo (figure 3). The tolerant child had a decrease in FEV1 once only but the allergic and inconclusive children showed a persistent FEV1 decline and throughout the challenge (figure 3). Furthermore these children also had a decline in FEV1/FVC ratio >10% as a sign of bronchus obstruction. The amount (%) of allergic, tolerant and inconclusive children that had any measurement with a 5 - 20% decrease in FEV1 from baseline or from the previous measurement during the peanut challenge and not on the placebo challenge is presented in table 3.

Characteristic of children with a decrease in FEV1

Overall 4/ 83 (5%) children had a decrease in FEV1 > 20% on the peanut day and not on the placebo day. One of those four children was known with (well controlled) asthma and used daily inhaled corticosteroids. In the three allergic children with a decrease in FEV1 >20% the challenge was stopped based on rhino-conjunctivitis and gastro-intestinal symptoms of Sampson grade 2-3. Those children did not have subjective or objective symptoms of the lower airways (i.e. cough, dyspnoea, wheeze) or other severe allergic symptoms.

Figure 1 Flowchart of food challenge outcome and follow-up



* Including n = 1 child with missing placebo day and missing open challenge due to poor asthma control
** Including n = 4 children with missing placebo day due to a severe reaction on day 1

Table 1 Baseline characteristics and available measurements

Baseline characteristics, n (%)	**Tolerant** n = 22	**Inconclusive** n = 16	**Allergic** n = 45	**Total** n = 83
Age (Mean, SD)	7.4 (2.8)	9.9 (4.5)	8.3 (3.3)	8.4 (3.5)
Sex	16 (73)	12 (75)	30 (67)	58 (70)
Asthma	8 (36)	7 (44)	25 (56)	40 (48)
Hay fever	10 (45)	10 (63)	28 (62)	48 (58)
Eczema	14 (64)	7 (44)	26 (58)	47 (57)
sIgE Peanut (kU_A/L) (Mean, SD)	3.1 (6.9)	3.9 (4.7)	56.3 (112.3)	32.1 (86.5)
sIgE Ara h 2 (kU_A/L) (Mean, SD)	0.2 (0.3)	1.0 (1.6)	31.1 (60.3)	17.1 (46.8)
FEV1 %pred (Mean, SD)	89.8 (9.6)	85.7 (9.5)	87.7 (10.1)	87.9 (9.8)
FEV1/FVC % (Mean, SD)	94.3 (7.9)	90.6 (6.5)	92.1 (8.4)	92.4 (7.9)
PEF %pred (Mean, SD)	92.0 (14.5)	89.1 (14.3)	87.4 (148)	88.9 (14.5)
Severity of allergy				
Sampson 1			4 (9)	4 (5)
Sampson 2			20 (44)	20 (42)
Sampson 3			11 (24)	11 (23)
Sampson 4			10 (22)	10 (21)
Available measurements, n (%)				
Peanut day	n = 22	n = 16	n = 45	n = 83
Oximeter	17 (77)	13 (81)	39 (87)	69 (83)
Respiratory sounds	17 (77)	14 (88)	40 (89)	71 (86)
Respiratory function	16 (73)	12 (75)	34 (76)	62 (75)
Blood pressure	22 (100)	16 (100)	45 (100)	83 (100)
Temperature	22 (100)	16 (100)	45 (100)	83 (100)
Placebo day	n = 22	n = 15	n = 41	n = 78
Oximeter	19 (86)	11 (73)	31 (76)	61 (79)
Respiratory sounds	18 (82)	13 (87)	37 (90)	68 (88)
Respiratory function	16 (73)	10 (67)	32 (78)	58 (75)
Blood pressure	22 (100)	14 (93)	41 (100)	77 (100)
Temperature	22 (100)	14 (93)	41 (100)	77 (100)

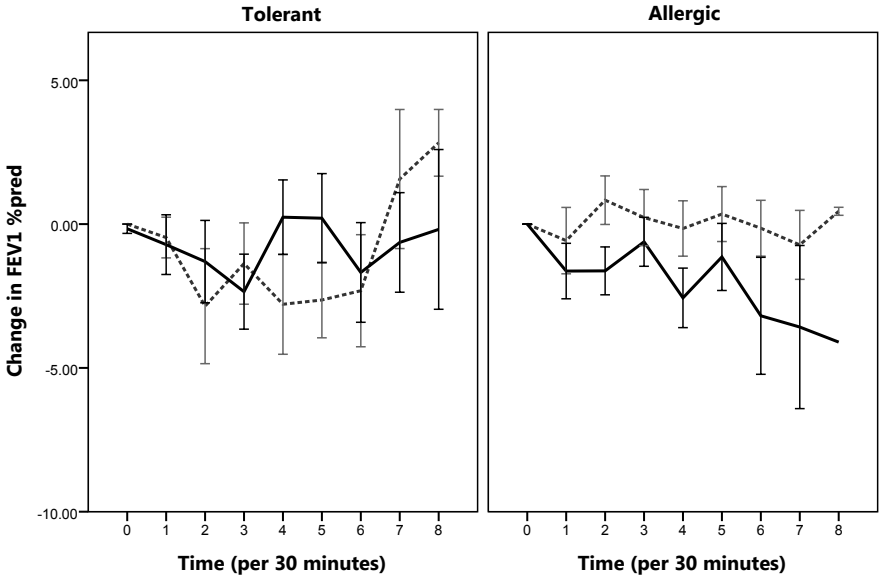
SD, Standard Deviation

Table 2 Mixed model analysis of parameters over time (per 30 minutes)

A. Vital signs	**RR sys** (% change)			**Temp**			**Saturation**			**Pulse-rate** (change)		
	B (95%CI)	df	p-value	B (95%CI)	df	p-value	B (95%CI)	df	p-value	B (95%CI)	df	p-value
Peanut*Allergic*Time vs.	Ref			Ref			Ref			Ref		
Placebo*Tolerant*Time	0.44 (-0.57;1.47)	169	0.391	0.006 (-0.02;0.03)	152	0.644	-0.01 (-0.24;0.2)	288	0.875	0.04 (-0.71;0.8)	251	0.907
Placebo*Allergic*Time	-0.22 (-0.77;0.32)	878	0.421	-0.008 (-0.02;0.01)	837	0.402	0.03 (-0.13;0.19)	595	0.703	0.61 (0.09;1.13)	589	**0.021**
Peanut*Tolerant*Time	0.15 (-0.79;1.11)	127	0.743	0.01 (-0.01;0.03)	93	0.398	-0.15 (-0.35;0.03)	210	0.111	-0.019 (-0.69;0.66)	182	0.955
Time	0.45 (-0.16;1.06)	195	0.149	0.02 (0.003;0.03)	186	**0.020**	-0.1 (-0.23;0.03)	318	0.134	0.83 (0.37;1.29)	277	**<0.001**
B. Respiratory function	**FEV1%pred** (change)			**Tiff%pred** (change)			**PEF%pred** (change)					
	B (95%CI)	df	p-value	B (95%CI)	df	p-value	B (95%CI)	df	p-value			
Peanut*Allergic*Time vs.	Ref			Ref			Ref					
Placebo*Tolerant*Time	-0.52 (-1.1;0.05)	107	0.074	0.37 (-0.07;0.82)	131	0.099	-0.63 (-1.51;0.23)	123	0.152			
Placebo*Allergic*Time	-0.58 (-0.92;-0.25)	657	**0.001**	0.14 (-0.13;0.43)	662	0.311	-0.01 (-0.57;0.54)	661	0.957			
Peanut*Tolerant*Time	-0.48 (-1.01;0.05)	79	0.077	0.31 (-0.09;0.71)	90	0.133	-0.1 (-0.89;0.69)	85	0.795			
Time	-0.45 (-0.82;-0.08)	167	**0.017**	0.32 (0.02;0.61)	212	**0.031**	0.03 (-0.53;0.61)	202	0.895			
C. Respiratory sounds	**CW IWR**			**CW EWR**			**TR IWR**			**TR EWR**		
	B (95%CI)	df	p-value	B (95%CI)	df	p-value	B (95%CI)	df	p-value	B (95%CI)	df	p-value
Peanut*Allergic*Time vs.	Ref			Ref			Ref			Ref		
Placebo*Tolerant*Time	0.16 (-0.12;0.44)	191	0.272	0.16 (-0.2;0.54)	160	0.377	0.05 (-0.25;0.36)	153	0.737	0.04 (-0.16;0.25)	158	0.652
Placebo*Allergic*Time	0.18 (0.01;0.35)	780	**0.031**	0.18 (-0.02;0.39)	775	0.078	0.02 (-0.14;0.19)	774	0.743	0.02 (-0.08;0.14)	775	0.625
Peanut*Tolerant*Time	-0.006 (-0.27;0.26)	152	0.960	-0.045 (-0.4;0.31)	131	0.804	-0.175 (-0.47;0.12)	127	0.246	-0.14 (-0.34;0.05)	129	0.168
Time	-0.07 (-0.2;0.09)	207	0.357	-0.08 (-0.3;0.14)	172	0.477	0.04 (-0.14;0.22)	164	0.651	0.03 (-0.08;0.16)	168	0.568

95%CI, 95% Confidence Interval of the B; B. Regression-coefficient; CW, chestwall sensor; df. Degrees of Freedom; EWR, expiratory wheeze rate; IWR, inspiratory wheeze rate; TR, tracheal sensor.
Peanut*Allergic*time = The three-way-interaction between challenge material, allergic status and time.
For example: The FEV1 of allergic children on peanut changes with an average of -0.58% from baseline per 30 minutes compared to allergic children on placebo.
Time = The overall mean course of the diagnostic parameter over time. For example: The mean pulse rate of children increases with 0,83 beats per minute from baseline per 30 minutes.
Bold faced p-values indicate a significant difference of the outcome parameter over time with allergic children on the peanut day.

Figure 2 Mean course of FEV1 over time



----- placebo ── verum
Mean values with error bars representing +/- 1 SE.

Figure 3 Course of FEV1 over time for children with a decrease in FEV1 > 20%

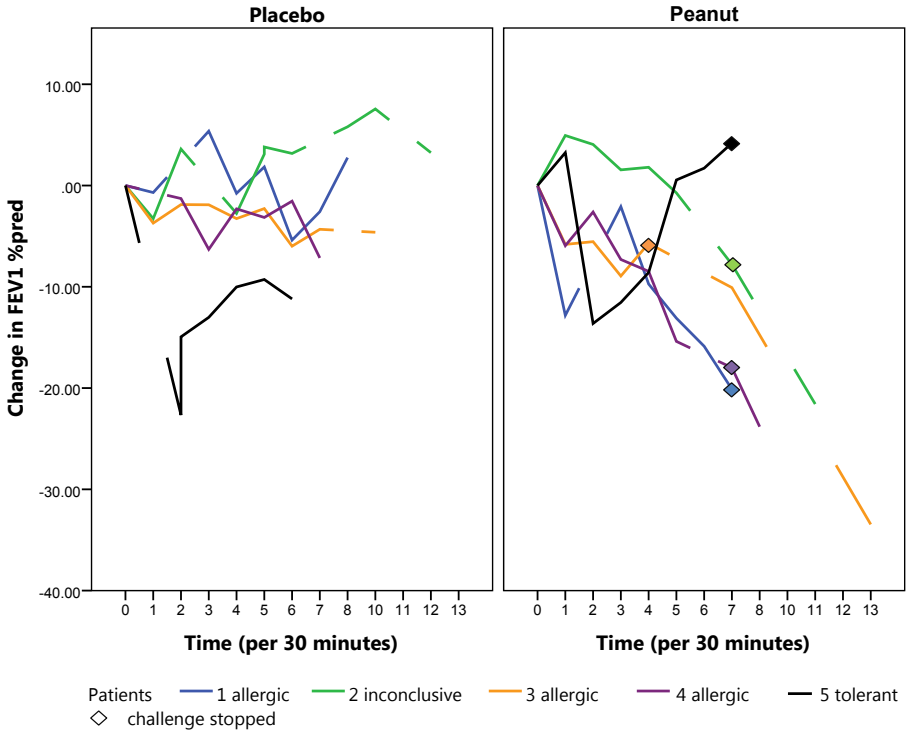


Table 3 Cut-off points for FEV1

	Tolerant (n = 16)	**Inconclusive** (n = 10)	**Allergic** (n = 32)
A Change from baseline			
FEV1-5%	4 (25)	2 (20)	7 (22)
FEV1-10%	1 (6,25)	3 (30)	8 (25)
FEV1-15%	2 (12,5)	2 (20)	5 (15)
FEV1-20%	0 (0)	1 (10)	3 (9)
B Change from previous measurement			
FEV1-5%	4 (25)	1 (10)	6 (19)
FEV1-10%	1 (6)	4 (40)	5 (1)
FEV1-15%	1 (6)	1 (10)	2 (6)
FEV1-20%	0 (0)	0 (0)	1 (3)

Numbers in this table represent the amount (%) of children that had any measurement with a 5 - 20% decrease in FEV1 from baseline (A) or from the previous measurement (B) during the peanut challenge and not during the placebo challenge.

The decline of FEV1 started early in challenge (up to 2,5 hours before the challenge was stopped) but did not reach 20% until the first objective symptoms occurred (figure 3). All children were treated with intravenous antihistamine and corticosteroids during challenges but this did not improve the decrease in FEV1. At the time of discharge, all children were symptom free and had a FEV1 >70% predicted and no late respiratory reactions were reported (during telephonic consults 24-48 hours after challenge) at home. The child with a decline in FEV1 and an inconclusive challenge outcome had intermitted oral symptoms, abdominal pain and felt dyspnoea during peanut challenge but no objective symptoms occurred. This child failed to reintroduce peanut in the diet and was diagnosed with a mild peanut allergy due to recurrent oral symptoms during follow-up. A history of asthma or allergic rhinitis had no effect on the decrease of lung function parameters (FEV1, PEF, FVC) over time. The challenge day did have significant influence: children had higher lung function parameters on day 2 compared to day 1 independent of challenge material or allergic status.

Vital signs

Mean temperature significantly increased over time for all children independent of allergic status or challenge material (B (95%CI) 0.02 (0.003;0.03) degrees of Celsius, p = 0.020). There was however no difference in temperature over time between placebo and peanut day for allergic children or between allergic and tolerant children. The mean pulse rate also significantly increased over time for all children (B (95%CI) 0.83 (0.37;1.29) beats, p < 0.001 per 30 minutes). In allergic children the pulse rate increased significantly more on peanut compared to placebo (B (95%CI) 0.61 (0.09;1.13) beats per 30 minutes, p =0.021). There was no significant change in the course of blood pressure and oxygen saturation over time and no difference between placebo and peanut day for allergic children. An age specific low systolic blood pressure according to the Anaphylaxis Guidelines was not specific for peanut allergy. It was found in 5% of children; on the

peanut day in one tolerant and in one allergic child and on the placebo day in two allergic children and in one tolerant child.

Respiratory sounds

There was a significant increase in CW-IWR (B (95%CI) 0.18 (0.01;0.35)% per 30 minutes, p = 0.03) over time for allergic children on the peanut day compared to placebo. However no significant differences in wheeze rate over time between tolerant and allergic children were found, table 2. Moreover, no significant difference in amount of children with relevant wheezing time (> 5%) between peanut and placebo day was seen CW-IWR (9/35 (26%) and 7/35 (20%), p = 0.754) seen in allergic children.

DISCUSSION

This is to our knowledge the first study that investigates multiple objective vital and respiratory parameters during DBPCFC in children with suspected peanut allergy. Our results indicate that an allergic reaction on peanut during food challenge is associated with a decrease in FEV1. However, other respiratory and vital signs could not be used to discriminate children with and without peanut allergy.

Respiratory symptoms were common and occurred in 53% of allergic patients which was comparable to previous reports.(15–17) Persistent and relevant spirometric changes were however rare and occurred only in 3/45 (7%) allergic children and in one inconclusive child. These three children, diagnosed with peanut allergy did not show a decrease in FEV1 on placebo and did not have objective lower respiratory symptoms. Interestingly, these cases demonstrate that in contrast to previous studies significant bronchoconstriction during food challenge can occur and is not necessarily accompanied with 'asthmatic symptoms' or an asthma diagnosis in history. Furthermore, decreases in FEV1 started early during challenge and therefore FEV1 could be a marker that detects an allergic reaction early. Two previous studies in children report that relevant FEV1 decreases were seen only in patients with wheeze and dyspnoea during food challenges.(15,16) Furthermore, asthmatic responses and decreases in FEV1 or increased bronchial responsiveness to inhaled methacholine after exposure occurred only 2-6 hours after challenge in several studies.(3,16,18) Our objective was to detect changes in objective parameters during food challenges. As we did not continue FEV1 monitoring after discharge we could have missed those late responses but no late respiratory symptoms or increased use of inhalants were reported at home. It can however not be excluded that treatment during challenge (antihistamines and corticosteroids) prevented the development of lower respiratory symptoms or that subjective respiratory symptoms like dyspnoea occurred without notice by the child or physician.

Despite using age specific criteria for low blood pressure and relative changes in pulse rate and temperature, variability of those measurements in and between patients was high. Occasional low systolic blood pressure for age was seen in 5% of all patients during

challenge but this was not specific for peanut allergy. It has been suggested before that hypotension is uncommon (especially as presenting symptom) during anaphylaxis and is seen in non-allergic children.(7) An increase in pulse rate and temperature was seen on both challenges days independent from peanut allergy or challenge material. This is possibility reflecting increased agitation and distress at the end the challenge day.

Several remarks have to be discussed when interpreting the results of this study. Discomfort during challenge like abdominal pain and nausea can contribute to a decrease in lung function parameters like the PEF. Although all children with a decrease in FEV1 reported abdominal pain during challenge it is unlikely that this influenced our results as the flow-volume curves were visually checked and only included when valid. Placebo data were missing in five patients as the second challenge day was cancelled due to severe reactions or poor asthma control at the start of challenge. Furthermore data of the pulse-oximeter and automatic wheeze detection had to be excluded in up to 17-25% of patients on one or both challenge days. This was due to technical problems (poor quality or loss of recordings) and unrelated to allergic status, challenge material or other patient characteristics. It is therefore unlikely that these missing data influenced our results. Furthermore, linear mixed model analysis does account for differences in timing and amount of measurements between patients.

Current food challenge guidelines and classification systems for anaphylaxis suggest that a relevant drop in blood pressure, a decrease in FEV1 and increase in heart rate are signs of an allergic reaction during DBPCFC.(1,10,19,20) However no standardized protocol for monitoring of those parameters or clear cut-off points that can be used to stop challenges are provided. It could be argued that in the child with inconclusive challenge outcome that was diagnosed with peanut allergy during follow-up, the decrease in FEV1 during challenge together with subjective symptoms did already indicated peanut allergy. Although changes in FEV1 were not related to severity of peanut allergy, relative rare and monitoring of lung function is intensive and can be burdensome for children, FEV1 might be measured during challenge for safety purposes. It could be argued that children with relevant decreases in FEV1 need additional medication during challenge (epinephrine / inhalants) and emergency medication at home (epinephrine auto-injector). Furthermore, relevant decreases of FEV1 during challenges can give reason to reconsider underlying asthma. Especially because children with (uncontrolled) asthma might be at risk to develop severe allergic reactions in daily life.(21,22)
Further exploration of other objective markers of allergy during challenge, like measurement of the fraction of exhaled nitric oxide, mast cell mediators and metabolic profiles in saliva and plasma should be further explored to objectify food challenge outcomes.(23–25) Until now the outcome of food challenges remains dependent on the clinical interpretation of symptoms.

CONCLUSION

In conclusion this study demonstrates that a decrease in FEV1 > 20% is indicative for an allergic reaction on peanut during DBPCFC. Monitoring of FEV1 during peanut challenges might be important for safety purposes. Monitoring of other respiratory and vital parameters has limited added value when interpreting food challenges. To provide adequate treatment and support during systemic anaphylaxis it remains important to monitor vital sings when symptoms occur.

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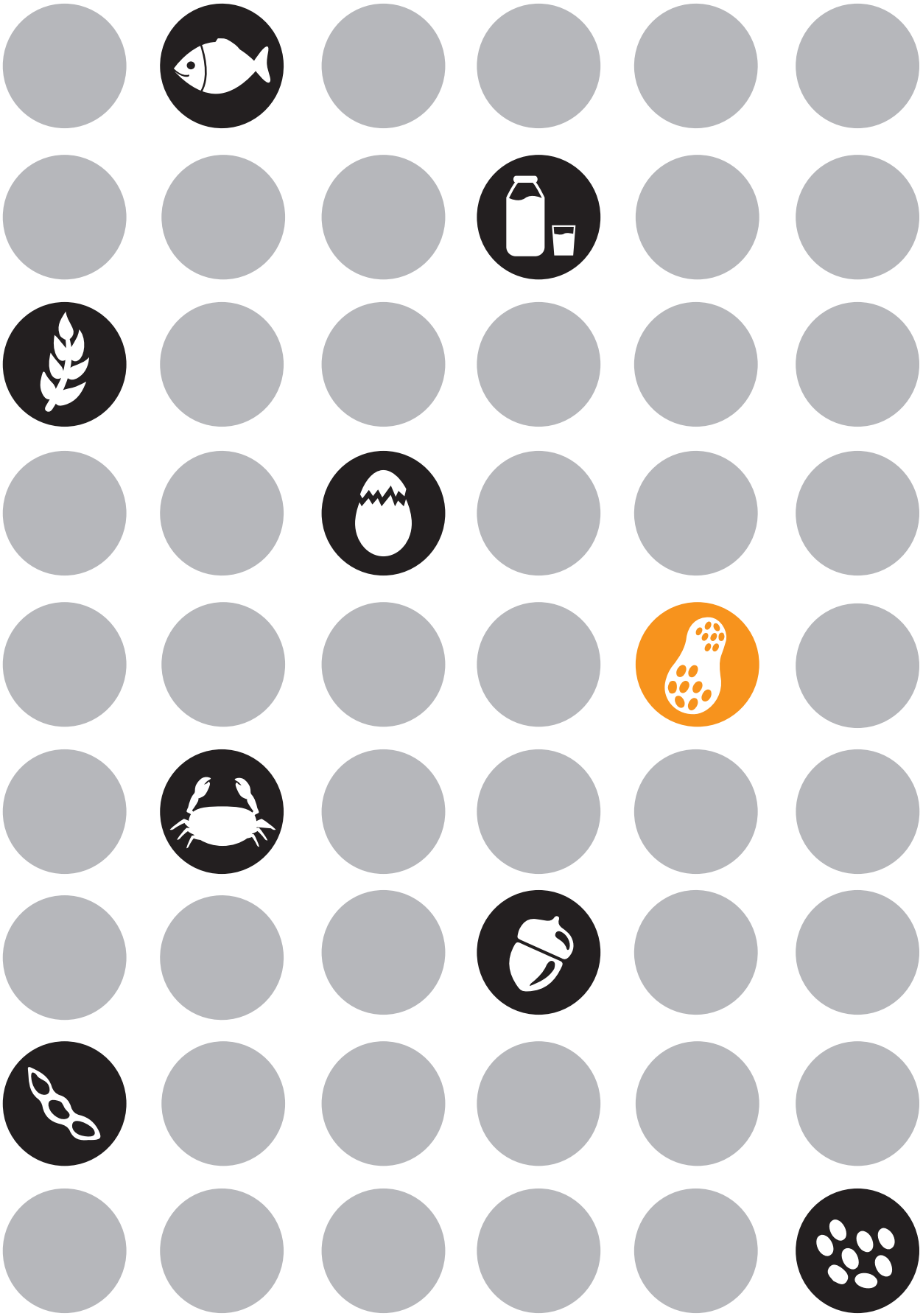
PART IV

Post-food challenge outcomes



CHAPTER 9

Outcome of open peanut challenges and guided introduction after negative double blind placebo controlled challenges

Submitted

Francine C. van Erp
André C. Knulst
Diana M.W. Gorissen
Irene L. Kok
Cornelis K. van der Ent
Yolanda Meijer

ABSTRACT

Background

Failure of introduction after a negative double blind placebo controlled food challenge (DBPCFC) is not rare and can indicate false-negative food challenge outcome. In this prospective study we investigated the outcome of subsequent open challenges and course and success rate of guided introduction of peanut after negative or inconclusive DBPCFC.

Methods

Children with suspected peanut allergy (n = 83) underwent a DBPCFC. Those with negative (n = 29) or inconclusive results (n = 18) subsequently underwent open peanut challenge. Children who were still considered negative (n = 37) introduced peanut at home using a standardized protocol and diary. During this introduction period they were guided by telephone calls of the physician.

Results

Peanut allergy was diagnosed after open challenge in another 10 patients: 2/29 (7%) with a negative and 8/18 (44%) with an inconclusive DBPCFC. Regular ingestion of peanut at home failed in 10/37 (27%) children. Reasons for not introducing were: aversion (n = 4), oral symptoms (n = 3), fear of the child (n = 1), an exacerbation of eczema that was considered peanut related by parents (n = 1) and uncertainty of parents about the challenge outcome (n = 1). Three children 3/37 (6%) were diagnosed with a mild peanut allergy during introduction at home.

Conclusion

Open challenges and subsequent introduction are indispensable to exclude or diagnose peanut allergy accurately in children with negative or inconclusive DBPCFC outcomes. Introduction after negative or inconclusive peanut challenges should be monitored to detect (peanut related) problems early.

INTRODUCTION

Children with suspected peanut allergy and their parents are faced with dietary restrictions, show high levels of anxiety and have an impaired quality of life comparable to children with diabetes.(1,2) A diagnostic double blind placebo controlled food challenge (DBPCFC) is the gold standard to confirm or exclude a (severe) peanut allergy.(3) After a negative food challenge parents and children report less fear of an allergic reaction and better quality of life.(4,5) Moreover a negative food challenge usually results in introduction of peanut at home and when successful no restrictions when eating out or buying and cooking food. Nevertheless, previous studies show that introduction at home fails in up to 25% of patients.(6–8) Reasons are refusal, behavioural or psychological factors and having symptoms during introduction. Failure of introduction (especially when symptoms occur) could indicate lack of diagnostic accuracy of the food challenge. Results of previous studies were based on retrospective questionnaires up to multiple years after food challenges often with unstandardized introduction procedures. This results in recall bias and in lack of data about the timing and start of introduction, amount of food eaten and symptoms and difficulties during introduction. Moreover, these data are usually not correlated to signs and symptoms during DBPCFC and the result of open challenges (if available) is often not taken into account. The current study is part of a prospective diagnostic study on peanut allergy that includes open challenges and subsequent standardized introduction at home with careful monitoring after negative food challenge. We aimed to improve the diagnostic procedure in children with suspected peanut allergy and investigated the usefulness of open challenges and outcome of introduction after DBPCFC.

METHODS

Study design and study population

This study is part of a prospective diagnostic study performed in the Wilhelmina Children's Hospital, a tertiary referral centre for food allergy in the Netherlands. All children with suspected peanut allergy referred to our hospital between January 2012 and May 2015 were selected for this study. Suspicion of peanut allergy was based on (1) a clinical history of a reaction to peanut with or without peanut sensitization or (2) peanut sensitisation without previous known ingestion to peanut. Peanut sensitization was defined as a peanut sIgE > 0.35 kUA/L or a peanut SPT mean wheal size of > 3 mm. All included children subsequently underwent clinical evaluation, sensitization tests, a two day DBPCFC for peanut, open challenge and a one month follow-up period. This study was reviewed and approved by the ethical committee of the University Medical Centre Utrecht. Written informed consent was obtained from parents and children before enrolment in the study.

Food challenges

Food challenges were performed and interpreted by a trained nurse under supervision

of an allergologist in a clinical setting equipped for resuscitation with monitoring of vital signs and lung function throughout the whole challenge. Intravenous access was established before the first portion. The two day DBPCFC challenge protocol consisted of 7 increasing portions of ginger bread containing 8 g defatted peanut flour (50% peanut protein) on the peanut day.(9) The second challenge day was subsequently followed by an open challenge with 10 g whole peanuts or an equivalent when the DBPCFC was considered negative or inconclusive.

All signs and symptoms during DBPCFC and open challenge were classified and recorded on a food challenge score sheet adapted from Nowak et al.(10) Challenges were stopped, treated according to protocol and considered positive when objective symptoms indicative for an allergic reaction occurred (i.e. rash, urticaria, angioedema, rhinitis, conjunctivitis, vomiting, diarrhoea, dyspnoea, wheezing and hypotension). Oral allergy symptoms (OAS), nausea, abdominal pain, change in behaviour were referred to as subjective symptoms. If subjective symptoms were severe and lasted for more than 45 minutes the challenge was considered positive. If doubtful symptoms (i.e. intermittent or transient oral itching or one or two urticae or abdominal pain) the outcome was considered inconclusive. Children were observed for two hours (no symptoms) or four hours (experiencing symptoms) after the last dose given during challenge.

The definitive diagnosis of peanut allergy was made with panel diagnosis after DBPCFC, open challenge and follow-up. An expert panel of three independent experienced allergologists, blinded for patient characteristics and sensitization tests individually classified children as peanut allergic or tolerant and determined severity of symptoms (by Sampson grade 1 - 5) and eliciting dose based on all available information of DBPCFC, open challenge and the follow-up period. In case of no consensus a face-to-face panel discussion meeting was held until consensus among all experts was reached.

Introduction at home

Children with a negative or inconclusive open food challenge introduced peanut at home during an introduction period of one month. This introduction consisted of a seven day introduction schedule, telephonic counselling with a physician (once a week) and a diary for the child and parents. Challenge outcome, de-blinding of the challenge material and the introduction protocol were explained to parents during a telephonic consult 24-48 hours after the second challenge day. The introduction schedule consisted of 5 steps with increasing amounts of peanut butter (or an equivalent) and subsequently two steps with 5 g and 10 g of peanuts (2.5 g peanut protein). After this introduction schedule children were encouraged to ingest products with peanut each week. The date and time, products with peanut consumed and symptoms of each introduction step and symptoms during follow-up were reported in the diary and recorded during weekly telephonic consults. Telephonic consults were performed by a trained researcher supervised by a dietician and an allergologist. During telephonic consults parents and

children were motivated, offered alternative products with peanut when necessary and coached if symptoms occurred. If introduction was not possible during the introduction period due to illness or other practical reasons the introduction was postponed and the follow-period extended. After the introduction period was completed, parents received a questionnaire about the child's current diet, reading labels regarding peanut and other foods, opinion about the food challenge and concern of an accidental reaction. The questions (1) "Are you convinced about the negative challenge outcome?" and (2) "Are you worried about a peanut allergic reaction at home / outdoors?" were scored on a 10 point likert scale and scored positive if parents scored > 8 points (1) and > 2 points (2). Successful introduction in to the diet was defined as the child was eating products containing peanut as ingredient on a regular basis at the time of the follow-up questionnaire.

Statistical methods

The outcome of open challenges and introduction was evaluated with descriptive statistics. Characteristics of children with and without introduction failure and symptoms during introduction were compared using the X_2-statistic for proportions and the Student's T-test or nonparametric Mann-Whitney U test for continuous data when appropriate. Analyses were performed using SPSS for Windows (version 21) and a p-value < 0.05 was considered statistically significant.

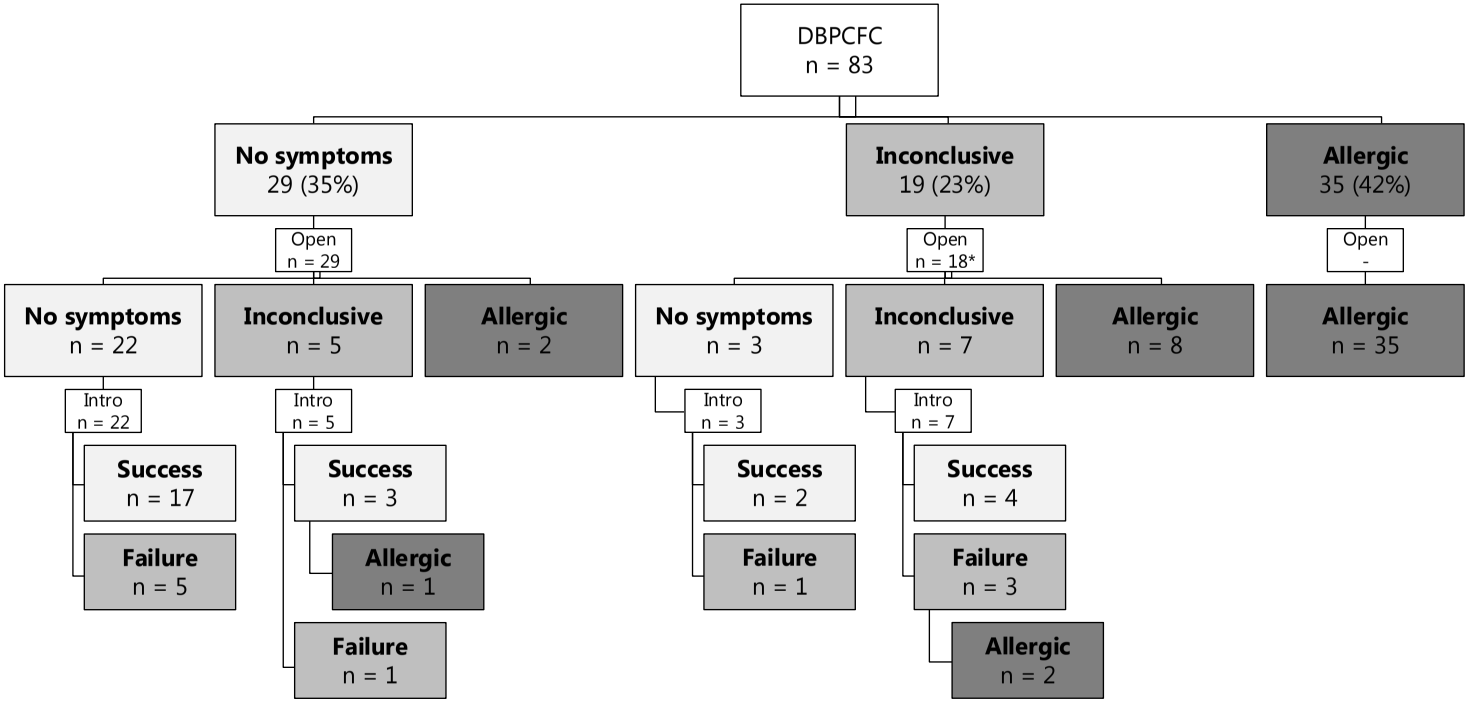
Table 1 Baseline characteristics, n = 83

Age, mean (SD)	8.4 (3.5)
Gender (male), n (%)	58 (70)
Atopy, n (%)	
Eczema	47 (57)
Food allergy	61 (74)
Asthma	40 (48)
Hay fever	48 (58)
Previous reaction, n (%)*	
Never ingested peanut	25 (30)
Grade 1	9 (11)
Grade 2	12 (15)
Grade 3	5 (6)
Grade 4	23 (28)
Grade 5	2 (2)
Contact	7 (8)
Sensitization to peanut (median IQR)	
sIgE peanut extract in kU_A/L	3.2 (0.8-17.1)
sIgE Ara h 2 in kU_A/L	0.98 (0.1-8.7)
SPT in mm	8.0 (6.0-11.2)

SPT, Skin Prick Test; SD, Standard Deviation

* According to the Sampson grade of anaphylaxis (11)

Figure 1 Flowchart of the outcome of DBPCFC, open challenge and introduction



DBPCFC, Double Blind Placebo Controlled Food Challenge; Intro, introduction protocol started
* In n = 1 the second challenge day and open challenge was cancelled due to poor asthma control

RESULTS

Study population

In the original study we included 83 children with suspected peanut allergy with a mean (SD) age of 8.4 (3.5) years. A previous reaction to peanut was reported in 58 (70%) children; the other children never ingested peanut before. Children were clearly atopic and often had eczema (57%), asthma (48%), allergic rhinitis (58%) or another food allergy (58%). Baseline characteristics of all children are shown in table 1.

The added value of open challenges

The DBPCFC was positive in 35/83 (42%) and negative in 29/83 (35%) children. The DBPCFC was inconclusive in 19/83 (23%) children due to symptoms on peanut and / or placebo. Symptoms considered inconclusive were: oral itching (n = 7), abdominal pain (n = 7), change of behaviour (n = 2), itch in face (n = 1) and symptoms after discharge (n = 3). In one child with a negative peanut day the second challenge day and open challenge were cancelled due to poor asthma control, this child was considered inconclusive and excluded from further analysis. Allergic symptoms (Sampson grade 2 – 3) during open challenge were observed in 2/29 (7%) children despite negative DBPCFC. In 5/29 (17%) the open challenge was inconclusive due to doubtful symptoms (oral itching and a solitary urtica, n = 2) and due to incomplete ingestion and aversion (n = 3). In children with inconclusive DBPCFC, 8/18 (44%) children developed allergic symptoms during open challenge including two children with severe respiratory symptoms that received epinephrine. In 7/18 (39%) the open challenge was inconclusive due to doubtful symptoms: oral itching (n = 5), eczema and changed stool at home (n = 1) or incomplete ingestion due to aversion (n = 1). Overall the open challenge detected peanut allergy in 10/47 (21%) children with negative or inconclusive DBPCFC. A flow-chart of the results of DBPCFC, open challenge and introduction is shown in figure 1. Characteristics of children with positive open challenge are shown in table 3.

The practical course of introduction

After open food challenge 37/83 (45%) children with an inconclusive or negative outcome entered the follow-up period. The follow-up questionnaire was completed in all but one child, in a median time of 42 (IQR 35-105) days after the last day of food challenge. In 19/37 (51%) children deviation from the original introduction schedule with peanut butter and the use of alternative products (i.e. M&Ms, peanut snacks) was necessary due to aversion for peanut butter or whole peanuts. In 2/37 (5%) children the introduction period was postponed due to a concomitant infection but completed successfully later on. Regular ingestion of peanut at home failed in 10/37 (27%) children (figure 1). Reasons for not introducing were: aversion (n = 4), oral symptoms (n = 3), fear of the child (n = 1), an exacerbation of eczema that was considered peanut related by parents (n = 1) and uncertainty of parents about the challenge outcome (n = 1). In the group with failed introduction 70% of parents were concerned about their child

Table 2 Children with and without introduction failure in the diet

Characteristic, n (%)	**Total (37)**	**Success (27)**	**Failure (10)**	p-value
Age (yrs.) mean, SD	8.46 (3.84)	8.48 (3.94)	8.39 (3.77)	0.853
Gender (male)	27 (73)	20 (74%	7 (70)	0.804
History of asthma	14 (38)	13 (48)	1 (10)	0.034*
History of allergic rhinitis	19 (51)	13 (48)	6 (60)	0.522
History of eczema	21 (57)	16 (59)	5 (50)	0.614
Elimination diet for > 1 other allergen	21 (58)	17 (65)	4 (40)	0.166
Nut free diet	17 (47)	14 (54)	3 (30)	0.199
Sensitization (median, IQR)				
Skin prick test (index)	1.36 (0.70-2.00)	1.33 (0.70-1.93)	1.56 (0.00-2.11)	0.674
sIgE to Peanut	0.76 (0.12-2.73)	0.76 (0.10-2.16)	1.14 (0.34-6.33)	0.448
sIgE to Ara h2	0.10 (0.10-.39)	0.10 (0.10-.39)	0.10 (0.10-1.17)	0.408
sIgE to Ara h8	0.62 (0.10-7.34)	0.31 (0.10-4.03)	5.77 (0.10-12.50)	0.271
History of peanut allergy, n (%)				
No (traces of) peanut in diet	13 (35)	9 (33)	4 (40)	0.706
Lifelong elimination in history	14 (38)	11 (41)	3 (30)	0.550
Severe reaction in history	13 (35)	10 (37)	3 (30)	0.690
Food challenge, n (%)				
Any symptom during DBPCFC (verum)	17 (46)	13 (48)	4 (40)	0.659
Any symptom during DBPCFC (placebo)	12 (32)	11 (41)	1 (10)	0.076
Any symptom during open challenge	12 (32)	8 (30)	4 (40)	0.550
Inconclusive challenge outcome	8 (22)	5 (19)	3 (30)	0.451
Convinced about challenge outcome[q]	28 (78)	20 (77)	8 (80)	0.842
Introduction, n (%)				
Aversion and deviation from schedule	19 (51)	16 (59)	3 (30)	0.114
Peanut related symptoms during introduction	11 (30)	6 (22)	5 (50)	0.101
Worried about exposure to peanut at home[q]	12 (33)	5 (19)	7 (70)	0.005*
Worried about exposure to peanut outdoors[q]	16 (44)	9 (35)	7 (70)	0.068
Buying and cooking different products[q]	22 (61)	19 (73)	3 (30)	0.018*
Reading food labels for peanut[q]	21 (58)	13 (50)	8 (80)	0.102

[q] Questionnaire missing n = 1

* Significant difference between failure and success group, $p < 0.05$

Table 3 Children with allergic symptoms during open challenge (n = 10) or introduction (n = 3)

ID	Age	AR	EC	sIgE pn	sIgE Ara h2	sIgE Ara h8	Birch	DBPCFC verum	DBPCFC placebo	Open FC	Symptoms open FC	Intro	Sampson	Peanut in diet
75	3.96	Yes	Yes	12.57	6.37	.77	4.70	ABP , tired	-	+	Vomiting, 1 urtica, wheeze	-	4	No
20	13.08	Yes	No	5.13	3.46	.66	43	-	-	+	ABP severe	-	2	No
23	5.43	No	Yes	2.06	1.39	10.71	12.50	Itch	-	+	Urticaria, rhinitis	-	3	No
29	11.15	Yes	Yes	22.79	23.01	12.24	37	ABP	-	+	Rhinoconjunctivitis, ABP	-	2	No
30	12.59	Yes	Yes	.68	.77	.53	2.17	OAS	-	+	OAS, feeling throat tightness	-	3	No
33	6.65	No	No	.39	.31	.19	7.30	OAS	-	+	Itch, swollen lip, 1 urtica	-	1	No
35	8.45	Yes	Yes	2.37	1.06	12.32	101	OAS, ABP	Rhinorrhoea	+	OAS, rhinitis	-	2	No
37	5.42	Yes	No	1.88	1.78	8.40	101	-	OAS	+	Rhinoconjunctivitis ABP	-	3	No
57	12.19	Yes	Yes	1.80	.68	4.52	34.50	ABP	-	+	OAS, urticaria, abp, dyspnoea	-	4	No
60	6.82	Yes	Yes	6.94	4.56	.10	5.30	Nausea, tired	OAS	+	Vomiting	-	2	No
25	6.32	Yes	No	.10	.10	1.21	41	-	OAS	+/-	OAS	OAS recurrent	1	Yes
80	10.81	Yes	Yes	5.50	4.80	18.50	101	OAS, nausea, ABP, sub dysp.	-	+/-	OAS	OAS recurrent	1	No
69	16.98	Yes	No	.37	.23	.10	.05	OAS	-	+/-	OAS	OAS recurrent	1	No

ABP, Abdominal pain; AR, Allergic Rhinitis in history; EC, Eczema in history; FC, Food challenge; OAS, Oral allergy symptoms; pn, Peanut; Sub Dysp, feeling dyspnoeic

being exposed to peanut compared to 19% of parents in the group with successful introduction, p = 0.005. Furthermore parents of children with failed introduction less often changed their habits with respect to buying and preparing meals (30% and 73%, p = 0.018) compared to children with introduction success. Nevertheless, parents of both groups were equally convinced about the challenge outcome (80% and 77%, p = 0.842). A fair amount of parents in both the failure and the success group reported to check food labels for peanut during follow-up (80% and 50%, p = 0.102). Children who failed introduction less often had asthma compared to children who succeeded introduction (10% and 74%, p = 0.034). No other differences with respect to patient characteristics (age, gender, allergic comorbidities, presence and duration of elimination diets), peanut sensitization and symptoms during food challenge were found between children with and without introduction failure (table 2).

Peanut related symptoms during introduction

Peanut related symptoms during introduction were reported in 11/37 (30%) children. Three children 3/37 (8%) reported repetitive and persistent oral itch within 5 minutes after all peanut ingestions in their diary and during telephonic consults. Those children were considered allergic by the expert panel. One of those children continued with regular ingestion of peanut during follow-up and therefore introduction was judged as successful. Other reported symptoms were: an exacerbation of eczema for several days (n = 2), (peri-) oral symptoms (n = 3), abdominal pain (n = 2), wheezing (n = 1). Those symptoms occurred once only or were transient and therefore were not considered allergic by the expert panel. No severe symptoms occurred and treatment was given in only one child (topical corticosteroids). Comparison of children with and without symptoms during introduction yielded no differences in patient characteristics, sensitization to peanut, symptoms during food challenge or timing and the course of introduction (data not shown).

Peanut allergy despite negative or inconclusive DBPCFC

Face-to-face panel discussion after DBPCFC, open challenge and introduction to determine peanut allergic status was necessary in 39 (48%) children. The expert panel classified 34 (41%) children as peanut tolerant and 48 (58%) children as peanut allergic. Open challenge and introduction were necessary to diagnose peanut allergy by the expert panel in 13/47 (16%) children with negative or inconclusive DBPCFC outcome. Characteristics and symptoms of those children are presented in table 3. Children that were considered peanut allergic (despite negative or inconclusive food challenge) more often reported (subjective) symptoms (77% and 24%, p < 0.001) during peanut day of DBPCFC compared to children that passed the open challenge and introduction. However, no difference in type, severity or frequency of those symptoms was found. Children considered allergic after open challenge or introduction had higher levels of sIgE to peanut (median (IQR): 2.2 (1.8-6.9) and 0.8 (0.1-2.7), p = 0.043) and Ara h 2 (1.6 (0.8-4.6) and 0.1 (0.1-0.4), p < 0.001) compared to tolerant children. No difference with

respect to other patient characteristics was found between children with and without peanut allergy after open challenge or introduction (data not shown).

DISCUSSION

This is the first prospective study that investigates the course and success rate of open challenges and standardized, carefully monitored introduction of peanut after DBPCFC. The results of our study indicate that open challenges are positive in 10/47 (21%) of children with negative or inconclusive DBPCFC. Our results are in line with two previous studies that reported 3-13% children with false-negative DBPCFC that appeared allergic after a subsequent open challenge.(12,13) Moreover, in this study the introduction was useful to confirm peanut allergy in 3/37 (8%) children with negative or inconclusive open challenge. The DBPCFC has several limitations that can explain false-negative outcomes.

Subjective symptoms like abdominal pain or oral itch can be attributable to aversion and therefore are difficult to interpret.(14,15) During challenge children are in stable condition and co-factors that can trigger allergic symptoms are minimized, in contrast to daily life.(16) It could be suggested that allergic reactions during DBPCFC are delayed or masked due to the matrix of the food ingested (ginger bread with relatively high fat content).(17) The induction of short-term tolerance during the up climbing doses of DBPCFC could also have concealed (severe) allergic symptoms during DBPCFC and explain positive open challenges or positive introductions.(18,19) Open challenges were performed on day two for logistic reasons, as a result children randomized for placebo/peanut received a higher cumulative dose of peanut on the second challenge day. Although not significant, a slightly higher percentage of positive open challenges occurred in this randomization group (placebo/peanut 27% and peanut/placebo 14%, $p = 0.293$). Although the total amount of peanut protein administered during open challenge and DBPCFC was comparable, it could be that in children with positive open challenges a higher top dose of DBPCFC was necessary to elicit symptoms.

Current guidelines mention the importance of the administration of a cumulative dose that reflects daily life during challenges but there is no clear recommendation for the procedure after negative or inconclusive DBPCFC.(3,20) Our results demonstrate the importance of a subsequent open challenge and guided introduction with portions up to at least 10 gram of whole peanuts to detect false-negative DBPCFC. If only subjective symptoms occur it is necessary to continue the challenge and/or start introduction to accurately diagnose or exclude peanut allergy. In our study this was a safe procedure as objective symptoms during open challenge were treated successfully and only very mild symptoms occurred at home.

Despite negative DBPCFC 10/37 (27%) children did not introduce peanut in the diet on a regular basis. The failure rate of introduction in the diet is comparable with that in studies not applying a standardized guided introduction.(6–8) In both the failure and

the introduction group a fair amount of parents reported that they did not change their shopping and cooking habits and continued to check food labels. This suggests that even extensive counselling during one month after DBPCFC was not effective in preventing introduction failure. The high rates of parents that worry about exposure suggest that families still have fear for a possible allergic reaction.

Aversion was also a common problem despite the offering of alternative products with hidden peanut. This can be caused by the unfamiliar taste and structure of (products with) peanut but also due to picky behaviour and fear of the child.(21) It could be that parents and children need other coaching techniques to reduce fear and reintroduce peanut. The usefulness of psychological guidance or further assistance by a dietician to encourage parents and children should be investigated.

A limitation of this study is that follow-up time was relatively short. Parents and children could need more time to get used to the introduction of peanut. Moreover, we studied a relatively small group of patients which did not allow us to perform multivariate analysis and determine the independent effect of predictors on introduction failure and symptoms after DBPCFC. Future studies should focus on the long term follow-up of included patients to investigate the development of tolerance and introduction success. Moreover further data of the effect of (long-term) elimination diets on the recurrence or development of peanut allergy is needed to give parents a thorough advice about the necessity to exposure their child to peanut after negative food challenge.

CONCLUSION

In conclusion this study shows that open challenges and subsequent introduction are indispensable to exclude or diagnose peanut allergy accurately in children with negative or inconclusive DBPCFC outcomes. Introduction after negative or inconclusive peanut challenges should be monitored to detect (peanut related) problems early.

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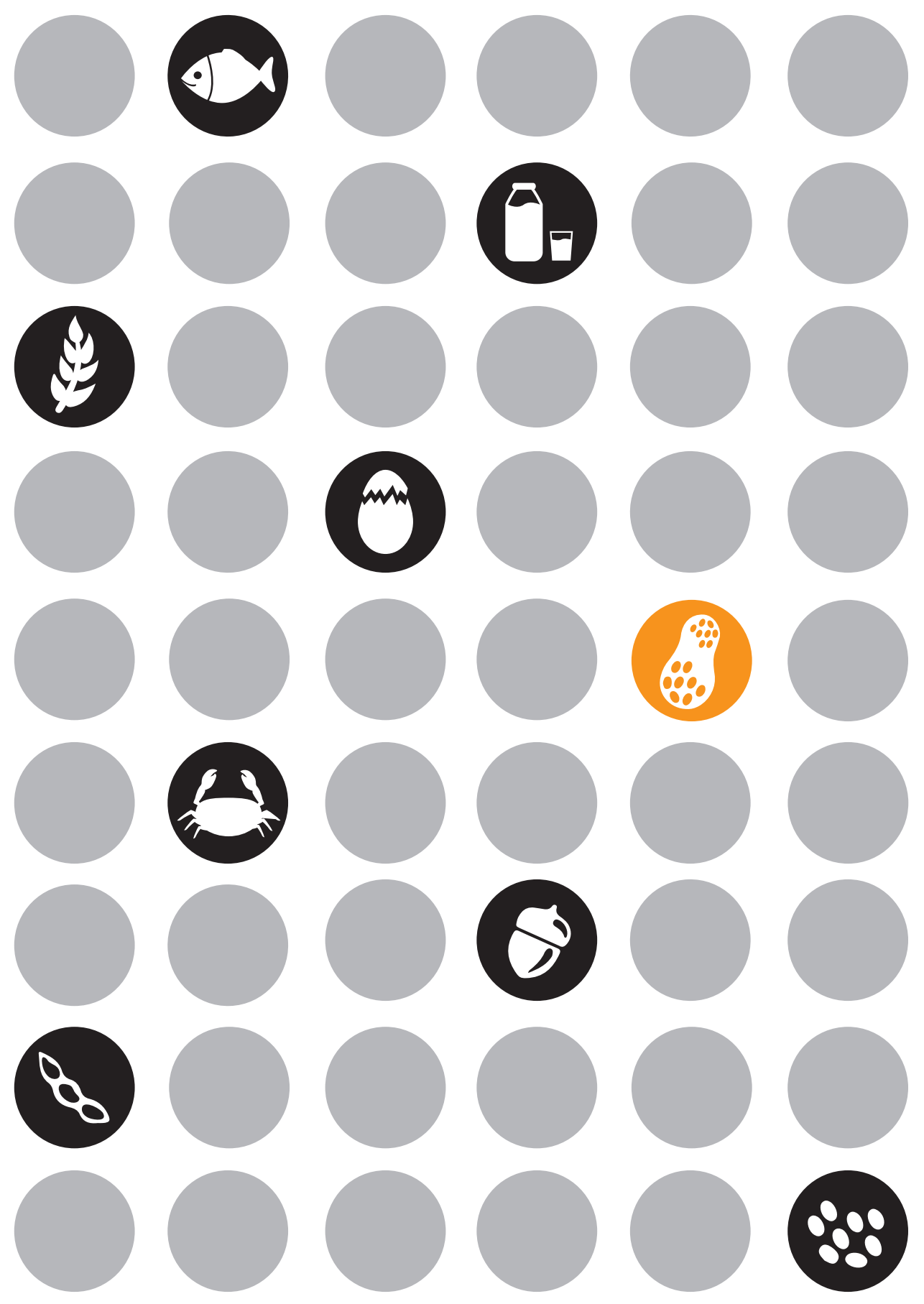

CHAPTER 10

Reintroduction failure after negative peanut challenges in children

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Francine C. van Erp
Jenneke Boot
André C. Knulst
Suzanne G.M. Pasmans
Cornelis K. van der Ent
Yolanda Meijer

ABSTRACT

Background

A negative double blind placebo controlled food challenge (DBPCFC) should normally be followed by reintroduction of the food. However, reintroduction fails in a subset of children. The observed reintroduction problems could be due to refusal of the food that long has been avoided, to other behavioural / psychological factors or to false negative DBPCFC outcome. This study analyses the frequency, causes and risk factors for reintroduction failure in children after negative peanut DBPCFC.

Methods

A retrospective study of children with a negative DBPCFC for peanut was performed. During follow-up after DBPCFC parents were systematically interviewed about the current diet, symptoms and problems during reintroduction and reactions to peanut after the reintroduction period. Successful reintroduction was defined as eating peanut or products containing peanut as ingredient on a regular basis.

Results

Follow-up data were obtained in 103 children with a negative peanut challenge. In 70 (68%) children reintroduction was successful (54 children tolerated peanut, 16 children tolerated peanut as ingredient). Reintroduction failed in 33 (32%) children. Food refusal (45%) and peanut related symptoms (33%) were the most reported reasons. Risk factors for reintroduction failure were an elimination diet for more than three other foods ($p=0.019$), a long elimination diet for peanut ($p=0.048$) and peanut related symptoms at home ($p=0.002$).

Conclusion

Reintroduction failure is a common problem in children after negative peanut challenge. To guide reintroduction and identify potential peanut related symptoms at home, careful follow-up after negative DBPCFC is advised. When symptoms occur or persist, food challenge outcome needs to be reconsidered.

INTRODUCTION

Children suspected of peanut allergy often present with a long-lasting elimination diet. To maintain this diet, parents of peanut allergic children change their way of life. They cautiously read labels, have to be alert when buying food and cooking, have to arrange a safe environment at school, being at family and friends or during holidays. Children are faced not only with dietary but not infrequently also with social restrictions.(1) In line with this, the majority of parents of children with suspected food allergy show high levels of anxiety about a potentially life treating reaction.(2) Moreover, quality of life in adults and children suffering from food allergy is impaired. (3)To prevent unnecessary (psychosocial) burden on families with food allergic children, it is therefore important to diagnose the culprit foods accurately.

To confirm food allergy, a double blind placebo controlled food challenge (DBPCFC) is considered to be the gold standard.(4) If no objective symptoms or severe or long lasting subjective symptoms to one of the active doses occur, a food challenge is considered negative and reintroduction of the challenged food is advised. However, there is evidence that reintroduction fails in a subset of children. Failure rates up to 25% were reported and even higher rates in children with previous suspected peanut allergy were found.(5,6) The observed reintroduction problems could be due to refusal of the food that long has been avoided or other behavioural / psychological factors. On the other hand, failure of reintroduction (especially when symptoms occur) could indicate lack of diagnostic accuracy and false negative outcome of the food challenge.

There is limited data on the success rate of reintroduction and risk factors for failure in a large and unselected group of patients with negative DBPCFC for peanut. We studied the variety of problems observed after reintroduction and aimed to identify risk factors of failure in children with negative peanut challenge outcome.

METHODS

Study population

We performed a retrospective study of children who underwent a DBPCFC for peanut in our tertiary allergy centre from 2008 – 2010. DBPCFC's were performed in all eligible children except for children with reactions requiring previous admission to an intensive care unit. Suspicion for peanut allergy was based on sensitization (a raised specific IgE (sIgE) or positive Skin Prick Test (SPT) to peanut) together with a history of (1) symptoms after ingestion of peanut (2) no previous known ingestion of peanut due to severe eczema or other food allergy or (3) allergic reaction with unknown origin. Children with a negative DBPCFC and available follow-up data were selected for this study. Data were collected as part of regular patient care, obtained by chart review and used in strictly anonymous form, according to the code of conduct for medical research approved by the hospital's Medical Ethical Committee.

Peanut challenges

The DBCPFC protocol was described earlier by Flinterman et al.(7) In short, increasing amounts of defatted peanut flour (50% peanut protein) from 0.01 to 3000 mg, were given with time-intervals of 15-30 minutes with randomly dispersed placebo's. An open challenge of 10 g peanuts was the last step in the protocol. Patients were observed for at least 2 hours. Challenges were discontinued and considered positive in case of objective symptoms or if suggestive subjective symptoms occurred at 3 subsequent doses or a severe subjective symptom lasted for more than 45 minutes. Oral allergy symptoms (OAS), abdominal pain and nausea were referred to as subjective symptoms. Objective symptoms were classified according to the Sampson classification (8). All signs and symptoms during challenge were recorded on a food challenge score sheets including timing and administration of doses. Twenty four hours after negative food challenge families were contacted by telephone. If no late symptoms occurred, parents were told to reintroduce peanut by means of a reintroduction schedule with increasing amounts of peanut (figure 1). The reintroduction schedule was used to assist parents and children in the reintroduction process and not (as physicians explicitly explained to parents) because peanut allergy was still suspected. After finishing the reintroduction schedule parents were advised to quit label reading regarding peanut and administer products containing peanut whenever they wanted.

Figure 1 Reintroduction schedule

Day	Product
1	Traces allowed*
2	Traces allowed*
3	½ sandwich with peanut butter
4	1 sandwich with peanut butter
5	2 sandwiches with peanut butter
6	5 peanuts
7	10 peanuts

* Product labelled with "May contain peanuts" or "Manufactured on a shared line with products containing peanut"

Follow-up after DBPCFC

Up to 4,5 years after the negative DBPCFC, parents were systematically interviewed by their physician based on a predefined questionnaire. The telephonic questionnaire contained questions about the current and previous diet concerning peanut and other allergens, timing and success of reintroduction and symptoms after peanut ingestion (supplementary figure A). Successful reintroduction of peanut was defined as the child eating peanut (e.g. whole peanuts, peanut butter) or products with peanut as ingredient (e.g. cookies or snacks) on a regular basis and no food labels were checked. We considered reintroduction as failed when children did not introduce peanut or only

introduced products containing possible traces of peanut e.g. labelled as "May contain peanut" or "Manufactured on a shared line with products containing peanut", since in this case it is unclear whether there is actual consumption of peanut.

Statistical analysis

Analyses were performed using SPSS for Windows (version 20.0 SPSS Inc., Chicago, IL, USA). Patient and reintroduction characteristics between children with successful and children with failed reintroduction were compared using univariable and multivariable logistic regression analysis. Variables significantly associated with failure in the univariable model were entered in a multivariable model to determine independent risk factors for reintroduction failure. A p-value < 0.05 was considered significant.

RESULTS

During the study period a total of 219 peanut challenges were performed of which 110 children had negative food challenge outcome. Follow up data were obtained by telephonic interview in 103 (94%) children. Patient characteristics of included children are shown in table 1. Time between interview and food challenge ranged from 1.4-4.4 years, with a mean (SD) time of 3.0 (0.90) years. Median age of children at food challenge was 7.1 years (range: 3.4 – 17.3 years). The study population consisted of predominantly boys (75%) and another food allergy was often present (76%). The majority of children had a long elimination diet for peanut (82%) and no previous history of peanut related symptoms (59%).

Outcome of reintroduction

Parents of 45 (46%) children confirmed that they used the reintroduction schedule during follow-up. Overall 70 (68%) of children successfully reintroduced peanut in their diet, including 54 (52%) children who consumed peanut and 16 children (16%) who consumed products with peanut as ingredient. The latter did not tolerate whole peanuts due to the taste / structure of peanut. Reintroduction failed in 33 (32%) children, parents of those children were still reading food labels for peanut. Thirty children (29%) with introduction failure did introduce products with (possible) traces of peanut, three children (3%) also eliminated those products.

Reasons for reintroduction failure

Despite the instructions after DBPCFC reintroduction was not started at all in 7 (7%) children. In 26 (25%) children reintroduction failed in the weeks after starting. Reported reasons for reintroduction failure are shown in table 2. Refusal of peanut (n = 15) was the most reported reason for failure. Further comparison of children with and without refusal revealed no significant differences with respect to patient characteristics, level of sIgE to peanut, severity of previous reaction, duration of previous peanut free diet, presence of other elimination diets or the presence of symptoms during challenge.

Table 1 Characteristics of children at time of challenge and during follow-up, n = 103

	Total (103)	**Failure** (33)	**Success** (70)	p-value	Adjusted p-value*
Median age [IQR] at food challenge in yrs.	7.1 [4.8-9.4]	7.6 [5.0-9.8]	6.8 [5.4-9.7]	0.740	
Mean time (± SD) between FC and Q in yrs.	3.0 ± 0.90	2.7 ± 0.81	3.2 ± 0.91	**0.019**	**0.039**
Male	77 (75)	24 (73)	53 (76)	0.745	
Atopy					
Eczema	84 (82)	56 (80)	56 (80)	0.554	
Hay fever	44 (43)	14 (42)	30 (43)	0.967	
Asthma	35 (34)	12 (36)	33 (33)	0.726	
Other food allergy	78 (76)	25 (76)	53 (76)	0.996	
Median sIgE to peanut [IQR] in kU_A/L	0.68 [0.35-2.44]	1.30 [0.38-2.80]	0.60 [0.38-2.80]	0.409	
Ara h2[1]	0.09 [0.03-0.42]	0.91 [0.04-2.64]	0.06 [0.02-0.18]	0.132	
Ara h8[1]	0.18 [0.01-1.26]	0.08 [0.01-1.59]	0.19 [0.00-1.19]	0.925	
Positive SPT[2]	49/69 (71)	14/17 (82)	35/52 (67)	0.358	
History of peanut allergy					
No previous known ingestion	60 (59)	19 (58)	41 (59)	Ref	
Previous reaction mild/moderate	30 (29)	11 (33)	19 (27)	0.636	
Previous reaction severe	13 (13)	3 (9)	10 (14)	0.543	
Duration peanut free diet before FC					
0 - 2 year	19 (18)	2 (6)	17 (24)	Ref	
> 2 year	84 (82)	31 (94)	53 (76)	**0.026**	**0.048**
Elimination diet for other food					
None	27 (26)	5 (15)	22 (31)	Ref	
1-2 other foods	53 (52)	16 (49)	37 (53)	0.152	
> 3 other foods	23 (22)	12 (36)	11 (16)	**0.015**	**0.019**
Current elimination diet for tree nuts	46 (45)	19 (58)	27 (39)	0.070	
Symptoms during food challenge[3]	39 (38)	16 (49)	23 (33)	0.129	
Refusal during challenge	9 (9)	3 (9)	6 (9)	0.931	
Reintroduction schedule used[4]	45/97 (46)	16/33 (49)	29/64 (45)	0.767	
Reported symptoms after challenge	21 (20)	13 (39)	8 (11)	**0.003**	**0.002**
Current diet					
No traces of peanut	3 (3)	3 (9)	-	-	
Products with traces of peanut[5]	30 (29)	30 (91)	-	-	
Products with peanut	16 (16)	-	16 (23)	-	
Peanut	54 (52)	-	54 (77)	-	

FC, Food Challenge; SD, Standard Deviation; IQR, Interquartile range; Ref, reference category.
Boldface variables were significantly related to reintroduction failure and entered in the multivariable logistic regression model.
[1] Missing n = 62 (60%) [2] Missing n = 34 (31%) [3] Transient subjective symptoms (not feeling well, change in behaviour) [4] Missing n = 6 (6%) [5] Product labelled with "May contain peanuts" or "Manufactured on a shared line with products containing peanut" * After multivariable logistic regression analysis

Table 2 Reported reasons for reintroduction failure, n = 33

	Not started reintroduction (7)	Started reintroduction (26)	Total (%) (33)
Fear of a reaction	1	1	2 (6)
Not convinced by test	1		1 (3)
Allergy family	2		2 (6)
Refusal	2	13	15 (45)
Habituation to the peanut free diet	1		1 (3)
Symptoms after ingestion of peanut		11	11 (33)
Autism child		1	1 (3)

Peanut related symptoms after negative food challenge

During follow-up 21 (20%) parents reported peanut related symptoms.. Based on the type of reported symptoms (Sampson grade 1 – grade 5), time between ingestion and reaction (0-30 minutes) and confirmation that the food contained peanut as ingredient, we suspected 13/21 children for having allergic reactions to peanut during follow-up. In those children reactions were often recurrent, the median number [IQR] during follow-up was 5 [3-10]. Severity of reactions ranged from Sampson grade 1 (oral allergy or localized angioedema) up to Sampson grade 4 (dyspnoea). The estimated peanut dose ingested did never exceed the maximum dose given during peanut DBPCFC. Symptoms developed most often at home in presence of parents. Parents of 6/13 children treated symptoms with antihistamines, in one case they also visited a doctor for dyspnoea. Allergic reactions to peanut were reason to avoid (products with) peanut in 9/13 children. Parents of 4/13 children continued to administer their child (products with) peanut, reasons not to avoid peanut were the presence of mild symptoms only (n = 2) or absence of symptoms after specific products containing (small amounts) peanut as ingredient (n = 2). No significant differences in patient characteristics, severity of previous reaction to peanut or level of sIgE were found between children with and without suspected peanut allergic reactions after challenge. Details of children with suspected peanut allergic reactions are shown in table 3. In 8/21 children, reported symptoms were considered nonspecific due to the type of symptoms (abdominal pain, eczema), time between ingestion and reaction (>60 minutes) or the ingestion of food without a substantial amount of peanut (traces). Nevertheless, parents of 3/8 children avoided (products with) peanut due to the nonspecific symptoms.

Potential risk factors for reintroduction failure

Univariable logistic regression analysis of patient characteristics showed that several patient and diet related factors were associated with reintroduction failure (table 1). In the multivariable model, time between food challenge and questionnaire (in years) p = 0.039, an elimination diet for more than three other foods (vs.0-2 eliminated foods) p = 0.019, a longer than 2 year elimination diet for peanut (vs.0-2 year elimination) p = 0.048 and the presence of peanut related symptoms during follow-up p = 0.002, were

Table 3 Children with suspected peanut allergic reactions, n = 13

Pt	Reactions (nr)	Symptoms	Grade[1]	Timing (min)	Label of food ingested	Location	Action taken	Current diet
12	20	Ang	1	5	Peanut as ingredient	Home	AH	Traces
13	2	Dys	4	-	Peanut	School	AH + doctor	Traces
30	3	Urt	2	2	Peanut	Home	AH	Traces
44	8	Abd, OAS	1	30	Peanut	Home	None	Peanut
47	30	OAS	1	10	Peanut as ingredient	Home	None	Traces
52	14	OAS	1	5	Peanut	Home	None	Peanut
68	-	OAS	1	10	Peanut as ingredient	Home	None	Peanut as ingredient
69	4	OAS	1	1	Peanut	Home	None	Traces
75	8	Ang, Dys, OAS, Thr	3	1	Peanut	Home	AH	Traces
77	3	Ang, Ecz, OAS	1	5	Peanut as ingredient	Home	AH	Traces
83	4	Thr,	3	5	Peanut as ingredient	Home	AH	Peanut as ingredient
90	-	Urt	2	5	Peanut	Home	None	Peanut as ingredient
91	5	Abd, Con, Rhi	2	10	Peanut	School	AH	Traces

Abd, abdominal pain; AH, antihistamines; Ang, angioedema; Con, conjunctivitis; Dys, dyspnoea; Ecz, eczema; Min, minutes; OAS, oral allergy syndrome; Pt, patient; Rhi, rhinitis; Thr, Throat tightness; Urt, urticaria;
[1] Sampson classification of anaphylaxis (8)

significantly associated with reintroduction failure. Children with reintroduction failure also tended to have an accompanying nut free diet more often although no significant association could be found. Other patient characteristics as age, atopic diseases, sensitization or occurrence of subjective symptoms during food challenge could not be identified as potential risk factors for reintroduction failure.

DISCUSSION

This study shows that reintroduction failure is a common problem in children after negative peanut challenge. Despite negative challenge outcome and the advice to start reintroduction, one third (32%) of the children were eliminating products with (traces of) peanut during follow-up. Moreover, parents of 13 (13%) children were suspected of (recurrent) peanut allergic reactions after peanut ingestion at home.

Our study shows that several patient related factors are associated with reintroduction failure. The presence of an elimination diet for more than three other foods was significantly associated with failure. In addition, children with reintroduction failure had a longer elimination diet for peanut in history. They also tended to have an accompanying nut free diet more often although no significant association could be found. This is in contrast with a previous small study about follow-up of challenges who could not identify risk factors for failure, possibly because only seven children with negative peanut challenge were included.(5) Seven parents did not start reintroduction at all and a subset was still concerned about the presence of peanut allergy. Practical difficulties, fear and uncertainty about the diagnosis were reported as the main reasons for not starting. These (parents of) patients continued to carefully read labels of products possibly containing peanut, suffer from dietary and social restrictions and may still have an impaired quality of life. Our results underline that changing dietary habits with regard to peanut avoidance needs more guidance and counselling than a negative challenge outcome and a reintroduction schedule alone, especially in patients with other dietary restrictions. Moreover, prior to the challenge parents should be questioned about their expectations of the challenge and the reintroduction procedure should be emphasized. Refusal of the child was the most important reported reason for reintroduction failure. Refusal could not be explained by patient related factors as young age, a previous long elimination diet or the presence of other dietary restrictions. Although refusal could be a sign of intolerance (e.g. OAS) we did not find differences in level of sensitization, occurrence of subjective symptoms during challenge or peanut related symptoms during follow-up between children with and without refusal. It is therefore most likely that these children have difficulties with the unfamiliar taste and structure of (products with) peanut. This is also demonstrated by the 16 children with reintroduction success who avoided whole peanuts and only consumed products with peanut as ingredient. It is debatable whether the prolonged peanut free diet in children with reintroduction failure due to aversion has a negative impact on quality of life as parents might well be satisfied with the negative challenge outcome itself.(2,9) Nevertheless, regular ingestion of (products containing) peanut should be encouraged because previously published data suggest that long term avoidance of allergen exposure might result in the occurrence of acute allergic reactions.(10)

Among the reported reasons for reintroduction failure were also symptoms after the ingestion of peanut at home, possibly representing the presence of peanut allergy despite a negative food challenge. It is unlikely that the ingested amount of peanut during challenge was insufficient as was suggested by previous authors (11), because an open provocation with 10 g whole peanuts was the last step of our DBPCFC protocol. Although all challenges were performed according to international guidelines in an expertise centre, very mild allergies could have been missed and resulted in a false negative food challenge.(12) Subtle subjective symptoms during challenge could have been only one end of the range of reactions (the very mild one) in the child's food allergy and therefore do not correspond to reactions observed during reintroduction at home

.(13) We could, however not find a significant association between the occurrence of mild transient symptoms during challenge and reintroduction failure. It has also been suggested that symptoms after negative peanut challenge can be caused by recurrence of peanut allergy and that food challenges with increasing amounts may induce specific oral tolerance that is only transient.(14–17) We cannot exclude or confirm this, because we do not have information about the exact timing of reactions, amount of peanut ingested during follow-up and (change in) level of sIgE to peanut. Moreover, in the future these children should be re-challenged to objectify symptoms and verify the presence and severity of a possible recurrent peanut allergy.

When interpreting the results of this study some limitations have to be discussed. This retrospective study is based on telephonic interviews after a relatively long follow-up time. Due to the retrospective nature we do not have data on the exact timing of failure. Recall bias cannot be ruled out as parents of children with reintroduction failure may have reported symptoms and reintroduction problems in the past more accurate compared to children with successful reintroduction. Follow-up time was significantly shorter in children who failed to reintroduce peanut (2.7 versus 3.2 years). We think however that this difference is unlikely to be clinically relevant as the minimum follow-up time of both groups was 1.4 years, which should be enough to accomplish reintroduction. The questionnaire used in this study was not validated but composed according to daily clinical practice. We cannot rule out that the content and way of asking may have influenced the results or our study. Furthermore, as is mentioned above peanut related symptoms after negative challenge were parent reported and unfortunately not objectified with re-challenges at the time of writing.

CONCLUSION

In conclusion, this study shows that despite negative peanut challenge continuation of the elimination diet occurred in a significant proportion of children. This might be due to patient related factors as refusal and the presence of other elimination diets or to (parent reported) peanut related symptoms after challenge. To guide parents and children and monitor the occurrence of symptoms, follow-up after food challenges should be intensified. When typical food allergic symptoms re-occur food challenge outcome needs to be reconsidered.

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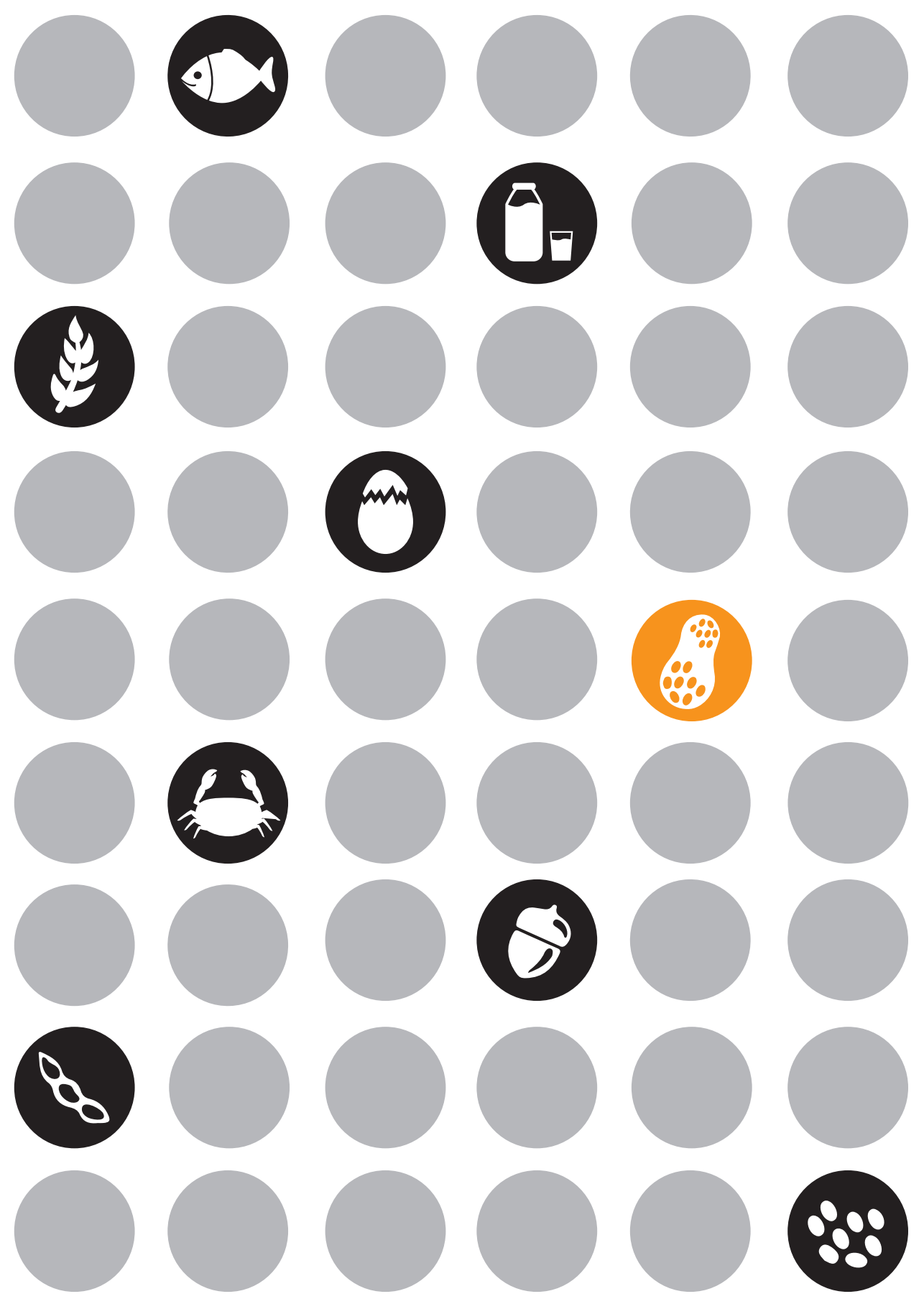
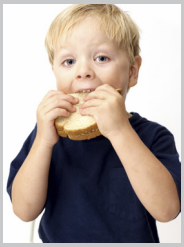


CHAPTER 11

General discussion

Box 1 Clinical examples



Tom is an eight year old boy with asthma. After eating a sandwich with peanut butter at age four, he had difficulties breathing and nearly chocked. Until the outpatient visit his family carefully checked labels, always carried an auto-injector and avoided eating in restaurants. His double blind placebo controlled food challenge (DBPCFC) was negative but during the open challenge he had oral itching, abdominal pain and vomited within minutes after the ingestion of 10 gram of whole peanut. He was diagnosed with peanut allergy and advised to avoid whole peanuts and products with peanut as ingredient.

Noa is a four year old girl with a history of cow's milk allergy and she is a picky eater. She did not like the smell of peanuts and developed abdominal pain after the first introduction of peanut butter. Parents are afraid of a severe peanut allergy. The peanut challenge was completed but inconclusive due to intermittent abdominal pain and nausea during both challenge days and aversion during the open challenge. However, guided introduction was started and successful without any symptom.

Emma is a six year old girl who has never ingested peanut before because she has atopic dermatitis. sIgE tests were performed by a previous doctor and were positive for peanut so he suspected peanut allergy and advised her parents not to introduce peanut among a list of other foods. While no symptoms occurred during DBPCFC and open challenge, an exacerbation of eczema was observed hours after eating a sandwich with peanut butter at home. Despite that peanut allergy was excluded, they decided to stop the peanut introduction and continued the elimination diet.

	sIgE Peanut	sIgE Ara h 2	Reason for suspicion	DBPCFC	Open challenge	Intro-duction	Diet
Tom	12.0	7.5	Severe respiratory reaction in history	Negative	Positive, vomiting Sampson 2	Not started	Traces of peanut
Noa	1.2	<0.1	Subjective symptoms, other food allergy	Abdominal pain	Aversion	Successful	Peanut
Emma	5.2	1.2	Eczema and sIgE	Negative	Negative	Failed	No peanut

GENERAL DISCUSSION

Diagnosing food allergy in children isn't peanuts. This thesis demonstrates that diagnostic procedures in children with suspected food allergy can be improved by: 1) prediction of food challenge outcomes using component resolved diagnostics; 2) objectifying food challenge outcomes; 3) open challenges and guided introduction after food challenges.

The implications, clinical recommendations and suggestions for future research regarding the main findings of this thesis will be discussed in this chapter based on three clinical examples (box 1).

MAIN FINDINGS OF THIS THESIS

I. The impact of food allergy

- The Electronic Portal is feasible to collect large amounts of patient reported and clinical data on respiratory and allergic diseases in a standardized way (**chapter 2**).
- Children referred for food allergy have an impaired generic and specific quality of life. Generic quality of life is most affected by respiratory and atopic comorbidities and food allergy specific quality of life is impaired by a severe allergic reaction in history and an extensive elimination diet (**chapter 3**).

II. Predicting peanut challenge outcomes

- sIgE to the peanut component Ara h 2 is the best predictor for peanut allergy (**chapter 4**).
- We cannot predict severe food challenge outcome with regular available patient data (**chapter 5**).
- sIgE to Ara h 2 can be used to exclude or diagnose peanut allergy in 62% of children with suspected peanut allergy (**chapter 6**).
- The basophil response to Ara h 2 and Ara h 6 has some added value in predicting peanut allergy but cannot be used to predict severity of peanut allergy (**chapter 6**).

III. The diagnostic accuracy of food challenges

- The DBPCFC, the current 'gold standard' for food allergy, is subject to variability within and between well trained clinicians, especially when subjective symptoms occur (**chapter 7**).
- A decrease in FEV1 during food challenge is related to peanut allergy but continuous monitoring of vital and respiratory signs during challenge has limited added diagnostic value (**chapter 8**).

IV. Post-food challenge outcomes

- False negative DBPCFCs occur and (severe) peanut allergy can be present despite a DBPCFC with inconclusive subjective symptoms (**chapter 9**).
- Reintroduction of peanut in the diet fails in up to 32% of children after negative food challenges especially due to food refusal and peanut related symptoms (**chapter 10**).

THE IMPACT OF FOOD ALLERGY ON SOCIETY AND PATIENTS

Food allergic reactions can range from mild local symptoms to severe or even fatal reactions. Daily physical symptoms are usually absent in children with food allergy, these are usually related to reactions due to unintended exposure. Patients especially suffer from the psychological and social consequences of having food allergy, fear of a (severe) allergic reaction and restrictions when buying foods, during social activities and eating out.(1,2) Besides its impact on children and their families, food allergy causes increasing hospital utilization and medical costs. A European case-control study showed that parents of children with suspected food allergy visit health care professionals 10.75 times per year (compared to 6.56 in controls).(3) Total direct health care costs for food allergy are $724 per year per child. These costs are about five times higher for asthmatics ($3259 per year per child). However, in food allergy, indirect costs and out-of-the pocket costs for medications, special diets, special child care arrangements and lost labour productivity are estimated to account for another $4184 per year per child.(4)

We are what we eat?

Peanut allergy awareness at schools

Until the 1980s food allergy was considered a rare disease that was occasionally fatal but not of much concern. After several publications about fatal reactions after the ingestion of peanut (5–7), allergy to this groundnut as well as other food allergies became a widely debated topic in society. This is illustrated by a rapid increase in interventions that aimed to decrease the risk and burden of severe food allergic reactions (i.e. allergen free public spaces and airlines, legislation of labelling on packed foods and food allergy support groups). Moreover, beliefs about food changed: what we eat became part of our identity and is connected to our physical, social and mental wellbeing. In last decades publications and recommendations (scientific or not) about a healthy diet have caused confusion about what to eat and especially what not to eat.(8) The increasing awareness about food allergy in combination with a changed attitude towards food can have caused that more individuals identified themselves as being food allergic which subsequently resulted in an increase in prevalence and even overdiagnosis.(8,9)

Box 2 Quotes of parents about diagnostic tests for peanut allergy

"I want to know what my child can eat and how to deal with allergy in daily life."

"By performing a food challenge my child knows what a peanut allergy feels like and has less fear about his allergy."

"Seeing other children react on peanut during food challenges is scary. "

"A blood test is less burdensome than two days in a hospital."

"A blood test can dissolve any remaining doubts about the presence of peanut "allergy."

This is illustrated by high self-reported food allergy rates: the lifetime self-reported prevalence of food allergy in children to common foods in Europe is currently estimated around 17% and the challenge proven prevalence around 1%.(10) Data from the Electronic Portal (**chapter 2**) also illustrate that elimination diets are common and often started based on self-reported food allergy. For example in 13% of children referred for asthma and 8% of children referred for infections one or more foods were eliminated without a doctor's diagnosis of food allergy. Additionally, the availability and use of sensitization tests (sIgE and skin prick tests) to diagnose food allergy could have caused a further increase the detection of food allergy.(11) This also points to overdiagnosis as many patients appear to have positive sensitization tests to food without having a clinically relevant allergy: an estimated 8.6% of the population is sensitized to peanut whereas the prevalence of challenge proven allergy is 0.2%.(10)

An accurate diagnosis of food allergy is important to prescribe adequate emergency medication and educate children and their families in avoiding allergens and other triggers to prevent fatal reactions and improve quality of life.(12,13) On the other hand, overdiagnosis can be harmful due to unnecessary elimination diets that can cause nutritional deficiencies and are accompanied with fear and a reduced quality of life.(14,15) Furthermore, it should be realized that diagnostic tests can have different advantages and disadvantages for individual patients: this is illustrated by quotes (box 2) of several parents of children that participated in our prospective diagnostic study (chapter 6).

In **chapter 3** it was demonstrated that children referred for food allergy have an impaired generic and food allergy specific quality of life. Especially children with reported severe allergic reactions (**Tom**) and extensive elimination diets (**Emma**) were at risk for a lower food allergy specific quality of life. Furthermore daily respiratory symptoms and active eczema were common (44% and 60%) and important determinants of generic quality of life in children referred for food allergy. Thorough examination and adequate management of allergic comorbidities in children referred for food allergy is therefore warranted. Especially because uncontrolled respiratory and atopic diseases could be related to more severe food allergic reactions in daily life.(16,17)

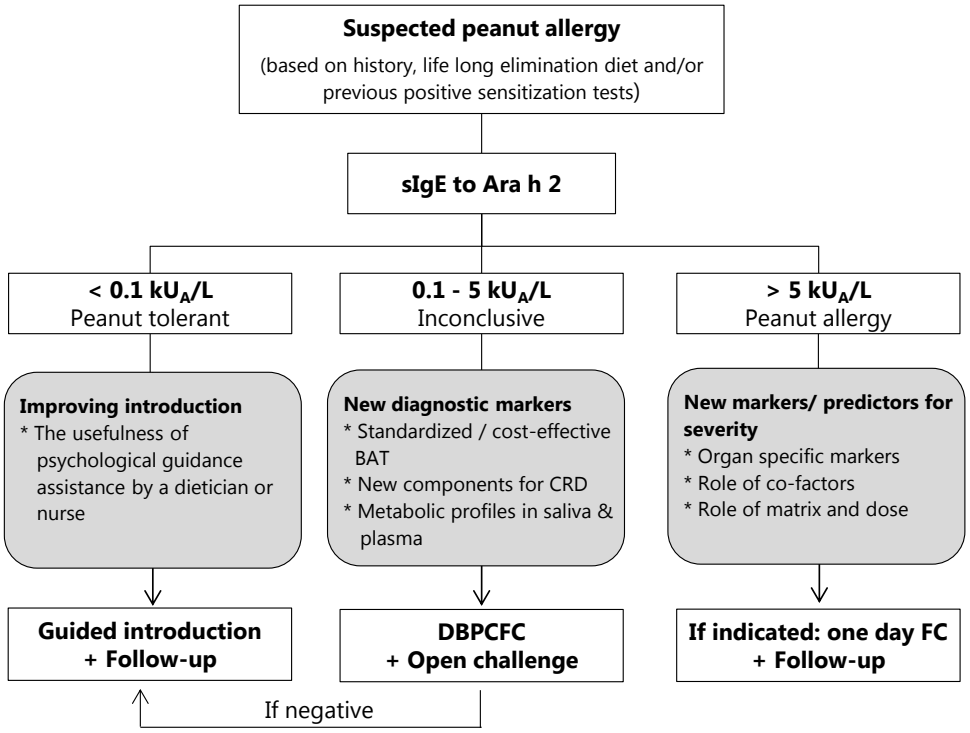
PREDICTION OF PEANUT CHALLENGE OUTCOME

In **Tom**, **Noa** and **Emma** peanut allergy was confirmed or excluded with food challenges. This thesis demonstrates that sIgE to one of the peanut components, Ara h 2 can be used to confirm or exclude peanut allergy in the majority of suspected patients without costly and burdensome double blind food challenges.

sIgE to Ara h 2 should be implemented in daily practice

Currently, the Dutch guideline for food allergy and food challenges states that implementation of sIgE to Ara h 2 is not yet possible due to variation in cut-off points between populations.(18) However, the findings in this thesis endorse use of sIgE Ara h 2 in daily practice: firstly to exclude peanut allergy and start guided reintroduction in children without sensitization to Ara h 2 (< 0.1 kUA/L) and secondly to select children with high probability for peanut allergy (> 5 kUA/L) (figure 1).

Figure 1 Proposed diagnostic algorithm for children with suspected peanut allergy and recommendations for future studies (grey boxes)



Indications for one day FC
1) More information about severity / eliciting dose (long elimination diet / subjective symptoms in history);
2) Decrease fear or explain allergy to child and parents.
DBPCFC, double blind placebo controlled food challenge; FC, Food challenge

Ara h 2 to exclude peanut allergy

A prospective study was performed in children with suspected peanut allergy, they all underwent DBPCFC and open challenges to diagnose peanut allergy accurately (**chapter 6**). All children with sIgE to Ara h 2 < 0.1 kUA/L were tolerant which validates our previous retrospective study of children that underwent peanut challenges in which almost the same cut-off point (0.07 kUA/L) for a 100% negative predictive value (NPV) was found.(19) By using this approach in up to 30% of children (including **Noa**) with suspected peanut allergy, burdensome double blind food challenges could be avoided (**chapter 6**). Other studies support our claim to start introduction in children with Ara h 2 < 0.1 kUA/L: even when a higher cut-off (< 0.35 kUA/L) was used a NPV ranging from 73-100% was found.(20) NPVs < 100% may be explained by rare cases of peanut allergy in which peanut components like Ara h 1, Ara h 3, Ara h 6 or Ara h 9 or even undiscovered allergens are relevant in absence or with very low levels of sIgE to Ara h 2.(21,22) Moreover, in older children and adults with suspected Bet v 1 related peanut allergy, elevated sIgE to Ara h 8 can explain (mild) peanut related symptoms.(23,24) This means that a careful history is always mandatory. Furthermore, introduction should be carefully guided to detect false negative Ara h 2 results early.

Ara h 2 to confirm peanut allergy

Patients with levels of Ara h 2 above 5 kUA/L are highly likely to be allergic for peanut: 100% in both the retrospective and prospective study of this thesis (**chapter 5 and 6)**. Previous studies demonstrate that sIgE to Ara h 2 > 0.35 kUA/L is already able to discriminate patients with peanut allergy (positive predictive value (PPV) ranging from 70-94%).(20) Those patients should eliminate peanut but there are several reasons why food challenges can still be indicated in this group. Firstly, more information about the severity of their allergy to guide the elimination diet (allowing traces or not) and other safety instructions (epinephrine auto-injector or not) can be necessary, especially in children with (life) long elimination diets or subjective symptoms in history (i.e. oral itch or 'not feeling well'). Furthermore it has been shown that challenges (also when positive) can reduce fear an improve quality of life.(25) Challenges may also be beneficial for parents and children to demonstrate what to expect when encountering peanut in daily life. Challenges in patients with high levels of sIgE to Ara h 2 will not have diagnostic purposes and can therefore be replaced by single day active challenges. The proposed diagnostic cut-off would have prevented challenges or half the amount of challenge days in 30% of patients (including **Tom**) with suspected peanut allergy in our prospective study (**chapter 6**). It should be noted that false positive tests can occur and children may develop tolerance despite high levels of Ara h 2.(26–28) Therefore, follow-up of patients that are diagnosed with peanut allergy by Ara h 2 is necessary. Follow-up should include monitoring of accidental reactions to reconsider safety instructions (in case of severe reactions at home) or the diagnosis (development of tolerance).

Instead of performing costly and burdensome new diagnostic studies in different patient's settings longitudinal implementation studies in both secondary and tertiary

care should be performed. Such implementation studies should include follow-up of patients to monitor the long term effects of using sIgE to Ara h 2 in practice and detect the occurrence of false negative and positive tests. Furthermore, future studies should focus on decision analytic modelling to evaluate the cost-effectiveness of the proposed diagnostic strategy in daily practice.(29)

Prediction of severe peanut challenge outcome

This thesis demonstrates that clinicians need to remain aware and prepared that any child can develop a severe allergic reaction during a food challenge. The levels of sIgE tot Ara h 2 and Ara h 6 were related to severe peanut allergy but prediction of individual children at high risk for severe food allergic reactions in our retrospective or prospective study was not possible (**chapter 5 and 6**). Also clinical characteristics (i.e. a severe reaction in history as was the case in **Tom**) were not related to a severity of food challenge outcome (**chapter 5**). Severe allergic reactions are part of a complex interplay between several host (i.e. genetics and comorbidities), event (i.e. co-factors like exercise) and allergen (i.e. dose, matrix) related factors.(30) Discrepancies between severity of symptoms in history and during challenge have been described in previous studies and can be due to the relative stable situation during challenge with absence of co-factors (i.e. uncontrolled comorbidities or exercise) which can aggravate reactions in daily life.(31) Furthermore, prediction of severity is difficult because challenges are usually stopped before a severe reaction occurs. It is necessary to identify new and accurate (organ specific) markers for severe allergy in future studies. For example low baseline levels of serum platelet activating factor (leading to increased PAF levels) have been associated with severe venom anaphylaxis.(32) Furthermore, it has been shown that severity of allergic symptoms is associated with increased plasma heparin levels and intensity of the plasma contact system activation.(33)

The added value of the BAT

The basophil response to Ara h 2 and Ara h 6 could further increase the proportion of correctly diagnosed patients from 62% to 80% mainly by detecting peanut allergic children in patients with 'inconclusive' (between 0.1 and 5 kUA/L) levels of sIgE to Ara h 2 (**chapter 6**). This reflects the more functional nature of the BAT which takes into account the concentration and affinity of sIgE and other (e.g. blocking) antibodies.(34) However, the added value of the BAT is restricted to a selective and relatively small patient population and several limitations of the BAT prevent current implementation in daily practice. Firstly, we could not reproduce previous findings that showed that the BAT had added value in predicting severe peanut allergy.(35) Additionally, the occurrence of non-releasers (10% in our study) is a major drawback as it leads to inconclusive results. Furthermore, the BAT needs to be performed on blood samples within 24 hours of blood collection and requires a specialized laboratory with expertise in functional cell activation testing and flow cytometry. Currently, no standardized procedure to perform and interpret the BAT is available.(36) Future studies should aim to improve the cost-

effectiveness of the BAT and focus on standardization of the test procedures. For now, in light of the limitations above, the BAT should not be performed in daily practice.

HOW GOLDEN IS THE 'GOLD STANDARD'?

It is advocated worldwide that the true diagnosis of food allergy can be established or ruled out by the 'gold standard' the double blind placebo controlled food challenge.(37–39) This thesis shows that food challenges, even double blind, have important limitations of which clinicians should be aware when performing them for diagnostic purposes.

Variability in the interpretation of symptoms

When presented with the same clinical information about symptoms during food challenges, clinical experts can disagree on whether to classify food challenge outcome as negative, positive or inconclusive (**chapter 7**). In particular, the occurrence of subjective symptoms (oral symptoms, abdominal complaints) or food aversion during challenge was associated with disagreement. To improve standardization and diminish variability in interpretation of food challenge outcomes clinicians should therefore not use those symptoms as stopping criteria for a DBPCFC and if ingestion is still possible continue challenges until clear objective symptoms occur. Despite continuation of the challenge some children may still have inconclusive symptoms during challenge (i.e. abdominal pain that prevents further ingestion or intermittent oral symptoms). In those children open challenges should be performed and guided introduction should be started to confirm or exclude peanut allergy. This is illustrated by **Noa**; disagreement about the food challenge outcome occurred but with guided introduction peanut allergy could be averted. Furthermore, consultation of an (independent) colleague or expert panel might be helpful to establish an unambiguous food challenge outcome. A future study that compares the outcomes of an expert panel that is provided with 1) DBPCFC only, 2) DBPCFC + diagnostic tests and 3) DBPCFC + diagnostic tests + patient characteristics might provide insight in the impact of additional information on the classification food challenge outcome.(40)

Objectification of food challenges

In this thesis the added value of objective parameters monitored during DBPCFC was investigated to decrease the subjective nature of the interpretation of food challenges. Continuous monitoring of the forced expiratory volume in one second (FEV1) during DBPCFCs demonstrated that a decrease in FEV1 >20% was discriminative for peanut allergy (**chapter 8**). This decline was always accompanied with objective symptoms indicative for peanut allergy but in contrast to previous studies not necessarily with 'asthmatic' symptoms.(41,42) Although the diagnostic value of lung function measurements during DBPCFC was low, measurement of FEV1 during challenge can be important for safety reasons and to provide adequate treatment during challenges. Other vital signs (blood pressure, heart rate, oxygen saturation and automatic wheeze detection) varied during both placebo and active challenges and could not be used to discriminate children with

peanut allergy from tolerant children (**chapter 8**). The diagnostic value of other objective markers of allergy during challenge, like mast cell mediators and metabolic profiles in saliva and plasma can be important targets for future studies to further objectify food challenges.(43,44)

THE PROOF OF THE PUDDING IS IN THE EATING

Open challenges

Results of this thesis illustrate that open challenges are a necessary part of the diagnostic procedure in children with suspected peanut allergy (**chapter 9**). **Tom** was one of the 2/29 (7%) children in which peanut allergy was diagnosed during open challenge after a DBPCFC without any symptom. Furthermore 8/18 (44%) children were diagnosed during open challenge after inconclusive DBPCFC. Positive open challenges have been reported by two previous studies (in 3% and 13% of negative DBPCFCs).(45,46) Current guidelines for food allergy and food challenges do mention the importance of an open challenge with the natural form of the allergen for confirmation of tolerance. However, there is no clear guideline for the dose and timing of these open challenges.(37,38,47) This thesis demonstrates that portions up to at least 10 gram of whole peanuts directly administered on the second day are feasible to detect false-negative DBPCFCs. Several explanations for false-negative DBPCFCs and positive open challenges have been suggested and should further be explored: a matrix or allergen concentration effect, the induction of short term tolerance and insufficient cumulative or top dose of DBPCFC.

Guided introduction

This thesis demonstrates that careful guided introduction is necessary: a) to confirm or exclude peanut allergy and b) to detect and deal with introduction problems in an early stage. Peanut related symptoms (like abdominal pain and eczema) were reported by 11/37 (30%) of parents during introduction. In 3/37 (8%) children a (mild) peanut allergy could be diagnosed based on the symptoms during introduction (repetitive and persistent oral itch within 5 minutes after all peanut ingestions (**chapter 9**). Reintroduction often failed after negative peanut challenges: in 32% children of the retrospective study (**chapter 10**) and in 27% of children in the prospective study (**chapter 9**). Reasons for failure of introduction in the diet included: aversion and food refusal, symptoms related to peanut by parents, fear for an allergic reaction, not convinced about the challenge outcome and practical reasons (habituation to the diet, a family member with food allergy). These findings highlight that concerns and perceptions about food and elimination diets seem deeply embedded in patients and their caregivers and cannot easily be changed by a negative food challenge only. The usefulness of psychological guidance to reduce fear and assistance by a dietician to improve success rates of introduction should be investigated.

Box 3 Recommendations for current clinical practice

1. Thorough examination and adequate management of allergic comorbidities in children referred for food allergy is warranted to improve generic quality of life.
2. Clinicians should be aware of high rates of self-reported food allergy and elimination diets in children referred for other allergic and respiratory conditions and diagnose food allergy accurately.
3. To diagnose peanut allergy accurately, sIgE to Ara h 2 should be implemented and used to start guided reintroduction and select children with peanut allergy for DBPCFC or one day challenges when indicated according to the proposed diagnostic algorithm (figure 1).
4. To establish unambiguous food challenge outcomes, challenges should be continued despite subjective symptoms if possible.
5. Food challenges require an open challenge and follow-up of patients to confirm or exclude peanut allergy definitely
6. As there are currently no accurate predictors of severe allergic reactions, clinicians should be aware and prepared that during food challenges any child can develop a severe allergic reaction and all measures to treat this are in place.

FOOD FOR THOUGHT

Implementation of new diagnostic strategies can be challenging in daily practice and management strategies regarding patients with food allergy vary among experts and centres.(48) A collaboration as in our Expert Network (**chapter 2**) can be useful to harmonize diagnostic work-ups and implement guidelines in a region. Furthermore, patient oriented educational programs like 'Making sense of allergies' that translate scientific knowledge to the public and explicate facts and myths about allergy should be encouraged.(49) For example, they clarify that allergy tests sold online have no scientific basis and refute myths like 'E-numbers and fast food cause allergies' and 'hypoallergenic means allergen-free'.

Furthermore, it should be realized that patients do not always expect absolute diagnostic certainty but sometimes prefer validation of their concerns and confirmation that their child's condition was appropriately managed.(50) See also box 2. This is illustrated by the fact that 32% of parents of children after a negative challenge were not fully convinced about the challenge test result (**chapter 9**). However, several of those parents including those of **Emma** still reported to be satisfied because at least a severe peanut allergy was excluded. The parents of **Tom** reported that they were not fully convinced about their child's peanut allergy because they expected to observe the same symptoms during challenge as they recognized from before (respiratory symptoms). In this example the

food challenge caused confusion and an extra outpatient visit was needed to convince parents about the serious nature of their child's disease. Additionally, in a study evaluation after our prospective diagnostic study (**chapter 6**), we asked parents what they preferred as a diagnostic test to confirm peanut allergy and what they preferred to exclude peanut allergy in their child. To confirm peanut allergy 15/16 (94%) of the respondents would have preferred an accurate blood test. Nevertheless, 10/15 (67%) of those parents preferred a food challenge to confirm true tolerance. These observations highlight the importance to discuss expectations and consequences of each diagnostic step during the diagnostic process with individual patients and their caregivers to get insight and if necessary manage their expectations.

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CHAPTER 12

Nederlandse samenvatting
Dutch summary

BELANGRIJKSTE BEVINDINGEN

I. De impact van voedselallergie

- Het Elektronische Portaal (www.luchtwegportaal.com) is geschikt om grote hoeveelheden patiënt gerapporteerde en klinische data op het gebied van luchtwegklachten en allergie op een gestandaardiseerde manier te verzamelen (**hoofdstuk 2**)
- Kinderen verwezen met voedselallergie hebben een verminderde algemene en voedselallergie specifieke kwaliteit van leven. De algemene kwaliteit van leven wordt vooral verminderd door respiratoire en allergische co-morbiditeit. De voedselallergie specifieke kwaliteit van leven wordt beperkt door ernstige reacties in de anamnese en een uitgebreid eliminatie dieet (**hoofdstuk 3**).

II. Het voorspellen van pinda provocatietest uitkomsten

- sIgE tegen het pinda component Ara h 2 is de beste voorspeller van pinda-allergie (**hoofdstuk 4**).
- Ernstige reacties tijdens voedselprovocaties zijn niet vooraf te voorspellen met klinische gegevens van patiënten (**hoofdstuk 5**).
- sIgE tegen Ara h 2 kan gebruikt worden om pinda-allergie aan te tonen of uit te sluiten in 62% van de patiënten met een verdenking van pinda-allergie (**hoofdstuk 6**).
- De basofiel respons tegen Ara h 2 en Ara h 6 heeft aanvullende waarde na sIgE tegen Ara h 2 maar kan niet worden gebruikt om ernstige reacties te voorspellen (**hoofdstuk 6**).

III. De beoordeling van voedselprovocaties

- Er is variabiliteit in de interpretatie van klachten tijdens voedselprovocaties tussen en binnen ervaren beoordelaars. Dit is in het bijzonder zo bij het optreden van subjectieve klachten (**hoofdstuk 7**).
- Een longfunctie daling tijdens een voedselprovocatie voor pinda is geassocieerd met pinda-allergie. Echter heeft continue monitoring van andere respiratoire en vitale parameters geen aanvullende diagnostische waarde (**hoofdstuk 8**).

IV. Follow-up van voedselprovocaties

- Vals negatieve voedselprovocaties komen soms voor. Een open provocatie na een dubbelblinde provocatie (zonder klachten of met alleen subjectieve klachten) kan een ernstige pinda-allergie alsnog opsporen (**hoofdstuk 9**).
- In 32% van de kinderen met een negatieve pinda provocatie test lukt herintroductie van pinda in het dieet niet. Dit komt door weigering van voedsel maar kan ook door pinda gerelateerde klachten komen (**hoofdstuk 10**).

NEDERLANDSE SAMENVATTING

De doelstellingen van dit proefschrift

Het doel van dit proefschrift is het verbeteren van de diagnostiek naar voedselallergie bij kinderen. De doelstellingen van het onderzoek waren: 1) het onderzoeken van de invloed van voedselallergie op de kwaliteit van leven; 2) het voorspellen van pinda provocatie test uitkomsten; 3) het onderzoeken en verbeteren van de diagnostische waarde van de voedselprovocatie testen en 4) het evalueren van lange termijn resultaten van voedselprovocatie testen. De betekenis van de belangrijkste begrippen uit deze samenvatting zijn te vinden in box 1.

Een allergische reactie

Een allergische reactie is een overdreven reactie van het afweersysteem. Het afweersysteem beschermt het lichaam normaal tegen lichaamsvreemde en ziekmakende stoffen. Dit zijn bijvoorbeeld virussen, bacteriën en giftige stoffen. Bij een allergische reactie komt het afweersysteem in actie tegen een normaal onschuldige stof. De eiwitten in de stoffen die kunnen zorgen voor een allergische reactie worden allergenen genoemd. Het pinda eiwit is een voorbeeld van een voedselallergeen. Als reactie op allergenen produceert het afweersysteem antistoffen (zoals IgE). Deze antistoffen kunnen zich binden aan afweercellen. Mestcellen en basofiele granulocyten zijn voorbeelden van deze afweercellen. Mestcellen bevinden zich op allerlei plekken in het lichaam zoals in de huid en in de longen. Als een allergeen zich vervolgens bindt aan twee van deze antistoffen op een afweercel kunnen er afweerstoffen vrijkomen uit de afweercel. Een van deze afweerstoffen is histamine. Van het vrij komen van histamine kun je klachten krijgen, zoals galbulten, jeuk, rode ogen en niezen. Er kunnen ook levensgevaarlijke klachten ontstaan zoals zwelling van de lippen en keel, benauwdheid en shock. Het is typisch voor reacties waarbij IgE is betrokken, dat de klachten snel ontstaan (meestal binnen minuten tot een uur na blootstelling).

Voedselallergie

Een voedselallergie is een reactie van het afweersysteem met IgE tegen een voedselallergeen. Tot in de jaren '80 maakte men zich niet veel zorgen over voedselallergie. Na een aantal publicaties over ernstige en zelfs dodelijke reacties na het eten van pinda veranderde dit echter. Voedselallergie werd steeds vaker opgemerkt, in het bijzonder in de Westerse wereld. Op dit moment wordt geschat dat ongeveer 17% van de kinderen in Europa een zelf gerapporteerde voedselallergie heeft. Het aantal kinderen met een bewezen voedselallergie ligt lager, rond de 1%. In dit proefschrift is vooral gekeken naar kinderen met (een verdenking op) pinda-allergie. Pinda is namelijk een van de meest voorkomende allergenen die kan zorgen voor ernstige reacties. Daarnaast is een pinda-allergie vaak levenslang aanwezig en groeien kinderen hier maar zelden overheen.
Een juiste diagnose van voedselallergie is belangrijk. Aan de ene kant is het belangrijk om voedselallergie aan te tonen om noodmedicatie voor te schrijven en ernstige reacties te voorkomen. Aan de andere kant is het ook belangrijk om een voedselallergie uit te

sluiten. Zo kunnen onnodig strenge diëten (die tot groeiproblemen kunnen leiden) en ongerustheid en angst voor reacties worden voorkomen.

I. De impact van voedselallergie

Het hebben van een voedselallergie heeft een negatieve invloed op de kwaliteit van leven. Een allergische reactie kan levensbedreigend zijn. Kinderen met voedselallergie en hun ouders leven met de continue angst voor een allergische reactie. Om reacties te voorkomen moeten kinderen met voedselallergie het allergeen vermijden. Het is voor de hele familie belangrijk om goed op te letten welke producten er gekocht en gegeten worden. Daarnaast lukt het soms niet om uit eten of naar verjaardagen te gaan. Ook moeten kinderen altijd noodmedicatie (zoals een adrenaline pen) bij zich hebben om een onverwachte reactie snel te behandelen.

Het Luchtwegportaal

In dit proefschrift wordt de inhoud en het doel van het elektronische portaal (Het Portaal) beschreven. Het Portaal is een digitaal registratie systeem gemaakt voor kinderen die luchtwegklachten, allergische klachten en/of eczeem hebben. In het Portaal bevinden zich gestandaardiseerde vragenlijsten over luchtwegklachten en allergie. Ouders en kinderen vullen deze vragenlijsten in voordat ze in het ziekenhuis komen. Ook kunnen aanvullende medische gegevens (zoals longfunctie testen en allergie onderzoek) worden ingevuld en ingezien. In **hoofdstuk 2** laten we zien dat het Portaal geschikt is om grote hoeveelheden data op het gebied van luchtwegklachten en allergie op een gestandaardiseerde manier te verzamelen. Deze data kunnen (anoniem) worden gebruikt voor onderzoek. Er kan bijvoorbeeld onderzocht worden welke klachten voor ouders en kinderen het belangrijkst zijn. Er kan ook gekeken worden wat de relatie is tussen verschillende ziektebeelden (zoals astma en voedselallergie. Met dit onderzoek kan de zorg voor kinderen met luchtwegklachten en allergie verbeterd worden.

Een verminderde kwaliteit van leven

In **hoofdstuk 3** worden de data van kinderen in het Portaal gebruikt. Er wordt gezien dat kinderen die voor voedselallergie in het ziekenhuis komen een verminderde kwaliteit van leven hebben. De algemene kwaliteit van leven wordt vooral verminderd door luchtweg klachten (zoals astma en verkoudheden). De voedselallergie specifieke kwaliteit van leven wordt vooral verminderd door ernstige reacties op voedsel in de anamnese (zoals shock) en een uitgebreid eliminatie dieet (het vermijden van meerdere voedingsmiddelen).

Peanuts?

Door een reactie na het eten kan bij kinderen de verdenking op een voedselallergie ontstaan. Er zijn dan klachten (zoals galbulten of benauwdheid) snel na de inname van een voedingsmiddel. Er zijn een aantal testen die in het ziekenhuis kunnen worden gedaan, om te kijken of er inderdaad sprake is van een voedselallergie. Met een bloed

test kan de hoeveelheid specifiek IgE worden bepaald. Met een huidtest kan er gekeken worden of het allergeen voor een lokale reactie zorgt in de huid. Beiden testen geven aan of een patiënt is gesensibiliseerd (sIgE maakt tegen het allergeen). Niet alle kinderen die gesensibiliseerd zijn hebben echter een allergie. De 'gouden standaard' voor het aantonen van een voedselallergie is de voedselprovocatie test. Hierbij wordt er in het ziekenhuis onder gecontroleerde omstandigheden gekeken of er een reactie optreedt na het eten van het allergeen. De voedselprovocatie test is echter een lastige test. De beoordeling van subjectieve klachten tijdens de test (zoals buikpijn) kan moeilijk zijn. De test is daarnaast duur en belastend omdat kinderen (soms twee dagen) moeten worden opgenomen in het ziekenhuis. Ook kunnen kinderen een levensgevaarlijke reactie krijgen tijdens de test. Tot slot blijkt uit eerder onderzoek dat het niet altijd lukt om het allergeen thuis te eten na een negatieve test. Het is daarom de vraag of deze gouden standaard wel echt zo goud is. Als we de uitslag van de voedselprovocatie test al van te voren zouden kunnen voorspellen, heeft niet ieder kind deze test meer nodig.

II. Het voorspellen van pinda provocatietest uitkomsten

Het eiwit in pinda is te verdelen in verschillende specifieke onderdelen, componenten. Het is mogelijk om specifiek IgE tegen deze individuele componenten te bepalen. In **hoofdstuk 4** is beschreven dat het specifiek IgE tegen componenten van pinda gebruikt kan worden bij de diagnostiek van pinda-allergie. Het pinda component Ara h 2 lijkt het meest te relateren aan pinda-allergie. Waarschijnlijk komt dit omdat dit eiwit een belangrijk bouwsteen van pinda is die erg goed tegen hitte en vertering kan.
In **hoofdstuk 5** is onderzocht of ernstige reacties tijdens voedselprovocaties te voorspellen zijn met patiënt gegevens, zoals de ernst van de eerdere reactie en astma. De resultaten laten zien dat we deze reacties niet goed kunnen voorspellen. De resultaten in **hoofdstuk 6** laten zien dat met sIgE tegen Ara h 2 bij 62% van de kinderen de aan of afwezigheid van pinda-allergie goed voorspeld kan worden. Daarnaast heeft een andere test, de basofiel activatie test respons tegen pinda componenten, een aanvullende waarde naast sIgE tegen Ara h 2. Door tevens deze test te gebruiken na sIgE tegen Ara h 2 kan een pinda-allergie bij 80% van de kinderen worden voorspeld voor ze wel of geen allergie hebben. Beide testen kunnen echter niet worden gebruikt om de ernst van de reacties tijdens de provocatietest te voorspellen. Kortom, met behulp aanvullende testen kunnen we bij een groot deel van de kinderen een voedselprovocatietest voorkomen.

III. De beoordeling van voedselprovocaties

Hoofdstuk 7 laat zien dat ervaren artsen het niet altijd eens zijn over de beoordeling van de klachten tijdens voedselprovocaties. Dit is in het bijzonder zo wanneer er subjectieve klachten (zoals buikpijn en jeuk in de mond) optreden. In **hoofdstuk 8** is onderzocht of aanvullende metingen (longfunctie, longgeluiden, zuurstofgehalte, hartslag en bloeddruk) tijdens provocatie testen kunnen bijdragen aan de beoordeling van de voedselprovocatie. Aangetoond werd dat een verminderde longfunctie kan wijzen op het hebben van een pinda-allergie. Het is voor veiligheidsoverwegingen

(reacties van de luchtwegen kunnen gevaarlijk zijn en hebben andere medicatie nodig) daarom belangrijk om de longfunctie tijdens voedselprovocaties te meten. De overige onderzochte metingen hadden geen aanvullende waarde bij het beoordelen van de voedselprovocatie.

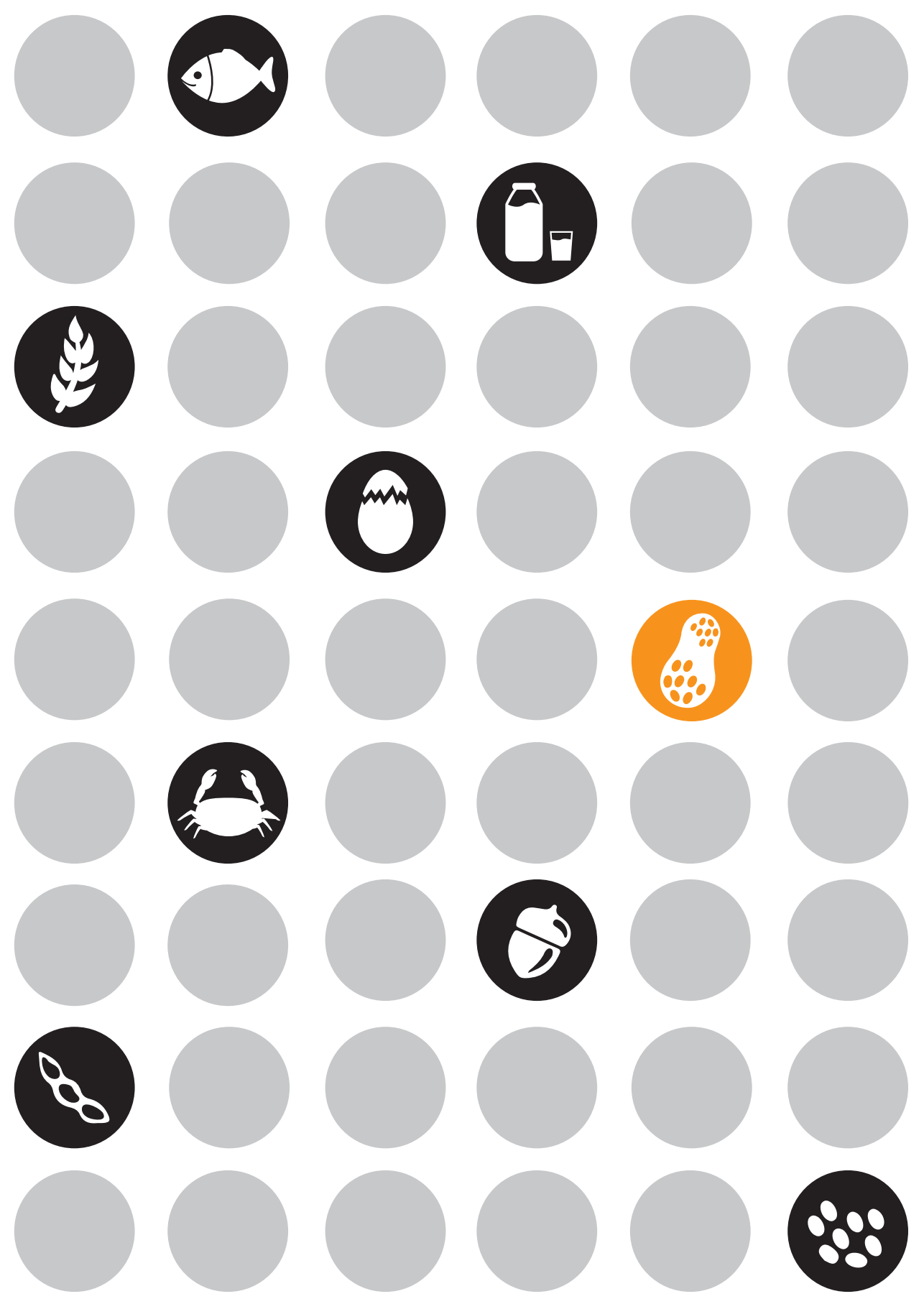
IV. Follow-up van voedselprovocaties

Bij sommige kinderen kan is de uitslag van de bloed test sIgE tegen Ara h 2 twijfelachtig en blijft een voedselprovocatie test nodig. In **hoofdstuk 9** zijn de uitkomsten van dubbel blinde voedselprovocaties onderzocht. Er is gevonden dat het nodig is om open provocaties uit te voeren na een dubbel blinde provocatie. Pas dan kan goed worden vast gesteld of er een pinda-allergie is of niet.
Na een negatieve test kan pinda weer gegeten worden en is een eliminatie dieet niet meer nodig. De resultaten van zowel **hoofdstuk 9 en hoofdstuk 10** laten echter zien dat het eten van pinda thuis niet altijd lukt. Bij 32% van de kinderen zijn er toch nog klachten van pinda of wordt pinda geweigerd door het kind. Deze resultaten tonen aan dat het opvolgen van kinderen na een negatieve test belangrijk is. Hiermee kunnen ouders en kinderen begeleid worden als er problemen zijn tijdens de introductie en kunnen vals negatieve testen worden opgespoord.

Box 1 Begrippenlijst

Allergeen	De eiwitten in een stof die kunnen zorgen voor een allergische reactie. Er bestaan inhalatie allergenen (zoals stof), contact allergenen (zoals nikkel), voedselallergenen (zoals pinda), geneesmiddelen (zoals penicilline) of insectengif (zoals bijen, wespen).
Anafylaxie	Een zeer heftige allergische reactie met een bloeddrukdaling of ernstige benauwdheid die levensbedreigend kan zijn.
Ara h 1 t/m 10	Een pinda bestaat uit ongeveer 25% eiwit. Vooral het eiwit in de pinda kan zorgen voor allergische reacties. Er zijn verschillende eiwit moleculen (allergenen) te onderscheiden in de pinda. Deze specifieke onderdelen noemen we pinda componenten en hebben de naam "Ara h" met een volgnummer. Ara h 2 is een voorbeeld van een pinda component en een specifiek pinda allergeen.
Basofiele granulocyten	Basofiele granulocyten zijn afweercellen. Net als bij mestcellen liggen in basofiele granulocyten prikkelende stoffen, zoals histamine, opgeslagen. Basofiele granulocyten bewegen zich naar weefsels waar allergische reacties plaats vinden.
Component	Een specifiek eiwit in een allergeen. Zie ook Ara 1 t/m 10.
DBPCFC	Dubbel blinde placebo gecontroleerde voedselprovocatie. Een voedselprovocatie van twee dagen waarbij de ene dag een placebo wordt gegeven en de andere dag het allergeen. Zowel de patiënt als de beoordelaar weet niet wat er welke dag wordt gegeven. Zie ook voedselprovocatietest.
Histamine	Prikkelende stof die ziekteverschijnselen kan veroorzaken, zoals jeuk en zwellingen. Histamine zit in afweercellen zoals mestcellen en in bepaalde (met name gegiste) voeding.
Huidpriktest	Bij de huidpriktest worden een allergeen op de huid gedruppeld en daarna in de huid geprikt. Bij een reactie ontstaat er roodheid en zwelling. Hoe groter de zwelling, des te groter is de kans op een allergie.
IgE	Afkorting van Immunoglobuline E. Door het afweersysteem gemaakte antistof. Het IgE bindt zich aan receptoren op afweercellen zoals mestcellen en basofiele granulocyten. Als een allergeen zich bindt aan twee IgE moleculen op de afweercellen wordt de cel geactiveerd. Er komt uit deze cel dan een reactieve stof vrij, zoals histamine. De hoeveelheid antistoffen voor een specifiek allergeen (specifiek IgE) kan gemeten worden in het bloed. Hoe hoger het specifiek IgE in het bloed is, des te groter is de kans op een allergie.

Pinda	De officiële naam van de pinda plant is: Arachis hypogaea. Pinda wordt ook wel aardnoot of grondnoot genoemd en is botanisch gezien géén noot maar een peulvrucht. De pindaplant groeit boven de grond maar de peul rijpt en ontkiemt onder de grond.
Mestcel	Afweercel die zich vooral bevindt in de huid, het maag-darmkanaal en de luchtwegen. In deze afweercel zitten korreltjes met daarin prikkelende stoffen zoals histamine.
Sensibilisatie	Het aanmaken van specifieke antistoffen (IgE) door het afweersysteem waardoor men gevoelig wordt voor een bepaald eiwit (allergeen). Sensibilisatie kan worden aangetoond door een bloed test (sIgE Test) of een huidpriktest.
Voedsel provocatie	Tijdens een voedselprovocatie wordt er getest of een patiënt een voedselallergie heeft. In ongeveer 8 stappen krijgt de patiënt elke 30 minuten steeds iets meer van het verdachte allergeen te eten. Het allergeen zit meestal verstopt in koek, cake of bijvoorbeeld appelmoes. Tijdens de test worden patiënten goed in de gaten gehouden. Er wordt goed gekeken of er allergische reacties ontstaan en zo nodig noodmedicatie gegeven.



APPENDICES A

Abbreviations
Contributing authors
Acknowledgements
List of publications
Curriculum vitae

ABBREVIATIONS

ACT	Asthma Control Test
AIRE	Asthma Insight and Reality
AUC	Area Under the Curve
BAT	Basophil Activation Test
BDR	Bronchodilator Response
BHR	Bronchial Hyper responsiveness
C-ACT	Child-Asthma Control Test
CRD	Component resolved diagnostics
CW	Chest wall
DBPCFC	Double Blind Placebo Controlled Food Challenge
EAI	Epinephrine auto-injector
ED	Eliciting dose
EN	Expert Network
eNO	exhaled Nitric Oxide
EP	Electronic Portal
EWR	Expiratory wheeze rate
FAQLQ	Food allergy specific quality of life
FC	Food Challenge
FCO	Food Challenge Outcome
FEV1	Forced expiratory volume in one second
FVC	Forced Vital Capacity
HRQL	Health related quality of life
ICS	Inhaled corticosteroids
IQR	Inter Quartile Range
IWR	Inspiratory wheeze rate
LTRAs	Leukotriene receptor antagonists
MARS	Medication Adherence Report Scale
MD	Maximum Dose
NPV	Negative Predictive Value
OAS	Oral allergy symptoms
OFC	Oral Food Challenges
PEF	Peak expiratory flow
PPV	Positive Predictive Value
RAND	RAND general health-rating index
ROC	Receiver Operating Characteristic
SD	Standard Deviation
sIgE	specific IgE
SPT	Skin Prick test
TR	Tracheal
WHISTLER	Wheezing Illnesses Study Leidsche Rijn

CONTRIBUTING AUTHORS

Walter A.F. Balemans, MD, PhD
St. Antonius Hospital, Department of Pediatrics, Nieuwegein, the Netherlands

Jenneke Boot, BSc
University Medical Centre Utrecht, Department of Pediatric pulmonology and allergology, Utrecht, the Netherlands

Cornelis K. van der Ent, MD, PhD
University Medical Centre Utrecht, Department of Pediatric pulmonology and allergology, Utrecht, the Netherlands

Bart E. van Ewijk, MD, PhD
Tergooi Hospital, Department of Pediatrics, Blaricum, the Netherlands

Carmelo Gabriele, MD, PhD
University Medical Centre Utrecht, Department of Pediatric pulmonology and allergology, Utrecht, the Netherlands

Diana M.W. Gorissen, MD
Deventer Hospital, Department of Pediatrics, Deventer, the Netherlands

Petra A. Kentie, NP
University Medical Centre Utrecht, Department of Pediatric pulmonology and allergology, Utrecht, the Netherlands

Rob J. B. Klemans, MD, PhD
Department of Dermatology and Allergology, University Medical Centre Utrecht, Utrecht, The Netherlands

Edward F. Knol, MD, PhD
University Medical Centre Utrecht, Department of Immunology & Department of Dermatology and Allergology , Utrecht, the Netherlands

André C. Knulst, MD, PhD
Department of Dermatology and Allergology, University Medical Centre Utrecht, Utrecht, The Netherlands

Irene L. Kok, MSc
University Medical Centre Utrecht, Internal Medicine and Dermatology, Department of Dietetics, The Netherlands

Yolanda Meijer, MD
University Medical Centre Utrecht, Department of Pediatric pulmonology and allergology, Utrecht, the Netherlands

Suzanne G.M. Pasmans, MD, PhD
University Medical Centre Utrecht, Department of (Pediatric) Dermatology and Allergology, Utrecht, The Netherlands

Bo Pontoppidan, PhD
Thermo Fisher Scientific, Uppsala, Sweden

Gordon Slabbers, MD
Bernhoven Hospital, Department of Pediatrics, Uden, the Netherlands

Maartje F. van Velzen, MD
Meander Medisch Centrum, Department of Pediatrics, Amersfoort, the Netherlands

Esther de Vries, MD, PhD
Jeroen Bosch Hospital, Department of Pediatrics, 's-Hertogenbosch, the Netherlands & Tranzo, Tilburg University, Tilburg, the Netherlands

Kim Zomer-Kooijker, MD, PhD
University Medical Centre Utrecht, Department of Pediatric pulmonology and allergology, Utrecht, the Netherlands

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CURRICULUM VITAE

Francine van Erp was born on September 14th 1986 in Huizen, the Netherlands. She graduated from secondary school in 2004 at the Baarnsch Lyceum in Baarn. At that same year she started her medical training at the University of Utrecht. During her medical school she was board member of the Faculty of Medicine Students Association, MSFU "Sams" in 2007 - 2008. She started working on food allergy during an elective research internship at the department pediatric pulmonology and allergology in the Wilhelmina Children's Hospital Utrecht. After graduation in 2011 her interest in allergic diseases in children sustained and she continued her research project as a PhD student. She performed the research described in this thesis under supervision of prof. dr. C.K. van der Ent, dr. A.C. Knulst and drs. Y. Meijer. In 2015 she obtained her Master of Science degree in Clinical Epidemiology at the University of Utrecht. In January 2016 she started the residency program Dermatology under supervision of Dr. V. Sigurdsson at the department of Dermatology at the University Medical Centre Utrecht. She will continue with her research on allergic diseases in children as a post-doctoral researcher at the department of pediatric pulmonology and allergology in the Wilhelmina Children's Hospital Utrecht. Francine lives in Utrecht together with her fiancé Joppe Nijman.