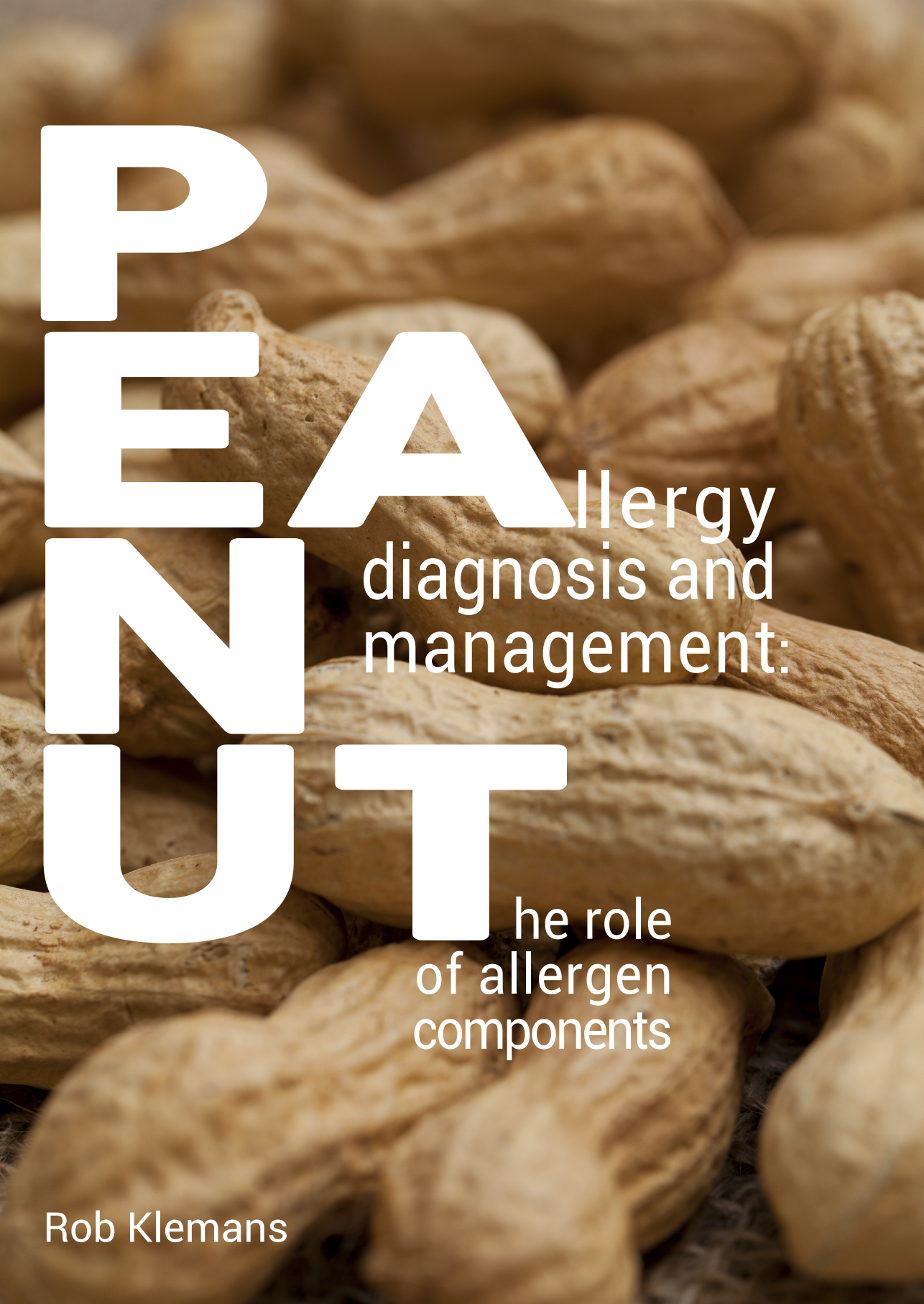

PEANUT

Allergy diagnosis and management: The role of allergen components

Rob Klemans

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Peanut allergy diagnosis and management: the role of allergen components

Pinda-allergie diagnose en management: de rol van allergeencomponenten
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op vrijdag 26 september 2014 des middags te 12:45 uur

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Chapter 1

General introduction

PREVALENCE OF PEANUT AND SOY ALLERGY

Peanut and soy are both legumes and therefore phylogenetically related. Serological cross-reactivity between both foods has been described.[1;2] Clinical cross-reactivity however, is generally thought to be low,[3;4] but data are very scarce. Numbers vary widely from 3% in a study conducted in peanut allergic patients to 5-67% in studies addressing soy allergic patients.[5-8] Therefore, both peanut and soy should be addressed in the diagnostic work-up of a suspected allergy to either of these foods.

The prevalence of peanut and soy allergy in the general population differs depending on geographical area. This is probably caused by dietary habits and the distribution of different pollen because of cross-reactivity between Bet v 1 in birch pollen and its homologues Ara h 8 in peanut and Gly m 4 in soy.[9-11] Recent research showed a self-reported peanut allergy of 0.67% in the general adult population in the Netherlands.[12] A probable peanut allergy however (positive history and specific IgE (sIgE) to peanut extract) was found in only 0.05%. The prevalence of confirmed peanut allergy by food challenge in 2 studies conducted in Denmark ranged from 0.2% (in children) to 0.4-0.6% (in adults).[13;14] The self-reported prevalence was also higher (5.3%).[14] The prevalence of confirmed peanut allergy in other westernized countries varied between 0.26-0.6% in adults and 0.3-2.0% in children.[15-23] Some studies showed an increasing prevalence during the last two decades.[15;16]

No reliable data are available regarding the prevalence of soy allergy in the Netherlands. A confirmed soy allergy in Denmark varied between studies from 0% (in children and adults) to 0.1% (adults).[13;14] This percentage was 0.5 in a study conducted in Berlin.[24] No other studies reported on the prevalence in the general population.[25]

THE IMPORTANCE OF AN ACCURATE DIAGNOSIS AND CURRENT PITFALLS

Allergic symptoms to soy and peanut can vary from mild oral allergy symptoms to severe respiratory and even cardiovascular symptoms.[8;26;27] Therefore, it is important that peanut or soy allergy is not missed in the diagnostic procedure and an adequate elimination diet is prescribed. On the other hand, having a food allergy significantly impairs the quality of life and increases the risk of impaired growth and inadequate nutrient intake in children.[28;29] This means that a false diagnosis and unnecessary elimination diets should be avoided as well. Because of the significant consequences of an incorrect diagnosis, accurate diagnostic tools are necessary.

Peanut or soy allergy should in general be diagnosed by a physician in case of:

1) A suggestive history (allergic symptoms after the ingestion of peanut or soy) in combination with sensitization (sIgE reactivity to peanut or soy extract as measured by in vitro assays or a skin prick test).
2) A positive food challenge with peanut or soy.

A well-taken history should address the amount that is ingested, the type of symptoms and duration of onset after ingestion. The history can be hindered in case it is unknown whether peanut, soy, or another causative allergenic food is ingested or when the ingested amount is unknown. This is for example the case when peanut or soy is just one of many ingredients. A reliable history is, in general, even more difficult in children, because many of them have never intentionally ingested the culprit food. Also, they can be too young to report subjective allergic symptoms, in which case one has to rely on their parents for the medical history. The most frequently used tests to measure sensitization are the in vivo skin prick test (SPT) and in vitro assays, of which the ImmunoCAP is most widely used, based on extracts of peanut or soy. With both methods the sIgE reactivity is measured and therefore they are often performed in the diagnostic work-up in subjects with a suggestive history of allergy. However, it is important to bear in mind that these tests measure sensitization, which is not the same as allergy. In two studies the prevalence of peanut sensitization among the general population was much higher (11.8% and 8.6%) than the true prevalence of peanut allergy as described in the first paragraph.[21;30] This means that sensitization is often not clinically relevant. As a result, the SPT or in vitro assays to measure sIgE to peanut or soy extract perform suboptimal when used as diagnostic tests, especially many false-positive outcomes occur.[31] A possible explanation is that many tolerant subjects are sensitized for Ara h 8 in peanut or Gly m 4 in soy via cross-reactivity with Bet v 1 in birch pollen. In this thesis, measuring sIgE reactivity with in vitro assays will be referred to as: measuring sIgE.

The reference standard for diagnosing peanut or soy allergy (or any food allergy) is the double-blind, placebo-controlled food challenge.[32;33] A food challenge has the additional advantage that the eliciting dose can be established and that the severity of the allergy can be estimated. In this way, an adequate diet can be prescribed in case of a positive food challenge. On the other hand, the food challenge also has some clear disadvantages: it is time-consuming, has high costs, is stressful for the patient, might result in severe clinical responses and it requires high standard hospital facilities.[34-36] Therefore, there is strong need for an accurate diagnostic test that is cheap, non-invasive and ideally can discriminate between mild and severe allergy as well. One way of improving diagnostics is by combining several more or less distinct parameters into a prediction model. DunnGalvin et al showed an excellent discrimination (AUC 0.97) between peanut allergy and peanut tolerance when they validated their prediction model consisting of 6 parameters in a population in the same center.[37] Another way

of improving diagnostics is to determine sIgE to peanuts' or soy's individual allergenic proteins, so-called components (see below), which were not included in the prediction model described by DunnGalvin et al.

PEANUT AND SOY COMPONENTS

Peanut and soy extract contain both allergenic as well as non-allergenic proteins. Instead of measuring sIgE to the whole extract, the diagnostic accuracy might be improved by measuring sIgE to these specific allergenic proteins, also called components. For peanut, 13 peanut components have been described at this moment (Ara h 1-13), with Ara h 4 being an almost identical isoform of Ara h 3 and therefore renamed to Ara h 3.02 (Table 1).[38;39] All components can be categorized into different protein families.[39] Ara h 1 (7S globulin) and Ara h 3/4 (11S globulin) are part of the cupin family. Ara h 1 was the first component to be identified as a major allergen in peanut allergic patients.[40] Ara h 2 and Ara h 6 are both conglutins (2S albumins) and share many homologue epitopes.[41]

Table 1. Peanut components

Allergen	Protein family	Molecular weight (kD)[38]	Important related proteins
Ara h 1	Cupin (7S globulin)	64	Gly m 5 (soy) Cor a 11 (hazelnut)
Ara h 2	Conglutin (2S albumin)	17	Gly m 8 (soy) Cor a 14 (hazelnut)
Ara h 3	Cupin (11S globulin)	60	Gly m 6 (soy) Cor a 9 (hazelnut)
Ara h 4	Cupin (11S globulin)	*see Ara h 3*	*see Ara h 3*
Ara h 5	Profilin	15	Bet v 2 (birch) Gly m 3 (soy)
Ara h 6	Conglutin (2S albumin)	15	*see Ara h 2*
Ara h 7	Conglutin (2S albumin)	15	*see Ara h 2*
Ara h 8	PR-10 protein	17	Bet v 1 (birch) Mal d 1 (apple)
Ara h 9	Lipid transfer protein (LTP)	9.8	Pru p 3 (peach) Art v 3 (mugwort) Pla a 3 (plane tree)
Ara h 10	Oleosin	16	Cor a 12 (hazelnut)
Ara h 11	Oleosin	14	Cor a 12 (hazelnut)
Ara h 12	Defensin	5.2	Gly m 2 (soy) Art v 1 (mugwort)
Ara h 13	Defensin	8.4	*see Ara h 12*

Table 2. Soy components

Allergen	Protein family	Molecular weight (kD)[38]	Important related proteins
Gly m 1	Hydrophobic soybean protein	7	-
Gly m 2	Defensin	8	Ara h 12 & 13 (peanut) Art v 1 (mugwort)
Gly m 3	Profilin	14	Bet v 2 (birch)
Gly m 4	PR-10	17	Bet v 1 (birch) Mal d 1 (apple)
Gly m 5	Beta-conglycinin (7S globulin)	48	Ara h 1 (peanut) Cor a 11 (hazelnut)
Gly m 6	Glycinin (11S globulin)	55	Ara h 3 (peanut) Cor a 9 (hazelnut)
Gly m 7	Seed biotinylated protein	76.2	Len c 2 (lentil)
Gly m 8	2S albumin	12	Ara h 2 & 6 (peanut) Cor a 14 (hazelnut)

They are very stable towards digestion, heat and roasting.[42-45] Previous studies showed that the majority of peanut allergic patients recognized Ara h 2 and Ara h 6, both in adults and children.[41;46-48] Ara h 7 is also a conglutin but no proper studies have been conducted with this allergen component. Possibly because of its small amount of the total protein content in peanut (around 0.5%), compared to Ara h 1, Ara h 2 and Ara h 3 (together 75%).[39;49] Ara h 5 is a profilin and together with Ara h 8 (PR-10 protein) and Ara h 9 (LTP protein) associated with cross-reactivity with different pollen (Ara h 5, 8 and 9) or peach (Ara h 9).[50;51] Ara h 10 and 11 are both oleosins, proteins of the oil bodies in the seeds, and Ara h 12 and 13 defensins. The clinical relevance of Ara h 10-13 is unknown yet.[49] In Chapter 2 of this thesis a selection of these peanut components will be further introduced from a diagnostic perspective.

To date, 8 components have been described for soy (Table 2).[38] Gly m 1 and Gly m 2 are associated with respiratory symptoms from soybean dust.[52;53] Gly m 3 is a profilin and together with Gly m 4 (PR-10 protein) often cross-reactive with different pollen allergens.[7;54] Gly m 5-8 are seed proteins. Gly m 8 was formerly known as Gly m 2S albumin and has recently been renamed to Gly m 8.

OUTLINE OF THIS THESIS

This thesis addresses the diagnosis and management of peanut allergy with a focus on sIgE to allergen components. Because of the serological cross-reactivity between peanut and soy, which results to some extent in clinical cross-reactivity, the diagnostic work-up of soy allergy will also be addressed.

In *Chapter 2* we systemically review the current literature as a further introduction on sIgE to peanut components. The discriminative ability of the SPT and sIgE to peanut extract will be compared with sIgE to peanut components. Since components can be measured by different techniques, *Chapter 3* compares four techniques with each other. A validation study of the prediction model of DunnGalvin et al[37] is performed in a pediatric population in *Chapter 4*. In addition, sIgE to peanut components are analyzed to their ability to further improve this prediction model. Consequently, the updated diagnostic pediatric prediction model is validated in an adult population in *Chapter 5*. Also in this adult population, the discriminative ability of sIgE to peanut components is analyzed as well as their relation with the severity of peanut allergy. *Chapter 6* directly compares the diagnostic value of measuring sIgE to Ara h 2 and Ara h 6, both 2S albumins, in diagnosing peanut allergy. In *Chapter 7* we address the diagnostic work-up of soy allergy; the discriminative ability of sIgE to soy components, their relation with the severity of soy allergy and their relation with the culprit soy source. The influence of sIgE to Ara h 2 and Ara h 8 on threshold distribution curves, derived from many individual eliciting doses during food challenges in both adults and children, is analyzed, together with other clinical characteristics, in *Chapter 8*. This information can be used for risk assessment to improve current labelling guidelines. Finally, all findings will be discussed in *Chapter 9* in the context of potential clinical implications.

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Chapter 2

Diagnostic accuracy of specific IgE to components in diagnosing peanut allergy: a systematic review

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ABSTRACT

Background: The diagnostic accuracy of skin prick test (SPT) and specific IgE (sIgE) to peanut extract in diagnosing peanut allergy is suboptimal. Recent studies have evaluated sIgE to peanut components as a possible new diagnostic tool.

Objective: To systematically search the literature to assess the diagnostic value of sIgE to peanut components in diagnosing peanut allergy.

Methods: A literature search was performed in PubMed, Embase and the Cochrane Library. Results were subsequently screened for in- and exclusion criteria. The quality of eligible studies was assessed using a standardized quality assessment tool (QUADAS-2). Data on sensitivity, specificity, and positive and negative likelihood ratios were extracted or calculated for a descriptive analysis.

Results: Twenty-two studies were eligible, of which 21 studies in pediatric populations. Most studies reported on sIgE to peanut extract (15) and sIgE to Ara h 2 (12), followed by SPT (9) and sIgE to Ara h 1 (7). All studies were at risk of bias or caused applicability concerns on at least one item of the quality assessment tool. The best combination of diagnostic accuracy measures of all diagnostic tests was found for sIgE to Ara h 2. This finding was independent of geographic location. Compared with SPT and sIgE to peanut extract, sIgE to Ara h 2 was mainly superior in diagnosing peanut allergy in case of a positive test result. Worst diagnostic accuracy measures were found in general for sIgE to Ara h 8 and sIgE to Ara h 9.

Conclusion and Clinical Relevance: sIgE to Ara h 2 showed the best diagnostic accuracy of all diagnostic tests to diagnose peanut allergy. Compared with the currently used SPT and sIgE to peanut extract, sIgE to Ara h 2 was superior in diagnosing peanut allergy and should therefore replace these tests in daily clinical practice, especially in children.

INTRODUCTION

Peanut allergy is one of the most common food allergies and the prevalence might be increasing.[1;2] Peanut allergic symptoms range from mild, for example oral allergy symptoms, to severe respiratory or cardiac symptoms, which can potentially be fatal.[3] Therefore, the diagnosis of peanut allergy should not be missed. On the other hand, overdiagnosis leads to unnecessary elimination diets, which significantly impairs quality of life.[4;5]

The diagnostic accuracy of the routine tests to diagnose peanut allergy, the skin prick test (SPT) and specific IgE (sIgE) to peanut extract, is suboptimal.[6-11] The double-blind, placebo-controlled food challenge (DBPCFC) is considered the gold standard for food allergy diagnosis and is often used as reference test.[12] In clinical practice however, many physicians report financial and logistic constraints, including lack of time and staff, and concerns with regards to the risk for the patient.[13] Therefore, the use of sIgE to peanut components has been increasingly studied to improve peanut allergy diagnostics and consequently reduce the need for DBPCFCs.

Until now, sIgE to peanut components Ara h 1, Ara h 2, Ara h 3, Ara h 5, Ara h 6, Ara h 8 and Ara h 9 have been analyzed in the context of diagnosis of peanut allergy.[8;10;11;14-17] Most of these studies showed a superior diagnostic accuracy of Ara h 2. It is important to summarize these findings to update guidelines regarding the diagnosis of peanut allergy. Therefore, the aim of this review was to systematically search the literature to assess the diagnostic value of sIgE to peanut components compared with the currently used SPT and sIgE to peanut extract in diagnosing peanut allergy (confirmed by food challenge) in subjects suspected of peanut allergy.

MATERIALS AND METHODS

Information sources and search

PubMed, Embase and the Cochrane Library were searched until October 15, 2013. The search combined the 4 elements of the PICO-question in this review: 'Patients' (suspected of peanut allergy), 'Index test' (specific IgE to peanut components), 'Comparison test' (skin prick test (SPT) and specific IgE (sIgE) to peanut extract) and 'Outcome' (peanut allergy assessed by food challenge, Table 1).[18] Two authors independently performed the search and consequently screened for in- and exclusion criteria (RK and HvOM). Disagreements were resolved by discussion. The references of the studies that met all criteria were reviewed to detect studies missing in the initial search.

Table 1. Search strategy

Database	Search
Pubmed	(((peanut*(Title/Abstract)) AND ((((((((((IgE(Title/Abstract)) OR sIgE(Title/Abstract)) OR CRD(Title/Abstract)) OR "skin prick test"(Title/Abstract)) OR "skin prick testing"(Title/Abstract)) OR SPT(Title/Abstract)) OR Component*(Title/Abstract)) OR Allergen*(Title/Abstract)) OR Ara(Title/Abstract)) OR Extract(Title/Abstract))) AND ((((((((((challenge(Title/Abstract)) OR DBPCFC(Title/Abstract)) OR double-blind(Title/Abstract)) OR "double blind"(Title/Abstract)) OR placebo-controlled(Title/Abstract)) OR "placebo controlled"(Title/Abstract)) OR OFC(Title/Abstract)) OR Oral(Title/Abstract)) OR Allerg*(Title/Abstract))
Embase	(peanut*:ab,ti) AND (ige:ab,ti OR sige:ab,ti OR crd:ab,ti OR 'skin prick test':ab,ti OR 'skin prick testing':ab,ti OR spt:ab,ti OR component*:ab,ti OR allergen*:ab,ti OR ara:ab,ti OR extract:ab,ti) AND (challenge:ab,ti OR dbpcfc:ab,ti OR 'double-blind' OR 'double blind':ab,ti OR 'placebo-controlled':ab,ti OR 'placebo controlled':ab,ti OR ofc:ab,ti OR oral:ab,ti OR allerg*:ab,ti) AND (embase)/lim NOT (medline)/lim
Cochrane Library	(peanut*:ti,ab,kw) AND (ige:ti,ab,kw OR sige:ti,ab,kw OR crd:ti,ab,kw OR "skin prick test":ti,ab,kw OR "skin prick testing":ti,ab,kw OR spt:ti,ab,kw OR component*:ti,ab,kw OR allergen*:ti,ab,kw OR ara:ti,ab,kw OR extract:ti,ab,kw) AND (challenge:ti,ab,kw OR dbpcfc:ti,ab,kw OR "double-blind":ti,ab,kw OR "double blind":ti,ab,kw OR "placebo-controlled":ti,ab,kw OR "placebo controlled":ti,ab,kw OR ofc OR oral:ti,ab,kw OR allerg*:ti,ab,kw)

ab, abstract; *ti,* title; *kw,* keyword

Eligibility criteria

Studies were included if patients were suspected of peanut allergy based on history and/or sensitization (as measured by SPT or sIgE to peanut extract, Figure 1). In addition, at least one index or comparator test had to be compared with the reference standard. Although the double-blind, placebo-controlled food challenge (DBPCFC) is the reference standard for food allergy, we also included studies that used an open food challenge as reference standard. At least 25% of the study population had to be challenged with a minimum of at least 5 positive and 5 negative outcomes. At last, sufficient information for a 2x2 table should be available or sensitivity, specificity and positive and negative likelihood ratio's should already be stated in the article.

Studies were excluded if they were performed in animals, if they were published over 10 years ago or if the written language was different than English. The timespan of 10 years was chosen because peanut components have only been widely used for the past few years. We preferred studies with a cross-sectional design as in those the tests under investigation are performed on the same day or same period as the reference standard. However, since in practice this does not always happen we also included cohort or case-control studies. Case-control studies were excluded however, if only healthy or atopic controls, who were not suspected of peanut allergy, had been included. Although interesting, studies addressing only diagnostic tests other than SPT with peanut extract and/or sIgE to peanut extract and/or peanut components, such as the basophil activation test (BAT) for example, were excluded as well. The final exclusion

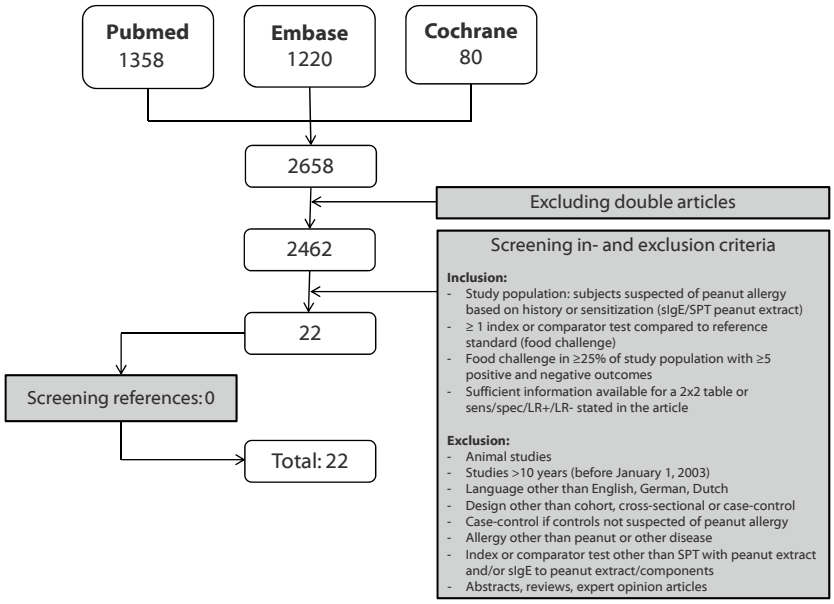

Figure 1. Flowchart

criteria were abstracts, reviews, expert opinion articles or studies addressing allergies other than peanut or other diseases. In this review, we did not select on age.

Quality assessment

We used the STARD Statement for Reporting Studies of Diagnostic Accuracy to systematically report all information of the primary studies necessary for quality assessment.[19] Consequently, the quality of the included studies was assessed using the QUADAS-2 checklist.[20] This checklist contains four domains: 1) patient selection, 2) index test, 3) reference standard and 4) flow and timing. For these items the risk of bias (all 4) and concerns regarding applicability (first 3) were scored; high, low, or unclear. The index and comparator test were both scored as 'index test' in this regard. Signaling questions with corresponding guidelines were developed to assess the risk of bias or concerns regarding applicability for the same four different domains. These questions and guidelines can be found in Table 3. For the patient selection domain, we defined 'suspicion of peanut allergy' as a positive history of allergic symptoms after ingesting peanut, regardless of sensitization, or as sensitization (based on SPT and/or sIgE to peanut extract) without previous ingestion. We focussed on secondary and tertiary care centres, since food challenges are usually only performed in these settings. The quality assessment was performed by 2 independent authors (RK and MB). Disagreements were resolved by discussion.

Data extraction and statistical analysis

Clinical heterogeneity between studies was first assessed by comparing clinical parameters by two authors (RK and MB). The most important parameter in this respect was the inclusion criteria on which the suspicion of peanut allergy was based, since this could highly influence the outcome measures by determining the a priori chance of having peanut allergy. If clinical heterogeneity was low, a statistical measure (I^2) would be assessed to decide whether statistical pooling was allowed. In case of clinical or statistical heterogeneity (I^2>40%), a descriptive analysis was performed.

Author, year of publication, country in which the study was conducted, setting, mean or median age, gender distribution of the study population and inclusion criteria of all studies were recorded. Sensitivity, specificity, positive and negative predictive values (PPV and NPV) and positive and negative likelihood ratios (LR+ and LR-) from all tests analyzed were extracted. If not stated in the article, 2x2 tables were developed for every index or comparator test to calculate these values. To calculate LR+ and LR-, sensitivity and specificity values with one decimal were used. The clinically used cutoff values for sIgE to peanut extract or peanut components (≥0.35 kU/L when measured by single-plexed assay or ≥0.3 ISU/L when measured by multi-plexed microarray) and SPT (≥3mm) were used for these calculations. Other cutoff values were used if this was not possible, always trying to utilize cutoff values as close to the clinical cutoff values mentioned above.

In case of studies with a case-control design with multiple control groups, data was used from the control group that most closely resembled the definition of subjects suspected of peanut allergy of our research question: positive history regardless of sensitization, or sensitization (based on SPT or sIgE to peanut extract) without previous ingestion.

RESULTS

Study selection

Our literature search identified 2658 articles in Embase, Pubmed and the Cochrane Library (Figure 1). After excluding duplicate articles and screening on in- and exclusion criteria, 22 articles were left to be further analyzed. Screening of the references of these articles did not result in additional relevant publications.

Study characteristics

Table 2 shows the characteristics of the 22 studies that were included. Half of the studies were performed in Europe (n=11), of which all but 1 in Northwest Europe. One study was performed in Central Europe (Switzerland). The remaining studies were performed in Australia (=5), the US (n=3), Canada (n=1), Japan (n=1) or Thailand (n=1).

The majority of studies (81.8%) were performed in tertiary centres/referral centres for food allergy (Table 2). Two studies reported a case-control design,[7;21] one study partially had a case-control design by artificially enhancing the peanut allergic group,[11] the

Table 2. Characteristics of included studies (n=22)

Characteristics	Number (%)	
Geographical area in which the study was conducted		
Europe	11	(50)
Australia	5	(22.7)
US/Canada	4	(18.0)
Asia	2	(9.1)
Setting		
Tertiary centre/referral centre for food allergy	18	(81.8)
Population setting	2	(9.1)
Other*	2	(9.1)
Design		
Cross-sectional	19	(86.4)
Case-control	2	(9.1)
Other^	1	(4.5)
Age		
Range mean pediatric studies	~13 mo – 8 yrs	
Range median pediatric studies	~13 mo – 12 yrs	
Mean adult study (SD)	30 yrs (±12.5)	
Diagnostic tests analyzed**		
SPT peanut extract	9	(40.9)
sIgE to peanut extract	15	(68.2)
sIgE to Ara h 1	7	(31.8)
sIgE to Ara h 2	12	(54.5)
sIgE to Ara h 3	6	(27.3)
sIgE to Ara h 5	2	(9.1)
sIgE to Ara h 8	5	(22.7)
sIgE to Ara h 9	4	(18.0)

**Combination of birth cohort and referral centre*

^ Combination of case-control and cross-sectional

*** All but two studies measuring sIgE to peanut components used recombinant components*

remaining studies had a cross-sectional design (usually not explicitly mentioned). All but one study were performed in pediatric populations. The age in the pediatric studies ranged from ~12 months to 8 years (mean) and ~12 months to 12 years (median). The mean age in the adult study was 30 years (SD ±12.5).[8] Characteristics regarding gender were reported in 19 studies. Of these, all pediatric studies reported a male predominance that ranged between 54-73% of the study population. In the adult study, 37% was male.

Of all studies, 15 (68.2%) reported the diagnostic accuracy of sIgE to peanut extract (Table 2). All but two studies made use of the ImmunoCAP (Uppsala, Sweden) to measure sIgE to peanut extract or recombinant peanut components. One study used an original enzyme allergosorbent test, using purified peanut proteins.[22] Another study spotted purified peanut proteins on the VBC ISAC (Immuno Solid-phase Allergen Chip; VBC Genomics, Vienna, Austria).[23] Keet et al[24] was the only study that measured sIgE to peanut components by using both ImmunoCAP (recombinant Ara h 2) and ImmunoCAP ISAC103 (natural Ara h 2, Uppsala, Sweden). SPT was performed with extracts from ALK-Abello (n=5), Hollister-Stier (n=3) or Stallergenes (n=1).

Quality assessment

The quality assessment of all studies can be found in Table 3. Twelve studies were scored as high risk of bias with regard to patient selection. In these studies, the main reason was that patients were not random or consecutively enrolled (n=8), a case-control design was used (n=2), or non-comparable cohorts were combined (n=2). Fifteen studies (68%) used open challenges as reference standard and were therefore at risk of bias for this domain. In addition, 2 out of these 15 studies used a combination of history and sensitization criteria as well as reference standard. In all studies, the diagnostic test that was used (referred as index test in Table 3) was not at risk of bias.

With regard to applicability concerns, in 12 studies (55%) there was a high concern for the patient selection domain. In 6 studies this was due to the fact that the criteria on which peanut allergy was suspected did not completely match the research question. In these studies suspicion was based on sensitization regardless of history (n=3,) on the combination of positive history and sensitization (n=2), or based on a positive SPT only (n=1). Other reasons for a high applicability concern for patient selection were a different setting of (part of) the study population (n=4) or a selection of suspected peanut allergic patients based on severity by excluding people with high sensitization values (n=2). Although the exact criteria for a positive food challenge varied widely between studies, the applicability concern regarding reference standard was low in general.

Heterogeneity

We did not statistically test for heterogeneity. The reason was that there was already too much heterogeneity based on clinical parameters to statistically pool the data. This

Table 3. Quality assessment. (+) = high risk of bias or high concern regarding applicability. (-) = low risk of bias or low concern regarding applicability. (?) = unclear risk or concern

	RISK OF BIAS				APPLICABILITY CONCERNS		
Author	**Patient selection**	**Index test**	**Reference standard**	**Flow/ timing**	**Patient selection**	**Index test**	**Reference standard**
Bernard[22]	?	-	+	+	-	+	-
Kagan[33]	-	-	+	-	+	-	-
Perry[29]	?	-	+	-	+	-	-
Roberts[34]	+	-	+	+	+	-	-
Nolan[9]	-	-	+	-	+	-	-
Wainstein[7]	+	-	+	-	+	-	-
v Nieuwaal[35]	+	-	-	-	-	+	-
DunnGalvin[36]	?	-	+	-	-	-	-
Johannsen[6]	-	-	+	-	-	-	-
Nicolaou[11]	+	-	+	+	+	-	+
Dang[21]	+	-	+	-	+	-	-
Ebisawa[14]	-	-	+	-	-	-	-
Glaumann[37]	?	-	-	-	+	-	-
Eller[10]	+	-	+	-	-	-	-
Keet[24]	+	-	+	-	+	-	-
Klemans/Otte[15]	+	-	-	-	-	-	-
Klemans/ Broekman[8]	+	-	-	+	-	-	-
Lieberman[17]	+	-	-	-	+	-	-
Lopes de Oliveira[23]	?	-	?	-	?	-	-
Ludman[38]	+	-	?	+	-	?	?
Peters[39]	-	-	+	+/-*	+	+	-
Suratannon[25]	+	-	+	+	+	-	+

RISK OF BIAS

Patient selection: (+) patients were not completely random or consecutively enrolled (because of missing data or serum not available for example), a case-control design was used, non-comparable cohorts were combined or >5% of the study population was excluded because of inconclusive outcomes of the reference standard

Index test: (+) cutoff value of index or comparator test in the article was not pre-specified

Reference standard: (+) reference standard was less likely to correctly classify peanut allergy (open challenge used in >50% of study population or reference test other than food challenge in >5% of study population)

Flow and timing: (+) more than one reference standard was used, >5% of the study population did not receive a reference standard and/or the index/comparator test

APPLICABILITY CONCERNS

Patient selection: (+) setting other than secondary or tertiary care, patients selected based on severity (other than excluding severe anaphylactic patients) or sensitization (other than used as acceptable inclusion criteria: IgE to peanut extract ≥ ~0.35kU/L or SPT ≥ ~3mm), suspicion peanut allergy other than positive history regardless of sensitization or sensitization (based on SPT or sIgE to peanut extract) without prior ingestion**

Index test: (+) index or comparator test was not standardized, non-commercially available products were used or cutoff values were only based on 95% PPVs

Reference standard: (+) criteria for positive food challenge highly likely to under- or overestimate the prevalence of peanut allergy or reference test other than food challenge in >5% of study population

*High risk for sIgE extract (missing data >5%), low risk for SPT

**Studies in which peanut allergy was suspected based on a positive history in combination with sensitization were scored as (+) because of a higher a priori chance of having peanut allergy

heterogeneity was mainly based on different inclusion criteria of the studies, which could also be found within studies. We therefore performed a descriptive analysis (Table 4).

Skin prick test and sIgE to peanut extract

Eight studies used a cutoff value of 3mm for SPT and showed a sensitivity ranging between 66-100% with a specificity of 0-95%. Corresponding LR+ and LR- were between 1-3.91 and 0-0.65, respectively. The specificity of 0% in 2 studies, with an LR- that could not be calculated, could be explained by the inclusion criteria; an SPT ≥3mm was required for all subjects.

Sensitivity for sIgE to peanut extract with a cutoff value of 0.35 kU/L ranged between 80-100% in 12 studies with corresponding specificity between 0-63%, LR+ 0.95-2.15 and LR- 0-0.56. In one study with a lower cutoff value (0.23 kU/L) these values were in the same range.[21] Two studies only provided high cutoff values that corresponded to the 95% PPV in those particular studies.

In studies with a higher cutoff value for SPT or sIgE to peanut extract, in general higher values for specificity, LR+, LR- and lower sensitivity values were found (Table 4).

sIgE to peanut components

All studies addressing the diagnostic accuracy of sIgE to peanut components used a cutoff value of 0.35 kU/L for the single-plexed assay (ImmunoCAP) or 0.30 ISU/L for the multiplexed microarray (ImmunoCAP ISAC). The best combination of diagnostic accuracy values was found for sIgE to Ara h 2; sensitivity, specificity, LR+ and LR- values ranged between 60-100%, 60-96%, 2.22-26.3 and 0-0.47, respectively (Table 4). The study that measured sIgE to Ara h 2 both by single-plexed assay and multiplexed microarray found comparable diagnostic accuracy among these tests.[24]

In general, sensitivity values for sIgE to Ara h 1 and Ara h 3 varied widely, between 26-92% and 21-84%, respectively, with low sensitivity values in the 2 Asian studies.[14;25] The range for specificity, LR+ and LR- between all studies was smaller but still within a wide range; 41-95%, 1.44-10.7 and 0.09-0.79 for sIgE to Ara h 1 and 44-91%, 1.40-4.80 and 0.23-0.87 for sIgE to Ara h 3.

Sensitivity values for sIgE to Ara h 5, Ara h 8 and Ara h 9 were low in all studies. A negative LR+ and positive LR- was found in 3 out of 5 studies analyzing sIgE to Ara h 8 and 2 out of 4 studies analyzing sIgE to Ara h 9, indicating that a value above the cutoff value was suggestive for peanut tolerance. Interestingly, the 2 studies with a positive LR+ and negative LR- for both sIgE to Ara h 8 and Ara h 9 were the 2 studies performed in Asian populations.[14;25]

Table 4. Diagnostic value per study and diagnostic test, studies per section graded on 1) lowest sum score on items quality assessment, 2) year of publication

	Study	Country	Criteria suspicion PA^	TP	FP	TN	FN	Se	Sp	LR+	LR-
Skin prick test											
Cutoff 3mm	Johannsen 2011	Australia	Pos SPT/sIgE without previous ingestion	24	14	11	0	100	44	1.79	0
	Kagan 2003	Canada	SPT ≥3mm without previous ingestion	23	24	0	0	100	0	1	-
	Nolan 2007	Australia	Pos history or pos SPT/sIgE without previous ingestion	17	11	16	1	94	95	2.32	0.09
	DunnGalvin 2011	Ireland and UK	Pos history or pos sIgE without previous ingestion	58	19	38	9	85	67	2.60	0.20
	Klemans/Br 2013	The Netherlands	Pos history, regardless of sensitization	31	16	17	16	66	52	1.38	0.65
	Wainstein 2007	Australia	Pos SPT, regardless of history	52	33	0	0	100	0	1	-
	Roberts 2005	UK	1)'Suspected PA' 2) Pos SPT and pos history or no previous ingestion	50	13	55	17	75	81	3.91	0.31
	Ludman 2013	Switzerland	Pos IgE/SPT without previous ingestion	9	8	3	1	90	27	1.24	0.37
Cutoff 8mm	Peters 2013*	Australia	SPT≥1mm compared with neg control	unk	unk	unk	unk	54	98	22.2	0.47
sIgE to peanut extract											
Cutoff 0.23 kU/L	Dang # 2012	Australia	SPT≥1mm compared with neg control	95	30	28	5	95	49	1.80	0.10
Cutoff 0.35 kU/L	Johannsen 2011	Australia	Pos SPT/sIgE without previous ingestion	23	18	6	1	96	25	1.28	0.17
	Ebisawa 2012	Japan	Pos history or pos sensitization	26	24	7	0	100	23	1.29	0
	DunnGalvin 2011	Ireland and UK	Pos history or pos sIgE without previous ingestion	50	31	26	17	75	46	1.37	0.56
	Glaumann 2012	Sweden	Pos history and pos SPT/sIgE	25	9	4	0	100	31	1.45	0
	Eller 2013	Denmark	Pos history	~166	~30	~0	~9	95	0	0.95	-
	Lieberman 2013	US	Pos history and pos SPT/sIgE (and pos FC in some patients)	99	51	10	7	93	17	1.12	0.41

Table 4. Continued

	Study	Country	Criteria suspicion PA^	TP	FP	TN	FN	Se	Sp	LR+	LR-
	Perry** 2004	US	Pos history (tolerance was suspected)	44	28	29	9	83	51	1.69	0.33
	Perry** 2004	US	Pos SPT/sIgE or unclear history (tolerance was suspected)	16	26	15	2	89	37	1.40	0.30
	Wainstein 2007	Australia	Pos SPT, regardless of history	51	22	11	1	98	33	1.47	0.06
	Lopes de O. 2013	Germany	?	34	27	0	0	100	0	1	-
	Bernard 2003	France	Pos history < 12 months	66	12	8	5	93	40	1.55	0.18
	Roberts 2005	UK	1)'Suspected PA' 2) Pos SPT and pos history or no previous ingestion	59	23	39	15	80	63	2.15	0.32
	Nicolaou 2011	UK	1)Pos SPT/sIgE regardless of ingestion 2) Pos history and (SPT ≥8mm and/or sIgE ≥15kU/l)	28	38	14	1	97	27	1.32	0.13
Cutoff 24.1 kU/L	V Nieuwaal 2010	The Netherlands	Pos history or pos sIgE/SPT without previous ingestion	27	1	46	29	48	98	23.0	0.53
Cutoff 34 kU/L	Peters 2013*	Australia	SPT≥1mm compared with neg control	unk	unk	unk	unk	14	99	11.2	0.87
sIgE to Ara h 1 single-plexed assay											
Cutoff 0.35 kU/L	Ebisawa 2012	Japan	Pos history or pos sensitization	10	7	24	16	38	77	1.70	0.79
	Glaumann 2012	Sweden	Pos history and pos SPT/sIgE	23	2	11	2	92	85	5.97	0.09
	Eller 2013	Denmark	Pos history	~101	~12	~18	~74	58	60	1.44	0.71
	Lieberman 2013	US	Pos history and pos SPT/sIgE (and pos FC in some patients)	59	8	53	47	56	87	4.31	0.51
	Bernard 2003	France	Pos history < 12 months	38	1	19	33	54	95	10.7	0.49
	Suratannon 2013	Thailand	Sensitization regardless of history/ ingestion	5	1	20	14	26	95	5.48	0.77

Table 4. Continued

	Study	Country	Criteria suspicion PA^	TP	FP	TN	FN	Se	Sp	LR+	LR-
sIgE to Ara h 1 multiplexed microarray											
Cutoff 0.30 ISU/L	Lopes de O. 2013	Germany	?	29	16	11	3	91	41	1.84	0.23
sIgE to Ara h 2 single-plexed assay											
Cutoff 0.35 kU/L	Ebisawa 2012	Japan	Pos history or pos sensitization	23	5	26	3	88	84	5.50	0.14
	Klemans/O. 2013	The Netherlands	Pos history or pos sIgE/SPT without previous ingestion	43	15	38	4	91	72	3.23	0.12
	Glaumann 2012	Sweden	Pos history and pos SPT/sIgE	24	2	11	1	96	85	6.23	0.05
	Eller 2013	Denmark	Pos history	~155	~12	~18	~20	89	60	2.22	0.19
	Klemans/B.2013	The Netherlands	Pos history, regardless of sensitization	32	5	28	21	60	85	4.00	0.47
	Lieberman 2013	US	Pos history and pos SPT/sIgE (and pos FC in some patients)	85	5	56	21	80	92	10	0.22
	Dang # 2012	Australia	SPT≥1mm compared with neg control	81	7	51	19	81	88	6.70	0.22
	Keet 2013	US	Pos sensitization, regardless of history	23	10	25	3	89	71	3.09	0.16
	Bernard 2003	France	Pos history < 12 months	50	3	17	21	71	85	4.69	0.35
	Nicolaou 2011	UK	1)Pos SPT/sIgE regardless of ingestion 2) Pos history and (SPT ≥8mm and/or sIgE ≥15kU/l)	29	2	50	0	100	96	26.3	0
	Suratannon 2013	Thailand	Sensitization regardless of history/ ingestion	13	1	20	6	68	95	14.3	0.33
sIgE to Ara h 2 multiplexed microarray											
Cutoff 0.30 ISU/L	Keet 2013	US	Pos sensitization, regardless of history	21	8	27	5	81	77	3.53	0.25
	Lopes de O. 2013	Germany	?	30	7	20	2	94	74	3.62	0.08

Table 4. Continued

	Study	Country	Criteria suspicion PA^	TP	FP	TN	FN	Se	Sp	LR+	LR-
sIgE to Ara h 3 single-plexed assay											
Cutoff 0.35 kU/L	Ebisawa 2012	Japan	Pos history or pos sensitization	11	6	25	15	42	81	2.18	0.72
	Glaumann 2012	Sweden	Pos history and pos SPT/sIgE	21	4	9	4	84	69	2.73	0.23
	Eller 2013	Denmark	Pos history	~67	~3	~27	~108	38	90	3.83	0.69
	Lieberman 2013	US	Pos history and pos SPT/sIgE (and pos FC in some patients)	51	6	55	55	48	90	4.80	0.58
	Suratannon 2013	Thailand	Sensitization regardless of history/ ingestion	4	2	19	15	21	91	2.22	0.87
sIgE to Ara h 3 multiplexed microarray											
Cutoff 0.30 ISU/L	Lopes de O. 2013	Germany	?	25	15	12	7	78	44	1.40	0.49
sIgE to Ara h 5 single-plexed assay											
Cutoff 0.35 kU/L	Ebisawa 2012	Japan	Pos history or pos sensitization	9	7	24	17	35	77	1.53	0.84
	Suratannon 2013	Thailand	Sensitization regardless of history/ ingestion	3	1	20	16	16	95	3.29	0.88
sIgE to Ara h 8 single-plexed assay											
Cutoff 0.35 kU/L	Ebisawa 2012	Japan	Pos history or pos sensitization	11	6	25	15	42	81	2.18	0.72
	Glaumann 2012	Sweden	Pos history and pos SPT/sIgE	9	9	4	16	36	31	0.52	2.08
	Eller 2013	Denmark	Pos history	~56	~13	~17	~119	32	57	0.74	1.20
	Lieberman 2013	US	Pos history and pos SPT/sIgE (and pos FC in some patients)	37	35	26	69	35	43	0.61	1.51
	Suratannon 2013	Thailand	Sensitization regardless of history/ ingestion	3	0	21	16	16	100	∞	0.84

Table 4. Continued

	Study	Country	Criteria suspicion PA^	TP	FP	TN	FN	Se	Sp	LR+	LR-
sIgE to Ara h 9 single-plexed assay											
Cutoff 0.35 kU/L	Ebisawa 2012	Japan	Pos history or pos sensitization	4	4	27	22	15	87	1.19	0.97
	Glaumann 2012	Sweden	Pos history and pos SPT/sIgE	3	2	11	22	12	85	0.78	1.04
	Eller 2013	Denmark	Pos history	~15	~5	~25	~160	8.6	83	0.51	1.10
	Suratannon 2013	Thailand	Sensitization regardless of history/ ingestion	5	1	20	14	26	95	5.48	0.77

PA, peanut allergy; *TP,* true positive; *FP,* false positive; *TN,* true negative; *FN,* false negative; *Se,* sensitivity; *Sp,* specificity; *LR+,* positive likelihood ratio; *LR-,* negative likelihood ratio; ∞, infinity; *unk,* unknown; *FC,* food challenge

^*Pos history,* positive history of adverse reaction to peanut; *pos SPT/sIgE,* IgE to peanut extract ≥ ~0.35kU/L and/or SPT ≥ ~3mm; *pos sensitization,* not further specified in article

* Study included 290 tolerant and 148 allergic patients. Exact values for TP,FP,TN,FP not known due to missing values for SPT and sIgE and use of logistic regression

Only peanut-sensitized subjects were used for tolerant group

**Subselection of patients within study: 1) Pos history, 2) Pos SPT/sIgE or unclear history

DISCUSSION

In this study we systematically searched the literature to assess the diagnostic value of sIgE to peanut components compared with the currently used SPT and sIgE to peanut extract in diagnosing peanut allergy. Twenty-two studies could be identified that addressed the diagnostic accuracy of SPT, sIgE to peanut extract and/or peanut components. Although the methodological quality of all studies was poor on at least one item, the results showed that sIgE to the peanut component Ara h 2 had a better diagnostic accuracy than all other tests analyzed in this review, including the currently used SPT and sIgE to peanut extract.

In the last two years many studies on the diagnostic accuracy of peanut components have been published. Therefore, we were allowed to set strict in- and exclusion criteria. Studies published before 2003 for example, were therefore excluded. Two of these studies however, that only addressed the diagnostic accuracy of SPT and sIgE to peanut extract, showed sensitivity and specificity results within the same range as shown in this review.[26;27] We also excluded case-control studies with controls not suspected of peanut allergy, such as the study of Codreanu et al,[16] because peanut components would not be used in daily clinical practice in these subjects. The study of Codreanu et al however, also suggested that sIgE to Ara h 2 is a better predictor for peanut allergy than the other peanut components. In addition, they showed that sIgE to Ara h 6 is a potential marker for peanut allergy. None of the studies in this review addressed the diagnostic accuracy of sIgE to Ara h 6.

Risk of bias in studies was often found in the domain of patient selection. Many studies in this review were retrospectively conducted. As a consequence, subjects were included based on availability of serum, no missing data, or were only included if they had underwent a food challenge. These subjects were most likely not completely random included among the entire population suspected of peanut allergy, which could have led to selection bias in these studies. In many studies, inconclusive food challenges were excluded from the analyses. This could have caused additional selection bias. As a consequence, the observed diagnostic outcome measures in these studies could differ from the actual diagnostic outcome measures in eligible subjects.[28]

The domain of patient selection also scored 'high' in many studies with regard to applicability concerns. We defined 'suspicion of peanut allergy' in our research question as a positive history, regardless of sensitization or sensitization without previous ingestion. In our opinion, this is the group of patients in which diagnostic tests are useful in clinical practice to diagnose or exclude peanut allergy. Studies that required subjects to have a positive history in combination with sensitization increased the pre-test probability of having peanut allergy. This could ultimately affect the outcome measures. In contrast, studies including subjects with sensitization regardless of history,

including even subjects ingesting peanut without any problem, decreased the pre-test probability of having peanut allergy. Although the outcome measures in studies with an increased or decreased pre-test probability of having peanut allergy are accurate for those particular patient groups, they cannot easily be applied to all subjects suspected of peanut allergy. It was remarkable however, that only minor differences in sensitivity and specificity values were found between studies with different inclusion criteria. The same finding was observed within studies. Perry et al[29] (in this review) and Codreanu et al[16] compared the outcome measures between groups of patients with and without a positive history to peanut and found similar results.

The reference standard was another domain of the quality assessment that was at risk of observer bias in many studies. This was always caused by the fact that at least 50% of the challenges was open. The choice for an open challenge could often be justified because of (young) children undergoing these challenges.[30] In addition, many studies used only objective symptoms to diagnose peanut allergy. Therefore, we believe that this possible source of bias was of minor influence on the outcome measures. It was remarkable however, that every study used a different challenge protocol, using a different dosing scheme, recipe, and definition for a positive challenge. Also, none of the studies that performed DBPCFCs, described how placebo reactions were handled. This illustrates the need for standardization of challenge procedures. This will help to better compare data among different studies.

Although the previous remarks regarding quality assessment are important to keep in mind when interpreting the results, sIgE to Ara h 2 had the best combination of diagnostic accuracy measures of all diagnostic tests. This finding was independent of the type of test that was used (single-plexed assay or multiplexed microarray) and also found in every study that directly compared sIgE to Ara h 2 with other diagnostic tests. In general, sIgE to Ara h 2 had the highest LR+, indicating that it was the best test to diagnose peanut allergy. The LR- was comparable to the SPT and sIgE to peanut extract. The LR+ and LR- allows to calculate the post-test probability given any prevalence of peanut allergy in a certain population (i.e., pre-test probability) using an easy nomogram.[31] For example, by using the lowest and highest LR+ for sIgE to Ara h 2 (2.22 and 26.3), a pre-test probability of 50% would result in a post-test probability of around 70 and 97%, respectively. The lowest and highest LR- (0 and 0.47) would decrease the post-test probability to around 0 and 30%, respectively.

The finding that sIgE to Ara h 2 had the best diagnostic accuracy measures appeared to be independent of the geographical areas that were represented in this review. Vereda et al[32] previously showed that there were differences in sensitization pattern in peanut allergic patients between countries. Spain, which had the most distinctive sensitization pattern in that study, with many patients recognizing the LTP protein Ara h 9, was not represented in this review. A diagnostic study addressing to peanut components

in Southern European countries would therefore be very helpful in improving the diagnostics of peanut allergy in these countries.

The most important geographical difference could be found between the 2 Asian studies performed in Thailand en Japan,[14;25] and the other studies with regard to sIgE to Ara h 8. Only in the Asian studies a value above the cutoff value for sIgE to Ara h 8 was indicative for peanut allergy, in the other studies it was indicative for peanut tolerance. This could probably be explained by the high specificity values in the Asian studies. Few peanut tolerant patients recognized sIgE to Ara h 8, indicating that birch-pollen related sensitization to Ara h 8 is uncommon in Asia. To some extent the same was true for sIgE to Ara h 9.

A limitation of this review was that all but 1 of the included studies were performed in children. Our previous study in adults showed the highest LR- for sIgE to Ara h 2.[8] This LR- value is caused by a significant amount of peanut allergic subjects who did not recognize sIgE to Ara h 2. Many of these subjects recognized sIgE to the PR-10 protein Ara h 8, so we therefore hypothesized that this is probably due to a pollen-related peanut allergy, which originates later in life. Additional studies in adults should be performed, preferably in non-birch endemic areas as well, to confirm this hypothesis.

In our opinion, this review shows strong evidence that sIgE to Ara h 2 is superior to SPT and sIgE to peanut extract and should replace these tests in daily clinical practice, especially in children and in the geographical areas that were represented in this review in both the SPT/sIgE to peanut extract group as the sIgE to peanut components group: Northwest Europe, North America and Australia. The concerns regarding methodological quality were equally found in both these groups. Future studies should focus on assessing clear cutoff values for sIgE to Ara h 2 in different settings.

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Chapter 3

IgE-binding to peanut components by four different techniques: Ara h 2 is the most relevant in peanut allergic children and adults

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ABSTRACT

Background: Several studies have analyzed the diagnostic value of specific IgE (sIgE) to individual peanut allergens. However, little is known about the concordance between different techniques available in both children and adults.

Objective: To evaluate the value of individual peanut allergens by different techniques, i.e. multi-plexed microarray, single-plexed IgE assay, skin prick test (SPT) and immunoblot in both peanut allergic adults and children.

Methods: Sensitization patterns to peanut allergens Ara h 1, 2, 3 and 8 were evaluated using four different techniques: multi-plexed microarray immunoassay, single-plexed IgE-assay, skin prick testing (SPT) and immunoblot. Twenty-two peanut allergic adults and 15 children scored on clinical severity according to double-blind, placebo-controlled food challenges and 27 atopic control patients were included.

Results: Comparable sensitivity values were found between all four techniques in adults, with the highest sensitivity for Ara h 2 (76.2-95.5%, compared with 100% with all techniques in children). The multi-plexed assay to Ara h 1 (93.3%) demonstrated a higher sensitivity compared with the other 3 techniques (P=0.04) in children, but absolute values were perfectly correlated. There were no differences between adults and children. The area under the receiver operating characteristic curve (AUC) of sIgE to Ara h 1 was higher with the multi-plexed assay compared with the single-plexed assay (0.91 vs. 0.75). In adults, sIgE to Ara h 1, 2 and 3 was correlated with clinical severity. No such correlation was found in children.

Conclusion and Clinical Relevance: In conclusion, the single- and multi-plexed assay, SPT and immunoblot perform equally in both peanut allergic adults and children, with Ara h 2 being most often recognized with all techniques. sIgE to Ara h 1, 2 and 3 in adults was correlated to severity.

INTRODUCTION

Peanut allergy is a common health problem affecting more than 1% of the population in the Western world.[1-4] Its prevalence may be on the rise, especially among children.[2;5] As peanut allergy is rarely outgrown and accidental exposure can elicit severe or even fatal reactions, accurate diagnostics is important.[6] The current gold standard, the double-blind placebo-controlled food challenge (DBPCFC), is expensive, laborious and burdensome. Routinely used diagnostic tools, such as skin prick test (SPT) and serum specific IgE (sIgE) tests often fail to discriminate between true allergy and asymptomatic sensitization.[6]

Component-resolved diagnostics (CRD) for peanut allergy might be more discriminative by using individual-specific allergens instead of whole peanut extracts, which contain both true peanut allergens and cross-reactive allergens (e.g. PR-10 family members) next to large amounts of non-allergenic proteins.[7;8] Until now, ten different specific peanut allergens have been characterized, e.g. Ara h 1 to 11, with Ara h 3 and 4 being the same protein. Among the three major peanut allergens (Ara h 1, 2 and 3/4), recent studies have demonstrated the superior value of Ara h 2.[9-17] However, none of these studies compared different techniques to analyze these peanut components. In addition, all these studies were performed in children. The sensitization pattern of peanut allergens in an adult peanut allergic population is largely unknown yet.

Therefore, our aim was to evaluate the value of individual peanut allergens by different diagnostic techniques, i.e. multi-plexed microarray, single-plexed IgE assay, skin prick test (SPT) and immunoblot in both peanut allergic adults and children. Peanut allergy was confirmed by DBPCFC and the sensitization profile of peanut allergic patients was compared with atopic control patients. Furthermore, we analyzed the possible association between IgE binding to peanut components and severity in both adults and children.

METHODS

Patients

In total 37 peanut allergic patients were included from the outpatient department of the University Medical Centre in Utrecht, The Netherlands. Twenty-two adults were included with a history of allergic reactions to peanut, sensitization and a positive DBPCFC.[18] The median age was 24 years (interquartile range (IQR) 21-29 years). Fifteen children were included based on sensitization to peanut and a positive DBPCFC.[19] Most of the children had a history of allergic reactions to peanut, but some had never consumed

peanut due to elimination diets. Median age was 6 years (IQR 4-7 years). The clinical characteristics of all peanut allergic subjects are summarized in Table 1.

In addition, 27 adult atopic controls, median age of 33 years (IQR 22-40 years), were included. At time of inclusion, all control patients indicated to consume peanut regularly without allergic symptoms. Ten patients had a mixed allergy to grass and birch pollen,

Table 1. Clinical characteristics of the peanut allergic patients (22 adults and 15 children)

ID	Sex	Age	Muller History	IgE peanut	SPT peanut	Lowest ED	Dose score	Symptoms DBPCFC	Muller DBPCFC	Severity Score
04	f	22	0	15.2	1.3	10	3	ap	2	3*(2+1)=9
16	m	21	0	<0.35	0.9	10	3	oas	0	3*(0+1)=3
20	f	21	0	<0.35	1.4	10	3	oas	0	3*(0+1)=3
01	m	21	1	3.6	1.9	300	1	oas	0	1*(0+1)=1
11	m	20	1	2.4	1.8	0.1	6	oas	0	6*(0+1)=6
19	f	35	1	3.4	1.4	1	4	rc	1	4*(1+1)=8
06	m	32	2	1.1	2.4	100	2	n, ap	2	2*(2+1)=6
14	f	27	2	16.1	6.8	10	3	n	2	3*(2+1)=9
18	f	29	2	0.9	2.9	10	3	ap, n, d	3	3*(3+1)=12
03	f	19	3	1.6	1.9	100	2	oas	0	2*(0+1)=2
05	f	37	3	8.7	3.8	10	3	dia	2	3*(2+1)=9
13	f	24	3	4.7	0.6	10	3	n, ap	2	3*(2+1)=9
15	f	23	3	91.4	5.9	0.1	6	d	3	6*(3+1)=24
21	m	33	3	100	6	1	4	ap, d	3	4*(3+1)=16
23	m	18	3	5.5	2.6	0.5	5	ap, n, v	2	5*(2+1)=15
25	f	29	3	1.9	8.8	100	2	n, ap	2	2*(2+1)=6
29	m	27	3	29.8	2	1	4	d, ap	3	4*(3+1)=16
02	m	16	4	101	1.9	100	2	d	3	2*(3+1)=8
09	m	25	4	28.1	6.4	0.1	6	n, v	2	6*(2+1)=18
10	f	22	4	101	6.2	0.1	6	n	2	6*(2+1)=18
22	f	24	4	9.2	4.2	10	3	d, h	3	3*(3+1)=12
26	f	28	4	18	14.8	0.1	6	oas	0	6*(0+1)=6
33	m	6	no	4	7	3000	2	rc, v	2	2*(2+1)=6
37	f	6	no	101	10	1000	3	rc, v, b	3	3*(3+1)=12
38	f	5	no	3.9	8	3000	2	oas, rc, ap, dia, d	3	2*(3+1)=8
40	m	4	no	9.5	1	1000	3	oas, fs, rc, ap, d	3	3*(3+1)=12
34	f	5	1	74.1	10	100	5	rc, d	3	5*(3+1)=20
35	m	6	1	101	6	10	6	oas, v	2	6*(2+1)=18
39	m	12	1	17.9	6	300	4	oas, rc, ap, v	2	4*(2+1)=12

Table 1. Continued

ID	Sex	Age	Muller History	IgE peanut	SPT peanut	Lowest ED	Dose score	Symptoms DBPCFC	Muller DBPCFC	Severity Score
42	f	4	1	24.7	2	1000	3	oas, ap, v	2	3*(2+1)=9
45	m	7	1	52.4	10	>3000	1	gu, rc, ap, v, dia	2	1*(2+1)=3
46	m	3	1	2	13	300	4	oas, v, rc, d	3	4*(3+1)=16
48	m	7	1	43	3	3000	2	oas, cu, fs	1	2*(1+1)=4
47	m	12	2	2.3	3	1000	3	n, v	2	3*(2+1)=9
41	m	4	3	58.7	10	1000	3	oas, gu, rc, b, t	4	3*(4+1)=15
43	m	7	3	101	3	10	6	oas, ap	2	6*(2+1)=18
44	m	12	3	2.4	9	100	5	oas, fs, urt, rc, ap	2	5*(2+1)=15

The solid line in the middle separates the adult (above) and pediatric (below) population. *No,* no symptoms*; oas,* oral allergy syndrome; *n,* nausea; *ap,* abdominal pain; *urt,* urticaria; *fs,* facial swelling; *rc,* rhinoconjunctivitis; *v,* vomiting; *dia,* diarrhea; *d,* dyspnea; *b,* bronchoconstriction; *t,* tachycardia. Severity score=dose score*(Mueller according to DBPCFC +1)

11 patients had a mono-allergy to grass pollen and 1 to birch pollen. The other 5 patients had a food allergy (other than peanut) in addition to their grass and birch pollen allergy.

The study was approved by the Medical Ethical Committee of the University Medical Center Utrecht. All patients gave written informed consent.

Design

Clinical information regarding history and results of earlier performed SPT, immunoblot and DBPCFC were obtained.[14;18;19] The DBPCFC was performed according to the international consensus protocol.[20] The challenge consisted of nine doses ranging from 0.01 mg to 3 g peanut flour. The recipe has been described in detail earlier.[18;19] The challenge was discontinued in case of objective symptoms or convincing subjective symptoms at three consecutive doses. The SPT was performed with purified Ara h 1, Ara h 2 and Ara h 3 in serial tenfold dilutions [14;18]. In this study we describe the SPT reactions on the highest dilution: 100 µg/ml, as this concentration gave the highest response. The preparation of the dilutions has been described in our previous studies of Flinterman et al. and Peeters et al.[18;19] Histamine dihydrochloride (10 mg/mL) and saline were used as positive and negative controls respectively. The SPT reactivity was measured after 15 minutes using computer scanning and expressed by the ratio of the wheal reaction to peanut in millimetres squared, divided by the area of the wheal reaction of the positive control.[21] A ratio ≥ 0.25 was regarded positive. For quantitative analysis of the immunoblots, a 5-points scale was used from 0 (negative) to 4 (strongly positive). A value ≥1 was considered positive. The severity of peanut allergy was expressed by a severity

score based on results of the DBPCFC. Specific IgE binding to whole peanut extract and recombinant peanut allergens Ara h 1, Ara h 2, Ara h 3 and Ara h 8 was measured using single-plexed IgE assay and a multi-plexed microarray as well.

Single-plexed IgE assay

Specific IgE to whole peanut extracts and four recombinant peanut allergen components were assessed using ImmunoCAP Specific IgE (Thermo Fisher Scientific, Uppsala, Sweden) according to manufacturer's instructions. A value of sIgE < 0.35 kU_A/L was considered negative.

Multi-plexed microarray immunoassay

Immuno Solid-phase Allergen Chip (ImmunoCAP ISAC, VBC Genomics and Thermo Fisher Scientific) with 103 allergen components was used following manufacturer's instructions.[22] A value of < 0.3 ISU was considered negative.

Clinical severity

Severity of allergic reactions according to history and during DBPCFC was graded according to Mueller.[23] Mueller grade 0 was defined as local symptoms of the oral cavity, grade 1 as symptoms of the skin and mucous membranes, grade 2 as gastrointestinal symptoms, grade 3 as respiratory symptoms and grade 4 as cardiovascular symptoms. Mueller grades do not take eliciting dose (ED) into account, which is an important clinical factor. Therefore, we applied a severity score which combines the severity of the reaction and the lowest ED during challenge in a similar way as previously described.[24;25] Severity score = (Mueller according to DBPCFC +1)*dose score (which is a 1-6 ranked score corresponding to ED from 300mg to 0.1 mg in adults and ED from >3g to 10mg in children). Mueller scores were increased by one to avoid multiplying with zero. Dose score was defined according to the range of ED in each of the two subpopulations. The range of ED in adults was from 300mg to 0.1 mg; therefore, the dose score was 1 for an ED of 300mg, 2 for 100mg, 3 for 10mg, 4 for 1mg, 5 for 0.5 mg and 6 for 0.1 mg. In children, the range of ED was from >3g to 10mg; therefore, the dose score was 1 for an ED of >3g, 2 for 3g , 3 for 1g , 4 for 300mg, 5 for 100mg and 6 for 10mg.

Statistics

All analyses were performed using non-parametric tests as the data were not normally distributed. Correlations were analyzed using Spearman's rank. Comparisons of two independent groups of continuous variables were tested by Mann-Whitney U test and comparisons of more independent groups were tested by Kruskal-Wallis test. Dichotomous variables were analysed using chi-square test. The area under the receiver operating characteristic (ROC) curve (AUC) was used to discriminate between peanut

allergic individuals and controls with a value of 0.5 indicating 'no discrimination' and a value of 1 indicating 'perfect discrimination.'

All analyses were performed using SPSS (version15, Chicago, IL, USA). P values < 0.05 were considered statistically significant.

RESULTS

Sensitivity values between four different techniques in both adults and children

Sensitivity values were assessed as the percentage of peanut allergic adults and children with a positive test result with any of the four different techniques to the peanut components Ara h 1, 2, 3 and 8 (Table 2). In the adult population, we found no significantly different sensitivity values between all four techniques for all peanut components. Ara h 2 was most often recognized by all tests with the highest sensitivity

Table 2. Sensitivity of 4 different techniques in both adults and children

	Sensitivity adults (%)	**Sensitivity children (%)**	**Children vs. adults (*P*-value)**
Ara h 1			
ImmunoCAP*	52.4	60.0	*0.74*
ImmunoCAP ISAC	68.2	93.3	*0.11*
SPT	59.1	50.0 (n=8)	*0.70*
Immunoblot	68.2	46.7	*0.31*
P-value	*0.66*	*0.04*	
Ara h 2			
ImmunoCAP*	76.2	100	*0.06*
ImmunoCAP ISAC	86.4	100	*0.26*
SPT	95.5	100 (n=7)	*1.00*
Immunoblot	90.9	100	*0.51*
P-value	*0.27*	*NA***	
Ara h 3			
ImmunoCAP*	42.9	60.0	*0.50*
ImmunoCAP ISAC	54.5	60.0	*1.00*
SPT	59.1	55.6 (n=9)	*1.00*
Immunoblot	68.2	53.3	*0.49*

Table 2. Continued

	Sensitivity adults (%)	Sensitivity children (%)	Children vs. adults (*P*-value)
P-value	*0.41*	*0.98*	
Ara h 8			
ImmunoCAP*	68.4	60.0	*0.72*
ImmunoCAP ISAC	59.1	53.3	*0.75*
P-value	*0.75*	*1.00*	

* ImmunoCAP was measured in 21 of 22 adults, ** Not able to calculate

established by SPT (95.5%). In addition, significant correlation coefficients between all techniques were found for individual components (data not shown).

In children a significantly higher sensitivity value for Ara h 1 was seen with the multi-plexed assay (93.3%) compared with the single-plexed assay, SPT and immunoblot (60%, 50% and 46.7%, respectively, P=0.04, Table 2). Still, the absolute value of the multi-plexed assay was perfectly correlated with the absolute value of the single-plexed assay (rho 0.86, P<0.001), SPT (rho 0.84, P<0.001) and immunoblot (rho 0.59, P=0.004). No significant difference in sensitivity was found between the four techniques with respect to Ara h 2, 3 and 8. The correlation coefficients between all techniques were also significant for these individual peanut components in children (data not shown). Also in children, Ara h 2 was most often recognized, with a sensitivity of 100% for all four techniques.

With all different techniques to measure Ara h 1, 2, 3 and 8 comparable sensitivity values were seen between adults and children, although there was a trend that Ara h 2 was more often recognized by the single-plexed assay in children compared with adults (100% vs. 76.2%, P=0.06, Table 2).

sIgE values comparable between the single- and multi-plexed assay in both adults and children

Potential differences between the techniques were in more detail analyzed between the single- and multi-plexed assay with regard to absolute values because of the comparable outcome measure and their frequent use in routine clinical practice.

No significantly different median sIgE levels were found between the single- and multi-plexed assay in both adults and children to all peanut components (Figure 1). Moreover, no significant difference was seen when the children and adult data were put together and analyzed as one group (data not shown).

In addition, the absolute values of the SPT reactivity and immunoblot between adults and children were comparable. The exact median values for both adults and children of all four techniques to all peanut components can be found in Table 3.

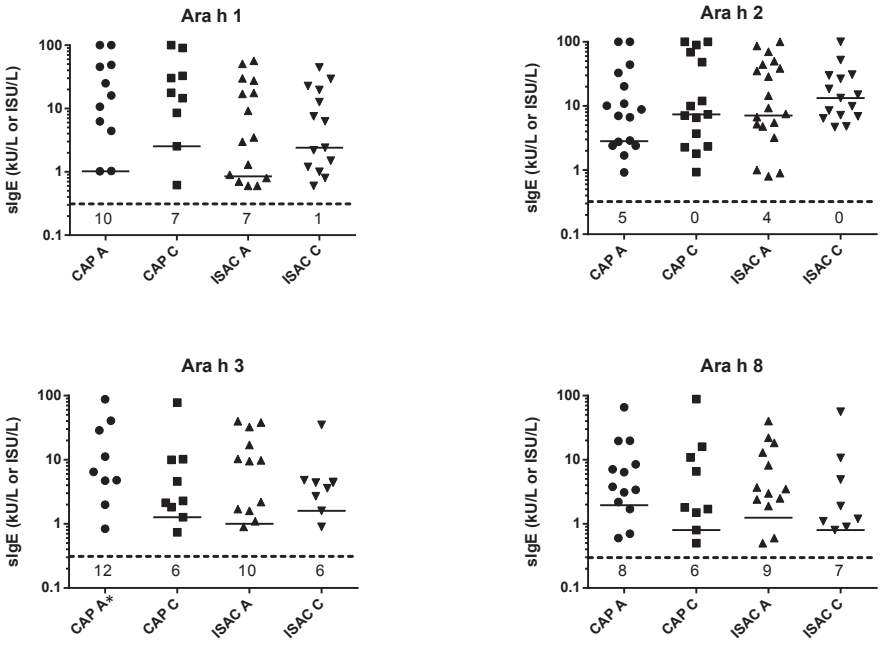

Figure 1. Median sIgE values measured by both single-plexed IgE assay (in kU/L) and multi-plexed microarray immunoassay (in ISU/L) in adults and children.
The dotted line indicates the cutoff value for the multi-plexed assay (0.3). The number below the dotted line indicates the number of patients having a value below this cutoff value. A = adults, C = children, CAP = single-plexed assay, ISAC = multi-plexed assay, * = median value lies below the cutoff value.

Table 3. Median values in adults and children of all 4 test methods to Ara h 1, 2, 3 and 8

		Adults	**Children**	***P*-value**
Single-plexed assay (sIgE in kU/L)	Ara h 1	1.0 (0.0 – 21)	2.5 (0.0 – 30)	0.70
	Ara h 2	2.9 (0.5 – 16)	7.4 (2.3 – 69)	0.16
	Ara h 3	0.0 (0.0 – 5.6)	1.3 (0.0 – 4.6)	0.70
	Ara h 8	2.2 (0.0 – 7.1)	0.8 (0.0 – 6.6)	0.49
Multi-plexed assay (sIgE in ISU/L)	Ara h 1	0.9 (0.0 – 17)	2.4 (1.0 – 20)	0.23
	Ara h 2	7.1 (0.9 – 40)	13 (6.9 – 30)	0.33
	Ara h 3	1.0 (0.0 – 9.9)	1.6 (0.0 – 4.4)	0.94
	Ara h 8	1.2 (0.0 – 4.8)	0.8 (0.0 – 1.9)	0.51

Table 3. Continued

		Adults	Children	*P*-value
SPT (ratio)	Ara h 1	0.3 (0.2 – 1.1)	0.3 (0.1 – 0.7)	0.70
	Ara h 2	1.6 (0.8 – 2.4)	1.3 (0.6 – 3.1)	0.90
	Ara h 3	0.4 (0.1 – 0.7)	0.3 (0.2 – 0.5)	0.54
Immunoblot (scale 0 – 4)	Ara h 1	1.0 (0.0 – 1.3)	0.0 (0.0 – 1.0)	0.32
	Ara h 2	1.0 (1.0 – 2.3)	2.0 (1.0 – 4.0)	0.28
	Ara h 3	1.0 (0.0 – 2.0)	1.0 (0.0 – 1.0)	0.30

Data are presented as median values with interquartile ranges

sIgE to Ara h 2 is the most capable to discriminate between peanut allergy and peanut tolerance with both single-plexed and multi-plexed assay

We analyzed the discriminative ability of peanut components as measured by both single- and multi-plexed assay to discriminate between peanut allergic patients (n = 37) and atopic controls (n=27). With both methods, sIgE to Ara h 2 had the best discriminative ability with an AUC of 0.92 (single-plexed assay) and 0.96 (multi-plexed assay) respectively (Figure 2). The AUC values for sIgE to Ara h 3 and Ara h 8 were comparable between both methods (0.74 vs. 0.78 and 0.67 vs. 0.66, respectively). A difference was seen for sIgE to Ara h 1 with an AUC of 0.75 when measured by single-plexed assay and 0.91 when measured by the multi-plexed assay.

sIgE to Ara h 1, 2 and 3 in adults correlated with clinical severity

Specific IgE titers measured by the multi-plexed assay to the four peanut components in challenged adults and children (n=37) were plotted against the severity score, indicating the clinical severity observed during DBPCFC (Figure 3). In adults, the sIgE level was positively correlated with clinical severity for Ara h 1 ($r = 0.74$, $P < 0.001$), Ara h 2 ($r = 0.63$, $P = 0.002$) and Ara h 3 ($r = 0.78$, $P < 0.001$). No significant correlation in children could be found between severity and sIgE to any of these components (P-values of 0.32, 0.36 and 0.25, respectively, Figure 3). Ara h 8 showed no significant correlation in both adults and children ($P = 0.28$ and $P = 0.07$, respectively) and even showed a negative correlation trend.

Similar results were obtained when sIgE levels were measured by the single-plexed assay.

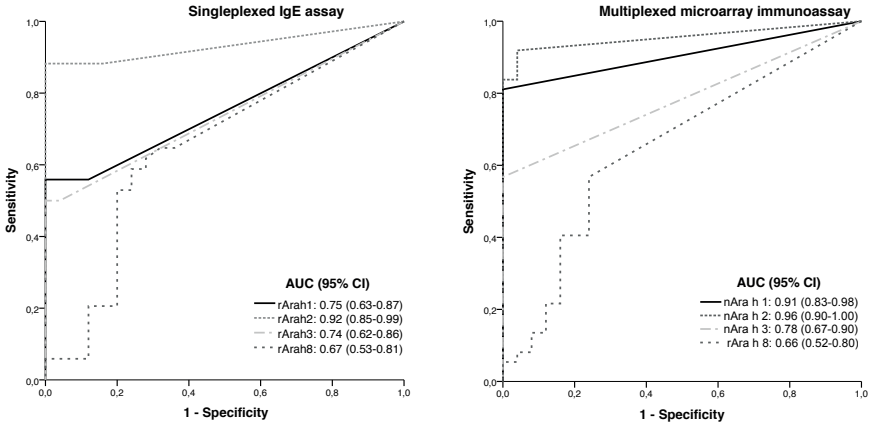

Figure 2. AUC values of sIgE to the peanut components between single-plexed IgE assay and multi-plexed microarray immunoassay. AUC = area under the curve, CI = confidence interval.

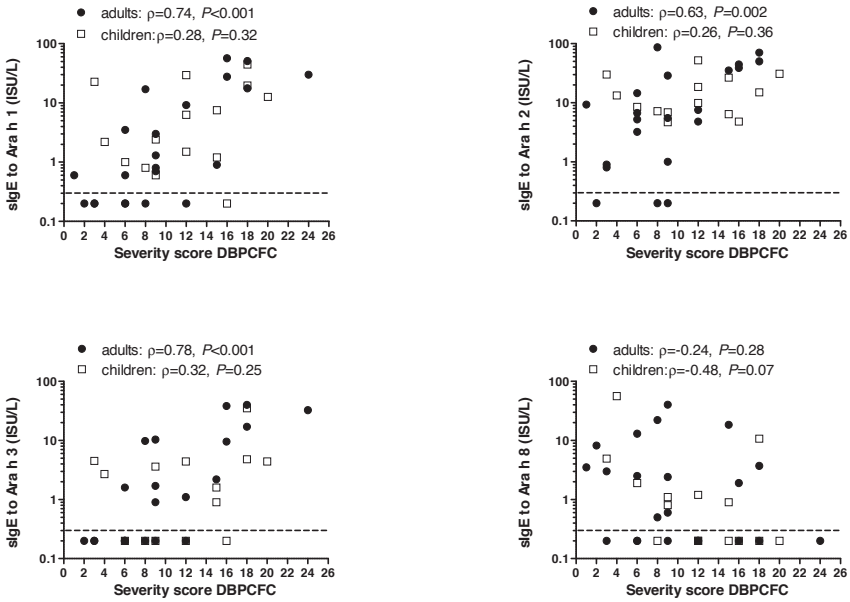

Figure 3. Peanut components measured by multi-plexed microarray immunoassay related to clinical severity in both adults and children.
Spearman's rho and it's corresponding p-value (2-tailed) is given for the two subgroups. The dotted line indicates the cutt-off value of 0.3. One circle or square may represent several patients with the same severity score and sIgE titer.

DISCUSSION

To our knowledge, this is the first study that thoroughly evaluated the use of the peanut components Ara h 1, 2, 3 and 8 in both peanut allergic adults and children with four different techniques: multi-plexed microarray, single-plexed IgE assay, SPT and immunoblot.

The sensitivity and median absolute values of all 4 techniques to all peanut components were not significantly different between children and adults. However, a trend of a higher sensitivity in children was found for Ara h 2 with the single-plexed assay (100% vs. 76.2%). We believe this can be explained by the fact that in adults more peanut allergic patients were also pollen allergic and therefore more often sensitized to the Bet v 1-related Ara h 8 instead of Ara h 2. This hypothesis was supported by higher sensitivity and median values to Ara h 8 in the adult population compared with the pediatric population. In our study population two peanut allergic adults but no children were mono-sensitized to Ara h 8. In addition, Ara h 8 was least often recognized of all peanut components by both single-plexed and multi-plexed assay in children, whereas in the peanut allergic adults this was Ara h 3.

With all techniques, Ara h 2 was most recognized in both peanut allergic adults and children and also had the highest median sIgE value. The significance of Ara h 2 was already suggested in an earlier study from 1998 on serological analysis and was in 2004 further underlined by the analysis of IgE binding to purified Ara h 2 on immunoblot, SPT and basophil activation tests.[9;26] Recently, in a Swedish pediatric population, 83% of the children sensitized to Ara h 2 reported allergic symptoms to peanut.[12] Three other studies, again in pediatric populations, showed that Ara h 2 was a very important predictor of clinical allergy.[13;15;16] A recently published Japanese study showed an AUC for sIgE to Ara h 2 of 0.91 with a sensitivity of 88% by using the ImmunoCAP in a pediatric population.[17] A good agreement was found with their immunoblot results. Our results further extend the evidence of Ara h 2 being the most important peanut allergen for diagnosis, by demonstrating the recognition of Ara h 2 in both a pediatric and adult DBPCFC confirmed peanut allergic population with 4 different techniques. Recently, the importance of Ara h 2 was also found with an additional technique, the basophil threshold sensitivity, by Glaumann et al.[27] They showed in peanut allergic children a CD-sens of Ara h 2 similar to peanut extract (92%). Unfortunately, because our results were retrospectively collected, we were not able to compare them with functional assays, such as the basophil activation test.

When comparing the discriminative ability of the single-plexed and multi-plexed assay to discriminate between peanut allergy and peanut tolerance, we found a higher AUC of sIgE to Ara h 1 with the multi-plexed assay (0.91 vs. 0.75). Although the median sIgE value measured with the multi-plexed assay was slightly higher than when measured with the single-plexed assay in the whole peanut allergic population, this difference was

not significant. More important probably was the fact that 3 of the 27 atopic controls recognized Ara h 1 with the single-plexed assay compared with none of the controls with the multi-plexed assay. We cannot explain this, but because two different preparations of Ara h 1 are used: a recombinant in the single-plexed versus a purified natural one in the multi-plexed assay, this might be a technical issue.

We would like to point out that the purpose of our study was not to assess a diagnostic value, which would need another design, but to compare the discriminative ability between different diagnostic techniques. As a result, the AUC values presented here should not be used for diagnostic purposes. In addition, we could not show specificity values since the control group we used was not suspected of peanut allergy and peanut allergy was not ruled out by a food challenge but by convincing history. A diagnostic study design should include subjects suspected of peanut allergy, which results in both positive and negative outcomes after food challenge.

Another possible limitation of our study was that the SPT and immunoblot data of the atopic control group were lacking. Therefore, we could not compare all four techniques with regard to their discriminative ability. In addition, all atopic control patients were adults because no pediatric controls could be included. When dividing the peanut allergic group into adults and children, higher AUC values where found for the pediatric population. Since the high diagnostic AUC values for Ara h 2 in literature were all established in pediatric populations,[13;15-17] this finding suggests that a diagnostic study in adults is essential to analyze whether these high AUC values can be reproduced in adults.

We have shown a significant positive correlation between clinical severity and the level of sIgE to Ara h 1, Ara h 2 and Ara h 3 in adults. In children, no such correlation could be found. Three earlier studies that mainly analyzed peanut allergic children also found no relation between the clinical severity and sIgE level or size of SPT reactions.[10;16;26] A possible explanation could be that none of the children experienced a Mueller 0 reaction during food challenge, whereas in adults 6 subjects did. Interestingly, the same analysis in our study performed with the severity score adapted from Mueller, assessed by both history or by food challenge, resulted in the same findings in adults and children.[23] This was also found when the ED was used to assess clinical severity or when the scoring system of Clark and Ewan was used.[28] Nevertheless, interpretation of this correlation for patients has to be approached cautiously since correlations at population levels do not necessarily apply to individual patients.

In conclusion, the single-plexed IgE assay, the multi-plexed microarray immunoassay, SPT and immunoblot have comparable sensitivity values compared with each other and between adults and children, with Ara h 2 being most often recognized and with the highest outcome measure among all techniques. However, the exact diagnostic value of all techniques should be compared in future prospective studies in larger populations in both adults and children.

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Chapter 4

The diagnostic value of specific IgE to Ara h 2 to predict peanut allergy in children is comparable to a validated and updated diagnostic prediction model

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ABSTRACT

Background: A diagnostic prediction model for peanut allergy in children was recently published, using 6 predictors: sex, age, history, skin prick test, peanut specific immunoglobulin E (sIgE), and total IgE minus peanut sIgE.

Objectives: To validate this model and update it by adding allergic rhinitis, atopic dermatitis, and sIgE to peanut components Ara h 1, 2, 3, and 8 as candidate predictors. To develop a new model based only on sIgE to peanut components.

Methods: Validation was performed by testing discrimination (diagnostic value) with an area under the receiver operating characteristic curve and calibration (agreement between predicted and observed frequencies of peanut allergy) with the Hosmer-Lemeshow test and a calibration plot. The performance of the (updated) models was similarly analyzed.

Results: Validation of the model in 100 patients showed good discrimination (88%) but poor calibration ($P < .001$). In the updating process, age, history, and additional candidate predictors did not significantly increase discrimination, being 94%, and leaving only 4 predictors of the original model: sex, skin prick test, peanut sIgE, and total IgE minus sIgE. When building a model with sIgE to peanut components, Ara h 2 was the only predictor, with a discriminative ability of 90%. Cutoff values with 100% positive and negative predictive values could be calculated for both the updated model and sIgE to Ara h 2. In this way, the outcome of the food challenge could be predicted with 100% accuracy in 59% (updated model) and 50% (Ara h 2) of the patients.

Conclusions: Discrimination of the validated model was good; however, calibration was poor. The discriminative ability of sIgE to Ara h 2 was almost comparable to that of the updated model, containing 4 predictors. With both models, the need for peanut challenges could be reduced by at least 50%.

INTRODUCTION

Peanut allergy can be life threatening, and its prevalence seems to be increasing.[1-3] It has an important influence on daily life for both children and their parents, especially because of the strict elimination diet that is generally prescribed.[4-6]

The gold standard to diagnose a peanut allergy is a double-blind, placebo-controlled food challenge (DBPCFC).[7] This is a costly and, especially for young children, burdensome test demanding specialized medical facilities. It can also result in severe reactions.[8] However, other diagnostic test methods, such as specific IgE (sIgE) to peanut and skin prick test (SPT) reactivity to peanut, perform suboptimally. Studies that investigated the diagnostic value of these methods showed an area under the receiver operating characteristic curve (AUC) of 0.79 to 0.86 for SPT and 0.77 to 0.87 for sIgE to peanut, respectively.[9-11]

DunnGalvin et al[12] found that one way of improving the diagnostic accuracy without using food challenges was to combine several diagnostic tests and patient characteristics into one prediction model. Their prediction model, including sex, age, history, SPT, sIgE to peanut, and total IgE minus specific IgE to peanut as predictors, was developed by using data from 94 patients. They all underwent an oral food challenge, both open and double blind, of which 53% were positive. When validated in the same center in 30 patients, the model showed an AUC of 0.97 to predict peanut allergy. As they already indicated, validation, preferably in a similar population in another pediatric center, is an important step to verify whether such a prediction model can be implemented in a broader setting.[13-16] It is known that prediction models tend to perform better on data on which the model was built compared with the performance of the model on new data derived from another population.[16]

Another way to improve the diagnostics of peanut allergy is the determination of sIgE to peanut specific components. Previous studies showed that sensitization particularly to Ara h 2 was useful to distinguish peanut allergic subjects from peanut tolerant subjects.[17-22] The AUC of sIgE to Ara h 2 in 2 of these studies ranged from 0.95 to 0.99.[19;21;22]

Given the strong need for improved diagnostics of peanut allergy without making use of food challenges, the aim of this study was to validate the promising prediction model as published by DunnGalvin et al.[12] The validation was performed in a pediatric population from a tertiary clinic, in which all patients suspected of having a peanut allergy underwent a food challenge. Furthermore, we evaluated whether additional candidate predictors such as atopic dermatitis, allergic rhinitis, and sIgE to the peanut-specific components Ara h 1, 2, 3, and 8 could increase the diagnostic accuracy. In addition, another prediction model was built in which only sIgE to the peanut specific components and peanut extract were analyzed as candidate predictors. The diagnostic value of this model was compared with the diagnostic value of the validated model of DunnGalvin et al.[12]

METHODS

Study population

All subjects (n = 261) with a suspected peanut allergy who visited the Centre of Pediatric Allergy in the Wilhelmina Children Hospital in Utrecht, The Netherlands, between 2008 and 2010 were considered for inclusion in this study if they had no missing data. Required data comprised sex, age, SPT, history, presence of allergic rhinitis and eczema, and the outcome of the food challenge; 200 patients had complete records in this regard. Suspicion was based either on a positive history irrespective of SPT or sIgE result or sensitization (positive sIgE or SPT to peanut extract) in case of unknown ingestion. The severity of the reaction was reported by the patients themselves or by their parents/caregivers. A suspected history was defined as an allergic reaction within 2 hours after the ingestion of peanut or a food product containing peanut. Because additional laboratory tests were needed, we randomly selected 100 of the 200 eligible patients for all analyses. No significant differences were found between the 3 groups (100 included, 100 excluded, 61 missing values) with regard to sex, age, sIgE to peanut extract, prior ingestion of peanut, presence of eczema, presence of allergic rhinitis, and outcome of food challenge. sIgE to Ara h 1, 2, 3, and 8 and total IgE were measured only in the 100 patients who were included in this study.

Outcome

All patients underwent a food challenge to confirm or exclude peanut allergy. The challenges were performed according to the international consensus protocol,[23] using the recipe described by Flinterman et al.[24] Oral allergy symptoms, nausea, abdominal discomfort, and throat tightness were considered subjective. Rhinoconjunctivitis, angioedema, generalized urticaria, emesis, diarrhea, hoarseness, stridor, wheezing, or significant drop in FEV_1 or blood pressure were referred to as objective symptoms. The challenge was discontinued and considered positive in case of objective symptoms, if suggestive subjective symptoms occurred at 3 subsequent doses, or if a severe, suggestive subjective symptom lasted for more than 45 minutes. None of the patients had an inconclusive food challenge. No late allergic reactions were reported the next day during a telephone call.

Predictors

To validate the model of DunnGalvin et al,[12] the same predictors were used as described in their study: sex, history, SPT, sIgE to peanut extract, total IgE minus sIgE to peanut extract, and age. Symptoms in history were similarly divided into 4 categories: 1, skin or oral or gastrointestinal or upper respiratory tract symptoms only; 2, upper respiratory

tract and gastrointestinal or 2 systems; 3, lower respiratory tract or 3 systems; 4, cardiovascular or 4 systems.[12]

An SPT was performed with peanut extract with a single-headed lancet (ALK-Abelló, Nieuwegein, The Netherlands). For the positive and negative controls, histamine dihydrochloride 10 mg/mL and glycerol diluent were used, respectively.

Allergic rhinitis, as diagnosed by a physician, atopic dermatitis, according to the criteria of Williams et al,[25] and sIgE to the most important peanut allergens (Ara h 1, 2, 3, and 8) were analyzed as additional predictors. sIgE to the aforementioned allergen components and peanut extract was determined by using the ImmunoCAP method (Phadia, Uppsala, Sweden) according to the manufacturer's instruction. The SPT and sIgE results were analyzed as continuous variables. All serum samples were collected during routine clinical practice. The local ethics committee approved the study.

Data analysis

This study consisted of 3 phases: (1) validation (discrimination and calibration) of the model published by DunnGalvin et al,[12] (2) analyzing whether the model could be updated with additional predictors, and (3) calculating the diagnostic value of a model by using only peanut-specific allergens (peanut extract and Ara h 1, 2, 3, and 8).

In phase 1, the existing model published by DunnGalvin et al[12] was validated. Therefore, we calculated for each patient the probability of having peanut allergy by using the regression coefficients as described by DunnGalvin et al with the following formula: probability of having peanut allergy = exp(−11.63 + (4.60 × sex) + (3.32 × history group 1) + (4.61 × history group 2) + (7.86 × history group 3) + (11.08 × history group 4) + (2.85 × SPT) + (0.50 × sIgE to peanut extract) + (−0.002 × total IgE − sIgE to peanut extract) + (−0.37 × age))/(1 + exp(−11.63 + (4.60 × sex) + (3.32 × history group 1) + (4.61 × history group 2) + (7.86 × history group 3) + (11.08 × history group 4) + (2.85 × SPT) + (0.50 × sIgE to peanut extract) + (−0.002 × total IgE − sIgE to peanut extract) + (−0.37 * age))).[12] The performance of the model was described by means of discrimination and calibration. Discrimination shows the degree of distinction between positive and negative outcomes of a model and was studied with a receiver operating characteristic curve. The AUC was calculated with this curve, with a value of 0.5 indicating "no discrimination" and a value of 1 indicating "perfect discrimination." Calibration refers to the agreement between predicted probabilities and observed frequencies of peanut allergy as assessed by a food challenge. This was tested with a calibration plot where a slope of 1, together with a Hosmer-Lemeshow test with a *P* value of .05 or more, indicates good calibration.

In phase 2, the additional predictors allergic rhinitis, atopic dermatitis, and sIgE to Ara h 1, 2, 3, and 8 were analyzed as to their ability to enhance the diagnostic value of the model. Therefore, a new multivariate logistic regression analysis was performed

entering all candidate variables (from the original model as well as possible additional predictors) simultaneously. This analysis was performed in the same way as described by DunnGalvin et al[12] (ie, stepwise forward with the probability of entering a variable .05 and removing a variable .06). The categorical predictor history was graded into 4 categories as described above with no symptoms/ingestion unknown as the reference. In addition, the diagnostic value was analyzed without entering the subjective predictor history and the labor-intensive SPT as possible predictors in a multivariate logistic regression analysis.

In phase 3, only sIgE to peanut allergens was used to develop a prediction model to examine the diagnostic value of these frequently investigated allergens. We again used multivariate logistic regression and a receiver operating characteristic curve, as earlier described.

All analyses were performed with SPSS (version 16.0; SPSS, Chicago, IL, USA). The calibration plot was made by using Microsoft Office Excel 2003 (Microsoft Corp, Redmond, Wash).

RESULTS

Patient characteristics

Table 1 shows the patient characteristics for both the peanut allergic (n = 47) and the peanut tolerant (n = 53) group. The predictors as used in the prediction model of DunnGalvin et al[12] are listed above the line. The median age of the total group was 6.0 years, and 65% were males. A DBPCFC was performed in 81 patients. In 19 patients, the food challenge was open because of difficulties with eating. As a consequence, these children tended to be younger (5.4 vs 7.1 years; $P = .05$).

From the predictors that were included in DunnGalvin's model,[12] sex, SPT, and sIgE to peanut extract differed significantly between the peanut-allergic group and the peanut-tolerant group in our population when analyzing the 95% CIs of the odds ratios (Table 1). From the additional candidate predictors, sIgE to Ara h 1 and Ara h 2 differed significantly between the allergic and tolerant groups (OR, 2.15; 95% CI, 1.08-4.28 and OR, 1.70; 95% CI, 1.27-2.27, respectively).

Phase 1: Validation of the existing model

Validation of DunnGalvins et al prediction model showed a discrimination of 0.88 (95% CI, 0.81-0.94; Figure 1). A cutoff point of the predicted probability of ≥0.5 led to a positive predictive value (PPV) and a negative predictive value (NPV) of 60.3% and 100%, respectively, with a sensitivity and specificity of 100% and 41.5%. This means that all patients in our study with a score of less than 0.5 (n = 22) were accurately predicted

Table 1. Demographic and clinical characteristics and odds ratios

	Total N = 100		Peanut allergic N = 47		Peanut tolerant N = 53		Odds ratio (95% CI)	
Male	65	(65%)	24	(51%)	41	(77%)	0.31	(0.13-0.72)
Age (year)	6.0	(4.0-8.0)	6.0	(4.0-7.0)	6.0	(4.5-8.5)	0.99	(0.88-1.11)
SPT wheal (mm)	10.0	(4.0-14.8)	14	(10.0-16.0)	5.0	(0.0-11.0)	1.27	(1.16-1.40)
sIgE (kUa/L) peanut extract	1.9	(0.4-15.3)	15.3	(2.2-68.0)	0.6	(0.4-2.1)	1.09	(1.03-1.16)
Total IgE-sIgE peanut extract	268	(105-832)	261	(104-774)	283	(102-937)	1.00	(1.00-1.00)
No symptoms / ingestion unknown*	71	(71%)	29	(62%)	42	(79%)	Reference category	
Skin or oral or GI or upper respiratory only	17	(17%)	9	(19%)	8	(15%)	1.63	(0.56-4.72)
Upper respiratory and gastrointestinal or 2 systems	5	(5%)	5	(11%)	0	(0%)	-	-
Lower respiratory or 3 systems	7	(7%)	4	(9%)	3	(6%)	1.93	(0.40-9.28)
Cardiovascular or 4 systems	0	(0%)	0	(0%)	0	(0%)	-	-
sIgE Ara h 1 (kUa/L)	0.06	(0.01-1.0)	0.96	(0.04-13.9)	0.03	(0.00-0.1)	2.15	(1.08-4.28)
sIgE Ara h 2 (kUa/L)	0.61	(0.07-6.3)	6.5	(0.85-38.3)	0.09	(0.03-0.6)	1.70	(1.27-2.27)
sIgE Ara h 3 (kUa/L)	0.07	(0.01-0.3)	0.19	(0.04-0.7)	0.02	(0.01-0.1)	2.12	(0.94-4.78)
sIgE Ara h 8 (kUa/L)	0.05	(0.00-1.1)	0.02	(0.00-1.8)	0.08	(0.00-1.1)	1.00	(0.97-1.03)
Atopic dermatitis	79	(79%)	37	(79%)	42	(79%)	0.97	(0.37-2.54)
Allergic rhinitis	40	(40%)	19	(40%)	21	(40%)	1.03	(0.46-2.31)

All data represent medians and interquartile ranges or numbers and percentages. Predictors above the line were included in the prediction model of DunnGalvin et al.
CI, confidence interval; *GI,* gastro-intestinal
*Sensitization for peanut tested as part of screening atopy syndrome: atopic dermatitis or food allergy other than peanut

(ie, had a negative food challenge). Because of the extreme calculated probabilities in all patients (93% had a probability of 0 or 1), cutoff values of ≥0.4 and ≥0.6 gave the same positive and negative predictive values with corresponding sensitivity and specificity.

Calibration of the prediction model by a calibration plot showed a line with a slope below 1, especially at a predicted probability of 1, which indicated poor calibration in our patient population (Figure 2). This was supported by a significant Hosmer-Lemeshow test ($P < .001$).

Phase 2: Updating the prediction model with additional predictors

Because of the poor calibration in our population and to analyze whether additional predictors could increase the diagnostic value, we updated the original model. None

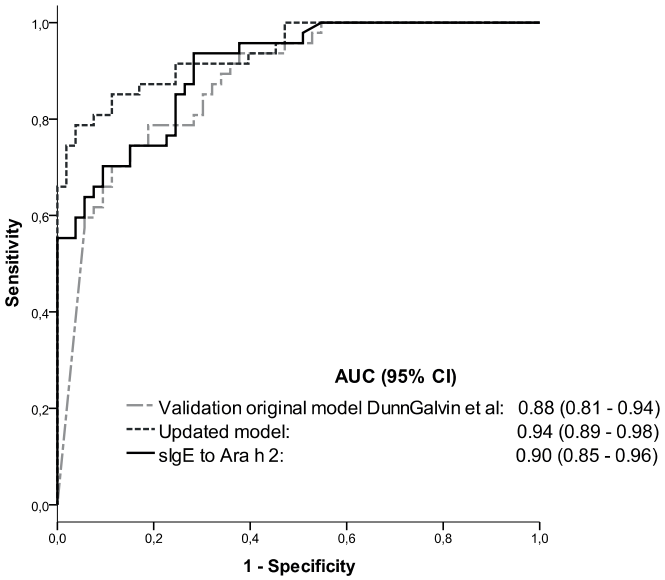

Figure 1. ROC-curves of 1) validation original model, 2) updated model, 3) sIgE to Ara h 2

of the additional predictors -allergic rhinitis, atopic dermatitis, and sIgE to Ara h 1, 2, 3, and 8- significantly increased the discriminative ability of the original model when performing a new analysis with all candidate variables (from the original model as well as possible additional predictors). In addition, the predictors history and age from the original model were excluded from the model in this analysis. This indicates that they did not significantly contribute to the discriminative ability of the updated model. Regression coefficients of the 4 remaining predictors after the updating process are shown in Table 2.

The updated model containing the 4 variables –sex, SPT reactivity to peanut, sIgE to peanut extract, and total IgE minus sIgE to peanut extract- showed an AUC of 0.94 (95% CI, 0.89-0.98; Figure 1). A cutoff point of a probability of ≥0.6 showed the best diagnostic accuracy with regard to the PPV and the NPV (93% and 83%, respectively; Table 3).

All patients with a predicted probability of more than 0.84 (n = 31) had a positive food challenge (ie, 100% PPV) while an NPV of 100% was reached at a cutoff point of less than 0.11, which accounted for 28 patients (data not shown). This means that in 59% of the patients in our population, the outcome of the food challenge could be predicted with 100% accuracy.

Calibration of the updated model was good, with the slope of the calibration plot approaching the reference line (Figure 2). In addition, the Hosmer-Lemeshow test showed a *P* value of .99.

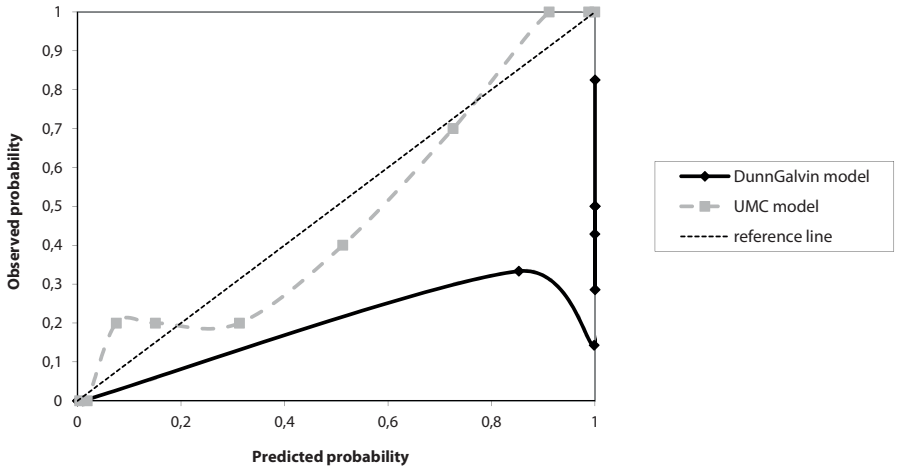

Figure 2. Calibration plot of the prediction model by DunnGalvin and the updated model.

Table 2. The regression coefficients of the validation data set compared with the original model

Predictors	Regression coefficient DunnGalvin et al.	Regression coefficient updated model	Standard error**	Odds ratio** (95% CI)
Male	4.60	-1.93	0.71	0.15 (0.04 - 0.58)
SPT (mm)	2.85	0.25	0.07	1.29 (1.12 - 1.47)
sIgE (kU/L)	0.50	0.15	0.05	1.16 (1.06 - 1.27)
Total IgE minus sIgE (kU/L)	-0.002	-0.002	0.001	1.00 (1.00 - 1.00)
History 1*	3.32	-	-	-
History 2*	4.61	-	-	-
History 3*	7.86	-	-	-
History 4*	11.08	-	-	-
Age (years)	-0.37	-	-	-
Intercept	-11.63	-1.77	0.77	NA

* History divided in 4 groups: 1, skin or oral or gastrointestinal or upper respiratory only; 2, upper respiratory and gastrointestinal or 2 systems; 3, lower respiratory or 3 systems; 4 cardiovascular or 4 systems. No symptoms / ingestion unknown was set as the indicator (0).
** Calculated for the regression coefficient of the updated model
NA, not applicable

When the same analysis was performed without entering SPT and history as candidate predictors, only sIgE to Ara h 2 and sex significantly contributed to the discriminative ability and were therefore included in the model, which showed an AUC of 0.95 (95% CI, 0.91-0.99).

Table 3. Diagnostic value of the updated prediction model for different cutoff points of the predicted probabilities

Cutoff point	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)
≥ 0.4	79	88	87	79
≥ 0.5	85	87	85	87
≥ 0.6	93	83	79	94

PPV, positive predictive value; *NPV*, negative predictive value

Phase 3: A prediction model using only sIgE to Ara h 1, 2, 3, 8, and peanut extract

Specific IgE to Ara h 2 was the only predictor that remained in the model when constructing a prediction model in which sIgE to Ara h 1, 2, 3, 8, and peanut extract were entered as candidate predictors in a new analysis. This means that sIgE to Ara h 1, 3, 8, and peanut extract did not significantly improve the diagnostic accuracy of sIgE to Ara h 2 alone.

The AUC of these separate allergens is displayed in Figure 3. As we could already expect from our multivariate analysis, sIgE to Ara h 2 showed the highest AUC: 0.90 (95% CI, 0.85-0.96). Therefore, we additionally calculated the NPV and PPV together with sensitivity and specificity for sIgE to Ara h 2 for different cutoff points (Table 4). A cutoff point of ≥5 kU/L gave the best combination of PPV and NPV (96% and 71%, respectively). A PPV of 100% was already found when using a cutoff point of >5.17 kU/L while an NPV of 100% was not reached until a cutoff point of >0.07 kU/L was used (data not shown). By using these cutoff points, in 50% of the cases (24 tolerant patients and 26 allergic patients) the outcome of the DBPCFC could be predicted with 100% accuracy. For comparison, the AUC of SPT as another single predictor was 0.83 (95% CI, 0.75-0.91).

Table 4. Diagnostic value of the predictor sIgE to Ara h 2 for different cutoff points

Cutoff point	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)
≥ 0.2 kU/L	71	92	94	66
≥ 0.35 kU/L	74	90	91	72
≥ 0.5 kU/L	75	83	83	75
≥ 1 kU/L	81	79	74	85
≥ 2 kU/L	87	77	70	91
≥ 5 kU/L	96	71	55	98
≥ 10 kU/L	100	66	43	100

PPV, positive predictive value; *NPV*, negative predictive value

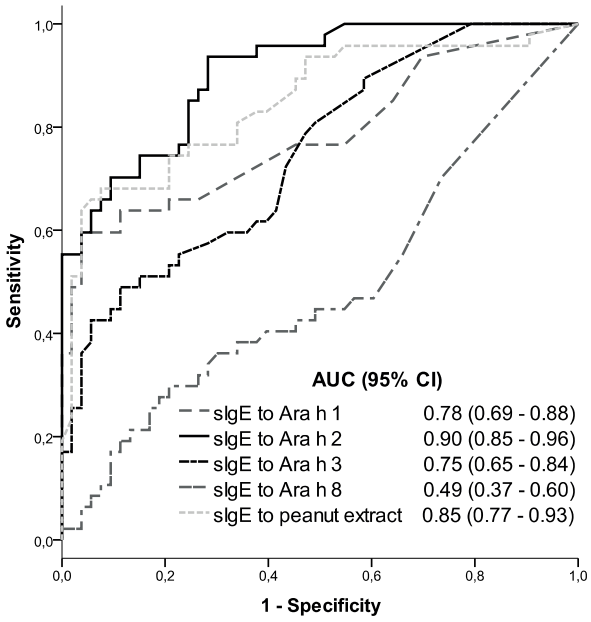

Figure 3. ROC-curves of the peanut specific allergens Ara h 1, 2, 3 and 8 and peanut extract

DISCUSSION

In this study, a promising diagnostic prediction model for peanut allergy, containing 6 predictors, was validated. This model showed good discrimination but poor calibration. After updating, the predictors sIgE to peanut extract, total IgE minus sIgE to peanut extract, sex, and SPT remained in the model. The discriminative ability of Ara h 2 by itself was almost comparable to that of the updated model.

Although many prediction models have been published in the medical literature, only a few of them have been validated.[15] External validation is very important because it shows the generalizability of a model.[15;26] Therefore, it is a significant step toward implementing a prediction model in daily clinical practice.[16]

Validation of the prediction model in our population showed an AUC of 0.88 compared with 0.95 in the development phase of DunnGalvin et al.[12] It should be noted that this AUC of 0.95 represents the diagnostic accuracy of all 3 models that were developed in that study (for peanut, egg, and milk allergy). The most likely reasons for the different AUC are population differences.

Because discrimination was good despite population differences, the poor calibration could not be explained only by these differences.[15] The fact that 93% of the patients had a predicted probability of 0 or 1 in our study suggests that there was overfitting of the regression coefficients in the development study of DunnGalvin et al.[15;27] This could

result in poor calibration. If regression coefficients are overfitted, predicted probabilities in the validation data set are often too low in the lower range, while in the higher range they are too high.[15;27] Overfitting can be introduced by including too many predictors relative to the number of patients and implies that the model is too closely adapted to the data.[28] We cannot rule out the possibility of overfitting in the updated model, because the number of predictors we used was comparable to that in the original model. Therefore, before physicians might use this updated model, the discrimination and calibration should be externally validated in different populations. In addition, it should be noted that the performance of the updated model was assessed in a tertiary population. Therefore, to apply this model in another population or to extrapolate our results to a secondary care or an adult population, the model should first be validated in these populations.

Excluding from the analysis SPT and history as possible predictors resulted in a model with sIgE only to Ara h 2 and sex, which appeared to have a comparable AUC (namely, 0.95 vs 0.94 for the updated model). Such a model would eliminate the potential disadvantages both of using the history, which might be subjective, and the SPT, which cannot always be assessed in patients with severe atopic dermatitis.

sIgE to Ara h 2 as the only predictor reached an AUC of 0.90 compared with 0.94 of the updated model. This is interesting because the AUC of the updated model will probably be lower than that of sIgE to Ara h 2 as the only predictor when validated in another population.[16] Another single predictor, the SPT, performed suboptimally compared with Ara h 2 with an AUC of 0.83. Cutoff points for sIgE to Ara h 2 with a 100% PPV and NPV could predict the outcome of the DBPCFC in 50% of the patients with 100% accuracy compared with 59% with the updated model. This means that with both models, the number of DBPCFCs could be reduced by challenging patients only with a value between these cutoff points. Previous studies have calculated 95% PPVs for SPT and sIgE to peanut extract when using cutoff values of ≥8 mm or ≥15 kU/L, respectively, thereby already reducing the need for food challenges.[11;29] Comparable PPV values could be calculated when these cutoff values were used in subsequent studies.[9;10;30] Using these cutoff values for SPT and sIgE to peanut extract in our study resulted in a PPV of 66% and 93%, respectively. Most of these studies however, did not address 95% or 100% NPVs.[10;11;30] Rance et al[9] found a 100% NPV for the SPT with a cutoff value of ≥3 mm, but it is not clear in how many patients a DBPCFC could be avoided for this reason. In both of our models, the updated prediction model and sIgE to Ara h 2, a 100% NPV could identify a quarter of all patients as peanut tolerant with 100% certainty (in 28 and 24 patients, respectively). In addition, we found a 100% NPV if both sIgE to peanut extract and Ara h 2 were lower than the clinically used cutoff value of 0.35 kU/L, which was the case in 23 patients.

Ara h 2 is the most frequently recognized peanut allergen in peanut allergic children and adults according to recent studies conducted in the United States, United Kingdom, France, Sweden,[18-21;31;32] and The Netherlands (data not published). One diagnostic study, prospectively performed in infants in Australia, found an AUC of sIgE to Ara h 2 of 0.95, which was better than the AUC of sIgE to whole peanut extract in the same study (0.89).[22] That study was well designed with clear inclusion and exclusion criteria. However, children were included only on the basis of the SPT result, while children with a suggestive history after the ingestion of peanut were not taken into account. This makes the results difficult to extrapolate to all patients with a suspected peanut allergy (ie, the group of patients with a suggestive history regardless of sensitization). The same was true for another well-designed diagnostic study in a birth cohort in which children were included on the basis of sensitization only.[19;21] This study showed an AUC of sIgE to Ara h 2 of 0.99. A third diagnostic study showed high sensitivities and specificities for sIgE to Ara h 2 but used a case-control design,[20] including controls who were not suspected of having a peanut allergy.

The strength of our study is that we assessed the diagnostic value of sIgE to Ara h 2 in patients who were included on the basis of a positive history or sensitization without known ingestion, which conforms to clinical practice. Furthermore, all patients suspected of having a peanut allergy were offered a DBPCFC; this remains the gold standard.

Our study had the additional advantage that both our study and the study of DunnGalvin et al were performed in comparable centers (ie, tertiary clinics). Therefore, the proportions of positive outcomes of peanut challenges were very similar (47% vs 53%). This is important because a different prevalence can be a reason for poor calibration.[15] In addition, the same SPT extract and tests to measure sIgE were used. No missing values existed in the validation data set, and so validation could be performed on the basis of real data only.

Although the findings in this study suggest that the number of food challenges to diagnose a peanut allergy can be reduced, it is also important to mention the advantages of a food challenge. A threshold can be established, the severity of the allergy can be estimated, and the outcome may be reassuring to patients and relatives. In our study population, sIgE to Ara h 2 could not discriminate between a mild, moderate, and severe allergy, as assessed by food challenge following the Sampson criteria (with Sampson 1 and 2 being mild, Sampson 3 moderate, and Sampson 4 and 5 severe).[33]

In conclusion, the validation of a promising diagnostic model predicting peanut allergy has shown a good discrimination but a poor calibration. The discriminative ability of sIgE to Ara h 2 was almost comparable to that of the updated prediction model, which contained 4 variables of the original model. Ara h 2 and the updated model could almost equally reduce the need for food challenges. These results implicate that sIgE to Ara h 2 can replace other diagnostic tests for peanut allergy with only a minimal loss

of diagnostic accuracy, if any. Future validation studies are needed to reproduce the importance of sIgE to Ara h 2 compared with the updated prediction model.

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Chapter 5

Ara h 2 is the best predictor for peanut allergy in adults

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ABSTRACT

Background: Specific IgE (sIgE) to Ara h 2 as a clinical predictor for peanut allergy in children has a diagnostic value comparable with a prediction model that contains sex, skin prick test (SPT), sIgE to peanut extract and total IgE minus sIgE. In adults, the diagnostic value of peanut components has not yet been studied.

Objectives: To validate a pediatric prediction model in an adult population; to define the diagnostic value of sIgE to peanut components.

Methods: Validation was performed by discrimination with an area under the receiver operating characteristic curve (AUC) and calibration with the Hosmer-Lemeshow test. The diagnostic value of the peanut components was assessed with the AUC.

Results: Validation of the pediatric model in 94 adults showed poor discrimination (AUC 0.64) but good calibration (P = 0 .48). sIgE to Ara h 2 was the best diagnostic predictor (AUC 0.76). By using a cutoff value with a 100% positive predictive value (≥1.75 kU/L), 28% of patients could be diagnosed with 100% accuracy. The highest negative predictive value (NPV) was 63%. A higher NPV could not be calculated for any other test. Although sIgE to Ara h 2 was significantly correlated with severity, it did not discriminate between mild and severe allergy in individual patients (AUC < 0.65).

Conclusion: sIgE to Ara h 2 has the best discriminative ability of all diagnostic tests and is correlated to clinical severity. It can accurately diagnose peanut allergy in 28% of patients, but cannot be used to exclude a peanut allergy in an adult population.

INTRODUCTION

Peanut allergy is worldwide one of the most common food allergies with sometimes severe consequences.[1;2] In the last decade, the prevalence seems to be increasing.[3;4] The reference standard to diagnose peanut allergy is the double blind, placebo-controlled food challenge (DBPCFC). This test is time consuming and stressful for the patient, has high costs and requires high standard hospital facilities.[2;5;6] Routine tests to diagnose peanut allergy, such as specific IgE (sIgE) and skin prick test (SPT) reactivity to peanut, perform suboptimally with an area under the receiver operating characteristic curve (AUC) ranging from 0.77 to 0.87 for sIgE to peanut extract and 0.79 to 0.93 for SPT.[7-10] Cutoff values for sIgE to peanut extract and SPT with a 95% positive predictive value (PPV) have been calculated and validated in the past, which could be used to reduce the need for DBPCFCs.[7,8,11]

One way of further improving diagnostics is to combine several clinical characteristics and diagnostic tests into one diagnostic prediction model, as recently published by DunnGalvin et al.[12] It is known that prediction models perform better in the population in which they were built.[9;13-15] Therefore, we validated the model of DunnGalvin et al[12] in a tertiary Dutch pediatric population of subjects with suspected peanut allergy.[9] Validation by discrimination was good, but calibration was poor. Discrimination (diagnostic value) was assessed with an AUC, calibration (agreement between predicted and observed frequencies of peanut allergy in the study population) with a calibration plot and Hosmer-Lemeshow test. Because of the poor calibration, the model was updated, which resulted in a model that contained 4 predictors from the original model: sex, SPT, sIgE to peanut extract and total IgE minus sIgE to peanut extract. sIgE to Ara h 2 did not add significantly to this updated model, with an AUC of 0.94. However, sIgE to Ara h 2 as a single predictor in the same study showed an AUC of 0.90.

In other recent studies, sIgE to Ara h 2 had an AUC of 0.90-0.99.[16-20] However, all these studies focused mainly on children. The diagnostic value of peanut components in an adult population is not known yet. In addition, our updated pediatric diagnostic prediction model should be validated in an adult population.[9]

The objective of the current study was to validate our updated pediatric diagnostic model in an adult population and, if necessary, to build a new diagnostic prediction model. We also analyzed the single and combined diagnostic values of the peanut components and their possible correlation with clinical severity.

METHODS

Study population

All adult subjects (n=136) who underwent a DBPCFC with peanut between 2002 and 2012 at the University Medical Centre Utrecht, the Netherlands, were included. One patient was excluded because of an inconclusive DBPCFC (Figure 1). A DBPCFC was conducted either with a diagnostic purpose if peanut allergy was suspected based on history, regardless of sensitization by SPT or sIgE to peanut extract (diagnostic group, n = 94) or as part of two previous studies performed at our centre (previous study group, n = 41, Figure 1).[21;22] In the diagnostic group, nineteen patients had a sIgE level < 0.35 kU/L to peanut extract and an SPT < 3mm, and, therefore, were not sensitized to peanut.

The 41 patients in the study group were preselected because sensitization was required for recruitment. This resulted in significant differences between the diagnostic group and the study group with regard to sIgE to peanut extract (median 2.1kU/L vs. 15.7 kU/L, $P = 0.001$) and SPT reactivity (median 5.0 mm vs. 9.3 mm, $P = 0.001$). To avoid selection bias, the 41 patients in the study group were only used to analyze the correlation between sIgE to peanut components and severity, and were excluded for analyses regarding the diagnostics of peanut allergy. As a consequence, three patients

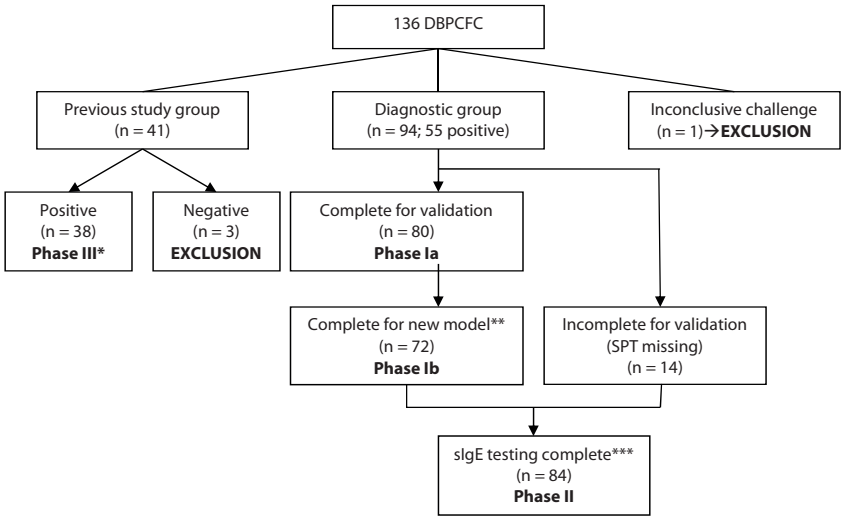

Figure 1. Patients used for different analyses.

* Together with positive challenges from diagnostic group used for Phase III analysis regarding correlation with severity, ** Both SPT data and sIgE to most peanut components available, in 8 patients sIgE to Ara h 2 could not be assessed. *** In 1 patient sIgE to Ara h 8 and in 1 patient sIgE to Ara h 9 could not be assessed.

with a negative challenge from the study group were excluded because they could not be used for any analysis.

Outcome

A DBPCFC was performed in all patients to diagnose or exclude a peanut allergy. The challenge was performed according to the international consensus protocol with the recipe described by Peeters et al.[22;23] The challenge was discontinued and considered positive in case of objective symptoms or if a severe, suggestive subjective symptom occurred on at least 3 subsequent doses or lasted for > 45 minutes.

Diagnostic predictors

Histories regarding previous reactions to peanut were drawn from patient records. The SPT was performed with peanut extract (ALK-Abello, Nieuwegein, The Netherlands), with histamine dihydrochloride 10 mg/mL and glycerol diluent as positive and negative controls. SPT reactivity was recorded after 15 minutes. Total IgE and sIgE to peanut extract and the peanut components Ara h 1, Ara h 2, Ara h 3, Arah 8 and Ara h9 was determined using the ImmunoCAP method (Thermo Fisher Scientific, Uppsala, Sweden) by using serum collected before the DBPCFC was performed. All measurements were performed using a uniform batch of reagents. The SPT and sIgE results were analyzed as continuous variables. In 9 patients, a new blood sample had to be taken because no serum from the past was left. The study was approved by the local ethics committee.

Severity

We used three different outcome measures for severity: an adapted Mueller score for history or food challenge and a severity score, based on the combination of the adapted Mueller score for the food challenge and the eliciting dose. The adapted Mueller score was divided into 5 categories as previously described: 0, oral symptoms; 1, cutaneous symptoms; 2, gastrointestinal symptoms; 3, respiratory symptoms; 4, cardiovascular symptoms.[24;25] The severity score was assessed as follows: the Mueller score at the DBPCFC, which ranged from 0 to 4 plus 1 (to avoid calculating with 0) times the dose score. The dose score ranged from 1 to 10, which corresponded with the 10 peanut doses that ranged from 1 µg to 10 g.[25;26] This resulted in a severity score that ranged from 1 to 50.

Study design

The study consisted of three phases: 1) validation of our updated pediatric diagnostic prediction model (Phase 1a) and, if necessary, building a new diagnostic prediction model for adults (Phase 1b), 2) assessment of the diagnostic value of sIgE to peanut specific components (Ara h 1, 2, 3, 8 and 9), both by univariate and multivariate analysis

compared with currently used SPT and sIgE and 3) correlation of severity to the different diagnostic tests.

Data analysis

For phase 1a (validation), data regarding sex, sIgE to peanut extract, SPT and total IgE minus sIgE to peanut extract was required. Of the 94 patients in the diagnostic group, SPT data were missing in 14 patients (Figure 1). No significant differences were found between these 14 patients and the remaining 80 patients with regard to the other 3 variables used in the prediction model.

For each patient, the probability of having peanut allergy was calculated by the following formula using the regression coefficients of the updated pediatric prediction model: exp(-1.77 + (-1.93 * sex) + (0.25 * SPT in mm) + (0.15 * sIgE to peanut extract) + (-0.002 * total IgE minus sIgE to peanut extract)) / (1 + exp(-1.77 + (- 1.93 * sex) + (0.25 * SPT in mm) + (0.15 * sIgE to peanut extract) + (-0.002 * total IgE minus sIgE to peanut extract))).[9] Discrimination and calibration of the model were analyzed as earlier described.[9,27] In short, discrimination was assessed with an AUC, which is a measure of how well a diagnostic test can distinguish between 2 diagnostic groups (in this case, patients with allergy and tolerant subjects). A value of 0.5 indicates "no discrimination," and a value of 1 indicates "perfect discrimination." Calibration was assessed with a calibration plot, which compares observed versus predicted outcomes, and the Hosmer-Lemeshow test, where a *P*-value < 0.05 indicates poor calibration.[28] Different cutoff points were used to calculate sensitivity, specificity, positive and negative predictive values (PPV, NPV) and positive likelihood ratio (LR+) and negative likelihood ratio (LR-).[29] In case of poor validation (phase 1b), a new multivariate logistic regression analysis would be performed by a stepwise forward method with the probability of entering a variable .05 and removing a variable .10. Discrimination and calibration of this possible new model would be assessed as described before.

In phase 2, we assessed the diagnostic value of sIgE to Ara h 1, 2, 3, 8 and 9 by the AUC and compared it with the most often used history, SPT and sIgE to peanut extract. In addition, a multivariate logistic regression analysis was performed as described before with sIgE to the peanut components as candidate predictors. In 10 patients of the diagnostic group, data on sIgE to at least one of these peanut components was missing (Figure 1). However, we found no significant differences between these 10 patients and the remaining patients of the diagnostic group with regard to sex, age, atopic features, sIgE to peanut extract, or SPT.

In phase 3, the different diagnostic tests were related to severity. Spearman's rho correlation was used for these analyses.

All analyses were performed with SPSS version 20.0 (Chicago, IL, USA). The calibration plot was made by using Microsoft Office Excel 2003 (Microsoft Corp, Redmond, Wash).

RESULTS

Patients

Patient characteristics of the diagnostic group are listed in Table 1. The mean (SD) age was 30 ± 12.5 years with 37.2 % being men. Significant differences between the group with peanut allergy and the peanut tolerant group were found for age (26.5 vs. 35 years), SPT wheal (8 vs 3.3 mm), sIgE to peanut extract (3.5 vs. 0.49 kU/L) and sIgE to Ara h 2 (1 vs 0.06 kU/L) when analyzing the 95% CIs of the odds ratios (Table 1). The patient characteristics of the peanut allergic patients of the previous study group (n = 38), who were only used for the severity analyses, can be found in Table E1.

Table 1. Patient characteristics of the diagnostic group

	Total Diagnostic N = 94		**Peanut allergic N = 55**		**Peanut tolerant N = 39**		**Odds ratio (95% CI)**	
Male	35	(37%)	21	(38%)	14	(36%)	1.1	(0.47- 2.58)
Age (year)	30	(± 12.5)	26.5	(± 9.5)	35	(± 16)	0.95	(0.91-0.98)
SPT wheal (mm)	5	(0-9.8)	8	(2.8-15)	3.3	(0-6.0)	1.2	(1.1-1.3)
sIgE (kUa/L) peanut extract	2.1	(0.35-11)	3.5	(0.66-18)	0.49	(0.35-3.8)	1.1	(1.0-1.1)
Total IgE-sIgE peanut extract (kUa/L)	316	(72-782)	381	(87-1013)	262	(51-488)	1.0	(1.0-1.0)
sIgE Ara h 1 (kUa/L)	0.02	(0.00-0.43)	0.05	(0.00-5.1)	0.01	(0.00-0.04)	1.7	(0.91-3.0)
sIgE Ara h 2 (kUa/L)	0.20	(0.04-2.9)	1.0	(0.06-8.1)	0.06	(0.03-0.13)	3.3	(1.2-9.0)
sIgE Ara h 3 (kUa/L)	0.02	(0.01-0.11)	0.03	(0.01-0.64)	0.01	(0.01-0.03)	8.6	(0.34-215)
sIgE Ara h 8 (kUa/L)	2.0	(0.04-9.0)	2.0	(0.1-8.8)	0.72	(0.03-9.9)	1.0	(0.98-1.0)
sIgE Ara h 9 (kUa/L)	0.02	(0.00-0.07)	0.02	(0.00-0.08)	0.01	(0.00-0.06)	0.74	(0.46-1.2)
Allergic Rhinitis	68	(72%)	43	(78%)	25	(64%)	2.0	(0.80-5.0)
Atopic Dermatitis	54	(57%)	35	(64%)	19	(49%)	1.8	(0.80-4.2)
Allergic Asthma	50	(53%)	32	(58%)	18	(46%)	1.6	(0.71-3.7)
History*								
Mueller 0	38	(40%)	26	(47%)	12	(31%)	reference category	
Mueller 1	6	(7%)	2	(4%)	4	(10%)	0.23	(0.04-1.4)
Mueller 2	19	(20%)	14	(26%)	5	(13%)	1.2	(0.35-4.1)
Mueller 3	14	(13%)	6	(11%)	8	(21%)	0.29	(0.08-1.1)
Mueller 4	6	(7%)	6	(11%)	0	(0%)	2.8	(0.30-26)

All data represent medians (interquartile ranges), means ± SDs, or no. (%).

* History not clear in 11 patients (12%), 1 (2%) in peanut allergic group and 10 (26%) in peanut tolerant group

Table E1. Patient characteristics study group (N=38)

Male	13	(34%)
Age (year)	26	(± 8)
SPT wheal (mm)	10 (n=37)	(7.8-14)
sIgE (kUa/L) peanut extract	17	(4.1-54)
Total IgE-sIgE peanut extract	430	(166-2434)
sIgEAra h 1 (kUa/L)	0.35	(0.28-13)
sIgEAra 2 (kUa/L)	2.4	(0.44-15)
sIgEAra h 3 (kUa/L)	0.35	(0.28-4.1)
sIgEAra h 8 (kUa/L)	3.8	(0.35-12)
sIgEAra h 9 (kUa/L)	0.04	(0.01-0.20)
Atopic Dermatitis	32	(84%)
Allergic Rhinitis	33	(87%)
Allergic Asthma	31	(82%)
History		
Ingestion unknown	1	(3%)
Muller 0	16	(42%)
Muller 1	6	(16%)
Muller 2	11	(29%)
Muller 3	2	(5%)
Muller 4	2	(5%)

All data represent medians (interquartile ranges), means ± SDs, or no. (%).

Phase 1: Validation of the pediatric diagnostic prediction model in the adult population was poor

The probability of peanut allergy for every patient was calculated by using the formula as described in the Methods section. Validation by means of discrimination showed an AUC of 0.64. The best combination of PPV and NPV was found when using a cutoff value of a probability of ≥ 0.7 with a PPV of 100% and an NPV of 48%. Corresponding sensitivity and specificity were 35% and 100%, respectively, with a LR+ of infinity and LR- of 0.65. The calibration plot, which compares predicted probabilities of peanut allergy with observed patients with peanut allergy in our study population, showed data points around the reference line, which indicates good calibration (Figure 2). This was also shown by the Hosmer-Lemeshow test with a *P*-value of 0.48.

Because of the poor validation by discrimination, we built a new model specific for an adult population. We chose a maximum of 4 candidate predictors based on literature and our univariate analyses to minimize the risk of overfitting: sIgE to peanut extract and Ara h 2, SPT reactivity and age. In addition to the 14 patients with missing SPT data, sIgE to Ara h 2 could not be assessed in eight patients because of a lack of serum, which left 72 patients of the diagnostic group (n=94) to build a new model (Figure 1).

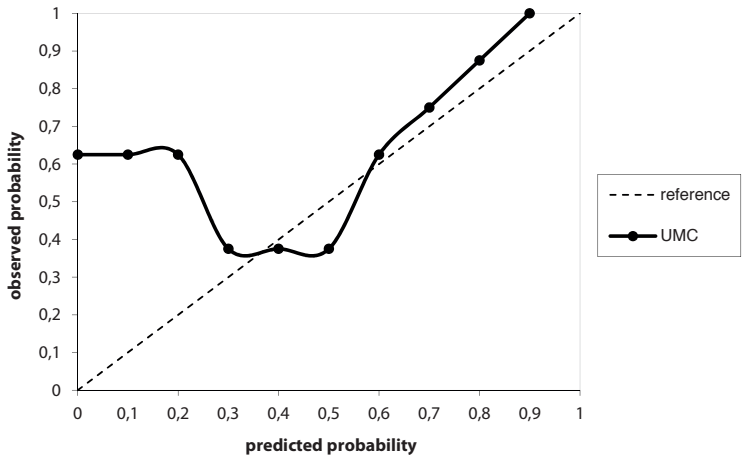

Figure 2. Calibration plot of the pediatric prediction model in our adult population

No significant differences were found between the 8 patients with missing Ara h 2 data and the remaining 72 patients with regard to the other 3 candidate predictors: SPT, sIgE to peanut and age. When entering the 4 candidate predictors in a multivariate logistic regression model, only SPT was included in the final model with an AUC of 0.72. The best combination of PPV and NPV was found when using a cutoff value of ≥ 15 mm with a PPV of 100% and a NPV of 49% (Table 2). Corresponding sensitivity and specificity were 26% and 100%, respectively, with LR+ of infinity and LR- of 0.74. By using this cutoff value with a 100% PPV, peanut allergy could be predicted in 12.5% of patients with 100% certainty.

Phase 2: sIgE to Ara h 2 has the best diagnostic value of all peanut components

Ara h 2 had the best discriminative ability of all peanut components (AUC 0.76, Figure 3). This value also was higher compared with the currently used history, SPT and sIgE to peanut extract (AUC's of 0.67, 0.72 and 0.70, respectively). The difference with SPT and

Table 2. Diagnostic value of the predictor SPT in mm for different cutoff points

Cutoff point	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	LR+	LR-
≥ 3 mm	66	52	66	52	1.38	0.65
≥ 5 mm	76	59	66	70	2.22	0.49
≥ 8 mm	88	56	51	86	3.64	0.57
≥ 10 mm	91	49	34	84	2.13	0.79
≥ 15 mm	100	49	26	100	infinity	0.74

PPV, positive predictive value; *NPV*, negative predictive value; *LR+*,positive likelihood ratio; *LR-*, negative likelihood ratio

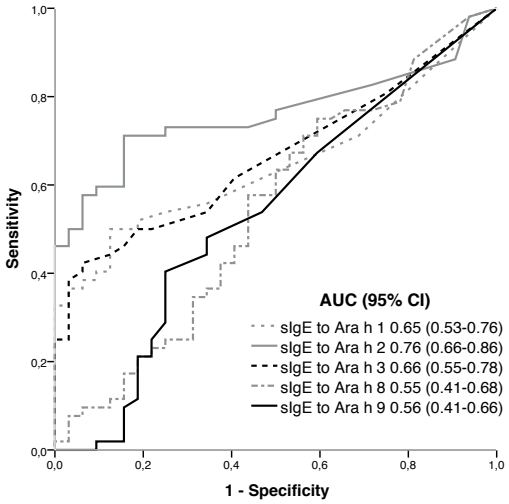

Figure 3. ROC-curves of sIgE to Ara h 1, 2, 3, 8 and 9

sIgE to peanut extract, however, was not significantly different (*P* values of 0.15 and 0.09, respectively).

Specific IgE to Ara h 2 was the only predictor that remained in the model when performing a multivariate analysis with sIgE to Ara h 1, 2, 3, and peanut extract as candidate predictors. Because the highest AUC with both univariate and multivariate analysis was found for sIgE to Ara h 2, we used several cutoff points to calculate PPV, NPV, sensitivity, specificity, LR+ and LR- (Table 3). The best combination of PPV and NPV was found by using a cut-ff point of ≥ 1.0 kU/L (93% PPV and 54% NPV) with corresponding sensitivity, specificity, LR+ and LR- of 57%, 94%, 9.5 and 0.46, respectively. A 100% PPV was found when using a cutoff value of ≥1.75 kU/L (Table 3). Twenty-eight percent of

Table 3. Diagnostic value of the predictor sIgE to Ara h 2 for different cutoff values

Cutoff point	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	LR+	LR-
≥ 0.1 kU/L	81	63	74	73	2.74	0.36
≥ 0.2 kU/L	84	63	70	82	3.89	0.37
≥ 0.35 kU/L	86	57	60	85	4.00	0.47
≥ 0.5 kU/L	88	56	57	89	5.18	0.48
≥ 1.0 kU/L	93	54	57	94	9.50	0.46
≥ 1.32 kU/l	95	54	49	97	16.33	0.53
≥ 1.75 kU/l	100	53	45	100	infinity	0.55

PPV, positive predictive value; *NPV*, negative predictive value; *LR+*, positive likelihood ratio; *LR-*,negative likelihood ratio

all the patients had a higher value than this cutoff and, therefore, could be predicted as being peanut allergic with 100% certainty. A 95% PPV was calculated at a cutoff value of ≥1.32 kU/L. The highest NPV was 63% when using a cutoff value for Ara h 2 of ≥ 0.1 kU/L, which was comparable with the SPT when a cutoff value of ≥5 mm was used (59%). It was not possible to calculate a higher NPV with any of the other diagnostic tests.

Phase 3: Ara h 2 was correlated to clinical severity as assessed in three different ways

In phase 3, all 93 patients with a positive food challenge to peanut (55 from the diagnostic group and 38 from the study group) were used to analyze the relationship between the diagnostic tests and clinical severity as assessed in three different ways (Figure 1). The sIgE to Ara h 2 was the only diagnostic test that significantly correlated with clinical severity with all 3 methods of scoring severity: Mueller based on history, Mueller based on food challenge and severity score (*P*-values 0.007, < 0.001 and 0.030, respectively; Spearman's rho, Table 4). However, also for sIgE to peanut extract, Ara h 1 and Ara h 3 there was a trend of higher sIgE values with a more severe reaction. In contrast, there was a significantly negative correlation between sIgE to Ara h 8 or Ara h 9 and clinical

Table 4. Correlation between all diagnostic tests and severity as scored in three different ways

	Mueller based on history	Mueller based on food challenge	Severity score*
Ara h 1			
Correlation coefficient	0.19	0.34	0.19
P-value	0.07	<0.001	0.08
Ara h 2			
Correlation coefficient	0.28	0.416	0.23
P-value	0.01	<0.001	0.03
Ara h 3			
Correlation coefficient	0.21	0.25	0.10
P-value	0.04	0.02	0.37
Ara h 8			
Correlation coefficient	-0.29	-0.09	-0.02
P-value	<0.001	0.43	0.85
Ara h 9			
Correlation coefficient	-0.22	-0.02	-0.04
P-value	0.04	0.89	0.68
sIgE to peanut extract			
Correlation coefficient	0.22	0.22	0.15
P-value	0.03	0.04	0.15
Skin Prick Test			
Correlation coefficient	0.13	0.17	0.09
P-value	0.23	0.12	0.43

Correlation coefficient = Spearman's rho

* Combination of Mueller score based on food challenge and eliciting dose (see also Methods section)

severity, but only when the adapted Mueller score based on history was used (*P*-values 0.004 for sIgE to Ara h 8 and 0.04 for sIgE to Ara h 9, respectively). All correlations are shown in Table 4. SPT reactivity was not correlated with clinical severity.

Based on these results, we calculated the discriminative ability of sIgE to Ara h 2 between mild and severe allergy based on both history and food challenge (with Mueller 0, 1 and 2 being mild and Mueller 3 and 4 being severe). This resulted in an AUC value of only 0.58 and 0.65, respectively, which indicates poor discrimination between mild and severe allergy. When a severe allergy was defined as a Mueller score > 0, these AUC values were 0.65 and 0.79, respectively.

DISCUSSION

In this study, we validated a pediatric prediction model for peanut allergy in an adult population suspected of peanut allergy. Because of poor validation, we analyzed a possible new model with 4 candidate predictors, which left only SPT as a predictor. The diagnostic value of sIgE to Ara h 2, however, was an even better predictor and also appeared to be correlated to clinical severity.

Validation of a prediction model is essential before it can be applied to a different patient group, in this case, an adult population.[13-15] Validation by discrimination showed a striking difference between the two populations with an AUC of 0.64 in adults vs. 0.94 in children, in which the original model was updated.[9] This is best explained by population differences. Therefore, our pediatric model could not be generalized to this adult population. In general, prediction models tend to perform better in the original data set on which the model was built than in the data set of another study population that is used for validation.[13]

A general rule when building a prediction model states that, for every candidate predictor, at least 10 patients with the smallest outcome are required.[30] To minimize the risk of overfitting, we only entered 4 candidate predictors in a multivariate logistic regression analysis: SPT, sIgE to Ara h 2 and peanut extract and age. Our choice of the 4 predictors was based on the literature and univariate analyses of our data. We found age to be negatively correlated with severity based on history, which meant that older subjects reported milder symptoms. This could probably explain the significantly higher age in the tolerant subjects because only mild symptoms ultimately led to a smaller chance of meeting the criteria for a positive food challenge. When analyzing the 4 candidate predictors, it was interesting that only SPT was left as a predictor in the model, since the diagnostic value of sIgE to Ara h 2 as a single predictor was higher (0.76 vs. 0.72). This can be explained by the type of logistic regression analysis we used.[31] We performed the stepwise forward method in which the model is built from the most significant

variable between the peanut allergic and peanut tolerant group, which was the SPT. Thereafter, variables that have a significant additional value in the model are included one by one. This is in contrast with the backward elimination method by starting with all candidate predictors and with every step eliminating the least contributing. Use of backward regression in our data set would result in Ara h 2 being the only predictor. The stepwise forward method was chosen to compare our results with our pediatric study, which was a validation study of the prediction model published by DunnGalvin et al,[12] in which the same method was used. This choice was supported by the fact that there is no consensus about the best method of selecting variables for a prediction model.

The diagnostic value of the SPT (AUC 0.72), in general, was lower than that found in other studies in which the AUC ranged from 0.79-0.93.[7-10] The cutoff value of 8 mm with a 95% PPV, as previously described by Sporik et al,[11] led to an 88% PPV in our population. The differences could be caused by the fact that all aforementioned studies were performed in children with sometimes different inclusion criteria. In some of these studies, many patients were included based on sensitization only, regardless of history.

The sIgE to Ara h 2 as single predictor had the best diagnostic value of all studied diagnostic tests (AUC 0.76), although not significantly different compared with SPT or sIgE to peanut extract. The major advantage of Ara h 2, however, is that it can be standardized. The AUC of sIgE to Ara h 2 was lower than the AUC found in recent diagnostic studies in children (AUC ranging from 0.90 to 0.99).[9;16-20] In addition, the cutoff value for a 100% PPV was much lower compared with our former study in children (> 1.74 kU/L vs. > 5.17 kU/L) but applicable to a similar number of patients (28 % vs. 26%).[9] This means that, both in children and adults, peanut allergy can be well predicted with Ara h 2. Predicting tolerance, however, seems difficult in adults, reflected by a lower AUC and very low NPV values. We think that this difference could be explained by the fact that many allergic patients recognized the Bet v 1 related Ara h 8 (62%, versus 36% in our pediatric database). This is probably because birch pollen allergy usually becomes clinically significant at an older age. Twenty-one patients in the adult population did not recognize Ara h 2 but did have a positive food challenge (22%). These patients mainly recognized Ara h 8 (61% monosensitized) and to a lesser extent the other peanut components. Most of these patients (84%) experienced only mild symptoms after the ingestion of peanut. However, Ara h 8 does not discriminate well between peanut allergy and peanut tolerance because, in the tolerant group, a large proportion of subjects reacted to Ara h 8 as well (AUC 0.55), which corresponds with other populations.[9;16-20] In addition, the different diagnostic value between children and adults could be caused by different selection criteria. Many subjects in pediatric studies are included based on sensitization only, whereas, in our study, all adult patients had a suggestive history, regardless of sensitization.

We used three different methods of measuring severity because, in literature there is no consensus about the best method and Hourihane et al[26] previously described only a weak correlation between severity based on history and food challenge. Ara h 2 showed a correlation with severity when using all methods. Interestingly, Ara h 8 and Ara h 9 showed a negative correlation with severity, but this was only significant when using the severity classification based on history. In general, a similar trend was seen among the three different scoring methods for all diagnostic tests. Previous studies that analyzed the relationship between sIgE to peanut allergens and severity had contradictory results. Peeters et al,[22] in general, found a larger SPT reactivity to Ara h 1, 2, 3 and 6 in the case of a more severe reaction in an adult population. A similar study in a pediatric population, however, showed no correlation between clinical severity and SPT reactivity or sIgE to Ara h 1, 2 and 3.[32] This finding is in agreement with our previously published study in children,[9] in which no significant correlation was found between clinical severity based on food challenge and sIgE to Ara h 1, 2, 3 and 8. Moverare et al[33] recently suggested that a positive sIgE value to the major peanut allergens Ara h 1, Ara h 2, and Ara h 3 is associated with severe reactions, but they did not take into account the exact sIgE values. The differences can possibly be explained by the fact that the study of Peeters et al[22] and our study were conducted in adult patients only. We would like to point out that, although a correlation was seen on a population level, sIgE to Ara h 2 could not be used to predict the severity of peanut allergy in an individual patient in our study.

A possible limitation of our study was that not every subject with a suspected peanut allergy who visited our outpatient department underwent a food challenge, which could have influenced the prevalence of peanut allergy in our study population and, therefore, the exact PPV and NPV values. However, 59% of the patients were peanut allergic, which was comparable with other studies performed in children in tertiary centres (prevalence of 47%-56%).[9;12] We also presented likelihood ratios, which are a diagnostic measure that is independent of the prevalence. With likelihood ratios, the difference between pre- and post-test probability can be assessed.[29] Another possible limitation to our study is that we had some missing data. However, no significant differences were found between the groups with or without missing values. The strength of this study was that we used a diagnostic design, comparable with clinical practice. In addition, we included a large group of adults, which, to our knowledge has not been previously studied. All patients in our study underwent a DBPCFC to diagnose or exclude a peanut allergy.

In conclusion, we showed that sIgE to Ara h 2 has the best diagnostic value to diagnose peanut allergy in adults. Specific IgE to Ara h 2, however, was not able to exclude a peanut allergy and could not discriminate between mild and severe allergy in individual patients, which indicates that patients with negative or low sIgE levels to Ara h 2 can still be peanut allergic and even have a severe peanut allergy. Future prospective studies should validate the cutoff values in other adult populations.

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Chapter 6

The diagnostic accuracy of specific IgE to Ara h 6 in adults is as good as Ara h 2

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ABSTRACT

Specific IgE (sIgE) to Ara h 2 is useful in diagnosing peanut allergy. Our aim was to assess the diagnostic value of sIgE to Ara h 6, another 2S albumin, in an adult population suspected of peanut allergy. Subjects with suspected peanut allergy between 2002 and 2013 were included if a diagnostic double-blind, placebo-controlled food challenge with peanut was performed. sIgE to Ara h 2 and Ara h 6 was measured by ImmunoCAP ISAC 112. Of 107 challenged subjects, 65 had a positive challenge (61%). The discriminative ability of sIgE to Ara h 2 and Ara h 6 was comparable: AUC 0.81 *vs* 0.82. Positive predictive value for both tests was 95% using a cutoff value ≥1 ISU/l with poor corresponding sensitivity values (58% for Ara h 2, 62% for Ara h 6), but good specificity values (95% for both tests). In conclusion, the diagnostic value of sIgE to Ara h 6 on population level was as good as sIgE to Ara h 2. On individual level, however, 5% of the subjects showed contradicting results between both tests using a cutoff of 0.3 ISU/l, leading to a risk of misdiagnosis if only one of both tests is used.

BRIEF COMMUNICATION:

Determining specific IgE (sIgE) to Ara h 2 seems promising to replace sIgE with peanut extract and skin prick test (SPT) as diagnostic tool for peanut allergy in most geographic locations, because recent studies have shown its important value in children and adults.[1-8] The diagnostic value of sIgE to Ara h 6 has so far only been studied in a case–control design.[9] Both Ara h 2 and Ara h 6 are 2S albumins. Some patients in our outpatient department were only sensitized to either Ara h 2 or Ara h 6, as was also described before by Asarnoj et al.[10] Therefore, the aim of this study was to assess the diagnostic value of determining sIgE to Ara h 2 and Ara h 6 in an adult population suspected of peanut allergy in a cross-sectional design. The study was approved by the local ethics committee.

All subjects (n = 124) with a suspected peanut allergy who underwent a diagnostic double-blind, placebo-controlled food challenge (DBPCFC) with peanut between 2002 and 2013 were included if there was leftover serum for sIgE measurements. Only 17 challenged subjects had to be excluded for this reason, and they were equally distributed among the positively and negatively challenged group. Suspicion of peanut allergy was based on history (history group, n = 86) or sensitization without previous ingestion (sensitized group, n = 21). The sensitized group was included based on SPT (≥3 mm, using ALK-Abello peanut extract) (n = 9), sIgE to peanut extract (≥0.35 kU/l) (n = 4), or both (n = 8). The distribution between positive and negative challenge outcomes was not significantly different between the history group and the sensitized group (54 *vs* 32 and 11 *vs* 10, P = 0.46). The protocol and recipe of the DBPCFC were described by Peeters et al.[11] The challenge was considered positive in case of objective symptoms or if a suggestive subjective symptom occurred on at least three subsequent doses, with a minimal twofold increase, or lasted for >45 min. Specific IgE to Ara h 6, as well as Ara h 1, 2, 3, 8, and 9, was measured by ImmunoCAP ISAC 112 (Uppsala, Sweden) using serum that was collected during diagnostic workup. The ImmunoCAP ISAC was used as Ara h 6 cannot be tested yet with the regular ImmunoCAP. The discriminative ability of the peanut components was assessed by the area under the receiver operating characteristic (ROC) curve (AUC) (version 20.0; SPSS Inc, Chicago, IL). Sensitivity, specificity, positive and negative predictive values (PPV and NPV), and positive and negative likelihood ratios (LR+ and LR–) were calculated for sIgE to Ara h 6 and Ara h 2 with cutoff values of 0.3 and 1 ISU/l.

The median age of the 107 challenged subjects was 24 (IQR 21–31), 36% were male, and 65 had a positive food challenge (61%). Ara h 6 was recognized (≥0.3 ISU/l) in 52 subjects (49%) and Ara h 2 in 49 subjects (46%). Four subjects (3.7%) recognized Ara h 6 and not Ara h 2. The other way around this was the case in only one subject. In three of these five non-matching cases, the clinical outcome of the DBPCFC was well

predicted by sIgE to Ara h 2. Specific IgE to Ara h 2 and Ara h 6 in the total group was very well correlated (Spearman's rho 0.94, $P < 0.001$), and median values (after excluding responses <0.3 ISU/l) were similar (4.3 *vs* 5.6 ISU/l, $P = 0.63$). The absolute values, as well as the percentage of positive outcomes (>0.3 ISU/l), of both sIgE to Ara h 2 and Ara h 6 were not significantly different between the history group and sensitized group (*P*-values of 0.31 and 0.47 for sIgE to Ara h 2 and *P*-values of 0.20 and 0.34 for sIgE to Ara h 6). The discriminative ability of sIgE to Ara h 2 and Ara h 6 in the total population was comparable: AUC 0.81 *vs* 0.82 (Figure 1). The AUC values of sIgE to Ara h 1, 3, 8, and 9 were 0.68, 0.63, 0.53, and 0.46, respectively (data not shown). When subjects who only reported oral allergy symptoms in their history ($n = 37$) were excluded, the AUC value of sIgE to Ara h 2 and Ara h 6 increased to 0.89 and 0.92, respectively. For this analysis, subjects without history were excluded as well ($n = 22$).

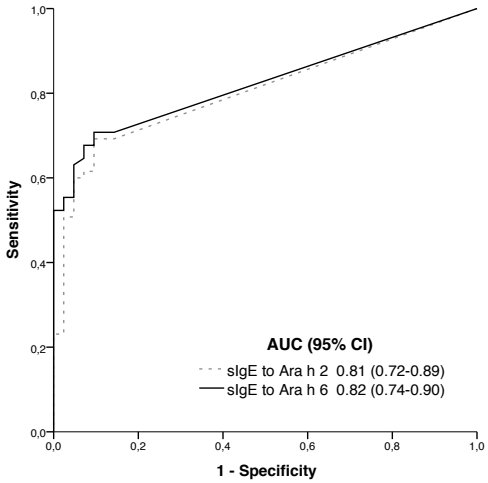

Figure 1. The AUC values of sIgE to Ara h 6 and Ara h 2 are comparable. CI, confidence interval.

Only some small differences were observed in the different diagnostic values of sIgE to Ara h 2 and 6 in the total population with a cutoff value of 0.3 ISU/l (Table 1). Sensitivity values were suboptimal, indicating that approximately 30% of peanut allergic patients neither recognized Ara h 2 nor Ara h 6. Specificity values were good (90% and 86%), indicating that only few tolerant patients recognized either of the two components. The good PPV and LR+ values and poor corresponding NPV and LR– values indicate that both tests are primarily useful in diagnosing peanut allergy in case of a positive test result. A negative test result, however, does not accurately rule out peanut allergy. By

Table 1. Diagnostic value of sIgE to Ara h 2 and Ara h 6 for different cutoff values

	Sensitivity	Specificity	PPV	NPV	LR+	LR-
≥0.3 ISU/L						
Ara h 2	69	90	92	66	6.9	0.34
Ara h 6	71	86	88	65	5.07	0.34
≥1.0 ISU/L						
Ara h 2	58	95	95	60	11.6	0.44
Ara h 6	62	95	95	62	12.4	0.40

PPV, positive predictive value; *NPV*, negative predictive value; *LR+*, positive likelihood ratio; *LR-*, negative likelihood ratio

increasing the cutoff value to 1 ISU/l, a 95% PPV could be established for both sIgE to Ara h 2 and Ara h 6 with LR+ values above 10 (Table 1).

A 100% PPV for sIgE to Ara h 6 could be calculated when using a cutoff value of >2.40 ISU/l. Thirty-four allergic patients (32% of the study population) had such a value and could therefore be classified as peanut allergic with 100% certainty as based on our population. For sIgE to Ara h 2, the cutoff value with a 100% PPV was >9.74 ISU/l and was applicable to 15 patients (14% of the study population). The high cutoff value for sIgE to Ara h 2 was caused by one patient with a sIgE value of 9.74 ISU/l and still a negative peanut challenge (no symptoms after 10 g open dose in addition to the negative DBPCFC). Lowering the cutoff value for a positive test did not result in an increase of the NPV. Combining sIgE to Ara h 2 and Ara h 6, considering them positive when either of these tests had a value ≥0.3 ISU/l, did not result in a better diagnostic accuracy.

It is important to realize that the diagnostic accuracy of sIgE to Ara h 6 in this study was assessed in an adult population. The percentage of subjects with a suggestive history would probably be lower in a pediatric population, which could affect the diagnostic value. Although we found no meaningful differences in AUC values between the history group and the sensitized group (data not shown), the sensitized group was too small to draw firm conclusions. Moreover, the present study was performed in a tertiary center. Validation of our findings in a pediatric population and first and second line hospitals is required before our results can be extrapolated to these patient groups. Eighteen peanut allergic patients neither recognized Ara h 2 nor Ara h 6. The majority of them, that is, 11/18 patients (61%), was monosensitized to Ara h 8, which can probably be explained by a birch pollen-related peanut allergy in these patients. All but one of these patients reported only oral allergy symptoms after ingestion of peanut. In 17 of these patients, sIgE to Ara h 2 was measured by ImmunoCAP as well, which also showed negative responses (cutoff 0.35 kU/l). Although ImmunoCAP and ImmunoCAP ISAC have different outcome measures, sIgE to Ara h 2 in 87 subjects showed a strong correlation between both tests (Spearman's rho 0.90, $P < 0.001$).

In conclusion, the diagnostic value of sIgE to Ara h 6 on population level, as measured by ImmunoCAP ISAC 112, was as good as sIgE to Ara h 2. Combining both tests did not result in a better diagnostic accuracy. On individual level, however, 5% of the subjects showed contradicting results between both tests using a cutoff of 0.3 ISU/l, leading to a risk of misdiagnosis if only one of both tests is used.

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Chapter 7

Components in soy allergy diagnostics: Gly m 2S albumin has the best diagnostic value in adults

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ABSTRACT

Background: Thus far, four soy allergens have been characterized. Their diagnostic value was assessed only using a case-control design with controls not suspected of soy allergy or in a soy-allergic population without controls. Our objective was to analyze the diagnostic value of specific IgE (sIgE) to Gly m 2S albumin, Gly m 4, 5, and 6, and their possible relation with severity or culprit soy product.

Methods: Adult patients suspected of soy allergy were included (n = 46). Allergy was confirmed by challenge (n = 19) or history (n = 16) and excluded by challenge in 11 patients. Soy components were analyzed by ImmunoCAP. Diagnostic value was assessed in the challenged patient group by an area under receiver operating characteristic (ROC) curve (AUC).

Results: sIgE to Gly m 2S albumin had the highest AUC (0.79), comparable to skin prick test (SPT) and sIgE to soy extract (0.76 and 0.77, respectively). All patients were sensitized to either soy extract or Gly m 4 (sIgE ≥ 0.35 kU/l). sIgE to soy extract, Gly m 5, and Gly m 6 was significantly higher in patients with mild symptoms (P = 0.04, 0.02 and 0.02, respectively). Patients only reacting to soy milk had higher sIgE levels to Gly m 4 (median 9.8 *vs* 1.1 kU/l, P = 0.01).

Conclusion: sIgE to Gly m 2S albumin had the best accuracy in diagnosing soy allergy. Gly m 5 and 6 were related to mild symptoms. Higher levels of sIgE to Gly m 4 were related to allergy to soy milk.

INTRODUCTION

The prevalence of soy allergy in adults has not been studied yet.[1] Symptoms may range from mild oral allergy to severe respiratory or cardiovascular reactions.[2;3] Frequently used diagnostic techniques to diagnose soy allergy, such as skin prick test (SPT) and specific IgE (sIgE) to soy extract, perform suboptimally.[2-4] Ballmer-Weber et al[2] found a sensitivity of 80% when using a prick-to-prick test with soy flour. With commercial soy extracts, sensitivity ranged from 69% to 76%.[2;3] Another study illustrated that SPT with soy flour was a sensitive tool to detect sensitization but was not able to discriminate between soy allergy and soy tolerance.[5] The sensitivity of sIgE to soy extract ranged from 45% to 77%.[2-4]

The role of various specific soy components was evaluated in an attempt to improve the diagnosis of soy allergy.[2-8] Most studies focused on sIgE to Gly m 4 and showed sensitivity ranging from 70% to 100%.[2-4] sIgE to Gly m 5 and 6 was detected in 5–67% and 5–58% of patients respectively.[4;6;8]. Remarkably, the sensitivity for sIgE to Gly m 5 and Gly m 6 was much lower in adults than children (5% *vs* 67% for Gly m 5 and 5% *vs* 58% for Gly m 6, respectively).[4;6] No diagnostic values could be extracted from the only study that addressed sIgE to Gly m 2S albumin in a soy-allergic population.[7]

Soy components were also studied in relation to the severity of soy allergy.[6;8] sIgE to Gly m 5 and Gly m 6 was identified as a potential diagnostic marker for severe soy allergy.[8] In contrast, high levels of sIgE to Gly m 4 were detected in patients with anaphylactic reactions to soy drinks.[9;10]

The use of soy-specific components was only investigated in studies with a case-control design, with controls not suspected of having a soy allergy,[4-7] or in a soy-allergic population without a control group.[2;3;8-10] This makes it difficult to translate diagnostic values into daily clinical practice.

The aim of this study was to analyze the diagnostic value of SPT and sIgE to soy extract, as well as sIgE to Gly m 2S albumin, Gly m 4, 5, and 6, in adults who were all suspected of soy allergy. Furthermore, we studied the relationship with severity of soy allergy or culprit soy product.

METHODS

Study population

All adult subjects who visited the outpatient Department of Dermatology/Allergology at the University Medical Center, Utrecht, between 2003 and 2011, and underwent a food challenge to diagnose or exclude soy allergy, were included. The challenged

patient group was used to assess the diagnostic value of all diagnostic tests because no selection was made in this group.

In addition, soy-allergic patients with a convincing history were included during the same period. These were patients with an anaphylactic reaction following the ingestion of any soy product or patients with a clear allergic reaction after the ingestion of soy milk. These patients were used, together with the group with a positive challenge, to study the relationship between soy components and severity or culprit soy product. The study was approved by the local ethics committee.

Questionnaire

A questionnaire was sent to all soy-allergic patients to differentiate between the type of symptoms after ingestion of soy milk products and other soy products. Furthermore, for both kinds of products, questions regarding the onset of the reaction and severity were asked. In addition, atopic dermatitis, allergic rhinitis, asthma, and peanut allergy were evaluated.

Food challenge

Both open food challenges and double-blind, placebo-controlled food challenges (DBPCFCs) were performed. Open food challenge consisted of increasing doses of soy milk (range 0.04–5.6 g soy protein). The DBPCFC consisted of either pancakes with increasing doses of soy milk (active doses, range 0.5–2 g soy protein) or water (placebo doses) or a drink with increasing doses of soy flour (active doses, range 0.3–6.1 g soy protein) or Protifar (placebo doses). Doses were given every 30 minutes, according to the international consensus protocol.[11] The challenge was considered positive when objective symptoms occurred or when consistent subjective symptoms occurred on at least 3 subsequent doses or lasted for at least 45 min.

SPT and measuring sIgE

Skin prick test was performed with commercial soy extract (ALK-Abelló, Nieuwegein, the Netherlands). As positive control, histamine dihydrochloride 10 mg/ml was used; negative control was glycerol diluent.

In all patients, sIgE to Gly m 2S albumin, rGly m 4, nGly m 5, nGly m 6, soy extract, rAra h 2, and rBet v 1 was measured using ImmunoCAP (Phadia, Uppsala, Sweden) according to manufacturer's instructions. sIgE to rAra h 2 and rBet v 1 was measured because in daily clinical practice, many soy-allergic patients report a peanut allergy or birch pollen (related food) allergy. Gly m 2S albumin was purified from soybean extract using ion exchange chromatography and gel filtration. Experimental ImmunoCAP tests with the Gly m 2S albumin was developed by conjugation of the allergens to

CNBr-activated ImmunoCAP matrix according to the standard method of Phadia AB, as described elsewhere.[12]

In part of the population, the Immuno Solid-phase Allergen Chip (ImmunoCAP ISAC, VBC Genomics and Phadia), containing the soy allergens rGly m 4, nGly m 5, and nGly m 6, was performed as well.[13] Values below the detection limit of 0.3 ISU/l were considered negative and given the value 0 for statistical analysis. ImmunoCAP ISAC results were used to compare with the ImmunoCAP results. For diagnostic analyses, sIgE measured by ImmunoCAP was used.

Clinical severity

To assess clinical severity, the most severe reaction of history or food challenge was chosen and divided into five categories as follows: (1) oral symptoms, (2) cutaneous symptoms, (3) gastrointestinal symptoms, (4) respiratory symptoms, and (5) cardiovascular symptoms. This range of categories (1–5) was correlated with the result of all diagnostic tests. In addition, clinical severity was analyzed by comparing the test results between patients with mild symptoms (category 1–3) and patients with severe symptoms (category 4–5).

Statistics

The descriptive statistics are presented as percentages or median values with interquartile ranges. Differences between groups were analyzed with independent *t*-tests for parametric data, Mann–Whitney U-test for nonparametric data, and chi-squared test for categorical data. A Spearman's rho was used to analyze correlations. A *P*-value <0.05 was considered significant for all tests.

The diagnostic value of the tests was assessed with an area under the receiver operating characteristic (ROC) curve (AUC). Different cutoff points were used to calculate sensitivity, specificity, positive and negative predictive values (PPV, NPV), and positive and negative likelihood ratios (LR+, LR–).

RESULTS

Patient characteristics

A total of 46 subjects were included as follows: 35 soy allergic (19 challenged, 16 convincing reaction to soy) and 11 soy tolerant (all challenged negative). Of the 16 patients with a convincing reaction to soy, 11 had a history of an anaphylactic reaction and five reported a convincing reaction immediately after the ingestion of soy milk. Clinical characteristics of the soy-allergic and soy-tolerant patient group are summarized in Table 1.

Table 1. Demographic and clinical characteristics

	Total N = 46		Soy allergic N = 35		Soy tolerant N = 11		*P*-value
Male	16	(35%)	11	(31.4%)	5	(46%)	0.39
Age (year)	31.5	(24.0 – 49.2)	31	(24.0 – 50.0)	35.0	(25.0 – 47.0)	0.83
sIgE soy extract (kU/L)	0.4	(0.09 – 3.4)	0.5	(0.2 – 6.1)	0.1	(0.01 – 1.4)	0.16
SPT (mm) (n = 40)	2.5	(0.3 – 6.8)	3.0	(2.0 – 7.0)	0.0	(0.0 – 3.5)	0.02
sIgE Gly m 2S albumin (kU/L)	0.3	(0.1 – 1.2)	0.6	(0.2 – 2.0)	0.2	(0.02 – 0.4)	0.03
sIgE Gly m 4 (kU/L)	2.0	(0.2 – 9.1)	3.6	(0.2 – 9.8)	1.0	(0.0 – 2.9)	0.06
sIgE Gly m 5 (kU/L)	0.02	(0.0 – 3.0)	0.02	(0.0 – 3.4)	0.01	(0.0 – 1.0)	0.58
sIgE Gly m 6 (kU/L)	0.03	(0.0 – 2.0)	0.03	(0.0 – 4.0)	0.01	(0.0 – 0.6)	0.31
sIgE peanut extract (kU/L)	2.2	(0.4 – 18.5)	2.4	(0.9 – 62.0)	1.3	(0.2 – 3.3)	0.09
sIgE Ara h 2 (kU/L, n = 44)	0.04	(0.01 – 4.5)	0.04	(0.01 – 17.0)	0.02	(0.01 – 3.3)	0.71
Peanut allergy*	26	(57%)	21	(60%)	5	(46%)	0.40
sIgE Bet v 1 (kU/L, n = 44)	10.4	(0.3 – 29.8)	15.7	(0.3 – 33.2)	5.0	(0.01 – 9.8)	0.07
Allergic rhinitis	34	(74%)	26	(74%)	8	(73%)	0.92
Atopic dermatitis	13	(28%)	11	(31%)	2	(18%)	0.37
Asthma	10	(22%)	8	(23%)	2	(18%)	0.61

All data represent medians (interquartile ranges) or no. (%).
CI, confidence interval
* Assessed by food challenge (n = 7) or by the combination of history (evaluated by a physician) and sensitization (sIgE to peanut extract ≥ 0.35 kU/L and/or SPT ≥ 3mm)

Table 2. Reported symptoms of soy allergic patients

ID	Sex	Age	Soy related symptoms		Food challenge	
			Soy milk	Other soy products	Type	Symptoms
Positively challenged						
2	M	65	oas, rc, ae, dys, as	none	open	oas, rc, dys
3	F	61	oas, ae, dys, h	unknown*	open	oas, ap
4	F	40	oas, ae, urt, dys	unknown	open	oas, rc, h
5	F	26	oas	unknown	open	oas**
7	F	24	oas, rc	unknown	open	oas, rc
9	F	24	oas, urt, n, dys	unknown	open	oas, n, ap
10	F	18	oas, rc, n	unknown	open	oas, n
11	F	18	oas, n, dys	unknown	open	oas**
12	M	25	oas	none	DBPCFC	oas
13	M	36	oas, n	oas	DBPCFC	oas
14	F	19	oas	unknown	DBPCFC	oas
15	M	26	unknown	oas	DBPCFC	oas

Table 2. Continued

ID	Sex	Age	Soy related symptoms		Food challenge	
			Soy milk	Other soy products	Type	Symptoms
16	M	26	unknown	oas, n, dys	DBPCFC	oas, n, dys
17	M	25	unknown	oas	DBPCFC	oas, n, ap
18	F	24	unknown	oas, rc, d, dys	DBPCFC	oas, rc, h
19	F	31	unknown	oas, n, dys	DBPCFC	oas, le
20	F	23	oas	oas, urt, ap	DBPCFC	oas, rc, dys
21	M	60	oas, n	oas, n	open (tahu)	oas, ae
32	M	47	oas, n, dys	none	open	oas, rc
Anaphylactic reaction						
33	F	55	oas, rc, urt, ae, dys, h	none	nt	NA
34	F	48	oas, rc, urt, ae, n, dys, as	oas, urt	nt	NA
35	F	27	oas, rc, dys, as	unknown	nt	NA
36	F	54	oas, urt, n, v, dys, h	none	nt	NA
37	M	22	oas, rc, ae, v, dys	none	nt	NA
38	F	59	oas, rc, dys	oas, n	nt	NA
41	F	55	oas, ae, n, v, dys	n	nt	NA
42	F	19	oas, dys	none	nt	NA
43	F	61	oas, dys	oas, dys	nt	NA
51	F	44	oas, urt, dys	none	nt	NA
53	F	38	oas, ae, dys	unknown	nt	NA
Convincing history (other than anaphylactic)						
45	M	20	oas	none	nt	NA
46	F	47	oas, urt	none	nt	NA
47	F	32	oas	none	nt	NA
48	F	65	oas	none	nt	NA
50	M	61	n,v	none	nt	NA

Oas, oral allergy symptoms; *rc,* rhinoconjunctivitis; *ae,* angioedema; *dys,* dyspnoea; *as,* anaphylactic shock; *h,* hoarseness; *ap,* abdominal pain; *urt,* urticaria; *n,* nausea; *d,* diarrhoea; *le,* laryngeal edema; *nt,* not tested; *NA,* not applicable; *v,* vomiting

*Unknown: never ingested

** Both patients reported more intense oral allergy symptoms with increasing doses. In addition, the duration of their symptoms prolonged, lasting > 45 minutes at the highest dose.

Thirteen patients from the soy-allergic population reported only reactions to soy milk while tolerating other soy products (three challenged, ten convincing history, of which five anaphylactic). Reported symptoms of all soy-allergic patients can be found in Table 2. Many subjects had a concomitant peanut allergy (n = 26, Table 1). The peanut allergic group had significantly more often sIgE to soy extract, Gly m 2S albumin, Gly m 5 and Gly m 6 (≥0.35 kU/l) compared with the peanut tolerant group (*P*-values

0.007, 0.007, <0.001 and <0.001). In addition, sIgE values to these components were significantly higher in the peanut-allergic group (all *P*-values < 0.001). We also found that sIgE to Ara h 2 was significantly correlated with sIgE to these soy components (soy extract: ρ = 0.75, *P* < 0.001; Gly m 2s albumin: ρ = 0.52, *P* < 0.001; Gly m 5: ρ = 0.81, *P* < 0.001; Gly m 6: ρ = 0.77, *P* < 0.001).

There was a trend of higher sIgE values to Bet v 1 in the soy-allergic group (15.7 *vs* 5.0 kU/l, *P* = 0.07). As expected, sIgE to Bet v 1 was strongly correlated with sIgE to Gly m 4 (ρ = 0.92, *P* < 0.001). In addition, we found weak negative correlations with sIgE to soy extract (ρ = −0.34, *P* = 0.02), Gly m 5 (ρ = −0.38, *P* = 0.01), and Gly m 6 (ρ = −0.37, *P* = 0.01).

Diagnostic value of sIgE to soy components compared with soy extract and SPT

Specific IgE to the soy component Gly m 2S albumin had the highest AUC to discriminate between soy allergic (*n* = 19) and soy tolerant patients (*n* = 11) in the challenged patient group, which was used to assess the diagnostic value (0.79, Table 3). sIgE to soy extract, Gly m 5, and Gly m 6 and SPT reactivity to soy extract had comparable AUC values, ranging from 0.74 to 0.77 (Table 3). AUC for Gly m 4 was poor (0.54).

When also adding the 16 patients with a convincing history of soy allergy (not challenged soy-allergic group) to the analysis (ten patients only reported symptoms to soy milk), the AUC for sIgE to Gly m 4 increased from 0.54 to 0.69, indicating an important role of sIgE to Gly m 4 in patients who solely react to soy milk.

Table 3. AUC of all diagnostic tests in the challenged population (n=30)

Diagnostic test	AUC (95% CI)	
Gly m 2S albumin	0.79	(0.62 – 0.96)
Gly m 4	0.54	(0.32 – 0.77)
Gly m 5	0.74	(0.54 – 0.94)
Gly m 6	0.77	(0.58 – 0.96)
Soy extract	0.77	(0.57 – 0.97)
SPT (n = 40)	0.76	(0.57 – 0.96)

CI, confidence interval

Diagnostic value of all tests for different cutoff values in the challenged patient group

All soy allergic patients of the challenged group (*n* = 19) had sIgE to either soy extract or Gly m 4 when using the clinical cutoff value of ≥0.35 kU/l. Therefore, the sensitivity of the combination of these tests, being positive when either of them was ≥0.35 kU/l, was 100%, with a specificity of 36%. Corresponding PPV and NPV were 73% and 100% with a LR+ of 1.57 and LR− of 0.

Table 4. Diagnostic value of SPT and sIgE to soy components and soy extract for different cutoff points in the challenged patient group (n=30)

Cutoff point	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	LR+	LR-
sIgE to Gly m 2S albumin						
≥ 0.1 kU/L	75	83	95	45	1.73	0.11
≥ 0.35 kU/L	81	57	68	73	2.52	0.44
≥ 1 kU/L	89	48	42	91	4.67	0.64
sIgE to Gly m 4						
≥ 0.1 kU/L	67	44	74	36	1.16	0.72
≥ 0.35 kU/L	61	33	58	36	0.91	1.17
≥ 1 kU/L	60	33	47	45	0.85	1.18
sIgE to Gly m 5						
≥ 0.1 kU/L	81	57	68	73	2.52	0.44
≥ 0.35 kU/L	80	53	63	73	2.33	0.51
≥ 1 kU/L	82	47	47	82	2.61	0.65
sIgE to Gly m 6						
≥ 0.1 kU/L	81	57	68	73	2.52	0.44
≥ 0.35 kU/L	81	57	68	73	2.52	0.44
≥ 1 kU/L	85	53	58	82	3.22	0.51
sIgE to soy extract						
≥ 0.1 kU/L	72	80	95	36	1.48	0.14
≥ 0.35 kU/L	79	64	79	63	2.14	0.33
≥ 1 kU/L	80	53	63	73	2.33	0.51
SPT						
≥ 1 mm	78	71	88	56	2.00	0.21
≥ 3 mm	73	43	50	67	1.52	0.75
≥ 5 mm	100	50	44	100	infinity	0.56

PPV, positive predictive value; *NPV*, negative predictive value; *LR+*, positive likelihood ratio; *LR-*, negative likelihood ratio

To diagnose a soy allergy, SPT with soy extract had the best diagnostic value (PPV 100%, LR+ infinity) when using a cutoff value of ≥5 mm, followed by sIgE to Gly m 2S albumin with a cutoff value of ≥1 kU/l (PPV 89%, LR+ 4.67, Table 4). The best diagnostic value to exclude a soy allergy was found for sIgE to Gly m 2S albumin with a cutoff value ≥0.1 kU/l (NPV 83%, LR– 0.11).

Correlation between single-plexed assay and multiplexed microarray

In 25 patients, the ImmunoCAP ISAC was performed as well (positive challenge (8), clear history to soy (10), and soy tolerant (7)). sIgE levels to Gly m 4, 5, and 6 measured both by ISAC and ImmunoCAP showed a significant correlation (rho values of 0.78, 0.78, and 0.82, respectively, $P < 0.001$). However, significantly higher sIgE levels were found when

using the ImmunoCAP for all 3 allergens ($P < 0.001$, $P = 0.02$, and $P = 0.02$, respectively). In 9 patients with a negative ImmunoCAP ISAC result for Gly m 4 (<0.3 ISU/l), for example, the ImmunoCAP result was positive (≥0.35 kU/l).

Predicting the severity of soy allergy

Of all 35 soy-allergic patients (19 challenged, 16 convincing reaction), 6 (17%) reported oral symptoms as most severe symptom, 4 (11%) cutaneous, 5 (14%) gastrointestinal, 17 (49%) respiratory, and 3 (9%) cardiovascular. Although a trend toward a negative correlation was seen between severity and sIgE to Gly m 5 and 6, no significant differences were found when correlating severity with any of the diagnostic tests results (Table 5).

When dividing all 35 soy-allergic patients into just two groups based on severity, 15 patients had a mild allergy and 20 patients a severe allergy. The median sIgE values to Gly m 5, Gly m 6, and soy extract were significantly higher in the mild soy-allergic group (0.87 *vs* 0.00 kU/l, $P = 0.02$, 1.74 *vs* 0.01 kU/l, $P = 0.02$, and 1.70 *vs* 0.35 kU/l, $P = 0.04$, respectively, Table 5).

Table 5. All diagnostic tests related to severity by 2 different methods in all soy allergic patients (n=35)

	Correlation	Comparison between mild and severe group*		
	Spearman's rho (P-value)	Mild allergic group (n = 15)	Severe allergic group (n = 20)	P-value
sIgE soy extract (kU/L)	-0.28 (0.10)	1.70 (0.35 – 12.70)	0.35 (0.03 – 0.83)	0.04
sIgE Gly m 2S albumin (kU/L)	-0.26 (0.14)	0.93 (0.28 – 3.44)	0.32 (0.12 – 1.23)	0.11
sIgE Gly m 4 (kU/L)	-0.03 (0.86)	1.96 (0.37 – 14.60)	4.71 (0.19 – 9.77)	0.97
sIgE Gly m 5 (kU/L)	-0.31 (0.07)	0.87 (0.01 – 16.40)	0.00 (0.00 – 0.12)	0.02
sIgE Gly m 6 (kU/L)	-0.33 (0.05)	1.74 (0.02 – 15.40)	0.01 (0.00 – 0.41)	0.02
SPT (mm) (n = 31)	-0.07 (0.70)	5.00 (1.25 – 8.00)	3.00 (2.00 – 7.00)	0.47

* Data are presented as median values (interquartile range)

Soy milk allergy vs allergy to other soy sources

Patients who solely reacted to soy milk ($n = 13$; three challenged, ten convincing history, of which five anaphylactic) had a higher median sIgE to Gly m 4 than the other patients (9.81 *vs* 1.09 kU/l, $P = 0.01$, Figure 1). No differences existed between these groups with regard to the other diagnostic tests. No correlation could be found between severity and culprit soy source ($P = 0.76$).

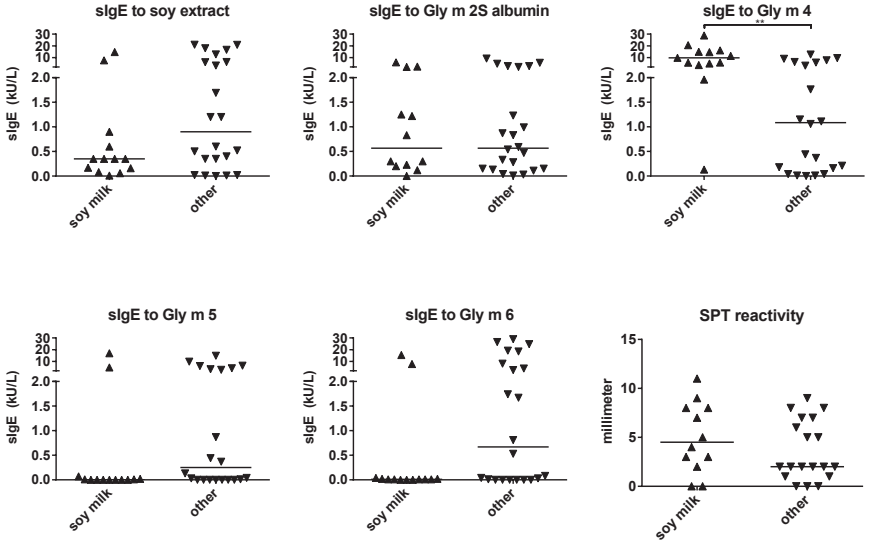

Figure 1. Soy milk (n=13) versus other soy products (n=22). Other: patients with allergic symptoms to other soy products than soy milk as well, ** *P*-value <0.001

DISCUSSION

This is the first study showing the diagnostic value of the SPT and sIgE to soy allergens in a population where all subjects were suspected of soy allergy. Previous diagnostic studies included controls not suspected of a soy allergy[4-7] or only described a soy-allergic population.[2;3;8] We studied a challenged population in which every subject was suspected of soy allergy, irrespective of sensitization. Not all patients with a suspected soy allergy presenting at our outpatient department were challenged. Therefore, we presented likelihood ratios (LRs) in addition to PPV and NPV. In contrast to PPV and NPV, the LR is a measure which is independent of the prevalence of soy allergy in the population and can be used if the exact prevalence of a disease in a certain population is unknown. With the LR+ and LR–, the post-test probability can be calculated given any prevalence (i.e., pretest probability).[14] sIgE to Gly m 2S albumin had the highest AUC (0.79) with the best LR+ and LR–. The diagnostic value of sIgE to Gly m 2S albumin has not been described before. AUC values of the currently used SPT or sIgE to soy extract were just slightly different (0.76 and 0.77, respectively). The main advantage of Gly m 2S albumin, however, is that it can be standardized. So Gly m 2S albumin has the potential to replace currently used SPT or sIgE to soy extract.

We found that many soy allergic patients had a concomitant peanut allergy. Given the high degree of co-sensitization between sIgE to Ara h 2 and sIgE to the soy components

Gly m 2S albumin, Gly m 5, Gly m 6, and soy extract, the presence of peanut allergy could theoretically have influenced the diagnostic value. Unfortunately, it was not possible to assess a separate diagnostic value for subjects with or without a concomitant peanut allergy because of the low number of peanut tolerant subjects in the challenged, soy allergic group (n = 4). We believe, however, that the significant number of subjects having a concomitant peanut allergy reflects daily clinical practice.

In our population, all soy-allergic patients had either sIgE to soy extract (54%) and/or to Gly m 4 (74%), using the usual cutoff value of ≥0.35 kU/l. Others also showed that most soy allergics who had no sIgE to soy extract had detectable sIgE to Gly m 4.[2] This suggests that Gly m 4 levels in the ImmunoCAP for soy are too low to detect all soy allergic patients.[3;15] Three other studies found a sensitivity of sIgE to soy extract or Gly m 4 that ranged from 45% to 77% and 70% to 100%, respectively, compared with 79% and 58% in our study.[2-4] A possible explanation for the poor sensitivity of sIgE to Gly m 4 could be that the group of patients who solely reacted to soy milk, who had significantly higher sIgE levels to Gly m 4, were underrepresented in the population used for the diagnostic part of the study. The reason for this was that these patients were not always challenged as most reactions to soy milk were so clear that a food challenge was not indicated.

We showed a trend toward higher sIgE levels to soy extract, Gly m 5, and Gly m 6 in patients with only mild symptoms. This is in contrast with two other recent studies. These studies, however, measured sIgE and severity in a different way[8] or based their conclusion regarding Gly m 5 and Gly m 6 on the comparison between severe allergic and soy tolerant patients.[6] Another explanation for the differences could be that the two other studies included only children[6] or a combination of adults and children.[2;8] Different sIgE patterns to Gly m 4, 5, and 6 between an adult and pediatric soy-allergic population were recently described.[4;6] In line with our findings, Vissers et al[7] found no sensitization for Gly m 5 and Gly m 6 in a group of 8 adult soy allergic patients with a history of anaphylaxis to soy. Altogether, the findings of the available studies, including our own, indicate that it is premature to predict severity based on the sIgE profile to currently known soy allergens.

Patients who solely reacted to soy milk had much higher sIgE levels to Gly m 4 compared with patients who also reacted to other soy products. A possible explanation could be a different way of processing of soy milk compared with other soy products. Two other studies also described high sIgE titers to Gly m 4 in patients reacting to soy milk (containing) products.[9;10]

In conclusion, the diagnostic value of sIgE to Gly m 2S albumin in diagnosing soy allergy equaled the currently used SPT or sIgE to soy extract and might be a good candidate to replace it. Higher sIgE levels to Gly m 5, Gly m 6, or soy extract were predictive for a

mild soy allergy in adults, while higher sIgE levels to Gly m 4 were found in patients specifically allergic to soy milk.

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Chapter 8

Objective eliciting doses of peanut allergic adults and children can be combined for risk assessment

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Submitted

ABSTRACT

Background: To improve food labeling strategies, information regarding eliciting doses (ED) and the effect of patient characteristics on these EDs is necessary.

Objective: Establish EDs for objective and subjective symptoms and analyze the effect of sensitization levels and other patient characteristics on threshold distribution curves (TDC).

Methods: Threshold data from 100 adults and 262 children with a positive food challenge were analyzed with Interval-Censoring Survival Analysis (ICSA) and fitted to a TDC from which EDs could be extracted. Possible influencing factors were analyzed as covariates by ICSA. A hazard ratio (HR) was calculated in case of a significant effect.

Results: TDCs for both objective and subjective symptoms were significantly different between adults and children ($P<0.001$). Objective ED05 values however were comparable (2.86mg peanut protein in adults and 6.38mg in children, respectively). Higher levels of sIgE to Ara h 2 and peanut extract were associated with a larger proportion of patient groups reacting to a certain ED with objective symptoms (adults and children) or subjective symptoms (adults, in children a trend). Age had a similar effect in children (HR 1.05 for objective symptoms and 1.09 for subjective symptoms). Gender had no effect on TDCs.

Conclusions: Subjective and objective TDCs were different between adults and children, but objective ED05 values were comparable, meaning that threshold data from children and adults can be combined for risk assessment. Higher sIgE levels to Ara h 2 and peanut extract were associated with a larger proportion of both patient groups to react to a certain ED.

INTRODUCTION

Peanut allergy accounts for most fatal food allergic reactions, both in children and in adults, and its prevalence seems to increase.[1-4] Since even trace amounts of peanuts can trigger allergic reactions, a strict elimination diet is generally prescribed.[5;6] Unfortunately, hidden or undeclared allergens in foods are a common cause of accidental reactions.[7]

Legislation in the European Union, the US, Australia and New Zealand requires that peanut has to be declared on all food products of which it is an ingredient.[8-10] However, an allergen may also be present unintentionally, e.g. in case of residues in transport equipment or food production facilities. Uncertainty herewith has led to an increase of voluntary use of so-called precautionary labeling (e.g. 'may contain peanut') on pre-packaged food products. Studies investigating to what extent this precautionary labeling was correctly used to inform consumers, showed that peanut was detected in only 4.5-25% of food products with such labeling.[11-14] Though peanut was also found in up to 10% of chocolate products carrying no such labeling.[14]

For risk assessment in food allergy, and to improve labeling guidelines, information on eliciting doses (ED) for peanut on population level is needed.[15-17] Such ED can be obtained by performing (low dose) food challenges in peanut allergic individuals. So far, several studies have provided ED data on population level.[18-22] In these studies however, a variety of different food challenge protocols were used and most data were based on challenges performed in children. Also, the effect of different patient characteristics on these values is important for risk assessment.[17] The effect of age, gender, sensitization or atopic features on clinical thresholds is not completely known yet.[17] Knowledge of these effects could also help in adjusting challenge dosing schemes.

The aim of our study was to provide data on ED values for both objective and subjective symptoms from a large number of low dose double-blind, placebo-controlled peanut challenges in both adults and children and to analyze whether data from these groups can be combined for risk assessment and elaboration of action levels. Furthermore, we analyzed whether age, gender, sIgE to peanut extract, Ara h 2 and Ara h 8, skin prick test (SPT), asthma, atopic dermatitis, or allergic rhinitis were associated with objective or subjective clinical thresholds. These data help to establish evidence based reference doses to improve labeling guidelines.[23;24]

METHODS

Patients

All patients (n=362; 100 adults and 262 children) with a positive double-blind, placebo controlled peanut challenge (DBPCFC) in the University Medical Center Utrecht between

January 2001 and May 2013 were included in this study. The vast majority underwent a DBPCFC for diagnostic purposes. The decision to undergo a peanut challenge was based on a positive history of allergic symptoms after the ingestion of peanut or sensitization (SPT ≥3mm and/or sIgE to peanut extract ≥0.35kU/L) without known prior ingestion. Twenty-six adult patients and 22 children were earlier described in detail in two threshold studies.[6;25] The study was approved by the local ethics committee.

Double blind, placebo-controlled food challenges

All DBPCFCs were conducted in line with the consensus protocol for threshold studies.[26] Twenty-six adults were challenged using the recipe and dosing scheme as described by Wensing et al.[6] All other DBPCFCs were conducted using the recipe and dosing scheme (9 doses ranging from 0.01mg-3g peanut flour with an additional open dose of 10g whole peanut) as described by Flinterman et al.[25]

All challenge materials were prepared by the hospital pharmacy and all challenges were conducted in the same hospital setting. Light roasted, partially defatted peanut flour (protein content 50%) was used for all challenges. Antihistamines and beta agonists were discontinued before challenge. The challenge was discontinued when objective symptoms were observed or when clear subjective symptoms occurred on at least three subsequent doses or lasted for more than 45 minutes.

Possible influencing factors on threshold distribution curves

Both subjective and objective threshold distribution curves were analyzed. Oral allergy, nausea and abdominal pain were considered subjective. Rhinoconjunctivitis, angioedema, urticaria, emesis, diarrhoea, hoarseness, dyspnoea, stridor and tachycardia were referred to as objective symptoms. Dyspnoea was considered objective only if it led to objective symptoms such as wheezing or a significant drop in FEV1.

The effect of gender, age, SPT, asthma, atopic dermatitis, allergic rhinitis and sIgE to peanut extract, Ara h 2 and Ara h 8 on the TDCs was analyzed. Specific IgE was measured using the ImmunoCAP System (Phadia, Uppsala, Sweden). A skin prick test was performed with peanut extract (ALK-Abelló, Nieuwegein, The Netherlands). Histamine dihydrochloride 10mg/mL and glycerol diluent were used as positive and negative control, respectively. After 15 minutes skin reactions were recorded as the mean of the longest diameter and the perpendicular diameter through the middle. Asthma, atopic dermatitis and allergic rhinitis were based on doctor's diagnosis.

Statistical analyses

The descriptive statistics were presented as percentages or median values with interquartile ranges. Differences between groups were analyzed with the Mann-

Whitney U test for not normally distributed measurement data, and the chi-square test for categorical data.

Individual discrete no observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs) for both subjective and objective symptoms were established for all patients as the highest dose to which patients did not react and the first dose to which patients reacted, respectively. These individual NOAELs and LOAELs were analyzed with Interval-Censoring Survival Analysis (ICSA) and fitted to threshold distribution curves (TDC) from which eliciting doses (EDs) could be extracted.[18;21] Using ICSA, the subjective and objective ED was analyzed as a value between the NOAEL and LOAEL. If the patient reacted to the first dose, left-censoring was applied: the LOAEL being the first dose while the NOAEL was set to zero. If the patient reacted to the highest dose, the NOAEL was considered the last dose and the LOAEL was right-censored.

Consequently, the subjective and objective ED in mg peanut protein were plotted against the probability that a person would react to a certain dose and fitted to subjective and objective TDCs with a Weibull distribution according to Crevel et al[27] We only found minor, non-significant differences when using the Log-Normal and Log-Logistic distribution. The ED05, ED10 and ED50 could be extracted from these TDCs as the discrete EDs in mg peanut protein to which respectively 5, 10 and 50% of the total patient population reacted. The association of gender, age, SPT, asthma, atopic dermatitis, allergic rhinitis and sIgE to peanut extract, Ara h 2 and Ara h 8 with the TDC was assessed by analyzing them as covariates with ICSA and calculating hazard ratios for statistically significant effects (HR). For all analyses, SAS 9.1 (SAS Research Institute) was used and a *P*-value of 0.05 was considered significant.

RESULTS

Patient characteristics

A total of 362 patients were included: 100 adults and 262 children. Table 1 shows the clinical characteristics of all patients. The median age for adults was 24 years (IQR 20-29), for children 7 years (IQR 5-9). The male versus female ratio was greater in children compared with adults ($P < 0.001$). In addition, significantly higher sensitization levels were found in children with regard to the SPT and sIgE to peanut extract and Ara h 2 (Table 1). sIgE to Ara h 8 was significantly higher in the adult population, which could possibly be caused by a higher percentage of birch pollen-related peanut allergy in the adult population. This was also reflected by significantly more subjects with allergic rhinitis in the adult population (85%, vs. 46% in children).

Table 1. Patient characteristics*

	Total (n=362)		Children (n=262)		Adults (n=100)		P-value
Age (year)	8	(5-18)	7	(5-9)	24	(20-29)	<0.001
Male	193	(53%)	157	(60%)	36	(36%)	<0.001
sIgE peanut extract (kU/L)^	14.7	(3.2-64.0)	16.1	(4.0-80.8)	8.5	(1.6-36.6)	0.003
sIgE Ara h 2 (kU/L)^	6.3	(1.0-34.1)	9.7	(2.0-51.1)	2.3	(0.3-9.7)	<0.001
sIgE Ara h 8 (kU/L)^	1.0	(0.05-9.3)	0.4	(0.03-9.0)	2.2	(0.4-11.7)	0.003
Skin prick test (mm)^	10.8	(8.0-15.0)	13.0	9.0-15.5)	8.5	(6.0-14.0)	<0.001
Asthma	210	(58%)	145	(55%)	65	(65%)	0.12
Atopic dermatitis	295	(82%)	224	(86%)	71	(71%)	0.002
Allergic rhinitis	205	(57%)	120	(46%)	85	(85%)	<0.001
Subjective symptoms	362	(100%)	262	(100%)	100	(100%)	NA
Objective symptoms	293	(81%)	242	(92%)	51	(51%)	<0.001

*Data represent median values (interquartile range) or no (%).
^Missing data for sIgE to peanut extract (10 adults), sIgE to Ara h 2 and Ara h 8 (41 children, 3 adults) and SPT (159 children, 11 adults)

There were no significant differences between the groups with or without missing data on SPT or sIgE to Ara h 2/Ara h 8 with regard to gender, age, asthma, atopic dermatitis, allergic rhinitis and sIgE to peanut extract in both children and adults.

Subjective and objective TDCs are different between children and adults

TDCs for both subjective and objective symptoms in the total population (children and adults) are shown in Figure 1. Subjective symptoms occurred at significantly lower doses than objective symptoms since the 95% confidence intervals of the curves were not overlapping. The ED05, ED10 and ED50 values for subjective symptoms in the total population were also significantly lower than for objective symptoms (Table 2).

The TDC for both objective and subjective symptoms was significantly different between adults and children (P<0.001, HR=0.49 for objective symptoms and P<0.001, HR=1.75 for subjective symptoms, respectively, Figure 2). This means that given a certain dose, a lower proportion of the adult population reacts to that dose with objective symptoms compared with the pediatric population. For subjective symptoms, which are difficult to report, especially for young children, the opposite is true. The objective ED05 and ED10 values however, were comparable between adults and children (Table 2). The ED05, ED10 and ED50 for subjective symptoms were significantly lower in the adult population compared with children.

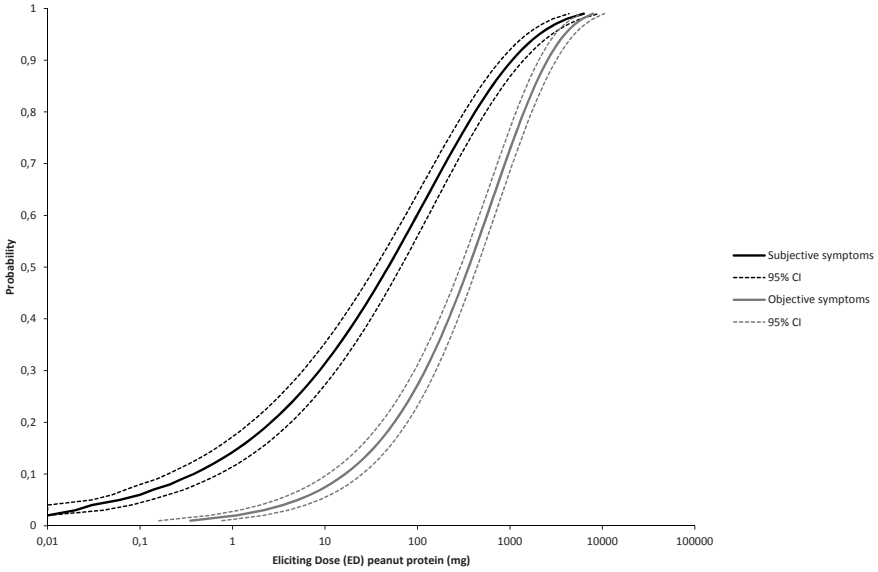

Figure 1. Subjective and objective threshold distribution curves of the total population

Table 2. ED05, ED10 and ED50 values (in mg peanut protein)

	ED05 (mg) (95% CI)	ED10 (mg) (95% CI)	ED50 (mg) (95% CI)
Objective symptoms			
Total population	5.08 (2.99-8.65)	10.8 (16.5-25.3)	291 (358-440)
Children	6.38 (3.68-11.1)	18.6 (11.9-29.0)	303 (243-376)
Adults	2.86 (0.66-12.4)	13.7 (4.37-42.8)	821 (451-1493)
Subjective symptoms			
Total population	0.03 (0.06-0.13)	0.20 (0.38-0.72)	35.1 (48.1-65.9)
Children	0.23 (0.10-0.52)	1.16 (0.60-2.27)	82.6 (59.6-114)
Adults	0 (0-0.02)	0.03 (0.01-0.13)	4.94 (10.2-21.1)

CI, confidence interval

Because both the objective and subjective TDCs were significantly different between adults and children, possible influencing factors were analyzed for adults and children separately.

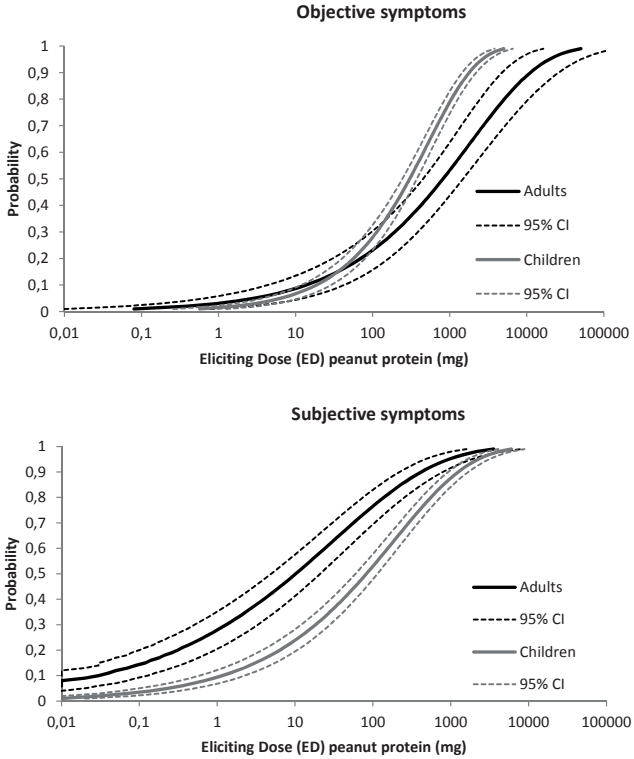

Figure 2. Objective and subjective threshold distribution curves in children vs. adults

Influencing factors on objective TDCs in children and adults

In children, age (P=0.01, HR=1.05), sIgE to peanut extract (P=0.02, HR=1.004) and sIgE to Ara h 2 (P=0.02, HR=1.004) had a statistically significant effect on the objective TDC (Table 3). In the adult population, a statistically significant effect was found for sIgE to peanut extract (P<0.001, HR=1.01), sIgE to Ara h 2 (P<0.001, HR=1.02) and SPT wheal (P=0.02, HR=1.06). These findings mean that subjects that are older (in children), have bigger SPT wheals (in adults) or have higher sIgE values to either peanut extract and/or Ara h 2 have a higher chance to react with objective symptoms to a certain dose.

No significant effect on the objective TDCs in both adults and children was found for gender, sIgE to Ara h 8, asthma, atopic dermatitis or allergic rhinitis (Table 3).

Influencing factors on subjective TDCs in children and adults

Age was the only factor with a significant effect on the subjective TDC in children (P<0.001, HR=1.09, Table 3). Although not statistically significant, there also was a clear trend towards an effect of sIgE to peanut extract (P=0.06, HR=1.003) and sIgE to Ara h 2 (P=0.08, HR=1.003) in the pediatric population. In adults, the effect of these two factors

Table 3. Effects on objective and subjective TDCs in children and adults. A hazard ratio (HR) is provided when P<0.05.

Effect of different factors on the TDC for objective symptoms

	Children		Adults	
	HR	**P-value**	**HR**	**P-value**
Gender		0.68		0.56
Age	1.05	0.01		0.08
sIgE peanut extract	1.004	0.02	1.01	<0.001
sIgE Ara h 2	1.004	0.03	1.02	<0.001
sIgE Ara h 8		0.57		0.18
SPT wheal		0.62	1.06	0.02
Asthma		0.28		0.10
Atopic dermatitis		0.53		0.98
Allergic rhinitis		0.82		0.61

Effect of different factors on the TDC for subjective symptoms

	Children		Adults	
	HR	**P-value**	**HR**	**P-value**
Gender		0.44		0.57
Age	1.09	<0.001		0.28
sIgE peanut extract		0.06	1.008	0.01
sIgE Ara h 2		0.08	1.01	0.005
sIgE Ara h 8		0.67		0.43
SPT wheal		0.99	1.04	0.01
Asthma		0.99	1.85	0.003
Atopic dermatitis		0.13		0.47
Allergic rhinitis		0.25		0.73

HR, hazard ratio; *TDC,* threshold distribution curve

was statistically significant (P=0.01, HR=1.008 for sIgE to peanut extract and P=0.01, HR=1.01 for sIgE to Ara h 2, respectively). In addition, SPT wheal (P=0.01, HR=1.04) and asthma (P=0.003, HR=1.85) had a statistically significant effect on the subjective TDC in the adult population as well.

The effect of all factors on the subjective TDCs in both children and adults can be found in Table 3.

DISCUSSION

In this study we presented data on threshold distributions for both objective and subjective symptoms to peanut in a large pediatric and adult patient group. To our knowledge, this study provides threshold doses for the largest peanut allergic population so far, involving both adults and children from a single centre, making a direct comparison possible. We found that both objective and subjective TDCs were different between adults and children. The ED values in the lower region of the objective TDC however, which are most important in the elaboration of reference doses and thus risk management,[23;24] were similar. We also found significant effects of sIgE to peanut extract and Ara h 2 in both adults and children on the clinical thresholds.

The objective ED05 and ED10 values presented in this study are in the same range compared with these values determined in other studies. Taylor et al.[19] found a cumulative objective ED05 and ED10 value of 7.3 and 14.4 mg whole peanut (corresponding to 1.8 and 3.6mg peanut protein) in 286 patients with a median of 7 years (compared with 5.08 and 10.8mg peanut protein in our population). In comparison, our results were displayed as discrete doses because it is the most conservative choice for risk assessment. The ED05 and ED10 values in other studies, all addressing children data, varied between 1.6-18.9mg (ED05) and 4.4-32.9mg (ED10) peanut protein.[20-22] The main reason for these small differences is probably due to a different study design or different challenge protocol with regard to challenge material, matrix and/or dosing scheme.[18;19]

The difference in objective TDC between adults and children would suggest that children react to lower doses with objective symptoms. When we had a closer look at the distribution of the thresholds we can conclude that this is only true for eliciting doses much higher than 100mg peanut protein. This means that for risk management and the elaboration of action levels, for which the low eliciting doses are most relevant, data from adults and children can be combined. A possible explanation for the difference with regard to the high eliciting doses could be the relatively high number of right-censored adults, which have caused a shift of the curve to the right. Compared with children, the challenges in adults were more often discontinued and called positive based on convincing subjective symptoms only. This can possibly be explained by significantly higher sIgE values to Ara h 8 in adults, reflecting a higher percentage of birch pollen-related peanut allergy in the adult population. Although not significant, there was a trend towards patients with higher sIgE levels to Ara h 8 having less chance to react to a certain objective ED. It is known that these patients usually report mild symptoms, which are most often subjective.[28;29] Another explanation for the different objective TDC could be that because of the lower weight in children, a smaller amount of peanut is necessary to elicit an allergic reaction, which could have led to a shift of the curve to the left. However, this explanation would be contradictory with the fact that higher age,

and therefore a higher weight, in children was associated with a higher proportion of children reacting to a certain ED.

An opposite difference was found with regard to the subjective TDC; adults reacted to lower doses compared with children. This finding was also observed in a large hazelnut allergic group of adults and children (manuscript in preparation). This might indicate that it is more difficult for children to note and/or report subjective symptoms, or for doctors to recognize it, while adults might report these symptoms much sooner. This hypothesis was supported by the fact that in the current study increasing age in children was associated with a higher proportion to report subjective symptoms to a certain dose. A similar association with age in children was found by van der Zee et al.[30]

Specific IgE levels to peanut extract and Ara h 2 were associated with objective and subjective clinical thresholds in both adults and children, although the association with the subjective TDC in children was not significant. Each 1kU/l increase in sIgE level led to a higher hazard to react to a certain dose, meaning that given a certain dose, the chance on an allergic reaction is higher in subjects with higher sIgE levels compared with subjects with lower sIgE levels. This is in line with previous studies performed in peanut allergic children.[30;31] To our knowledge, it has not been shown before in an adult peanut allergic population. The influence of these patient-related factors on threshold distribution curves is important for risk assesment.[17] It does not mean however, that high sIgE levels will ultimately lead to more severe reactions during food challenge. Although it was described that sIgE to peanut extract predicted severe food challenge outcome,[32] other studies showed opposite results.[33-35]

Another important finding in our study was that asthma, allergic rhinitis and atopic dermatitis had no significant effect on the objective and subjective TDCs. The only exception was that adult patients with asthma had a higher chance to experience subjective symptoms at a certain ED. However, more importantly, in both adults and children, having asthma was not associated with a higher chance to react with objective symptoms to a certain eliciting dose. In line with this finding, several other studies concluded that patients with asthma did not have an increased risk of developing a severe reaction during food challenge.[32;34;35] Asthma has been described as a risk factor for fatal food allergic reactions in daily life.[36;37;38] A possible explanation that having asthma is not associated with a low eliciting dose (this study), or a severe reaction during food challenge (other studies),[32;34;35] is that only patients with controlled asthma are being challenged.

Although our study included a large group of peanut allergic patients, the fact that many adult patients did not experience objective symptoms during their challenge could be a possible limitation. In these cases, the ED was right-censored and shifted the TDC to the right. This could possibly have led to the significant difference with the

objective TDC in children. However, there was no significant difference in response percentages between adults and children in the lower dose range of the objective TDCs.

Strength of the current study is that both objective and subjective eliciting doses are addressed, in adults as well as children. Furthermore, the association of different factors on TDCs with the distinction between objective and subjective TDCs has not been studied before in the same population. Another strength of this study is that all patients underwent a low dose DBPCFC. In addition, we used an interval censoring survival analysis which had two advantages. One, all data could be analyzed because of the left and right censoring, even when the NOAEL or LOAEL was not known. Second, the threshold for each patient was most accurately predicted because of the interval censoring, i.e. the threshold was set as a fictive value between the NOAEL and LOAEL.

In conclusion, we found that objective threshold data of both adults and children could be combined for elaboration of reference doses for use in risk management. In both adults and children, higher sIgE levels to Ara h 2 and peanut extract were associated with a larger proportion of the patient groups to react to a certain ED, which is important information for risk assessment.

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Chapter 9

General discussion

The aim of this thesis was to assess the diagnostic accuracy of sIgE to allergen components in diagnosing a peanut allergy. In addition, their diagnostic accuracy in diagnosing a soy allergy was analyzed because of possible serological and clinical cross-reactivity with peanut. Several aspects have to be taken into account when interpreting the results of diagnostic studies. These aspects will be discussed below. In addition, the use of sIgE to components in predicting (the severity of) peanut or soy allergy and recommendations for future research will be discussed:

1. **Factors that can influence the diagnostic accuracy of sIgE to peanut and soy components**
2. **sIgE to peanut components as diagnostic test**
3. **The use of sIgE to peanut components in predicting a severe peanut allergy**
4. **sIgE to soy components as diagnostic test and as predictor for a severe soy allergy**
5. **Recommendations for future research**

1. FACTORS THAT CAN INFLUENCE THE DIAGNOSTIC ACCURACY OF SPECIFIC IGE TO PEANUT AND SOY COMPONENTS

When interpreting the diagnostic accuracy of sIgE to peanut or soy components, it is important to know which factors regarding selection of the study population, the tests under investigation and the way the outcome peanut or soy allergy was measured could have had an influence on our results. The study population in our diagnostic studies consisted of subjects suspected of peanut or soy allergy. This suspicion was based on a suggestive history regardless of sensitization, or on sensitization as measured by SPT and/or sIgE to peanut/soy extract without known prior ingestion. The test under investigation was sIgE to peanut or soy components, which was compared with sIgE and SPT to peanut or soy extract. The outcome peanut or soy allergy was based on a food challenge. On all three levels (study population, tests under investigation and outcome), several possible influencing factors will be discussed below.

1.1 Study population

Both subjects with a suggestive history, as well as sensitized subjects without known prior ingestion, were included in the pediatric diagnostic study in Chapter 4 and in the adult diagnostic studies in Chapter 5 and 6. Seventy-one percent of the children in Chapter 4 were included based on sensitization only. This percentage was much lower in the adult study populations, were 12% and 20% were included based on sensitization only as described in Chapter 5 and 6, respectively. A similar difference in inclusion

criteria between children and adults was found in hazelnut allergy.[1] The explanation for this finding is simple; many children have never ingested the culprit food intentionally, but are often tested for sensitization in the context of other atopic features (other food allergies or atopic dermatitis for example). We chose to include both groups into our diagnostic studies (Chapters 4, 5 and 6) because both groups are similarly tested in clinical practice for a possible allergy. This is also the reason why the same inclusion criteria were used in other diagnostic studies as well.[2-5]

Since the 2 ways of inclusion are slightly different, one might argue whether this could have influenced our results. Therefore, we performed a sub-analysis in both groups of the pediatric population described in Chapter 4 and we found only a minor difference in the AUC value of sIgE to Ara h 2: 0.89 in the group with suggestive history vs 0.91 in the group with only sensitization. A comparable sub-analysis in the adult population in Chapter 6 showed a similar minor difference: 0.76 vs 0.74. In the adult population in Chapter 5, a sub-analysis was not possible because of the low number of subjects who was only sensitized (12%). Other studies that performed a similar sub-analysis also found no significant difference between both groups with regard to diagnostic values.[6;7] In addition, van Erp et al[8] showed that a suggestive history was not associated with a higher chance of a positive food challenge compared to sensitization only. We therefore have no reason to believe that the slightly different inclusion criteria influenced our results.

Another important issue to address is that our diagnostic studies were performed in a tertiary centre. From daily clinical practice we know that of our population suspected of peanut or soy allergy, around 50% appears to be truly allergic during food challenge. This percentage is comparable to other studies with similar inclusion criteria performed in tertiary centres.[3;4;9] In contrast, Nicolaou et al[10] found a prevalence of 22.4% among sensitized subjects in a population-based birth cohort. Positive and negative predictive values (PPV and NPV) are very much dependent on the prevalence of allergy in a certain population and can therefore not easily be translated to other settings. To overcome this matter, positive and negative likelihood ratio's (LR+ and LR-) can be used. LR's are independent of the prevalence of allergy in a certain population and can be applied to calculate the chance of allergy in an individual patient, which is different from the population-specific predictive values.[11] If the pre-test probability is known, the post-test probability can readily be calculated by using a frequently used nomogram. When the LR's of sIgE to Ara h 2 with a cutoff value of ≥0.35kU/l were calculated from the sensitivity and specificity as described in the pediatric population in Chapter 4, we found that a pre-test probability of 50% increased to a post-test probability of 76% for example by applying the LR+ of 3.23 (Figure 1). The LR- of 0.12 decreased the probability from 50% to 10%. However, since the pre-test probability in primary or secondary care is

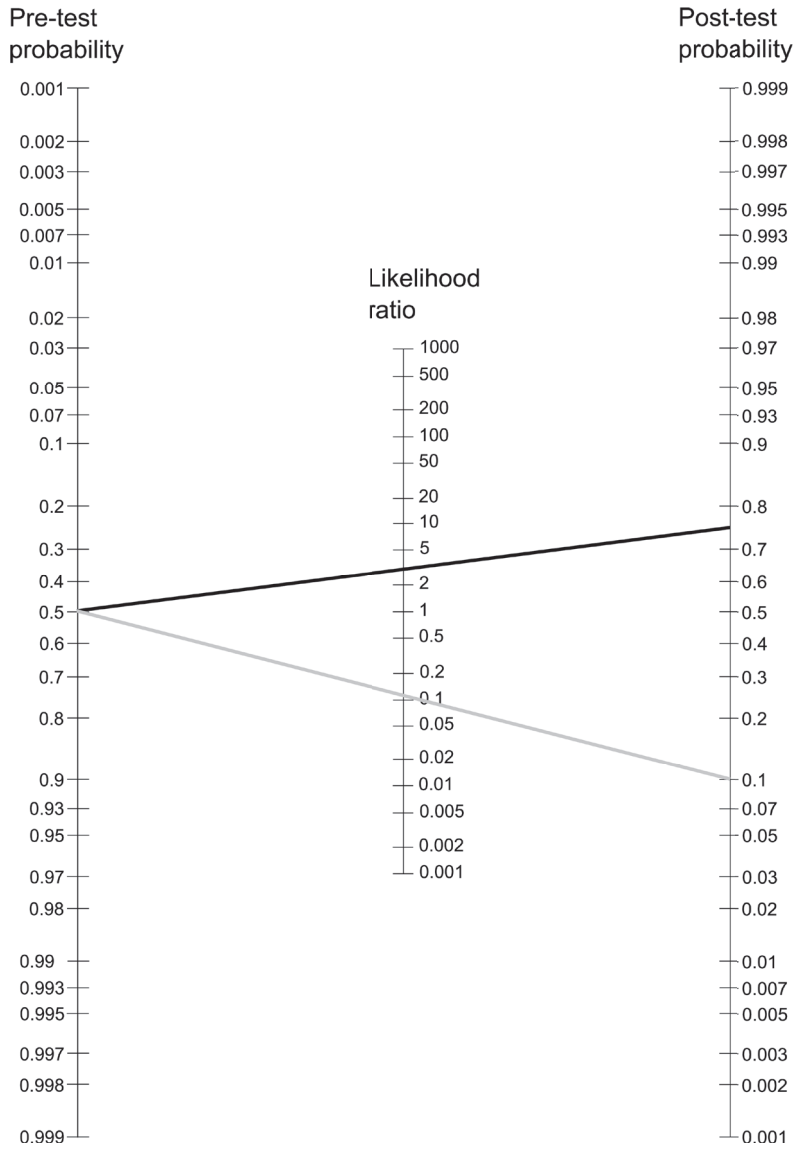

Figure 1. Nomogram for Bayes' Theorem.[12] The lines represent the increase or decrease from pre- to post-test probability by using the LR+ (black line) or LR- (grey line) as derived from sensitivity and specificity values in the pediatric population in Chapter 4.

not known, this does not mean that these LR's can directly be applied in these settings. Validation studies should be performed first.

1.2 Tests under investigation

Specific IgE patterns to peanut components can be different between geographical areas. Vereda et al[13] found that sIgE to Ara h 2 was most common in peanut allergic individuals in the United States and Sweden, whereas sIgE to Ara h 9 was most often found in Spanish proven peanut allergic patients. A logical explanation would be a different distribution of pollen exposure in these areas, resulting in different sensitization patterns, or a difference in dietary habits. The importance of sIgE to Ara h 9 in Spain was also shown by two other studies.[14;15] One of these studies concluded that in many of these patients Pru p 3 in peach, another lipid transfer protein (LTP), was the primary sensitizer.[15] Sensitization to one or more LTP proteins is common in Southern Europe.[16] Cross-reactivity between Pru p 3 and both mugwort LTP (Art v 3) and plane tree LTP (Pla a 3) has been described, but it is still matter of debate whether these pollen components can be primary sensitizers, like Bet v 1 in birch pollen.[17-21]

In this thesis, sIgE to Ara h 9 could only be measured and analyzed in the adult peanut allergic population because the test was not available at the time of conducting our study in the pediatric population. In both the allergic and tolerant group, there was hardly any sensitization to Ara h 9. The fact that sIgE to Ara h 9 in Spain was not only found in 82.4% of the peanut allergic patients in the study of Javaloyes et al,[15] but also in 75% of sensitized subjects without allergy, suggests a poor discriminative value. A recent study conducted in Spain showed that sIgE to Ara h 2, as measured by the basophil activation test (BAT), had a far better discriminative ability than sIgE to Ara h 9.[22] Remarkably, sensitivity of sIgE to Ara h 2 decreased dramatically in this study when it was measured by ImmunoCAP instead of BAT (73% vs. 17%), while specificity increased from 80% to 100%. For sIgE to Ara h 9 these two values were comparable between both techniques. In addition, controls consumed peanut regularly and were therefore not suspected of peanut allergy. A well conducted diagnostic study should include subjects who are all suspected of peanut allergy since this reflects clinical practice. Another study conducted in Spanish children also found a good diagnostic value of sIgE to Ara h 2 (AUC 0.86) but did not address sIgE to Ara h 9 and also included controls who were not suspected of peanut allergy.[23] Hence, the diagnostic value of sIgE to Ara h 2 should be further analyzed in diagnostic studies in Spain and other Southern European countries including subjects who are all suspected of peanut allergy by using different techniques. With regard to sIgE to Ara h 9, not only many peanut allergic patients in Spain recognized sIgE to Ara h 9, but also many tolerant subjects, and it is therefore a poor predictor for peanut allergy.

In Chapter 4 and 5 we found that many peanut allergic patients were sensitized to Ara h 8 (36.2% of children, 62% of adults). As Ara h 8 is homologue with Bet v 1 in birch, which is endemic in Northwest Europe, many peanut allergic patients will be sensitized via serological cross-reactivity between these two PR-10 proteins.[24] As a consequence, many tolerant subjects often recognized sIgE to Ara h 8 as well, which made it a poor predictor for peanut allergy. This finding was supported by other studies.[25-27] Despite the fact that sensitization pattern depends on geographical area, all studies so far addressing the diagnostic accuracy of sIgE to peanut components in populations suspected of peanut allergy, showed sIgE to Ara h 2 having the best diagnostic accuracy.[4;10;25-31] These studies were performed in Northwest Europe, Asia (Thailand and Japan), the United States, and Australia. In summary, we can conclude that the geographical area clearly influences the overall sensitization pattern, but does not have on impact on the diagnostic accuracy of sIgE to Ara h 2 in these areas.

With regard to soy components, no clear differences in sensitization patterns were found between studies from different geographical areas addressing the diagnostic value of soy components.[32-36] Gly m 4 is a PR-10 protein, like Ara h 8 in peanut and Bet v1 in birch pollen. Specific IgE to Gly m 4 is therefore often recognized by soy allergic patients by serological and/or clinical cross-reactivity with Bet v 1.[36;37] Since the same is true for soy tolerant subjects, as described in Chapter 7, sIgE to Gly m 4 was a poor predictor for soy allergy. In general, allergens that are highly cross-reactive with their primary sensitizers, such as Ara h 8 with Bet v 1 in birch pollen and Ara h 9 with Pru p 3 in peach, are poor predictors for true allergy because many tolerant subjects are sensitized to these cross-reactive allergens as well.

Another factor that influenced the diagnostic test results of our studies, was the technique by which sIgE to components was measured. The results of the ImmunoCAP (a single-plexed assay) were displayed as kilo units per liter (kU/l), while the results of the ImmunoCAP ISAC (a multiplexed microarray) were displayed as arbitrary 'International Standardized Units per liter' (ISU/L), which makes it a semi-quantitative method.[38] Another important difference is that the amount of allergens on the ImmunoCAP ISAC is approximately a 100.000 fold lower than the amount of allergens on the ImmunoCAP. It has been described that the ISAC chip is less sensitive than ImmunoCAP at low sIgE levels.[38;39] As a result of less allergen on the ISAC chip for IgE to bind to, an explanation for the lower sensitivity could be that there is a heavier competition between allergen specific IgG and IgE to bind to the low amount of immobilized allergens on the ISAC chip.[38] In Chapter 3 we compared the ImmunoCAP with the ImmunoCAP ISAC 103 in peanut allergic patients. Despite the differences between the two techniques described above, we only found one significant difference between both tests, concerning the sensitivity values of sIgE to Ara h 1. However, this might be explained by a technical issue, since two different preparations of Ara h 1 were used in these tests: a recombinant one in

the ImmunoCAP and a purified one in the ImmunoCAP ISAC. In the new version of the ISAC, the ImmunoCAP ISAC 112, all peanut components but Ara h 6 are recombinant. In all chapters, except for Chapter 6, the ImmunoCAP was used to assess the diagnostic accuracy of the peanut components. The ImmunoCAP ISAC 112 was only used to assess the diagnostic accuracy in Chapter 6 because the test under investigation, sIgE to Ara h 6, was not commercially available yet on the ImmunoCAP. However, a comparative analysis in this chapter between ImmunoCAP and ImmunoCAP ISAC 112 with regard to Ara h 2 in 81 subjects showed a high correlation coefficient (spearman's rho 0.90, P<0.001) and comparable median values: 4.3 kU/l (IQR 1-17.3) vs. 5.6 ISU/l (IQR 1.4-12.9), P=0.89. Therefore, we think the influence of the technique we used to measure sIgE was negligible in the peanut allergy studies. In addition, Keet et al[29] found similar diagnostic values (sensitivity, specificity, LR+ and LR-) for sIgE to Ara h 2 as measured by ImmunoCAP and ImmunoCAP ISAC.

With regard to soy components however, significantly higher sIgE values were found for Gly m 4, Gly m 5 and Gly m 6 when using the ImmunoCAP method. In 9 out of 25 subjects for example, sIgE to Gly m 4 was undetectable with ImmunoCAP ISAC (<0.3 ISU/L), while positive with ImmunoCAP (>0.35kU/L). A possible explanation could be that all these 9 patients had low sIgE levels (<6 kU/l) as measured by ImmunoCAP, of whom 3 had levels <1 kU/l. It seemed unlikely that the difference could be caused by the quality of the allergens since there were no differences between both methods with regard to recombinant or natural allergens. Other *in vitro* methods to measure sIgE reactivity, such as immunoblot, SPT, or basophil activation test (BAT) are far more difficult to standardize, and are therefore as yet not preferred over measuring of sIgE.

1.3 Outcome

The reference standard in diagnosing food allergy is a double-blind, placebo-controlled food challenge.[40-43] Although a DBPCFC is preferred, an open food challenge (OFC) is sometimes performed instead. The latter is usually performed in young children, because of difficulties with eating the bigger portions and more doses during DBPCFC. In Chapter 4, 19 children who underwent an OFC were significantly younger than the 81 children who underwent a DBPCFC. Different protocols on how food challenges should be conducted have been published.[40-42;44] Although these protocols resulted in improved standardization of performing food challenges, there still are considerable differences between different centres and countries with regard to dosing scheme, dosing interval, handling of placebo reactions and criteria for a positive food challenge. Although all these items can have an influence on the outcome of the food challenge, two items are important when interpreting the food challenge result. First, dealing with placebo reactions. Two major studies showed that 2.8%-12% of placebo challenges were positive (of which 43%-53% immediate reactions), if placebo doses were given on a different day

than the verum doses.[45;46] Giving placebo and verum doses on different days was advised by a recent consensus report on conducting DBPCFCs.[42] Others suggested however, that a 1 day DBPCFC with interspersed placebo doses is as effective as a 2-day DBPCFC.[47] For practical reasons, the placebo doses during the food challenges in this thesis were randomly interspersed on both days; it could lead to a reduction of the DBPCFC of 1 day. Also, in this way challenged subjects were not influenced by the knowledge that one day would consist of only placebo doses. If it became clear after unblinding the challenge that there was a placebo reaction, the severity of this reaction was compared with the severity of the symptoms following the verum doses. The physician who evaluated the challenge also took the possibility into account that the placebo reaction was a late reaction to a verum dose. A definite diagnosis in case of placebo reactions was made based on the judgment of the physician. Unfortunately, placebo reactions in our studies were not systematically reported because of the retrospective study design. A second and most important issue that can influence the interpretation of the outcome of the challenge is that many of the protocols do not clearly state the criteria for a positive food challenge.[48] The most recent consensus report states that a food challenge should be stopped and called positive in case of objective symptoms.[42] This report also suggests however, that subjective symptoms would be sufficient in some cases. In this thesis, a food challenge was stopped in case of objective symptoms. The challenge was called positive when objective symptoms occurred, in case of suggestive, subjective symptoms on at least 3 subsequent doses, or if a severe suggestive, subjective symptom lasted >45 minutes. In practice all subjects with a severe suggestive, subjective symptom lasting >45 minutes also fulfilled the criterium of subjective symptoms at 3 subsequent doses as well. Food challenges in some other studies were handled similarly.[3;5] However, in most other studies only objective symptoms were sufficient to judge a food challenge positive.[4;9;27;28;30] The advantage of objective symptoms is of course that there is less doubt about a positive food challenge compared to a food challenge that was called positive based on subjective symptoms only. A recent study in our centre by van Erp et al[49] showed that there was more inter and intra-observer variability in food challenges with subjective symptoms or even mild objective symptoms compared to food challenges with objective symptoms. The disadvantage however, is that food allergy might be underdiagnosed, since food challenges with only subjective symptoms would be considered negative. Remarkably, hardly any differences regarding the prevalence of peanut allergy were encountered in Chapter 2 among studies with different criteria for a positive food challenge. A possible explanation would be that in all different centres the criteria to stop a food challenge were objective symptoms and therefore most challenges were also called positive based on objective symptoms. Despite this fact, consensus on clear criteria for a positive food challenge would further improve food allergy diagnostics.

In summary, several factors have to be taken into account when interpreting the results of this thesis and when implementing the results in clinical practice. These factors were related to study population (inclusion criteria, setting), tests under investigation (geographical area, different techniques) and the way the outcome peanut or soy allergy was measured (DBPCFC vs open challenge, handling of placebo reactions and criteria for a positive challenge).

2. SPECIFIC IGE TO PEANUT COMPONENTS AS DIAGNOSTIC TEST

In chapter 4 and 5 the diagnostic accuracy of peanut components was assessed in a pediatric and adult population. In both chapters we found that sIgE to Ara h 2 had the best diagnostic accuracy of all peanut components, and clearly better than the currently used SPT and sIgE to peanut extract. This finding was in concordance with other diagnostic studies listed in Chapter 2. It was remarkable that the good predictive value was regardless of geographical area. A possible explanation would be that Ara h 2 is not cross-reactive with pollen allergens. The good diagnostic accuracy of sIgE to Ara h 2 was not surprising since it was already identified as the most important peanut allergen in peanut allergic adults by Koppelman et al[50] in 2004. This finding was reproduced in 2007 in our centre in both peanut allergic adults and children and could be explained by the fact that Ara h 2 is heat and digestion stable.[51-55] This thesis adds to these findings by not only confirming the importance of sIgE to Ara h 2 in peanut allergic patients, but also its ability in discriminating between allergy and tolerance in subjects suspected of peanut allergy. It is important that in a diagnostic study all subjects are suspected of the disease that is studied, because the study population should be similar to the population in which the diagnostic test is used. In this way, the diagnostic accuracy of the test obtained reflects clinical practice. Although the diagnostic accuracy of sIgE to Ara h 2 in Chapter 4 was comparable to a validated and updated prediction model, sIgE to Ara h 2 has the advantage over the model of being a simple, cheap and less-invasive test. In addition, in Chapter 6 we found that the diagnostic value of sIgE to Ara h 2 and Ara h 6 on population level was comparable in the adult population. This also was not surprising since a recent study showed that Ara h 2 and Ara h 6 independently can account for most of the allergenic activity in a crude peanut extract.[56] Some individual patients in our study however, were sensitized to Ara h 6 while negative for sIgE to Ara h 2. A similar case was described by Asarnoj et al.[57] Therefore, we would suggest to measure sIgE to Ara h 6 in patients with a highly suggestive history but negative sIgE levels to Ara h 2. At the moment however, sIgE to Ara h 6 can only be measured by the

more expensive ImmunoCAP ISAC. We will discuss the clinical applicability of sIgE to Ara h 2 in a pediatric and adult population below.

2.1 Specific IgE to Ara h 2 in a pediatric population

By using sIgE to Ara h 2, cutoff values with a 100% PPV and NPV could be calculated. Twenty-six patients (26% of the study population) had a value above the cutoff of 5.17kU/L and could therefore all be diagnosed as peanut allergic (Figure 2). In addition, 24 patients (24% of the study population) had a value lower than 0.07 kU/L, and were therefore all peanut tolerant. This would mean that only the subjects with a value between these two cutoff values (50%) should undergo a food challenge for diagnosis. The question is however, whether 100% certainty is necessary in daily practice, or whether it is acceptable to have a limited number of false-positive and false-negative results. On one hand, this would further reduce the need for the costly and invasive food challenges. On the other hand, this would lead to unnecessary elimination diets in case of false-positive results or an increased risk of an allergic reaction in case of false-negative results by not following an elimination diet. Although an unnecessary elimination is a burden for the patient and his or her relatives,[58] especially when more allergens have to be avoided, it does not put the person at risk of an allergic reaction. By preliminary results of current research, we know that peanut allergic patients react on average 1.2 times a year despite their diet. If patients are followed-up regularly, the diagnosis of peanut allergy could therefore be reconsidered if no allergic reaction occurred in the follow-up period. False-positive outcomes seem therefore acceptable to a certain extent, especially given the many disadvantages of a food challenge; high costs, only possible in

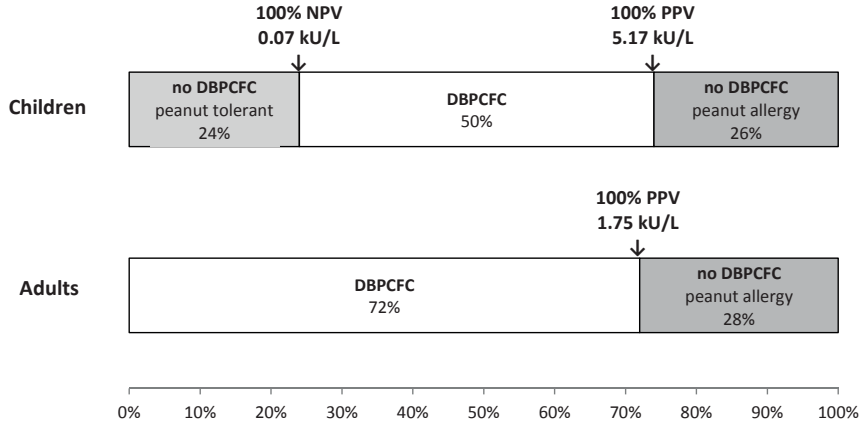

Figure 2. Reduction of DBPCFCs by applying 100% NPV and PPV in the pediatric and adult population as described in Chapters 4 and 5, respectively.

specialized centres and a burden for the patient.[59-61] Accepting false-negative results on the other hand, might put a person at an increased risk of an allergic reaction. To find out what the possible consequences would be, it is important to get more insight in which patients would receive a wrong diagnosis when making use of a higher cutoff value than >0.07kU/L. When the clinically often used cutoff value of 0.35kU/L was applied for example, 38 of 42 subjects with a sIgE level to Ara h 2 lower than this value were tolerant (NPV 90%). One of these 4 subjects who was misdiagnosed (with sIgE to Ara h 2 0.07 kU/L) experienced respiratory symptoms during food challenge, the other 3 subjects experienced only mild symptoms. Although only one subject experienced a severe allergic reaction, the reaction was possibly life-threatening and therefore too severe to accept a false-negative test result.

2.2 Specific IgE to Ara h 2 in an adult population

In the adult population a 100% PPV could be calculated as well, by using a cutoff value ≥1.75kU/l (Figure 2). Although lower compared to the pediatric population, the percentage of patients with a value higher than this cutoff value was comparable (28% of the adult population vs. 26% in the pediatric population). Peanut allergic adults in Chapter 5 had significantly lower sIgE levels to Ara h 2 than the children in Chapter 4 (median 1.01 kU/l vs. 6.48 kU/l, P<0.001, Figure 3). This was probably caused by a selection in the adult population. In general, many adult peanut allergic patients are probably already diagnosed in their childhood and hence do not visit our outpatient department again for diagnosis in adulthood. Although we did not survey the age of onset of peanut allergy in the adult population in Chapter 5, we expect that many of these subjects had an onset later in life. In addition, many adults in Chapter 5 were

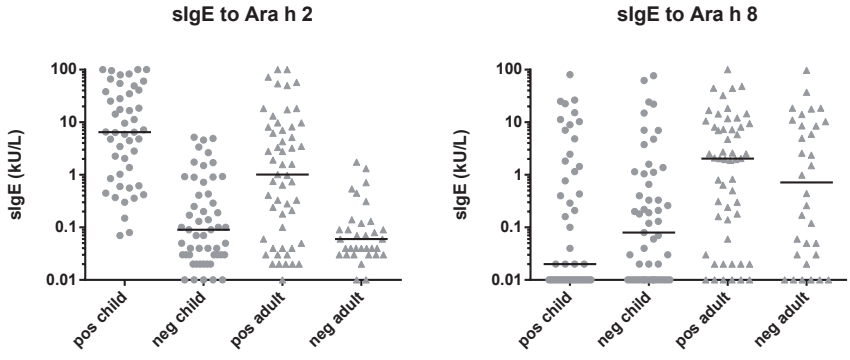

Figure 3. Significantly higher sIgE levels to Ara h 2 and significantly lower sIgE levels to Ara h 8 in children compared to adults.

sensitized to Ara h 8, probably via cross-reactivity with Bet v 1 in birch pollen. This was reflected by higher sIgE levels to Ara h 8 in the adult population compared to the pediatric population (median 0.72 vs 0.08 kU/l, P=0.04 in the allergic population and 2.03 vs 0.02 kU/l, P<0.001 in the tolerant population, Figure 3). As a result, a 100% NPV for sIgE to Ara h 2 could not be calculated. Therefore, still many food challenges have to be conducted for a reliable diagnosis (72%, Figure 2). The need for food challenges could be lowered when accepting false-positive results to a certain extent as in the pediatric population. Combining sIgE to Ara h 2 with other diagnostic tests did not result in a better NPV. Possibly the NPV could be improved by including the basophil activation test (BAT). A study of Glaumann et al showed a good negative predictive value by using the BAT with peanut in a group of children suspected of peanut allergy.[25] Although this study was performed in children, it would be worthwhile to analyze in an adult population. Unfortunately, to our knowledge no other studies were performed in adults addressing the diagnostic value of peanut components. It would be interesting to analyze the diagnostic accuracy of sIgE to Ara h 2 in a non-birch endemic area to study the role of sensitization to Bet v 1 and Ara h 8 in the differences between adults and children.

In summary, sIgE to Ara h 2 has the best diagnostic accuracy in discriminating between peanut allergy and peanut tolerance in both adults and children and reduces the need for food challenges. Given the fact that this finding has been reproduced by other studies, it should be implemented in clinical practice, at least in tertiary centres. The cutoff values with a 100% PPV and NPV are population dependent and should therefore be applied with caution. Validation in primary or secondary care is necessary before sIgE to Ara h 2 can be implemented in these settings.

3. THE USE OF SPECIFIC IGE TO PEANUT COMPONENTS IN PREDICTING A SEVERE PEANUT ALLERGY

3.1 Difficulties in scoring the severity of peanut allergy

Next to diagnosing peanut allergy, sIgE to peanut components should ideally discriminate between mild and severe allergy. In Chapter 3 we found that sIgE to Ara h 1, Ara h 2, and Ara h 3 was significantly correlated with severity using a severity score in the adult population, but not in the pediatric population. This severity score was a combination of eliciting dose during food challenge and an adapted Mueller score based on symptoms during food challenge. The patient numbers in that study however, were relatively small. In Chapter 4 we found no significant correlation between sIgE to Ara h 2 and severity, as based on the Sampson criteria,[62] in a pediatric study population. A similar analysis was

performed in Chapter 5 in an adult population. sIgE to Ara h 2 was significantly correlated with severity, as scored by three different severity classifications: an adapted Mueller score based on history, the same score based on symptoms during food challenge, and the severity score based on the combination of the eliciting dose and adapted Mueller score during challenge. Significant, but weak, correlations were also found for sIgE to Ara h 1, Ara h 3 and peanut extract (positive correlation) and sIgE to Ara h 8 and Ara h 9 (negative correlation), but only with 1 or 2 of the 3 severity classifications. This negative correlation meant that higher sIgE levels to either Ara h 8 or Ara h 9 were correlated with milder symptoms. Since both Ara h 8 and Ara h 9 are highly cross-reactive, this finding was not surprising and with regard to Ara h 8 described before by Asarnoj et al.[63;64]

The severity analyses in the adult population in Chapter 5 raised two important questions: 1) Which severity classification should be used?, and 2) If based on allergic symptoms, should this classification be based on symptoms by history or during food challenge? Most severity classifications in literature are based on allergic symptoms.[62;65-68] However, there is no clear consensus in literature on which classification system should be applied. In our hospital the adapted Mueller score is used. It is easy to use and symptoms can be clearly attributed to a certain class, since all symptoms can be classified into one of 5 categories: 0; oral allergy symptoms, 1; cutaneous/mucosal symptoms, 2; gastrointestinal symptoms, 3; respiratory symptoms, 4; cardiovascular symptoms. In children, the more extensive Sampson classification is used as well in our hospital. In general, the Mueller and Sampson classification methods are based on symptoms during food challenge. An advantage is that symptoms during food challenge can be objectified by qualified personnel. On the other hand, a disadvantage is that a food challenge is usually discontinued before the most severe symptoms have evolved. The alternative however, a classification method based on symptoms in history, has the disadvantage that symptoms in history might be exaggerated by patients. We notice in routine clinical practice for example that if patients report dyspnea, this cannot always be objectified by spirometry or auscultation of the lungs during food challenge. With both methods the severity of allergy can still be unclear because a significant amount of patients has never intentionally ingested peanut before (symptoms based on history), or they are not challenged (symptoms based on food challenge). The eliciting dose during food challenge is another factor that can be combined into a severity score, as we used in Chapter 3. Although this gives a more complete image of the individual patient, it is more difficult to interpret since the continuous eliciting dose cannot easily be categorized into mild or severe allergy.

In summary, all severity classifications have their pro's and con's. The analyses in the Chapters of this thesis regarding the correlation between sIgE to components and severity were all performed in a peanut allergic population. For clinical practice however, one ideally wants to discriminate between severe allergy, and mild or no allergy, in a

population suspected of peanut allergy. Therefore, we performed some additional analyses for both the pediatric population in Chapter 4, as well as the adult population in Chapter 5. Because all these subjects were challenged, we used the adapted Mueller score based on food challenge.

3.2 Discriminating between severe and no/mild allergy in children vs. adults

For the additional severity analyses, the same study populations were used as described in Chapter 4 (children, N=100) and Chapter 5 (adults, N=94). The diagnostic group was used in the adult population, to prevent an artificial distribution between subjects with no/mild allergy and severe allergy. In all subjects the adapted Mueller score was applied with one additional category for no symptoms, because tolerant subjects were included as well. This resulted in 6 categories (0-5): 0; no symptoms/peanut tolerant, 1; oral allergy symptoms, 2; cutaneous/mucosal symptoms, 3; gastrointestinal symptoms, 4; respiratory symptoms, 5; cardiovascular symptoms. Subjects were categorized as no/mild allergy (Mueller score 0-3) or severe allergy (Mueller score 4-5). Logistic regression analysis was used to univariately analyze the association between SPT and sIgE to peanut components/peanut extract, and severity. The association was displayed as odds ratio's (OR) with 95% confidence intervals (CI). In addition, a multivariable logistic regression (stepwise backward with the probability of entering a variable .05 and removing a variable .10) was performed entering candidate variables based on literature and univariate analyses to adjust the OR for the other variables. Calibration of the final model was analyzed by the Hosmer-Lemeshow test, where a p-value >0.05 indicates good calibration. The discriminative ability of the model was assessed by an area under the receiver-operating characteristics curve (AUC).

The distribution of the severity categories in both adults and children can be found in Table 1. The number of severe peanut allergic patients (Mueller score 4 or 5) was comparable between children and adults (12% vs 16%, P=0.43). However, children had significantly higher Mueller scores than adults (P=0.003) and were therefore analyzed separately.

Table 1. Distribution of severity categories in children and adults

Mueller score	Children (N=100)	Adults (N=94)	Total
0	53	39	92
1	1	14	15
2	7	10	17
3	27	16	43
4	11	15	26
5	1	0	1

In the pediatric population, none of the predictors (sIgE to peanut components, sIgE to peanut extract and SPT) was significantly associated with a severe peanut allergy, since the 95% CI of the OR of all predictors included the value 1 (Table 2). In the adult population, sIgE to Ara h 2 (OR 1.03, 95% CI 1.01-1.06) and sIgE to peanut extract (OR 1.02, 95% CI 1.003-1.05) were significantly associated with a severe peanut allergy. A multivariable logistic regression analysis was not possible due to the low number of severely allergic patients. To perform such an analysis 10 subjects with the smallest outcome are necessary per candidate predictor to avoid overfitting of the model.[69] Since the severe pediatric and adult peanut allergic patients consisted of only 12 and 15 patients, respectively, this meant that only one predictor could be included in the model. The AUC values of sIgE to Ara h 2 and sIgE to peanut extract in the adult population were 0.81 (95% CI 0.69-0.93) and 0.70 (95% CI 0.54-0.86), respectively (compared to 0.77 (0.64-0.89) and 0.69 (0.53-0.85) in the pediatric population).

The same analyses were performed when a severe allergy was defined as a Mueller score 2-5. In this way we were able to analyze whether subjects with no allergy or only oral allergy symptoms could be discriminated from allergic subjects with more than oral allergy symptoms. This analysis was not possible in the pediatric population, since only 1 patient experienced only oral allergy symptoms. The discriminative ability would therefore be comparable to the one already shown in Chapter 4 to discriminate between allergy and tolerance. In the adult population, sIgE to Ara h 1 (OR 2.21, 95% CI 1.05-4.61), sIgE to Ara h 2 (OR 4.90, 95& CI 1.77-13.56), sIgE to peanut extract (OR 1.17,

Table 2. Univariate association of possible predictors and severity

	Children			**Adults**		
	No/mild allergy	**Severe allergy**	**OR (95% CI)**	**No/mild allergy**	**Severe allergy**	**OR (95% CI)**
sIgE to Ara h 1	0.05 (0.01-0.63)	0.68 (0.03-25)	1.01 (0.99-1.04)	0.02 (0.00-0.14)	3.5 (0.02-15)	1.02 (0.995-1.05)
sIgE to Ara h 2	0.42 (0.04-4.55)	6.8 (2.73-38)	1.02 (0.996-1.04)	0.10 (0.03-0.92)	7.1 (1.5-22)	1.03 (1.01-1.06
sIgE to Ara h 3	0.05 (0.01-0.23)	0.33 (0.05-3.9)	1.00 (0.95-1.05)	0.01 (0.00-0.07)	0.09 (0.02-2.2)	1.06 (0.99-1.13)
sIgE to Ara h 8	0.04 (0.00-0.97)	0.79 (0.01-4.2)	0.99 (0.94-1.05)	1.9 (0.03-8.5)	2.04 (0.12-19)	1.01 (0.98-1.04)
sIgE to Ara h 9	-	-	-	0.02 (0.00-0.07)	0.04 (0.01-0.13)	0.60 (0.12-3.0)
sIgE to peanut extract	1.61 (0.35-13)	12 (1.6-67)	1.01 (0.997-1.03)	1.3 (0.35-7.8)	11 (2.8-50)	1.02 (1.003-1.05)
SPT	10 (3-15)	13 (9.3-15)	1.09 (0.98-1.21)	6 (0-14)	15 (6-21)	1.00 (0.998-1.00)

Data are presented as medians with interquartile ranges. *CI*, confidence interval. *OR,* odds ratio

95% CI 1.07-1.28) and SPT (OR 1.00, 95% CI 1.00-1.00) were significantly associated with a severe peanut allergy as defined as a Mueller score 2-5. When all these 4 predictors were entered in a multivariable logistic regression analysis (53 negative and 41 positive outcomes), only sIgE to Ara h 2 was included in the final model, with a good calibration (P-value Hosmer-Lemeshow test 0.31). The AUC of sIgE to Ara h 2 was 0.90 (95% CI 0.82-0.97). However, there were still 9 severely allergic patients with a low sIgE value to Ara h 2 (<0.35 kU/L). These values would result in an underestimation of the severity of peanut allergy and therefore expose these patients to an increased risk of a severe allergic reaction.

In summary, in the pediatric population none of the diagnostic tests were significantly associated with a severe peanut allergy as defined by a Mueller 4-5 score. In the adult population, the highest association was found for sIgE to Ara h 2, with an AUC of 0.81. This AUC value increased to 0.90 when a severe peanut allergy was defined as a Mueller score 2-5. Despite this high AUC value, individual patients with low sIgE values could still have a severe peanut allergy. Therefore, sIgE values to Ara h 2 are not reliable enough to draw firm conclusions regarding the severity of peanut allergy in individual subjects suspected of peanut allergy.

4. SPECIFIC IGE TO SOY COMPONENTS AS DIAGNOSTIC TEST AND AS PREDICTOR FOR A SEVERE SOY ALLERGY

4.1 Diagnostics

In Chapter 7 we found that sIgE to Gly m 2S albumin had the best diagnostic accuracy of all soy components in diagnosing soy allergy (AUC 0.79). The diagnostic accuracy in our study however, was comparable to the currently used SPT and sIgE to soy extract. Yet, the main advantage of sIgE to Gly m 2S albumin is that it can be better standardized. Therefore, it might replace the currently used tests in the near future. Another advantage was that the diagnostic accuracy as measured by AUC was hardly influenced by adding soy allergic patients who solely reacted to soy milk to the study population. The initial study population consisted only of subjects suspected of soy allergy who underwent a food challenge. Some patients who solely reacted to soy milk were included based on history and therefore not used for the initial analysis to calculate the diagnostic accuracy of soy components. Unlike the AUC value of sIgE to Gly m 2S albumin, the AUC value of sIgE to Gly m 4 increased from 0.54 to 0.69 when these patients were added. Since the importance of sIgE to Gly m 2S albumin was highlighted by our study, it was renamed to Gly m 8 by the IUIS allergen nomenclature sub-committee.[70] The diagnostic accuracy of Gly m 2S albumin in diagnosing soy allergy was lower than that of Ara h 2, the 2S

albumin in peanut in diagnosing peanut allergy, as described in the studies in Chapter 2 and in this thesis. However, the AUC value of sIgE to Gly m 2S albumin was similar to the AUC value of sIgE to Ara h 2 in the adult population in Chapter 5 (0.79 vs 0.76). A possible explanation would therefore be that we assessed the diagnostic accuracy of Gly m 2S albumin in an adult population, while all peanut allergy studies, except our own study in Chapter 5, were performed in pediatric studies. Nevertheless, a similar diagnostic value of sIgE to Gly m 2S albumin was found by Ebisawa et al[34] in Japanese children (AUC 0.75). Thus, it seems unlikely that age could explain the difference with the high diagnostic accuracy of the 2S albumin in peanut, Ara h 2. In addition, because the study of Ebisawa et al was performed in Japan, where soy is regularly consumed, geographical area and dietary habits could not explain this difference. A possible explanation is that there still are some important allergens missing in soy. For peanut for example, 13 allergens have been described, while for soy there are 8 allergens known at this moment. It is important to highlight however, that both our study population, as well as the study population of Ebisawa et al[34] was relatively small. To analyze whether the diagnostic accuracy could further be improved by combining soy components for example, a larger study population would be necessary. Because soy allergy has a low prevalence, which makes it difficult to conduct large studies, sIgE to Gly m 2S albumin should be assessed in clinical practice next to the currently used tests to further analyze its value in diagnosing soy allergy.

4.2 Severity

In Chapter 7 we showed that sIgE levels to soy extract, Gly m 5 and Gly m 6 were higher in the mild soy allergic patients (Mueller score 0-2, with 0 being oral allergy symptoms). For clinical practice however, it is more useful to be able to predict severity in subjects suspected of soy allergy. In this way, a physician is able to discriminate between no/mild allergy and severe allergy. Therefore, we performed the same additional analyses as described in section 3.2 of this general discussion regarding peanut allergic patients. In short, logistic regression was used to analyze the association between sIgE levels to soy components/extract and severity in all subjects (N=46). The Mueller score with an extra category for no symptoms (score 0) was used as described in section 3.2. Severe allergy was defined as a score 4-5 (respiratory or cardiovascular symptoms).

Twenty subjects had a severe allergy, 26 subjects no/mild allergy. We found no statistically significant association for any diagnostic test (SPT, sIgE to soy extract, Gly m 2S albumin, Gly m 4, Gly m 5 and Gly m 6). Because of this finding and the contradictory results between our results in Chapter 7 and the study of Vissers et al[71] on one hand, and three other studies on the other hand,[32;35;72] we believe that SPT, sIgE to soy components or sIgE to soy extract should neither be used to predict severity in patients suspected of soy allergy, nor in patients already diagnosed with soy allergy.

5. FUTURE RESEARCH

5.1 Validation and impact studies

The excellent diagnostic accuracy of sIgE to Ara h 2 in diagnosing peanut allergy was reproduced by others as described in Chapter 2. The next step before implementation in daily practice in tertiary centres would therefore be to perform an impact study.[73] In such a study sIgE to Ara h 2 would be used by clinicians to make clear-cut decisions regarding further testing with a DBPCFC. Dependent on the accepted amount of false-positive and false-negative outcomes, clinicians should use the corresponding cutoff values for sIgE to Ara h 2 for their decision making. This means that some subjects with a value lower or higher than a certain cutoff value will not be challenged anymore. These subjects should be followed up to analyze whether the right decision was made. Instead, subjects with a negative test result could be offered to ingest peanut in a hospital setting.

A validation study would be needed before sIgE to Ara h 2 can be applied in other settings, for example in primary or secondary care.[74;75]

5.2 Basophil activation test and new biomarkers

Specific IgE to Ara h 2 improves the diagnostics of peanut allergy in both adults and children. However, there is still room for improvement, especially in the adult population. In addition, the diagnostic value of specific IgE to soy components was not significantly better than the currently used tests. Therefore, next to optimizing diagnostics using sIgE measurements, future research should focus on new methods to improve diagnostics. These methods should also address better prediction of the severity of allergy and should preferably be evaluated in combination with the current tests to measure sIgE reactivity. We will shortly address two of these methods below.

1) Basophil activation test (BAT)
2) New biomarkers

The BAT is a method to measure activation of the basophils of a patient upon stimulation with an allergen in different concentrations. The degranulation of the basophil can be assessed by measuring CD63, an intracellular basophil marker.[76] Glaumann et al[25] showed that the BAT can be useful in diagnosing peanut allergy. Next to diagnostic properties, the BAT showed to be useful in evaluating the effect of immunotherapy.[77;78] Future diagnostic studies should further evaluate the diagnostic accuracy of BAT.[79]

Another possibility to improve diagnostics is by using newly identified biomarkers. Mast cell and basophil mediators should be extensively evaluated. Vadas et al[80] for example showed that platelet-activating factor (PAF) was directly correlated to the severity of anaphylaxis. They also showed that serum PAF acetylhydrolase was inversely correlated with the severity of anaphylaxis. This finding was later reproduced in patients with an anaphylactic reaction to wasp venom.[81] In addition, previous studies showed an

important role for the complement system, coagulation factors and the contact system in an allergic reaction.[82;83] In a previous study we found differences in the metabolic profiles in plasma and saliva samples between peanut allergic patients and healthy controls using NMR spectroscopy.[84] Also, differences were found before and after food challenge in the peanut allergic patients. Therefore, next to an aimed approach for specific biomarkers, -omics techniques, such as metabolomics or proteomics, could be useful to detect new biomarkers. Ideally, new biomarkers should not only discriminate between allergy and tolerance, but also predict the severity of allergy. Studies addressing new biomarkers can still not attribute these biomarkers to specific allergies. Therefore, biomarkers should be studied in the context of sIgE levels, which are allergen specific.

In summary, although components increase the diagnostic accuracy in discriminating between allergy and tolerance, there is a need for further improvement of food allergy diagnosis. Assays like the BAT or identification of new biomarkers could lead to a better prediction of allergy, or the severity of allergy.

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Chapter 10

Summary / Samenvatting

SUMMARY

Peanut and soy are both legumes and therefore phylogenetically related. Both peanut and soy should be addressed in the diagnostic work-up of a suspected allergy to either of these foods because of possible cross-reactivity. Allergic symptoms to both foods can range from mild oral allergy symptoms to severe respiratory or even cardiovascular symptoms. Therefore, it is important that peanut or soy allergy is not missed in the diagnostic procedure and an adequate elimination diet is prescribed. An incorrect diagnosis of food allergy on the other hand significantly impairs the quality of life and increases the risk of impaired growth and inadequate nutrient intake in case of children. Skin prick test (SPT) and IgE reactivity to peanut extract in serum are two methods that are generally used to measure sensitization. Since sensitization is often not related to clinical symptoms, test results can be 'false-positive' when used as a diagnostic test for peanut or soy allergy. The reference standard on the other hand, a double-blind, placebo-controlled food challenge, has many disadvantages: it is time-consuming, has high costs, is stressful for the patient, might result in severe clinical reactions and it requires highly dedicated hospital facilities. Therefore, there is strong need for an accurate diagnostic test that is cheap, non-invasive and ideally can discriminate between mild and severe allergy as well. One possibility of improving diagnostics is by combining several predictors into one prediction model. Another possibility is by measuring serum IgE (sIgE) to specific allergenic peanut proteins, also called components. At the moment, 6 peanut components are commercially available and can therefore be used in clinical practice: Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8, and Ara h 9. Their diagnostic accuracy can be analyzed individually or in combination with each other or other clinical predictors.

In Chapter 2 we systematically reviewed current literature for studies addressing the diagnostic accuracy of the current diagnostic tests (SPT and sIgE to peanut extract) or measuring sIgE to one or more peanut components in diagnosing peanut allergy. All eligible studies were scored for risk of bias and concerns regarding applicability. Our results showed that sIgE to Ara h 2 had the best diagnostic accuracy of all diagnostic test methods that were analyzed; it was best in both diagnosing as well as excluding peanut allergy, while SPT and sIgE to peanut extract were primarily useful in excluding peanut allergy.

The diagnostic accuracy of peanut components can be analyzed by different techniques. In Chapter 3 we compared four different techniques with each other; i.e. multi-plexed microarray immunoassay, single-plexed IgE-assay, SPT and IgE immunoblot. All techniques were analyzed in both peanut allergic adults and children. Comparable sensitivity values were found between all four techniques in both adults and children, with the highest sensitivity for Ara h 2. One exception was found however; the multi-plexed assay to Ara h 1 demonstrated a higher sensitivity compared with the

other 3 techniques in children, but this might be explained by a technical issue, since two different preparations of Ara h 1 were used in these tests.

In Chapter 4 we validated a previously published, promising diagnostic prediction model in a pediatric population suspected of peanut allergy. This prediction model consisted of 6 predictors: sex, history, SPT and sIgE to peanut extract, total IgE minus sIgE to peanut extract, and age. Validation of the model in 100 subjects from our clinic showed good discrimination between peanut allergy and peanut tolerance but poor calibration (agreement between predicted and observed frequencies of peanut allergy). We updated the model because of the poor calibration and to analyze whether sIgE to peanut components could increase the diagnostic accuracy of the prediction model. In the updating process, age, history and sIgE to peanut components did not significantly increase discrimination, leaving only 4 predictors of the original model: sex, SPT and sIgE to peanut extract, and total IgE minus sIgE to peanut extract. However, when building a model with only sIgE to peanut components as candidate predictors, Ara h 2 was the only predictor that was left in the model, with a discriminative ability almost equal to the updated prediction model. Cutoff values with 100% positive and negative predictive values could be calculated for both the updated model and sIgE to Ara h 2. In this way, the need for peanut challenges in a pediatric population could be reduced by at least 50%.

Subsequently, the diagnostic accuracy of the updated pediatric prediction model was validated in an adult population suspected of peanut allergy in Chapter 5. Validation showed poor discrimination between peanut allergy and peanut tolerance but good calibration. We also showed in this adult population that sIgE to Ara h 2 had the best diagnostic accuracy of all peanut components, and even better than the diagnostic accuracy of sIgE to peanut extract and SPT. It could diagnose peanut allergy in 28% of patients with 100% certainty, but could not be used to exclude a peanut allergy in an adult population because of the low negative predictive value.

The diagnostic value of sIgE to peanut components in Chapters 4 and 5 was analyzed by the ImmunoCAP. However, specific IgE to Ara h 6, a 2S albumin like Ara h 2, can currently only be tested by the ImmunoCAP ISAC. Therefore, we compared the diagnostic value of sIgE to Ara h 2 and sIgE to Ara h 6, as measured by the ImmunoCAP ISAC, in an adult population suspected of peanut allergy in Chapter 6. The diagnostic value of sIgE to Ara h 6 on population level was as good as sIgE to Ara h 2. Combining both tests did not result in a better diagnostic accuracy. On the individual level, however, 5% of the subjects showed contradicting results between both tests using a cutoff of 0.3 ISU/l, leading to a possible risk of misdiagnosis if only one of both tests is used.

The diagnostic accuracy of sIgE to soy components in diagnosing soy allergy was addressed in Chapter 7. Adults with a suspected soy allergy were included. Specific IgE to Gly m 2S albumin (Gly m 8) had the best diagnostic accuracy, which was comparable

to SPT and sIgE to soy extract. All patients were sensitized to either soy extract or Gly m 4. Specific IgE to soy extract, Gly m 5, and Gly m 6 was significantly higher in patients with mild symptoms. Patients only reacting to soy milk products had significantly higher levels of sIgE to Gly m 4.

For risk assessment in food allergy, and to improve labeling guidelines, information on eliciting doses (ED) for peanut on population level is needed. In Chapter 8, we established EDs for objective and subjective symptoms and analyzed the effect of sensitization levels and other patient characteristics on threshold distribution curves. Threshold data from 100 adults and 262 children with a positive peanut challenge were analyzed and fitted to a threshold distribution curve from which the ED05 value, the value to which 5% of patients react, could be extracted. Subjective and objective threshold distribution curves were different between adults and children, but objective ED05 values were comparable, meaning that threshold data from children and adults can be combined for risk assessment. Higher sIgE levels to Ara h 2 and peanut extract were associated with a larger proportion of both patient groups to react to a certain ED.

In conclusion, this thesis shows that sIgE to Ara h 2 has the best diagnostic accuracy of all diagnostic tests in both adults and children to diagnose peanut allergy. In clinical practice this means that sIgE to Ara h 2 should be measured in all subjects with a suspected history of peanut allergy. In case of unknown prior ingestion, SPT or sIgE to peanut extract can be used as a screening tool to assess whether there is a suspicion of peanut allergy, since both tests have high sensitivity values. Consequently, sIgE to Ara h 2 should be measured in case of a positive SPT or sIgE to peanut extract to assess the risk of peanut allergy. In children, this sIgE to Ara h 2 measurement can be used to accurately diagnose or exclude a peanut allergy, while in adults sIgE to Ara h 2 should primarily be used to accurately diagnose peanut allergy. A negative sIgE result for Ara h 2 in adults does not accurately exclude a possible peanut allergy. Therefore, in case of a negative sIgE measurement for Ara h 2 in adults with a highly suspected peanut allergy, it is advisable to include testing sIgE for Ara h 6 and Ara h 8. Although sIgE to Ara h 8 was found to be a poor predictor for peanut allergy, some patients with mild symptoms were monosensitized to Ara h 8. In suspected soy allergy, sIgE to Gly m 2S albumin (Gly m 8) has the best diagnostic accuracy in diagnosing soy allergy, which is comparable to the current SPT and sIgE to soy extract. The advantage of Gly m 8 however, is that it can be better standardized.

SAMENVATTING

Pinda en soja zijn beide peulvruchten en daarom fylogenetisch verwant aan elkaar. Vanwege mogelijke kruis-reactiviteit moet in de diagnostiek bij verdenking op pinda- of soja-allergie aandacht worden geschonken aan zowel pinda als soja. Allergische symptomen na inname van beide voedingsmiddelen kunnen variëren van milde orale allergie symptomen tot ernstige respiratoire of zelfs cardiovasculaire symptomen. Het is daarom belangrijk dat de diagnose pinda of soja-allergie niet wordt gemist en dat een adequaat dieet wordt voorgeschreven. Echter, een onjuiste diagnose, oftewel het onterecht stellen van de diagnose voedselallergie, vermindert significant de kwaliteit van leven en vergroot het risico op groeivertraging en onvoldoende inname van de benodigde voedingsstoffen, met name bij kinderen. De huidpriktest en het meten van IgE-reactiviteit tegen pinda-extract in serum zijn twee methoden die routinematig worden gebruikt om sensibilisatie te meten. Aangezien sensibilisatie vaak niet gerelateerd is aan klinische symptomen kunnen de testresultaten 'vals positief' zijn indien deze methoden worden gebruikt als een diagnostische test om pinda- of soja-allergie vast te stellen. De gouden standaard daarentegen, de dubbel-blinde, placebo-gecontroleerde voedselprovocatie, heeft een aantal nadelen: deze is tijdrovend, duur, is een belasting voor de patiënt en het kan resulteren in een ernstige allergische reactie. Er zijn speciale faciliteiten voor nodig in het ziekenhuis en ervaren personeel. Er is daarom grote behoefte aan een accurate diagnostische methode die bij voorkeur goedkoop is, niet invasief en idealiter zou kunnen discrimineren tussen een milde en ernstige allergie. Een mogelijkheid om de diagnostiek te verbeteren is door verschillende klinische predictoren en patiëntkarakteristieken te combineren in een predictiemodel. Een andere mogelijkheid is om specifiek IgE (sIgE) tegen specifieke, allergene pinda-eiwitten, ook wel componenten genoemd, te analyseren. Op dit moment zijn 6 pindacomponenten commercieel beschikbaar en deze kunnen dus gebruikt worden in de klinische praktijk: Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8 en Ara h 9. De diagnostische waarde van deze componenten kan individueel worden bepaald of in een combinatie met andere componenten of met andere klinische predictoren.

Hoofdstuk 2 beschrijft het resultaat van een systematisch literatuuronderzoek naar studies die betrekking hadden op de diagnostische waarde van de huidige diagnostische testen (huidpriktest en sIgE tegen pinda-extract) of op 1 of meerdere pindacomponenten bij het diagnosticeren van pinda-allergie. Alle geschikte studies werden beoordeeld op het risico op bias en eventuele tekortkomingen ten aanzien van toepasbaarheid. De resultaten lieten zien dat sIgE tegen Ara h 2 de beste diagnostische waarde had van alle diagnostische testmethoden die geanalyseerd waren. Specifiek IgE tegen Ara h 2 scoorde het beste in zowel het aantonen als het uitsluiten van pinda-

allergie, terwijl de huidpriktest en sIgE tegen pinda-extract voornamelijk nuttig waren voor het uitsluiten van pinda-allergie.

De diagnostische waarde van de pindacomponenten kan met verschillende technieken worden geanalyseerd. In Hoofdstuk 3 werden vier van deze technieken met elkaar vergeleken: de 'multiplexed microarray immunoassay,' de 'singleplexed IgE-assay,' de huidpriktest en de IgE immunoblot. Alle technieken werden bestudeerd in zowel pinda-allergische volwassenen als kinderen. Vergelijkbare waarden voor de sensitiviteit werden gevonden voor alle vier de technieken, met de hoogste sensitiviteit voor Ara h 2. Er werd één uitzondering gezien: Ara h 1 gemeten met de 'multiplexed assay' had een hogere sensitiviteit dan de andere 3 technieken in kinderen. Dit zou verklaard kunnen worden door een technisch probleem, aangezien 2 verschillende vormen van Ara h 1, recombinant en natuurlijk, in de testen werden gebruikt.

In Hoofdstuk 4 werd een recent gepubliceerd model gevalideerd. Dit leek een veelbelovend diagnostisch predictiemodel bij kinderen met verdenking op een pinda-allergie. Dit predictiemodel bestond uit 6 predictoren: geslacht, anamnese, huidpriktest en sIgE tegen pinda-extract, totaal IgE min sIgE tegen pinda extract en leeftijd. Validatie van dit model in 100 kinderen uit onze kliniek liet een goede discriminatie zien tussen pinda-allergie en pinda-tolerantie maar een slechte calibratie (overeenkomst tussen de voorspelde en geobserveerde frequentie van pinda-allergie). Vanwege de slechte calibratie en om te analyseren of sIgE tegen pindacomponenten de diagnostische waarde van het predictiemodel zou kunnen vergroten, werd het model geüpdatet. Tijdens het update proces bleek dat leeftijd, anamnese en sIgE tegen pindacomponenten geen significant toegevoegde waarde hadden bij de discriminatie, waardoor slechts 4 predictoren van het originele model overbleven: geslacht, huidpriktest en sIgE tegen pinda-extract, en totaal IgE min sIgE tegen pinda-extract. Echter, wanneer een predictiemodel werd gemaakt met alleen sIgE tegen pindacomponenten als kandidaatpredictoren, was Ara h 2 de enige predictor die overbleef in het model, met een discriminerend vermogen dat bijna gelijk was aan dat van het geüpdate model. Afkapwaarden met 100% positief en negatief voorspellende waarden konden worden berekend voor zowel het geüpdate model en voor sIgE tegen Ara h 2. Op deze manier kon het aantal benodigde pindaprovocaties in een kinderpopulatie worden teruggebracht met tenminste 50%.

Vervolgens werd de diagnostische waarde van het geüpdate predictiemodel in de kinderpopulatie gevalideerd in een volwassen populatie met verdenking op pinda-allergie in Hoofdstuk 5. Validatie liet een slecht discriminerend vermogen zien tussen pinda-allergie en pinda-tolerantie maar een goede calibratie. We toonden in deze volwassen populatie ook aan dat sIgE tegen Ara h 2 de beste diagnostische waarde had van alle pinda-componenten, zelfs beter dan de diagnostische waarde van de huidpriktest en sIgE tegen pinda-extract. Specifiek IgE tegen Ara h 2 kon pinda-allergie

in 28% van de gevallen met 100% nauwkeurigheid aantonen, maar het kon in de volwassen populatie niet worden gebruikt om pinda-allergie uit te sluiten vanwege de lage negatief voorspellende waarde.

De diagnostische waarde van sIgE tegen pindacomponenten in de Hoofdstukken 4 en 5 werd geanalyseerd met de ImmunoCAP. Specifiek IgE tegen Ara h 6, net als Ara h 2 ook een 2S albumine, kan momenteel alleen getest worden met de ImmunoCAP ISAC. Dit is de reden dat de diagnostische waarde van Ara h 2 en Ara h 6 in Hoofdstuk 6 met elkaar werd vergeleken met de ImmunoCAP ISAC. Dit werd gedaan in een volwassen populatie met verdenking op pinda-allergie. De diagnostische waarde van sIgE tegen Ara h 6 op populatieniveau was net zo goed als die van sIgE tegen Ara h 2. Het combineren van beide testen resulteerde niet in een betere diagnostische waarde. Op individueel niveau echter, had 5% van de personen tegenstrijdige uitslagen tussen beide testen (met de geadviseerde afkapwaarde van 0.3 ISU/l) wat kan resulteren in een foute diagnose als slechts 1 van beide testen wordt gebruikt.

De diagnostische waarde van sIgE tegen sojacomponenten in het diagnosticeren van soja-allergie werd onderzocht in Hoofdstuk 7. Volwassenen met verdenking op soja-allergie werden geincludeerd. Specifiek IgE tegen Gly m 2S albumine (Gly m 8) had de beste diagnostische waarde, vergelijkbaar met de huidpriktest en sIgE tegen soja-extract. Alle patiënten waren gesensibiliseerd voor soja-extract of Gly m 4. Specifiek IgE tegen soja extract, Gly m 5, en Gly m 6 was significant hoger in patiënten met milde symptomen. Patiënten die alleen reageerden op sojamelkproducten hadden significant hogere sIgE waarden tegen Gly m 4.

Informatie over drempelwaarden ('eliciting dose' (ED)) op populatie niveau is nodig voor risicobeoordeling bij voedselallergie en om richtlijnen ten aanzien van etikettering te verbeteren. In Hoofdstuk 8 werden EDs voor objectieve en subjectieve symptomen vastgesteld en het effect van sensibilisatie en andere patiëntkarakteristieken op de bijbehorende drempelwaardeverdelingen geanalyseerd. Drempelwaarden data van 100 volwassenen en 262 kinderen met een positieve pindaprovocatie werden geanalyseerd en met behulp van een statistische methode 'gefit' in een drempelwaardeverdeling, van waaruit de ED05 waarde, de waarde waarop 5% van de patiënten reageert, kon worden afgeleid. Subjectieve en objectieve drempelwaardeverdelingen waren verschillend tussen volwassenen en kinderen, maar objectieve ED05 waarden waren vergelijkbaar. Dit betekent dat drempelwaarden gegevens van kinderen en volwassenen kunnen worden samengevoegd voor risicobeoordeling. Hogere sIgE waarden tegen Ara h 2 en pinda-extract waren geassocieerd met een grotere kans om op een bepaalde ED te reageren met allergische symptomen.

Concluderend toont dit proefschrift aan dat sIgE tegen Ara h 2 de beste diagnostische waarde heeft van alle op dit moment beschikbare diagnostische testen bij zowel volwassenen als kinderen om pinda-allergie te diagnosticeren. In de klinische praktijk

betekent dit dat sIgE tegen Ara h 2 bepaald zou moeten worden bij iedereen met een anamnese verdacht voor pinda-allergie. In het geval dat voorafgaande ingestie niet bekend is, kunnen de huidpriktest of sIgE tegen pinda-extract dienen als een screeningstest om vast te stellen of er een verdenking is op pinda-allergie, aangezien beide testen een hoge sensitiviteit hebben. Indien de huidpriktest of sIgE tegen pinda-extract positief is, zou sIgE tegen Ara h 2 vervolgens bepaald moeten worden om het risico op pinda-allergie te bepalen. Bij kinderen kan deze sIgE waarde tegen Ara h 2 gebruikt worden om pinda-allergie met grote nauwkeurigheid aan te tonen of uit te sluiten, terwijl in volwassenen sIgE tegen Ara h 2 voornamelijk gebruikt zou moeten worden om pinda-allergie aan te tonen. Een negatieve sIgE test tegen Ara h 2 in volwassenen sluit een pinda-allergie niet nauwkeurig genoeg uit. Daarom wordt geadviseerd om in het geval van een negatieve sIgE test tegen Ara h 2 in volwassenen met een hoge verdenking op pinda-allergie ook te testen op sIgE tegen Ara h 6 en Ara h 8. Hoewel sIgE tegen Ara h 8 een slechte predictor was voor pinda-allergie, waren sommige patiënten met milde symptomen alleen gesensibiliseerd voor Ara h 8. Bij de verdenking op soja-allergie heeft sIgE tegen Gly m 2S albumine (Gly m 8) de beste diagnostische waarde, vergelijkbaar met de huidige huidpriktest en sIgE tegen soja-extract, om soja-allergie te diagnosticeren. Voordeel van een test met Gly m 8 is dat deze beter is te standaardiseren.

Chapter 11

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Chapter 12

Appendices

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Curriculum Vitae

List of publications

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CURRICULUM VITAE

Rob werd geboren op 19 april 1985 te Meerlo-Wanssum. Na cum laude zijn Gymnasium diploma behaald te hebben in 2003 aan het Raayland College in Venray, begon hij in datzelfde jaar met de opleiding Geneeskunde aan de Universiteit Utrecht. In het laatste jaar van de opleiding heeft hij onder leiding van Thuy-My Le en André Knulst onderzoek gedaan naar de behandeling van voedselallergische reacties door huisartsen op de afdeling Dermatologie/Allergologie. Na het behalen van zijn arts-examen in 2009 heeft hij 5 maanden bij de afdeling longziekten gewerkt in het Amphia ziekenhuis in Breda. Hierna is hij begonnen als arts-onderzoeker bij de afdeling Dermatologie/Allergologie, hetgeen heeft geleid tot dit proefschrift. In 2014 is hij begonnen met de opleiding tot dermatoloog in het UMC Utrecht met Vigfús Sigurdsson als opleider.

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