

ORIGINAL ARTICLE

BIOTECHNOLOGY AND IN VITRO DIAGNOSTICS

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

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To cite this article: Klemans RJB, Knol EF, Michelsen-Huisman A, Pasmans SGMA, de Kruijf-Broekman W, Bruijnzeel-Koomen CAFM, van Hoffen E, Knulst AC. Components in soy allergy diagnostics: Gly m 2S albumin has the best diagnostic value in adults. *Allergy* 2013; DOI: 10.1111/all.12259.

Keywords

diagnostics; Gly m 2S albumin; soy allergy; soy components; soy milk.

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Accepted for publication 5 August 2013

DOI:10.1111/all.12259

Edited by: Reto Crameri

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 immunoglobulin E (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: Specific immunoglobulin E 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: Specific immunoglobulin E 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 Gly m 4 were related to allergy to soy milk.

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

Abbreviations

AUC, area under the curve; DBPCFC, double-blind, placebo-controlled food challenge; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic; sIgE, specific immunoglobulin E; SPT, skin prick test.

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

Patients

All adult patients 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 min, 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 and lasted for at least 45 min.

SPT and measuring sIgE

Skin prick test was performed with commercial soy extract (ALK-ABELLO, 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 cut-off 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 patients 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.

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 (≥ 0.35 kU/l) significantly more often sIgE to soy extract, Gly m 2S albumin, Gly m 5 and Gly m 6 compared to 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: $\rho = 0.75$, $P < 0.001$; Gly m 2s albumin: $\rho = 0.52$, $P < 0.001$; Gly m 5: $\rho = 0.81$, $P < 0.001$; Gly m 6: $\rho = 0.77$, $P < 0.001$).

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 ($\rho = 0.92$, $P < 0.001$). In addition, we found weak negative correlations with sIgE to soy extract ($\rho = -0.34$, $P = 0.02$), Gly m 5 ($\rho = -0.38$, $P = 0.01$), and Gly m 6 ($\rho = -0.37$, $P = 0.01$).

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

Specific immunoglobulin E 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.

Diagnostic value of all tests for different cut-off 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

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

CI, confidence interval.

All data represent medians and interquartile ranges or numbers and percentages.

*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 ≥ 3 mm).

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
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, diarrhea; 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 min at the highest dose.

cut-off 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.

To diagnose a soy allergy, SPT with soy extract had the best diagnostic value (PPV 100%, LR+ infinity) when using a cut-off value of ≥ 5 mm, followed by sIgE to Gly m 2S albumin with a cut-off 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 cut-off 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

Table 3 Area under the curve (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; SPT, skin prick test

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

Cut-off 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.

(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).

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, $P = 0.01$, Fig. 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 ($P = 0.76$).

Discussion

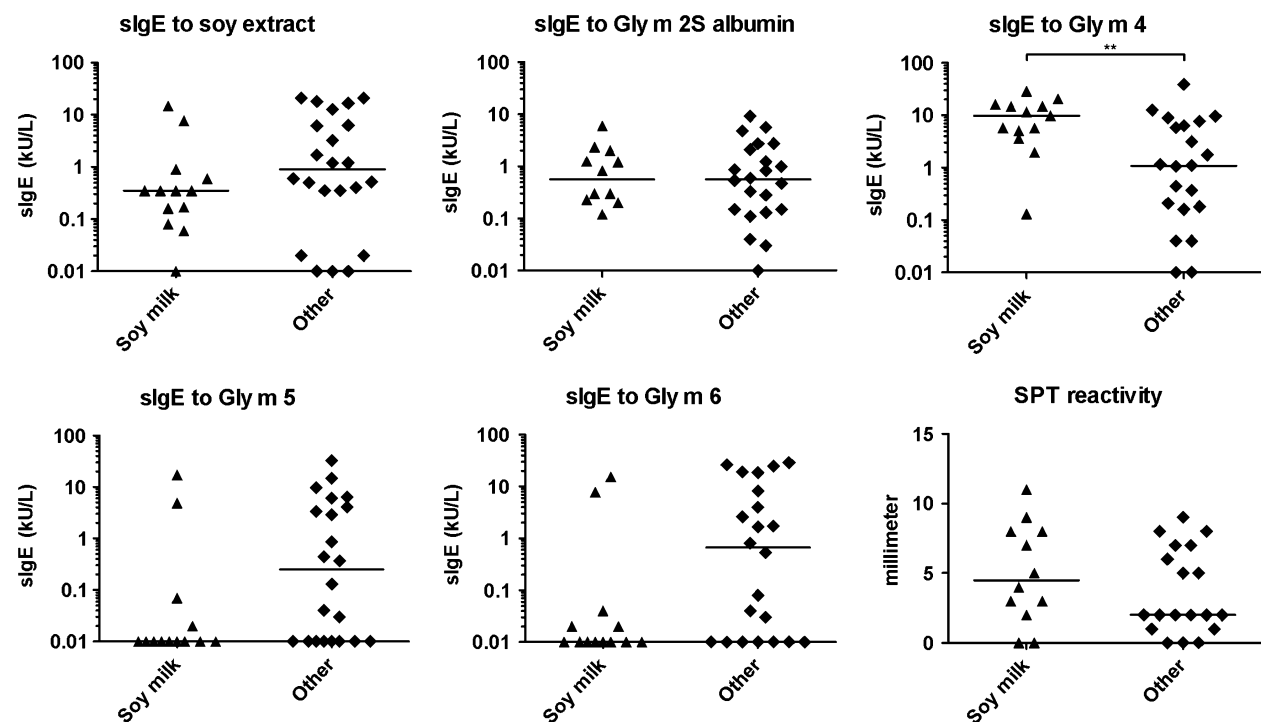
This is the first study showing the diagnostic value of the SPT and sIgE to soy allergens in a population where all subjects were suspected of soy allergy. Previous diagnostic studies included controls not suspected of a soy allergy (4–7) or only described a soy-allergic population (2, 3, 8). We studied 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., pre-test 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 cosensitization 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

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
slgE soy extract (kU/l)	-0.28 (0.10)	1.70 (0.35–12.70)	0.35 (0.03–0.83)	0.04
slgE Gly m 2S albumin (kU/l)	-0.26 (0.14)	0.93 (0.28–3.44)	0.32 (0.12–1.23)	0.11
slgE Gly m 4 (kU/l)	-0.03 (0.86)	1.96 (0.37–14.60)	4.71 (0.19–9.77)	0.97
slgE Gly m 5 (kU/l)	-0.31 (0.07)	0.87 (0.01–16.40)	0.00 (0.00–0.12)	0.02
slgE 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 with interquartile range.



Other: patients with allergic symptoms to other soy products than soy milk as well, ** P -value < 0.001

Figure 1 Soy milk ($n = 13$) vs other soy products ($n = 22$).

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 cut-off value of ≥ 0.35 kU/l. Others also showed that most soy allergies 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 to 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 to patients who also reacted to other soy products. A possible explanation could be a different way of processing of soy milk compared to 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.

Acknowledgments

The authors would like to thank Sigrid Sjolander from Thermo Fisher Scientific, Uppsala, Sweden, for providing

experimental Gly m 2S Albumin ImmunoCAP material and Ans Lebens for her help in collecting all patient data.

Author contributions

RJB Klemans made substantial contributions to conception and design, acquisition, analysis and interpretation of data, drafting article, and final approval of version to be published. EF Knol, E van Hoffen, and AC Knulst made substantial contributions to conception and design, interpretation of data, revising the article critically for important intellectual content, and final approval of version to be published. A Michelsen-Huisman and SGMA Pasmans made substantial contributions to conception and design, revising the article critically for important intellectual content, and final approval of version to be published. W de Kruijf-Broekmans made substantial contributions to acquisition of data, revising the article critically for important intellectual content, and final approval of version to be published. CAFM Bruijn-zeel-Koomen made substantial contributions to conception and design, revising the article critically for important intellectual content, and final approval of version to be published.

Conflicts of interest

University Medical Center Utrecht received an unrestricted grant from ThermoFisher Scientific, Sweden, and the Netherlands.

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