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Specific IgE to Jug r 1 has no additional value compared to extract based testing in diagnosing walnut allergy in adults

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Conflict of interest:

None

Summary:

Specific IgE to walnut 2S albumin Jug r 1 was superior compared to Jug r 2 and 3 in diagnosing walnut allergy in adults, but did not have additional value compared to sIgE to walnut extract or SPT with commercial walnut extract.

Key words:

Walnut allergy; Jug r 1; Jug r 2; Jug r 3; 2S albumin; 7S globulin; LTP; SPT; specific IgE; DBPCFC

Abbreviations:

AUC:	Area under the curve
DBPCFC:	Double blind placebo controlled food challenge
nsLTP:	Non-specific Lipid Transfer Protein
OAS:	Oral allergy symptoms
PR-10:	Pathogenesis-related class 10
ROC:	Receiver Operating Curve
sIgE:	Specific Immunoglobulin E
SPT:	Skin Prick Test

Word count:

1048 words, when considering component names (e.g. Jug r 1) as a single word instead of three.

To the editor:

Three English walnut (*Juglans regia*) components are currently commercially available for specific IgE (sIgE) testing: a 2S albumin (Jug r 1), a vicilin-like 7S globulin (Jug r 2) and a lipid transfer protein (Jug r 3). Their value in diagnosing walnut allergy remains unknown. The aim of this study was to prospectively investigate the predictive value of sIgE to walnut components Jug r 1, 2 and 3 in subjects suspected of walnut allergy and to compare the results with skin prick test (SPT) and sIgE to walnut extract. In addition, their ability to predict the severity of walnut allergy was assessed.

Adult subjects (n=66) with a suspected walnut allergy were included between November 2012 and October 2015. Diagnostic tests included SPT with three different commercial walnut extracts (ALK-Abelló, Stallergenes and GREER), sIgE detection on ImmunoCAP (walnut extract, Jug r 1 and 3, hereafter called CAP) and ImmunoCAP ISAC (Jug r 1, 2 and 3, hereafter called ISAC). A double blind placebo controlled food challenge (DBPCFC) with raw walnut was performed to establish walnut allergy or tolerance. Ethical approval was given by the local ethics committee. Detailed methods are reported in supplement 1 in the Online Repository.

Walnut allergy was confirmed in 33 of 55 challenged subjects (60%) (see figure E1 in the Online Repository for study flow chart). Median age of all subjects was 32 years (IQR 25-38) and 22% was male. Age and sex distribution did not differ between the in- and excluded subjects. Subject characteristics are listed in table E1 in the Online Repository. Sensitization to Jug r 1 (2S albumin) was most prevalent, with more than half of the walnut allergic subjects demonstrating sIgE on both CAP and ISAC. Sensitization results are listed in table 1. All subjects sensitized to Jug r 2 were also co-sensitized to Jug r 1 on ISAC, except for one subject, who had a negative challenge (data not shown). Sensitization to either native or recombinant walnut nsLTP (Jug r 3) was only observed in a few subjects, which was comparable with nsLTP from peanut and hazelnut in previous studies from our center [1,2]. A strong correlation was observed between rJug r 1 on CAP and ISAC ($r_s = 0.89$, $p < 0.001$), and also between rJug r 1 on both CAP and ISAC and the walnut extract on CAP ($r_s = 0.90$ and 0.83 respectively, both $p < 0.001$).

Of all walnut components, sIgE to Jug r 1 had the best discriminative ability, with an area under the curve (AUC) of 0.78 on CAP (table 1). However, results were similar to routinely used sIgE to walnut extract (AUC: 0.79) and SPT with the different extracts (AUC: 0.78-0.80) (table 1). Logistic regression analyses showed no additional value of combining sIgE to the three components in a prediction model for walnut allergy (data not shown). For use in daily practice, it must be considered that serological sIgE tests are standardized, but methods for performing SPT may differ and SPT results and cutoff values are not interchangeable between manufacturers of walnut extracts.

For Jug r 1 on CAP and ISAC, walnut extract on CAP and SPT with the ALK extract, as the best discriminative tests (table 1), a cutoff value with 100% positive predictive value (PPV) and specificity was calculated (table 2). Using these cutoff points, it was possible to correctly classify subjects as walnut allergic with 100% certainty: 24% of subjects for Jug r 1 on CAP, 25% for walnut extract on CAP and 16% for ALK SPT extract. It was not possible to reach 100% negative predictive value (NPV) and sensitivity using any of these tests. The highest positive likelihood ratio was found for the SPT, followed by walnut extract on CAP, using the calculated optimal cutoff levels. Sensitivity, specificity, PPV, NPV, positive and negative likelihood values for different cutoff points are listed in table 2.

Six subjects experienced only oral allergy symptoms (OAS) up to the highest dose of the challenge. When stratifying subjects into either a tolerant or OAS-only phenotype (n=28) or a more moderate to severe phenotype (symptoms more than only OAS, n=27) based on symptoms observed during the challenge, AUC values increased in ROC curve analyses for Jug r 1 and walnut extract on CAP as well as the ALK SPT extract (AUC 0.85, 0.86 and 0.83, respectively, table 1). The 100% PPV cutoff value and the amount of subjects that could be correctly classified remained the same for all three tests (table 2), thus allowing for the correct prediction of not only walnut allergy, but also allergy with allergic symptoms more than only OAS.

The relevance of sIgE to 2S albumins was previously established for peanut and hazelnut allergy in children and adults [1,3,4], as well as in cashew, pistachio and Brazil nut, mostly in children [5,6]. The fact that sIgE to the walnut 2S albumin Jug r 1 was not superior compared to sIgE to walnut extract on CAP or SPT is in contrast to previous results in peanut and hazelnut allergy [1,2,7]. Other walnut allergens, like the 11S globulin Jug r 4 or a not yet identified Bet v 1-like (PR-10) protein, might play a role in some subjects not sensitized to the currently available components [8]. In hazelnut, 11S globulin Cor a 9, homologous to Jug r 4, was an important marker for severe allergy [1]. While pollen related allergens have been identified in hazelnut and almond, none have been officially recognized in walnut so far. Nevertheless, we encountered patients with a birch pollen (PR-10) related food allergy, sensitized to PR-10 proteins from birch pollen (Bet v 1) and hazelnut (Cor a 1), that report OAS after ingestion of walnut. This suggests the presence of a PR-10 protein in walnut, although this has been disputed before [9].

Strengths of this study include the prospective design and the large cohort investigated, which was comparable to the population in which you would measure walnut sensitization in daily practice. In addition, walnut allergy was assessed using the reference standard DBPCFC. Validation studies in children, as well as in other (non-tertiary) centers should be a focus of future research in walnut allergy.

In conclusion, sIgE to Jug r 1 had the highest discriminative ability of the walnut components, but did not have additional value compared to sIgE to walnut extract or SPT with commercial walnut extract. When distinguishing tolerant or only OAS from moderate to severe walnut allergy (i.e. more than OAS), a higher discriminative ability was found for all available tests, allowing for a risk estimation of the severity of walnut allergy.

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Table and figure legends:**Table 1:** Sensitization characteristics and discriminative ability of the diagnostic tests**Table 2:** Diagnostic values of SPT and sIgE for walnut allergy versus tolerance using different cutoff points**Figure E1:** Study flow chart**Table E1:** Subject characteristics

Tables and figures

Table 1: Sensitization characteristics and discriminative ability of the diagnostic tests

Walnut sensitization	Overall (n=55)	Walnut tolerant (n=22)	Walnut allergic (n=33)	p value†	Tolerant versus allergic	Tolerant/OAS versus moderate to severe
	n positive* (%)	n positive* (%)	n positive* (%)		AUC (95% CI)	AUC (95% CI)
ImmunoCAP						
- Walnut extract	26 (47%)	3 (14%)	23 (70%)	<0.001	0.79 (0.68-0.91)	0.86 (0.76-0.96)
- rJug r 1	20 (36%)	2 (9%)	18 (55%)	0.001	0.78 (0.66-0.90)	0.85 (0.74-0.96)
- rJug r 3	2 (4%)	0 (0%)	2 (6%)	0.51	0.44 (0.29-0.60)	0.46 (0.31-0.62)
ImmunoCAP ISAC						
- rJug r 1	22 (40%)	2 (9%)	20 (61%)	<0.001	0.75 (0.63-0.88)	0.80 (0.68-0.93)
- nJug r 2	10 (18%)	1 (5%)	9 (27%)	0.07	0.63 (0.48-0.77)	0.67 (0.53-0.82)
- nJug r 3	3 (6%)	1 (5%)	2 (6%)	1.00	0.49 (0.33-0.64)	0.47 (0.31-0.62)
Skin Prick Test						
- Stallergenes	30 (55%)	7 (32%)	23 (70%)	0.006	0.79 (0.68-0.91)	0.83 (0.71-0.94)
- ALK	35 (64%)	8 (36%)	27 (82%)	0.001	0.80 (0.69-0.92)	0.83 (0.72-0.94)
- Greer	40 (73%)	12 (55%)	28 (85%)	0.01	0.78 (0.66-0.90)	0.80 (0.68-0.92)

AUC: Area under the curve. CI: confidence interval. OAS: oral allergy symptoms

* Using clinical cutoff values. † p value of difference in number of positive tests between tolerant and allergic subjects (chi-squared test).

Table 2: Diagnostic values of SPT and sIgE for walnut allergy versus tolerance using different cutoff points

Test cutoff	Sensitivity		Specificity		PPV		NPV		LR+		LR-	
	% (95% CI)		% (95% CI)		% (95% CI)		% (95% CI)		(95% CI)		(95% CI)	
CAP walnut (kUA/L)												
≥0.1	73	(54-87)	77	(55-92)	83	(64-94)	65	(44-83)	3.2	(1.4-7.1)	0.4	(0.2-0.6)
≥0.35*	70	(51-84)	86	(65-97)	88	(70-98)	66	(46-82)	5.1	(1.7-15)	0.4	(0.2-0.6)
≥0.46†	67	(48-82)	91	(71-99)	92	(73-99)	65	(45-81)	7.3	(1.9-28)	0.4	(0.2-0.6)
≥2.02	42	(25-61)	100	(85-100)	100	(77-100)	54	(37-69)	-	-	0.6	(0.4-0.8)
CAP Jug r 1 (kUA/L)												
≥0.1†	61	(42-77)	91	(71-99)	91	(71-99)	61	(42-77)	6.7	(1.7-26)	0.4	(0.3-0.7)
≥0.35*	55	(36-72)	91	(71-99)	90	(68-99)	57	(39-74)	6.0	(1.5-23)	0.5	(0.3-0.7)
≥1.49	39	(23-58)	100	(85-100)	100	(75-100)	52	(36-68)	-	-	0.6	(0.5-0.8)
ISAC Jug r 1 (ISU-E)												
≥0.3*†	61	(42-77)	91	(71-99)	91	(71-99)	61	(42-77)	6.7	(1.7-26)	0.4	(0.3-0.7)
≥2.85	30	(16-49)	100	(85-100)	100	(69-100)	49	(34-64)	-	-	0.7	(0.6-0.9)
SPT ALK (mm)												
≥3*	82	(65-93)	64	(41-83)	77	(60-90)	70	(46-88)	2.3	(1.3-4.0)	0.3	(0.1-0.6)
≥5†	55	(36-72)	95	(77-100)	95	(74-100)	58	(41-74)	12	(1.7-84)	0.5	(0.3-0.7)
≥8	27	(13-46)	100	(85-100)	100	(66-100)	48	(33-63)	-	-	0.7	(0.6-0.9)

PPV: positive predictive value, NPV: negative predicting value, LR+: positive likelihood ratio, LR-: negative likelihood ratio, CI: confidence interval, SPT: skin prick test

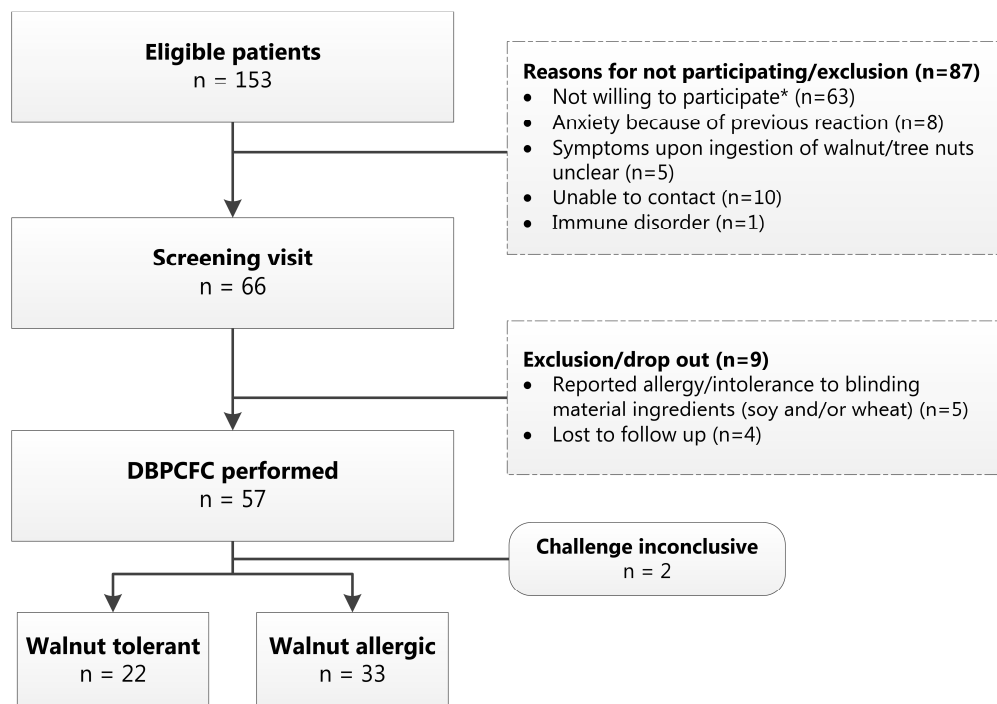
* Clinically used cutoff value, † Calculated optimal cutoff points

Table E1: Subject characteristics

Characteristic	Overall (n=55)		Walnut tolerant (n=22)		Walnut allergic (n=33)		p value †
Age (median [IQR])	32	(25-38)	32	(25-44)	30	(25-35)	0.45
Sex (n male [%])	12	(22%)	6	(27%)	6	(18%)	0.51
Patient history							
- Symptoms according to Mueller (n [%])							
-- Mueller 0	15	(27%)	8	(36%)	7	(21%)	0.31
-- Mueller 1	13	(24%)	7	(32%)	6	(18%)	
-- Mueller 2	6	(11%)	2	(9%)	4	(12%)	
-- Mueller 3	19	(35%)	5	(23%)	14	(42%)	
-- Mueller 4	2	(4%)	0	(0%)	2	(6%)	0.48
- Symptoms certainly due to walnut (n [%])	37	(67%)	13	(59%)	24	(73%)	
- Atopic comorbidity* (n [%])	52	(95%)	21	(96%)	31	(94%)	1.00
-- Asthma (n [%])	21	(38%)	8	(36%)	13	(39%)	0.82
-- Atopic dermatitis (n [%])	27	(49%)	7	(32%)	20	(61%)	0.04
-- Allergic rhinitis (n [%])	47	(86%)	21	(96%)	26	(79%)	0.13

IQR: Interquartile range

* One or more reported atopic diseases (asthma, atopic dermatitis, allergic rhinitis). † p value of difference in subject characteristics between tolerant and allergic subjects.



* E.g. too time consuming, large traveling distance, not interested.