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ORIGINAL ARTICLE

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Measurement of IgE to hazelnut allergen components cannot replace hazelnut challenge in Dutch adults

Sarah A. Lyons[1] | Paco M. J. Welsing[2] | Mariam Hakobyan[1] | Hannah M. Kansen[1,3] | Edward F. Knol[1,4] | Henny G. Otten[4] | Ronald van Ree[5,6] | André C. Knulst[1,4] | Thuy-My Le[1,4]

[1]Department of Dermatology and Allergology, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands

[2]Division of internal medicine and dermatology, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands

[3]Department of Pediatric Pulmonology and Allergology, Wilhelmina Children's Hospital, University Medical Center, Utrecht University, Utrecht, the Netherlands

[4]Center of Translational Immunology, University Medical Center Utrecht, Utrecht, the Netherlands

[5]Department of experimental immunology, Amsterdam University Medical Centers, Amsterdam, the Netherlands

[6]Department of otorhinolaryngology, Amsterdam University Medical Centers, Amsterdam, the Netherlands

Correspondence
Sarah A. Lyons, Department of Dermatology and Allergology, University Medical Centre Utrecht Heidelberglaan 100, 3584 CX, UTRECHT, The Netherlands.
Email: s.a.lyons-2@umcutrecht.nl

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Abstract

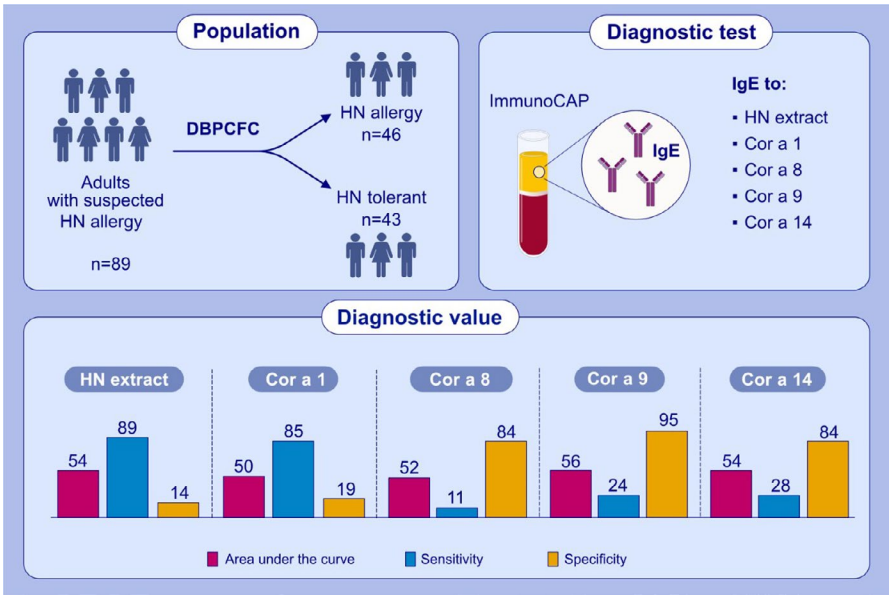
Background: Component-resolved diagnostics (CRD) help predict hazelnut allergy (HA) in children, but are of unknown diagnostic value in adults. This study aimed to evaluate the diagnostic accuracy of IgE to hazelnut extract and components in adults.

Methods: A Dutch population of consecutively presenting adults suspected of HA, who underwent a double-blind placebo-controlled food challenge, were included. Serum IgE to hazelnut extract and Cor a 1, 8, 9, and 14 was measured on ImmunoCAP. Diagnostic accuracy was assessed by area under the curve (AUC) analysis.

Results: Of 89 patients undergoing challenge, 46 had challenge-confirmed HA: 17 based on objective and 29 based on subjective symptoms. At commonly applied cut-offs 0.1 and 0.35 kU_A/L, high sensitivity was observed for IgE to hazelnut extract and Cor a 1 (range 85–91%), and high specificity for IgE to Cor a 8, 9 and 14 (range 77–95%). However, the AUCs for hazelnut extract and components were too low for accurate prediction of HA (range 0.50–0.56). Combining hazelnut extract and component IgE measurements did not significantly improve accuracy. Higher IgE levels to Cor a 9 and 14 were tentatively associated with HA with objective symptoms, but the corresponding AUCs still only reached 0.68 and 0.63, respectively.

Conclusions: Although hazelnut allergic adults are generally sensitized to hazelnut extract and Cor a 1, and hazelnut tolerant adults are usually not sensitized to Cor a 8, 9, or 14, challenge testing is still needed to accurately discriminate between presence and absence of HA in adults from a birch-endemic country.

KEYWORDS
adults, component-resolved diagnostics, diagnostic value, hazelnut allergy, IgE



GRAPHICAL ABSTRACT

Dutch hazelnut allergic adults are usually sensitized to hazelnut extract and Cor a 1 (sensitivity 85-91%). Dutch hazelnut tolerant adults are usually not sensitized to Cor a 8, 9, or 14 (specificity 77-95%). Measurement of IgE to hazelnut allergen components (AUC 50-56%) cannot replace hazelnut challenge for diagnosis of hazelnut allergy in adults in birch-endemic regions.

Abbreviations: AUC, area under the curve; DBPCFC, double-blind placebo-controlled food challenge; HN, hazelnut.

1 | INTRODUCTION

As hazelnut is the tree nut most commonly reported to cause food allergic reactions in European adults,[1-4] accurate diagnosis of hazelnut allergy (HA) is essential. Double-blind placebo-controlled food challenge (DBPCFC) is the reference standard for diagnosis. However, DBPCFC is resource-intensive, burdensome, and carries the risk of severe reactions. In addition, certain patients decline or are excluded from DBPCFC (eg, patients with a history of severe anaphylaxis, patients with chronic urticaria, or pregnant women).[5,6]

Other diagnostic tests in the evaluation of HA, which are less invasive and can be performed on anyone, include measurement of serum IgE levels to hazelnut extract, and more recently, hazelnut allergen components.[7,8] Such serology tests, commonly referred to as component-resolved diagnostics (CRD), are readily available for hazelnut storage components Cor a 9 (11S globulin) and Cor a 14 (2S albumin), and hazelnut cross-reactive components Cor a 1 (pathogenesis related [PR]-10 protein) and Cor a 8 (lipid transfer protein [LTP]). A recent systematic review and meta-analysis concludes that IgE to hazelnut extract, Cor a 9 and Cor a 14 can contribute to accurate identification of children with HA.[9] Some studies suggest that hazelnut CRD sensitization profiles are also linked to specific clinical allergy phenotypes and may predict the risk of a severe reaction to hazelnut.[8,10-12] Data on adults are scarce and have been obtained from case-control studies[10,13] or studies in mixed adult and pediatric populations.[11,14] Findings based on a cohort of consecutively presenting adults are not yet available.

The aim of this study was to evaluate the diagnostic performance of ImmunoCAP tests with hazelnut extract and components Cor a 1, Cor a 8, Cor a 9, and Cor a 14, individually and combined, for distinguishing between presence and absence of HA in adults. As has already been established for children, such data could reduce the need for DBPCFC for HA, and give hazelnut CRD a prominent place in food allergy diagnostic guidelines for adults.

2 | METHODS

2.1 | Study population

All consecutive adult patients with suspected HA who underwent DBPCFC between August 2012 and January 2019 at the University Medical Centre Utrecht (UMCU), the Netherlands, were eligible for inclusion. Prior to DBPCFC, all patients were evaluated in the UMCU outpatient clinic. Patients with an inconclusive DBPCFC, or with incomplete IgE results, were excluded from analyses. The study was approved by the research ethics committee of the UMCU (protocol number 18–428). Exemption with regard to obtaining informed consent was granted according to the GDPR. Analyses were performed with completely pseudonymized data.

2.2 | Data collection

Data on DBPCFC results; serum IgE levels to hazelnut extract and hazelnut components Cor a 1, 8, 9, and 14; patient demographics (age, sex); and allergic comorbidities (asthma, atopic dermatitis, allergic rhinitis) were collected retrospectively from patients' medical files. IgE

levels were determined using the ImmunoCAP platform (*Thermo Fisher Scientific Uppsala, Sweden*). In patients with missing IgE results, IgE levels were obtained using leftover serum stored in the department's serum bank and the UMCU's biobank. The median time between blood collection and DBPCFC was 7 months (interquartile range 4–10 months).

DBPCFC was performed according to international consensus protocols.[5,6] During a 2-day approach in a hospital setting, hazelnut protein or placebo was administered orally in portions increasing every 20–30 minutes, following a logarithmic increment. Blinding was achieved by incorporating hazelnut or placebo into apple sauce or a spiced cake. The doses given during the DBPCFC ranged from 1 to 450 mg of hazelnut protein, with minor changes to the dosing regimen over time. A negative DBPCFC was followed by an open challenge test with raw hazelnut with doses ranging from 300 to 3000 mg of hazelnut protein on the second challenge day. The maximum total cumulative dose was between 3242 and 4360 mg, depending on the protocol at the time. The outcomes of the challenges were discussed among local food allergy experts. The test was considered positive upon occurrence of objective symptoms, subjective symptoms in response to a minimum of three doses, subjective symptoms lasting at least 45 minutes,[5,6] or based on the conclusion of the expert discussion. Objective symptoms included urticaria, erythema, angioedema, objective conjunctivitis, objective rhinitis, vomiting, diarrhea, cough, wheezing, stridor, hoarseness, objective dyspnea, cyanosis, respiratory arrest, tachycardia, dysrhythmia, hypotension, or cardiac arrest. Subjective symptoms included oral allergy syndrome, sensation of oral swelling, difficulty swallowing, local or generalized pruritus, subjective eye symptoms (pruritus, irritation or burning of the eyes), sensation of nasal congestion, nausea, abdominal pain, subjective dyspnea, or dizziness. These criteria were agreed upon prior to data collection and statistical analysis.

2.3 | Statistical analysis

Data on patient characteristics for those with HA *vs* those without HA, and for those with HA with objective symptoms *vs* those with HA with subjective symptoms or without HA, were presented in absolute number and percentage for categorical variables, and mean and standard deviation or median and interquartile range for continuous variables, and compared using the chi-square test, independent samples t test, or Mann-Whitney U test.

The diagnostic accuracy of IgE levels to hazelnut extract and each of the individual components was assessed by the area under the curve (AUC) of the receiver-operating characteristic (ROC) and corresponding 95% confidence interval (CI). DeLong's test was used for statistical comparison of AUCs.[15] Sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) were obtained for cutoffs most commonly used in clinical practice: 0.1 and 0.35 kU_A/L. In case of sufficiently large AUCs indicative of accurate discrimination, cutoffs for IgE levels corresponding to positive or negative predictive values >95% were to be determined.

To evaluate the diagnostic value of all the ImmunoCAP results combined (hazelnut extract, Cor a 1, 8, 9, and 14) for prediction of HA, multivariable logistic regression was applied. After determining the AUC of the full model including all ImmunoCAPs, least absolute shrinkage and selection operator (Lasso) regression was used to determine the most discriminative combination of hazelnut extract and components. Lasso regression is a form of penalized regression, which selects only the most contributive predictors, and applies shrinkage of regression coefficients through cross-validation, to limit overfitting.[16] No multivariable analyses were performed for prediction of HA with objective symptoms because of the low number of patients with this outcome.

Analyses were conducted with SPSS version 25 (IBM Corporation, Armonk, NY) and R version 3.4.1 (R Core Team, Vienna, Austria).

3 | RESULTS

3.1 | Clinical characteristics

A total of 139 adults underwent hazelnut DBPCFC during the period of inclusion, of which 50 were excluded from analyses due to inconclusive DBPCFC (N=19) or a lack of serum for obtaining complete data on IgE levels (N=31). There were no statistically significant differences between included and excluded patients, except that included patients were more likely to have atopic dermatitis (58% *vs* 36%, Table S1).

Of the 89 included adults, 57 (64%) had a clear history of prior immediate reactions to hazelnut, and 19 (21%) had a history suggestive of anaphylaxis (Table 1). The other 32/89 (36%) subjects had all preventatively avoided hazelnut for years (often since early childhood) because of suspected hazelnut allergy. Birch pollen sensitization was detected in 90% of subjects. Based on challenge, 46/89 (52%) were classified as hazelnut allergic, and 17/46 (37%) hazelnut allergic patients had objective symptoms. In 16/46 (35%) hazelnut allergic patients, allergic symptoms were elicited during the open challenge part of the protocol. Clinical characteristics of the evaluated patients are shown in Table 1. Allergic rhinitis was significantly more common in hazelnut allergic than in hazelnut tolerant patients. There were no statistically significant differences in characteristics between the patients with objective symptoms and the patients with no symptoms or subjective symptoms during challenge. The median cumulative dose of hazelnut protein consumed during challenge was 1080 mg (IQR 1080–4360 mg) for all patients with a positive challenge test, 1080 mg (IQR 1080–4360 mg) for patients with only subjective symptoms during challenge, and 1080 mg (IQR 870–2652 mg) for patients with objective symptoms during challenge (p=0.218 for subjective *vs* objective symptoms).

The most commonly occurring sensitization pattern (IgE ≥0.35kU_A/L) comprised sensitization to hazelnut extract and Cor a 1 (N=48/89, 54%, Table S2). Sensitization to Cor a 8, 9, or 14 without co-sensitization to Cor a 1 was detected in 4/89 subjects (4%, Table S2). Overall, 10 of the 89 challenged subjects with complete IgE data were not sensitized to hazelnut extract or any of the components (or 9 subjects based on an IgE cutoff of 0.1 Ku_A/L). Four of these

TABLE 1 Patient characteristics of adults with and without HA, and adults with and without HA with objective symptoms

Characteristics	Total	HA	No HA	P-value†	Objective HA	Subjective/ No HA	P-value†
	N=89	N=46	N=43		N=17	N=72	
Age (years), *mean* (±*SD*)	33 (±13)	32 (±11)	35 (±15)	0.282	32 (±11)	33 (±14)	0.789
Male sex	26 (29)	10 (22)	16 (37)	0.109	2 (12)	24 (33)	0.079
Asthma	47 (53)	25 (54)	22 (51)	0.764	12 (71)	35 (49)	0.103
Atopic dermatitis	52 (58)	31 (67)	21 (49)	0.076	11 (65)	41 (57)	0.559
Allergic rhinitis	79 (89)	46 (100)	33 (77)	0.001	17 (100)	62 (86)	0.103
Birch pollen sensitization*	77 (90)	41 (91)	36 (88)	0.617	14 (82)	63 (91)	0.280
sIgE level in kU_A/L, *median (IQR)*							
Hazelnut extract	4.60 (1.32–14.05)	5.00 (1.33–15.20)	4.30 (1.22–12.90)	0.543	3.46 (0.34–8.85)	5.15 (1.46–15.45)	0.226
Cor a 1	4.70 (0.74–17.70)	4.60 (0.69–18.00)	4.70 (0.76–18.80)	0.974	2.93 (0.22–7.85)	7.30 (1.35–21.78)	0.098
Cor a 8	0.00 (0.00–0.05)	0.00 (0.00–0.02)	0.00 (0.00–0.09)	0.657	0.00 (0.00–0.02)	0.00 (0.00–0.08)	0.313
Cor a 9	0.00 (0.00–0.14)	0.01 (0.00–0.34)	0.00 (0.00–0.08)	0.306	0.14 (0.00–1.59)	0.00 (0.00–0.08)	0.012
Cor a 14	0.00 (0.00–0.09)	0.00 (0.00–0.53)	0.00 (0.00–0.05)	0.472	0.05 (0.00–0.87)	0.00 (0.00–0.06)	0.067

Note: Values are expressed as n (%) unless otherwise specified. *Sensitization to birch pollen as determined by skin prick testing, ImmunoCAP (IgE to birch extract ≥0.35 KU/L) or ISAC (IgE to Bet v 1 ≥0.3 IU/L). Data were available for 86/89 subjects. †Explorative analyses, no correction for multiple testing. HA, hazelnut allergy; SD, standard deviation; IQR, interquartile range.

subjects had HA according to challenge, 3 with objective symptoms. Skin prick test results for hazelnut could be obtained for 7/10 subjects, of which 1 was positive (allergen/histamine wheal ratio ≥0.5), though this patient was not one of those with a positive challenge.

3.2 | Diagnostic accuracy of serology-based testing for HA

There were no significant differences in levels of IgE to hazelnut extract, Cor a 1, 8, 9, or 14 between patients with and without HA, though higher levels of IgE to Cor a 9 and 14 were observed in patients with objective symptoms than in those with no symptoms or subjective symptoms (p=0.01 and p=0.07, respectively) (Table 1, Figure 1).

Subsequently, neither IgE to hazelnut extract nor IgE to individual hazelnut components was found to discriminate well between presence and absence of HA, with AUCs ranging from 0.50 to 0.56 (Figure 2A). The full multivariable logistic regression model containing all IgE variables (hazelnut extract, Cor a 1, 8, 9, and 14) had an AUC of 0.61, and the Lasso regression model, which selected all IgE variables as the optimal predictive combination, had an AUC of 0.58, but these AUC values were not significantly larger than those of any of the individual serology tests ($P_{\text{De Long's test}}$ >0.05). Because of the low AUC values, no cutoffs with optimum positive or negative predictive values were explored.

Table 2 reveals high sensitivity of hazelnut extract and Cor a 1 (range 85–91%) and high specificity of Cor a 8, 9, and 14 (range 77–95%) for HA when considering commonly used cutoffs (0.1 or 0.35 kU_A/L). In clinical practice, this means that hazelnut allergic adults are likely to be sensitized to hazelnut extract and Cor a 1, and hazelnut tolerant adults are unlikely to be sensitized to Cor a 8, 9, and 14. The positive and negative predictive values of hazelnut extract and components were mostly low, and close to the prevalence of HA (46/89, 52%) and hazelnut tolerance (43/89, 48%) in the study population (no matter the cutoff), as expected based on the finding that IgE levels to hazelnut extract and components had limited association with HA (Table 1, Figure 2A).

3.3 | Diagnostic accuracy of serology-based testing for HA with objective symptoms

IgE to Cor a 9 and 14 tended toward an association with objective symptoms, but the corresponding AUCs of 0.68 for Cor a 9 and 0.63 for Cor a 14 still indicated poor discrimination, and were not significantly larger than the AUCs of the other serology tests, which ranged from 0.57 to 0.62 (Figure 2B). Regarding sensitivity, specificity, and positive predictive value, the observations for HA with objective symptoms *vs* HA with subjective symptoms/no HA were similar to those for presence *vs* absence of HA (Table 3). The highest sensitivity was observed for hazelnut extract and Cor a 1 (77–83%), the highest specificity for Cor a 8, 9, and 14 (78–93%),

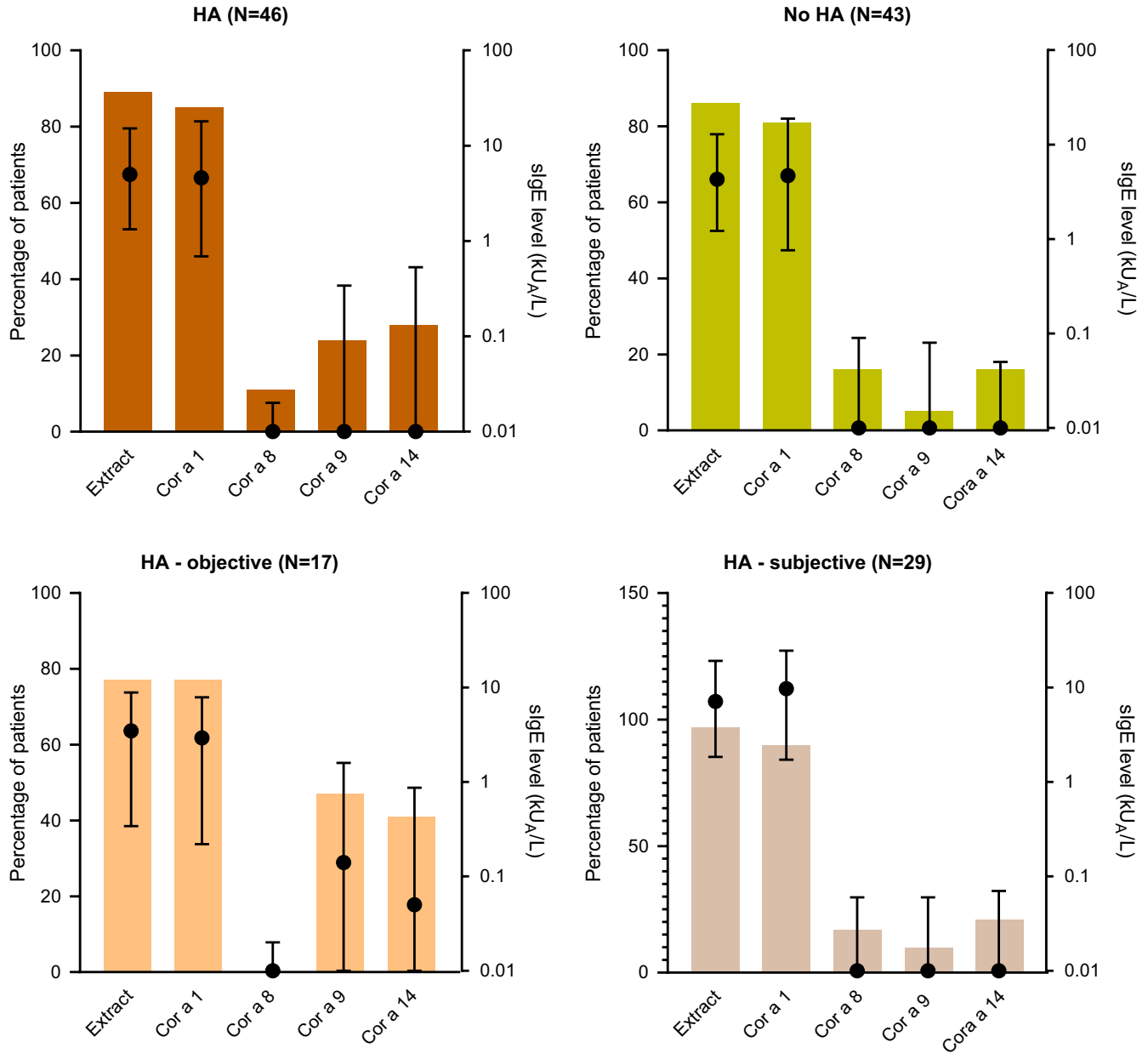


FIGURE 1 Percentage of patients with sensitization to hazelnut extract or components and corresponding IgE levels, stratified to hazelnut challenge outcome. Sensitization was considered present if IgE ≥0.35kU$_A$/L. For IgE levels, medians and interquartile ranges are displayed on a logarithmic scale (base 10)

and positive predictive values of all IgE measurements were low. Although negative predictive values appeared higher (70–88%), they approximately correspond to the prevalence (and therefore a priori probability) of no HA or HA with subjective symptoms in our study population (72/89, 81%) indicating limited added diagnostic value.

4 | DISCUSSION

Testing for IgE sensitization to hazelnut extract, and increasingly often for IgE sensitization to hazelnut allergen components, is standard practice in the diagnostic work-up of HA in adults. However, according to the current study, neither IgE to hazelnut extract nor IgE to hazelnut components Cor a 1, 8, 9, or 14 can accurately predict hazelnut challenge outcomes in Dutch adults with suspected HA.

4.1 | Findings for Cor a 9 and 14 in adults contrast with findings in children

Although IgE levels to hazelnut storage proteins Cor a 9 and Cor a 14 in our data tended to reach higher values in hazelnut allergic than in hazelnut tolerant adults (Table 1, Figure 1A), and in adults with

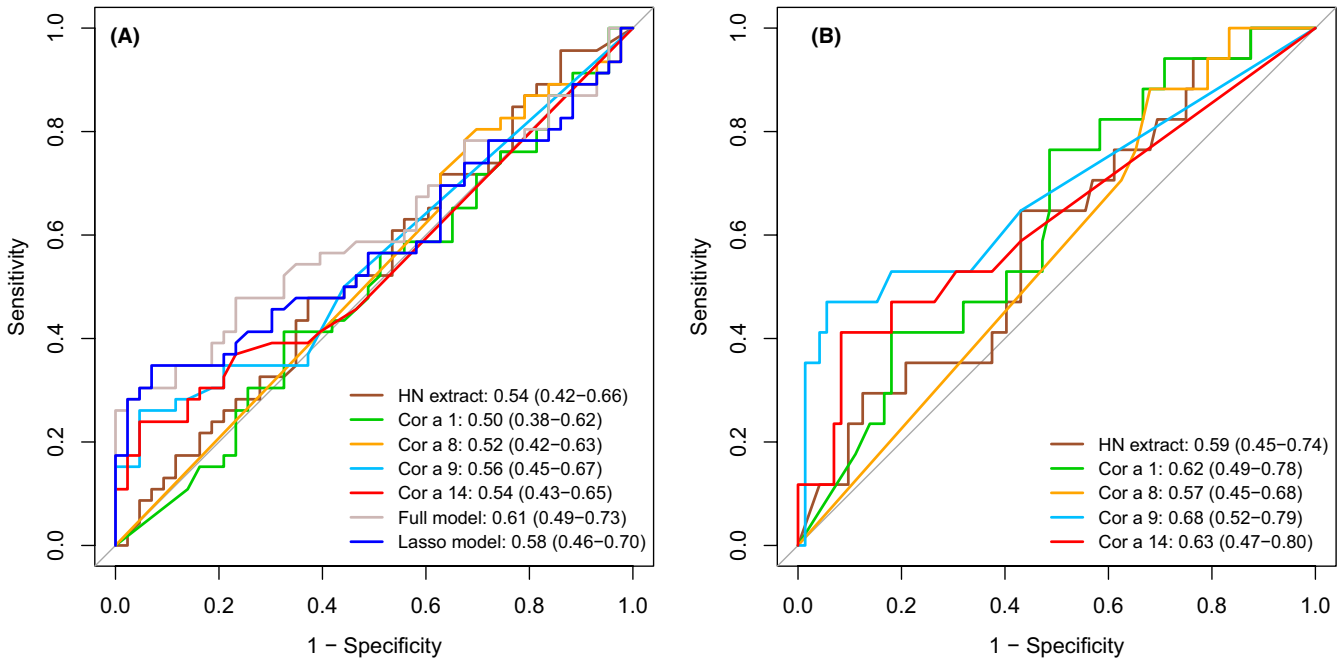


FIGURE 2 ROC curves of serology tests for predicting presence of allergy (A) and objective symptoms (B) during hazelnut challenge. Area under the curve (95% confidence interval) is presented in the plot legends. In Figure A, the full model contains IgE to hazelnut extract, Cor a 1, Cor a 8, Cor a 9, and Cor a 14. All IgE variables were also selected in the Lasso regression model. No multivariable models were developed for outcome B due to the small number of subjects with the outcome of interest (N=17 with objective symptoms). ROC, receiver-operating characteristic

TABLE 2 Measures of diagnostic accuracy for HA of hazelnut extract and CRD at cutoffs applied in daily practice

sIgE to:	Cutoff (kU/L)		HA	No HA	PPV	NPV	Sensitivity	Specificity
Hazelnut extract	0.10	>	42	37	53.2 [42.3–63.8]	60.0 [31.3–83.2]	91.3 [79.7–96.6]	14.0 [6.6–27.3]
		<	4	6				
	0.35	>	41	37	52.6 [41.6–63.3]	54.5 [28.0–78.7]	89.1 [88.0–95.3]	14.0 [6.6–27.3]
		<	5	6				
Cor a 1	0.10	>	39	35	52.7 [41.5–63.7]	53.3 [30.1–75.2]	84.8 [71.8–92.4]	18.6 [9.7–32.6]
		<	7	8				
	0.35	>	39	35	52.7 [41.5–63.7]	53.3 [30.1–75.2]	84.8 [71.8–92.4]	18.6 [9.7–32.6]
		<	7	8				
Cor a 8	0.10	>	8	10	44.4 [24.6–66.3]	46.5 [35.4–58.0]	17.4 [9.1–30.7]	76.7 [62.3–86.8]
		<	38	33				
	0.35	>	5	7	41.7 [19.3–68.0]	46.8 [36.0–57.8]	10.9 [4.7–23.0]	83.7 [70.0–91.9]
		<	41	36				
Cor a 9	0.10	>	16	9	64.0 [44.5–64.8]	53.1 [41.1–64.8]	34.8 [22.7–49.2]	79.1 [64.8–88.6]
		<	30	34				
	0.35	>	11	2	84.6 [57.8–95.7]	53.9 [42.8–64.7]	23.9 [13.9–37.9]	95.3 [84.5–98.7]
		<	35	41				
Cor a 14	0.10	>	14	8	63.6 [43.0–80.3]	52.2 [40.5–63.7]	30.4 [19.1–44.8]	81.4 [67.4–90.3]
		<	32	35				
	0.35	>	13	7	65.0 [43.3–81.9]	52.2 [40.6–63.5]	28.3 [17.3–42.5]	83.7 [70.0–91.9]
		<	33	36				

Note: The positive predictive value (PPV), negative predictive value (NPV), sensitivity, and specificity are presented (% [95% confidence interval]) for each of the investigated serology tests at cutoffs most commonly used in daily practice: 0.1 kU/L and 0.35 kU/L. N, number of patients included; HA, Hazelnut allergy.

TABLE 3 Measures of diagnostic accuracy of hazelnut extract and CRD for objective HA at cutoffs applied in daily practice

sIgE to:	Cutoff (kU/L)		Objective HA	No/Subjective HA	PPV	NPV	Sensitivity	Specificity
Hazelnut extract	0.10	>	14	65	17.7 [10.9–27.6]	70.0 [39.7–89.2]	82.4 [59.0–93.8]	9.7 [4.8–18.7]
		<	3	7				
	0.35	>	14	65	17.7 [10.9–27.6]	70.0 [39.7–89.2]	82.4 [59.0–93.8]	9.7 [4.8–18.7]
		<	3	7				
Cor a 1	0.10	>	13	61	17.6 [10.6–27.8]	73.3 [48.0–89.1]	76.5 [52.7–90.4]	15.3 [8.8–25.3]
		<	4	11				
	0.35	>	13	61	17.6 [10.6–27.8]	73.3 [48.0–89.1]	76.5 [52.7–90.4]	15.3 [8.8–25.3]
		<	4	11				
Cor a 8	0.10	>	2	16	11.1 [3.1–32.8]	78.9 [68.0–86.8]	11.8 [3.3–34.3]	77.8 [66.9–85.8]
		<	15	56				
	0.35	>	0	12	0.0 [0.0–24.2]	77.9 [67.5–85.7]	0.0 [0.0–18.4]	83.3 [73.1–90.2]
		<	17	60				
Cor a 9	0.10	>	9	16	36.0 [20.2–55.5]	87.5 [77.2–93.5]	52.9 [31.0–73.8]	77.8 [66.9–85.8]
		<	8	56				
	0.35	>	8	5	61.5 [35.5–82.3]	88.2 [79.0–93.6]	47.1 [26.6–69.0]	93.1 [84.8–97.0]
		<	9	67				
Cor a 14	0.10	>	8	14	36.4 [19.7–57.0]	86.6 [76.4–92.8]	47.1 [26.2–69.0]	80.6 [70.0–88.0]
		<	9	58				
	0.35	>	7	13	35.0 [18.1–56.7]	85.5 [73.3–91.9]	41.2 [21.6–64.0]	81.9 [71.5–89.1]
		<	10	59				

Note: The positive predictive value (PPV), negative predictive value (NPV), sensitivity, and specificity are presented (% [95% confidence interval]) for each of the investigated serology tests at cutoffs most commonly used in daily practice: 0.1 kU/L and 0.35 kU/L. N, number of patients included; HA, Hazelnut allergy.

objective symptoms than in adults with no or subjective symptoms to hazelnut (Table 2, Figure 1B), the corresponding AUCs indicated poor discriminatory value. This appears to contrast with literature on children, according to which Cor a 9 and 14, in particular, are associated with HA.[9,12,17-21] Regarding prediction of HA in adults, no previous study has, to our knowledge, reported AUC values for hazelnut extract or components for discriminating between presence and absence of HA. However, in agreement with our findings, Hansen et al. found no difference in levels of IgE to Cor a 9 between hazelnut allergic adults and hazelnut tolerant pollen-allergic controls from Denmark, Switzerland, and Spain.[13] Regarding prediction of HA with objective symptoms in adults, Masthoff et al. obtained AUC values of 0.66 and 0.67 for Cor a 9 and 14, respectively, and Datema et al. found AUCs of 0.70 and 0.71, which were similar to our respective AUC estimates of 0.68 and 0.63 (Figure 2B).[10,11] The slightly higher AUCs found by Datema et al. could be explained by their inclusion of children and adults. In comparison, AUC values of Cor a 9 and 14 for entirely pediatric populations from similar parts of Europe as the adults in the current study, are much higher: up to 0.80 for Cor a 9 and 0.89 for Cor a 14 for prediction of HA,[17,20] and 0.87 for Cor a 9 and 0.80 for Cor a 14 for prediction of HA with objective symptoms.[10]

4.2 | Interpretation of AUC values

The accuracy of a test as measured by AUC is a tradeoff between sensitivity and specificity,[22] as was also observed in the results of this study. At cutoffs frequently applied in clinical practice (0.1 and 0.35 kU_A/L), Dutch hazelnut allergic adults are mostly sensitized to hazelnut extract and to Cor a 1 (high sensitivity), but so are hazelnut tolerant adults (low specificity). On the contrary, hazelnut tolerant adults are generally not sensitized to Cor a 9 or 14 (high specificity), but neither are the majority of hazelnut allergic adults (low sensitivity). Although AUC values were not always available, similar patterns of high sensitivity (but low specificity) of hazelnut extract and Cor a 1, and high specificity (but low sensitivity) of Cor a 9 and 14, were observed for prediction of HA or HA with objective symptoms in previously published data in predominantly adult populations from Europe.[10,11,13,14]

4.3 | Cor a 1 sensitization affects the diagnostic value of hazelnut CRD in adults

Patterns of hazelnut component sensitization in pediatric populations differ considerably from those in adults, particularly in that the majority of hazelnut allergic children are sensitized to Cor a 9 or 14, but not to Cor a 1.[9] Eighty-three percent of adults in the current study were sensitized to Cor a 1 (IgE ≥0.35 kU_A/L). Cor a 1 sensitization occurs as a result of cross-reactivity with major birch pollen allergen Bet v 1 and likely affects the diagnostic value of CRD in several ways. First of all, Cor a 1 sensitization itself is poorly associated with hazelnut challenge outcome, because symptoms in subjects with so-called birch pollen-related HA are generally mild or subjective and therefore difficult to interpret, and often depend on the degree of (heat) processing and sometimes on season.[23-25]That said, pollen-related food allergy can sometimes present with objective symptoms and a more severe phenotype,[23,24,26] which may be the case in the 5 subjects who were only sensitized to hazelnut extract and Cor a 1, but still had objective symptoms (Table S3). Secondly, the majority of hazelnut allergic adults in this study (54%) had isolated Cor a 1 sensitization, leading to a much lower sensitivity (and inherently AUC) of Cor a 9 and/or 14. Furthermore, in those subjects with poly-sensitization to hazelnut components, we do not know which component is responsible for symptoms observed during challenge. We did investigate the independent association of each component with HA by including all components as covariates in multivariable analysis, but the power to explore interaction between the different components was lacking. It would be interesting to repeat our research in an even larger population of adults from birch-endemic regions, so as to have more subjects with mono-sensitization to Cor a 9 and 14 for study, and perhaps to explore whether the ratio between IgE level to hazelnut storage proteins and Cor a 1 or birch affects prediction of hazelnut challenge outcome. This would also provide the opportunity to explore the hypothesis that sensitization to birch and related PR-10 proteins may in some way inhibit (the clinical presentation of) sensitization to other plant food allergens, such as storage proteins and LTP.[11,13,27] In addition, research in adult populations from non-birch-endemic European regions could further improve our understanding of hazelnut components' clinical usefulness. Based on aforementioned considerations, AUC values of Cor a 9 and 14 in adults outside birch territory, where Cor a 1 sensitization is uncommon, may be more comparable to those in pediatric populations.

4.4 | Blocking IgG antibodies may affect the diagnostic value of hazelnut CRD in adults

One also ought to realize that ImmunoCAP quantifies allergen-specific IgE levels, but does not take presence of allergen-specific IgG antibodies into account.[28] IgG against food allergens indicates repeated exposure.[29] It is therefore conceivable that food-allergen-specific IgG levels may be higher in adults than in children. Food allergen-specific IgG antibodies, particularly IgG_4 antibodies, have the potential to counteract symptom induction through IgE.[24,30] If Cor a 9 or Cor a 14-specific IgG antibodies block an IgE-induced allergic response in some (but not all) adults with IgE sensitization to Cor a 9 or 14, this phenomenon may also play a role the finding that IgE levels to Cor a 9 or 14 do not predict hazelnut allergy in adults, in contrast to children. Although the necessary data were lacking to explore this hypothesis in current study, further insight could be gained in future studies by assessing allergen-specific IgE/IgG_4 ratios.[31]

4.5 | Findings regarding 2S albumins in HA contrast with findings on 2S albumins in peanut

Another interesting observation deserving attention because of contrast with our findings regarding HA is that IgE to 2S albumins is strongly associated with peanut allergy in Dutch adults, even to the degree that cutoffs for Ara h 2 and 6 with 100% positive predictive values could be obtained.[32,33] On the one hand, this could be because a much larger proportion of peanut allergic than hazelnut allergic adults is sensitized to 2S albumins, and IgE to peanut PR-10 protein Ara h 8 is less clinically relevant for peanut allergy than Cor a 1 for HA. Alternatively, IgE to 2S albumins may be less clinically relevant for HA than for peanut allergy, for example if Cor a 14 sensitization was due to cross-reactivity with 2S albumins in other food sources to which the patient is actually allergic. Cross-reactivity between Cor a 14 and Ara h 2 is low,[34] but between Cor a 14 and walnut 2s albumin Jug r 1 is high.[35] Perhaps the Cor a 14 sensitized individual is really walnut allergic?

4.6 | Strengths and limitations

A limitation of the current study was the retrospective data collection, as well as the necessary selection of patients with conclusive challenge and available serology results. The comparability of included and excluded patients (Table S1) makes it unlikely that this selection resulted in bias, but a certain degree of selection bias due to patients with a history of severe anaphylactic reactions being more likely to refuse challenge testing is a possibility. Another aspect, which may be considered a limitation, is the designation of patients who only displayed allergic symptoms during the open part of the challenge protocol as hazelnut allergic. However, patients with birch-pollen-related HA, a relevant and prevalent phenotype in the Netherlands, often only react to raw hazelnut. Besides, Figure S1 shows that designating these subjects as non-allergic did not result in improved discriminative power of IgE to hazelnut components. Furthermore, considering the small number of patients with objective symptoms in our study population, it is important to realize that our analyses with regard to severity of HA were merely explorative and should be interpreted as such. We also acknowledge that IgE to minor hazelnut allergens, such as 7S globulin Cor a 11, oleosins Cor a 12 and 13, or profilin Cor a 2, was not measured in the current study, but may be present in some patients.[36] The clinical relevance of these allergens in adults is presently unclear[36] and would be an interesting topic for future exploration. Perhaps, such components can help explain the 4 subjects without sensitization to hazelnut extract or components in our study who had positive challenge, of which 3 had objective symptoms (Table S2).

Nonetheless, this study investigated all commercially available ImmunoCAP tests for hazelnut components in a large sample of consecutively presenting adults, who all underwent standardized double-blind placebo-controlled hazelnut challenge. We demonstrate that, although hazelnut allergic adults were generally sensitized to hazelnut extract and Cor a 1, and hazelnut tolerant adults were generally not sensitized Cor a 8, 9, or 14, neither IgE to hazelnut extract nor IgE to hazelnut components can accurately discriminate between presence and absence of HA in adult individuals with suspected HA from birch-endemic regions. Where some studies have been able to present cutoff levels of IgE with optimal positive or negative predictive values for food allergies and therefore the ability to reduce the need for DBPCFC,[32,33,37] the current findings indicate that such IgE cutoffs cannot be determined for HA in adults from birch-endemic regions. Some previous studies suggest exclusion of pollen-allergic subjects to gain true insight into the importance of storage protein sensitization in hazelnut allergic adults,[4,13] but the clinical implications of such a study in birch-endemic Europe would be limited due to the fact that the vast majority of presenting patients are, in fact, allergic to birch pollen. For now, challenge testing is required to diagnose (severity of) hazelnut allergy in adults in birch territory, though future studies increasing the sample size to include more subjects with Cor a 9 or 14 mono-sensitization or more subjects with objective HA or taking the blocking potential of IgG antibodies into account could expand our knowledge on the diagnostic value of hazelnut CRD in adults. Furthermore, it is worth acknowledging that alternative and upcoming diagnostic modalities, such as the basophil activation test (BAT), may be of particular interest in the study population at hand. The BAT is reported to be potentially useful for assessing clinical relevance of sensitization to PR-10 proteins and could help identify whether Cor a 1 sensitization accounts for a hazelnut allergic reaction.[31,38-40]

5 | CONCLUSION

In conclusion, IgE to currently known and commercially available hazelnut allergen components does not accurately predict HA in adults from birch-endemic regions, and DBPCFC currently remains the tool of choice for final diagnosis of HA in this particular population.

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CONFLICTS OF INTEREST

ImmunoCAP material was provided by Thermo Fisher Scientific. All authors declare no further conflicts of interest in relation to this study. Outside submitted work, Dr. Knol reports personal fees from Thermo Fisher Scientific, and Dr. Van Ree reports personal fees from HAL Allergy BV, Citeq BV, Angany Inc., Thermo Fisher Scientific; and grants from the Dutch Science Foundation, European Commission, and Health Holland.

AUTHOR CONTRIBUTIONS

SAL, PMJW, MH, HMK, ACK, RvR, and TML were involved in conceptualization of the study design, data analysis, and article. SAL and

MH were involved in database design, data collection, data cleaning, statistical data analyses and writing of the manuscript. PMJW was involved in supervision of the statistical data analyses. PMJW, HMK, EFK, HGO, RvR, ACK, and TML were involved in critical review of the manuscript.

ORCID

Sarah A. Lyons https://orcid.org/0000-0001-7128-2699
Hannah M. Kansen https://orcid.org/0000-0003-1446-7704

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SUPPORTING INFORMATION

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