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Title:

For hazelnut allergy, component testing of Cor a 9 and Cor a 14 is relevant also in birch endemic areas **Short title: Cor a 9 and Cor a 14 should be used in diagnostics of hazelnut allergy in birch endemic areas**

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Key words: hazelnut allergy, reintroduction, birch pollen sensitization, Cor a 1, whole hazelnut extract

Abbreviations:

AH; Antihistamine

AUC; Area under curve

CI; Confidence interval

NPV; Negative predictive value

PPV; Positive predictive value

ROC; Receiver operating characteristic

SPT; Skin prick test

To the Editor:

Diagnosis of nut allergy is usually based on history with a relevant sensitization to the allergen in question and confirmed by allergen challenge. The diagnosis of hazelnut allergy in birch pollen allergic patients is hampered by cross-reactivity from birch pollen. (1,2) Earlier studies have concluded that Cor a 9 and 14 are the most accurate components for hazelnut allergy diagnostics.(3-5)

Avoidance of hazelnut and a fear of an allergic reaction may affect the quality of life, therefore improved in-vitro diagnosis and in the case of a negative challenge, reintroduction of hazelnuts into the diet is the goal.

The aim was to study component diagnostics for severe hazelnut allergy in children and adolescents in a region with heavy birch pollination. Further, we assessed the success of reintroduction in patients with a negative challenge.

Eighty-two children and adolescents (aged 1-to-19-years) with suspected hazelnut allergy were recruited at the Helsinki University Skin and Allergy Hospital. Eligible patients were sensitized to hazelnut (SPT ≥ 3 mm with ground unpeeled nut or IgE ≥ 0.35 kU/L ImmunoCAP whole hazelnut).

Specific IgE was measured against whole hazelnut extract; Cor a 1 (PR-10 protein), 8 (lipid transfer protein), 9 (11 S globulin), and 14 (2S albumin); birch pollen extract. Skin prick tests were performed with ground hazelnut mixed with 0.9 % saline and birch pollen extract.

At the double-blind placebo-controlled challenges (n=56), on two days, each patient received 5, 50, 200, and 540 mg hazelnut protein, or placebo. (Cumulative dose 795 mg, 6 hazelnuts). At open challenges (n= 26) doses were 5, 25, 50, 100, and 500 mg (Cumulat. 680 mg). Reaction severity was assessed by a threshold-adjusted score.

Supplemental table I. A questionnaire of hazelnut reintroduction was sent to the challenge-negative patients.

The study (326/13/03/03/2010) was approved by the ethics committee at the Helsinki University Hospital of Children and Adolescents. One of the parents and the patient (≥ 6 years old) signed written informed consents. See details on methods in the supplemental text.

Of the 82 patients, 33 (40%) showed reaction to hazelnut. Of the challenge reactions, 6 (18%) were severe, 7 (21%) moderate, and 20 (61%) mild. Four patients received adrenaline, and three of them needed more than one dose of adrenaline (2 to 3).

Birch pollen sensitization (SPT and/or IgE) was present in 68 (83%) patients (s-IgE ≥ 0.35 kU/L and/or SPT ≥ 3 mm) (median sIgE 33.8 kU/L [range 0 to 918], SPT 5 mm [0 to 13]).

(Table I.) Correlations between sIgE and SPT to hazelnut, Cor a 1, and birch pollen were moderate to strong. **(Supplemental table II.)**

Although the challenge result correlated to some extent with IgE to whole hazelnut

extract, moderate-to-severe hazelnut allergy could be most sensitively diagnosed by measuring IgE to both Cor a 9 and 14 (**Figure 1, Table I**) Higher levels of specific IgE to Cor a 9 and 14, predicted lower eliciting dose at the challenge: Spearman's rho -0.514, P= 0.002; and -0.413, P = 0.017, respectively. Cor a 14 was superior to Cor a 9 in predicting moderate-to severe reaction (**Figure 1**). For the cut-off of 2.04 kU/l, the likelihood ratio was 15.9 and the positive predictive value was 75%. Cor a 9 sensitization without Cor a 14 was present in 29 (35%) patients, of whom 25 (86 %) were tolerant at the challenge. By combining the IgE levels of Cor a 9 and 14, diagnostic performance was equally good as with Cor a 14 alone. (**Figure 1, Table I**)

Forty-seven of the 49 challenge-negative patients responded to the questionnaire on reintroduction of hazelnut. Of the respondents, 18 (38%) had introduced hazelnut to their diet (full introduction), 24 (51%) had not actively introduced, but reported not specifically avoiding hazelnut (partial introduction), and 5 (11%) reported avoiding hazelnut carefully (no introduction).

This study suggests that asymptomatic sensitization to hazelnut is common and testing for whole extract specific IgE is not a reliable tool when evaluating suspected hazelnut allergy in an area with heavy birch pollination.

Serum IgE to whole hazelnut extract and Cor a 1 associate with diagnostic markers of birch pollen allergy rather than hazelnut allergy. Even high IgE to whole hazelnut can be explained by cross-reactivity from birch pollen. In this study, patients with high whole hazelnut-IgE- up to 400 kU/L- without concurrent storage protein sensitization tolerated hazelnut. All Cor a 1 monosensitized patients were asymptomatic or experienced only mild oral allergy symptoms. That specific IgE to whole hazelnut correlated strongly with Cor a 1, can be explained by the fact that hazelnut IgE ImmunoCAP is spiked with Cor a 1. (10) Therefore, in a birch-endemic region, high serum specific IgE concentration to hazelnut is most often caused by cross-reactivity from birch pollen. The association of Cor a 1-sensitization and oral allergy syndrome is in line with studies from Central Europe and birch-endemic areas from the US. (3, 4, 6) Obviously, measuring Cor a 1 adds no value to the diagnosis of hazelnut allergy.

Studies from regions with less birch pollen exposure have reported Cor a 14-IgE as the best diagnostic marker for hazelnut allergy. Our results and the cut-off of 2.04 kU/L are in agreement with these reports. (8, 9) However, the diagnostic performance of Cor a 14-

IgE was superior to Cor a 9-IgE, which is in line with most previous studies including children and adults both from regions with and without birch pollination. (5-9) In our patients, sensitization to Cor a 9, without Cor a 14-sensitization, resulted in moderate-to-severe symptoms only in rare cases. By negative IgE testing for Cor a 9 and Cor a 14, true hazelnut allergy can be most accurately ruled out In patients with a history of birch pollen allergy or sensitization to birch pollen, hazelnut sensitization is frequent due to cross-reactivity (PR-10 proteins). Sensitization to hazelnut or Cor a 1 is a poor discriminator for hazelnut allergy in this group. This study however, showed that Cor a 14 and to a lesser extent Cor a 9 was a good predictor for true hazelnut allergy. The combination of Cor a 9 and 14 improves allergenic risk assessment. A follow up on challenges showed that most patients were reassured by a negative challenge and discontinued the unnecessary avoidance of hazelnut.

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Dr. Uotila has nothing to disclose.

Dr. Röntynen has nothing to disclose.

Dr. Pelkonen has nothing to disclose.

M.Sc. Voutilainen has nothing to disclose.

Dr. Kukkonen has nothing to disclose.

Dr. Mäkelä has nothing to disclose.

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Table I.

Baseline clinical characteristics and baseline values of the study population by challenge outcome

	Challenge negative or mild (n=69)	Challenge moderate or severe (n=13)	P value
Gender, female, n (%)	32 (46)	4 (31)	0.37

Age, mean (range)	9.5 (1.9-18.5)	10.0 (4.5-17.4)	0.57
Atopic dermatitis, n (%)	51 (74)	8 (62)	0.50
Asthma, n (%)	34 (49)	5 (39)	0.55
Other food allergy except nuts, n (%)	38 (55)	4 (31)	0.14
Previous history of reacting to some nut, n (%)	46 (67)	9 (69)	0.86
Birch pollen allergic rhinitis	52 (77 %)	6 (46 %)	0.03
Serum total IgE kU/L ^a	490 (17-14830)	346 (56-1225)	0.21
Hazelnut IgE kU/L ^b	18.5 (0.3-470)	30.9 (0.4-521)	0.58
Hazelnut skin prick wheal size mm ^c	6 (0-18)	9 (0-16)	0.02
Birch pollen IgE kU/L ^b	43.3 (0.03-918)	4.52 (0.02-542)	0.050
Birch pollen skin prick wheal size mm ^d	5 (0-13)	4 (0-13)	0.10
Blood eosinophil count E9/I ^d	0.39 (0.08-2.28)	0.27 (0.16-1.39)	0.75
Blood eosinophil percentage ^d	6 (1-29)	5 (3-16)	0.71
IgE to Cor a 1 kU/L ^e	19.1 (0-481)	4.74 (0-230)	0.050
IgE to Cor a 8 kU/L ^a	0.03 (0-6.4)	0.02 (0-0.24)	0.27
IgE to Cor a 9 kU/L	0.21 (0-27.4)	1 (0.07-251)	0.001
IgE to Cor a 14 kU/L	0.04 (0-10.5)	6.3 (0-126)	<0.001
Sensitization to Cor a 9 and Cor a 14, n (%)	5 (7)	8 (62)	<0.0001
Sensitization to Cor a 14 without Cor a 9, n (%)	7 (10)	1 (8)	1
Sensitization to Cor a 9 without Cor a 14, n (%)	25 (36)	4 (31)	0.76
No sensitization to Cor a 9 or Cor a 14, n (%)	32 (46)	0	0.001
Cor a 1 monosensitization	27 (39 %)	0	0.008
Cor a 8 positive	9 (13 %)	0	0.34

Significant P value < 0.05 is calculated by Pearson Chi-square, Fisher's exact, t-test or Mann-Whitney-U test. Values are represented as medians (range) unless specified.

Sensitization is defined as IgE \geq 0.35 kU/L. Number of available results

^a n=75 ^b n=78, ^c n=80, ^d n=76, ^e n=74, ^f n=79

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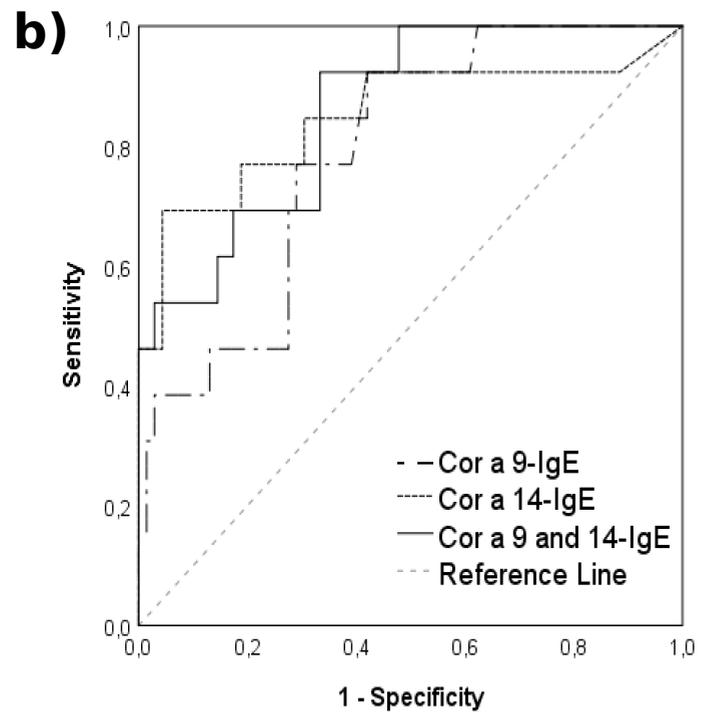
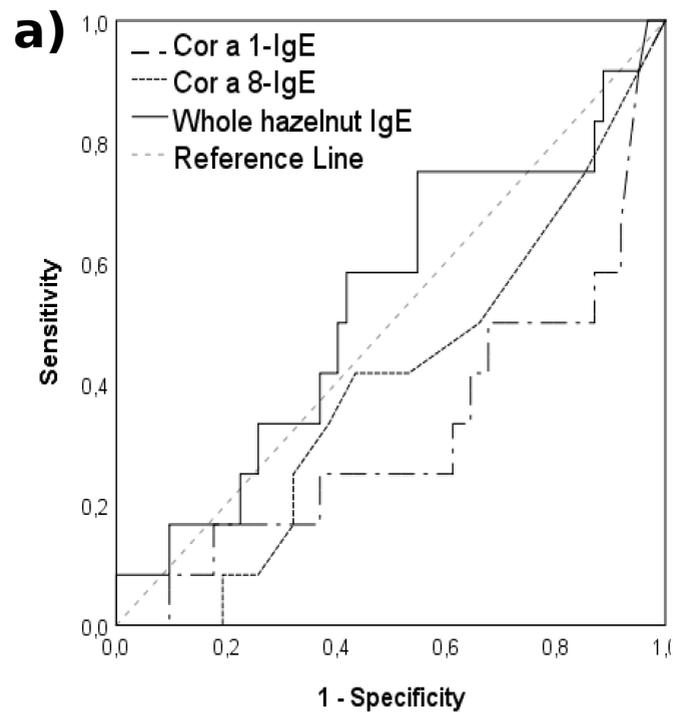
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Figure legends

Figure 1. Receiver operating characteristic (ROC) analysis of IgE to **a)** whole hazelnut, Cor a 1, Cor a 8, and **b-c)** Cor a 9, Cor a 14, and sum of IgE to Cor a 9 and 14 to discriminate negative and mild from the moderate and severe challenge reactions. CI; confidence interval, PPV; positive predictive value, NPV; negative predictive value



c)

	Area under the curve	P value	Asymptotic 95% CI		Cut-off (kU/L)	Sensitivity (%)	Specificity (%)	Likelihood ratio	PPV%	NPV%
			Lower Bound	Upper Bound						
Cor a 9	0.789	0.001	0.67	0.91	0.64	77	71	2.7	33	94
Cor a 14	0.848	<0.001	0.70	0.99	2.04	69	96	15.9	75	94
Cor a 9					0.35	92	57	2.1	29	98
Cor a 14					0.35	69	83	4.0	43	93
Cor a 9					6.27	39	96	8.8	63	89
Cor a 9 + 14	0.860	<0.001	0.76	0.96	10.1	54	96	12.5	70	92