

Suitability of Different Glycoproteins and Test Systems for Detecting Cross-Reactive Carbohydrate Determinant-Specific IgE in Hymenoptera Venom-Allergic Patients

Melanie Mertens Randolph Brehler

Department of Dermatology, University of Münster, Münster, Germany

Key Words

Ascorbic acid oxidase · Bromelain · Cross-reactive carbohydrate determinants · Horseradish peroxidase · MUXF · Venom double positivity

Abstract

Background: In hymenoptera venom allergy, about 75% of detected in vitro double positivity to yellow jacket and honeybee venom is ascribed to specific IgE (sIgE) directed against cross-reactive carbohydrate determinants (CCDs). To date, for the detection of CCD-sIgE, different carbohydrate antigens and methods are used. The most suitable one still has to be identified. **Methods:** Eighty-seven patients with confirmed hymenoptera venom allergy and venom sIgE values of ≥ 0.7 kU/l were investigated. Sixty-five patients showed sIgE reactivity to both yellow jacket and honeybee venom, 22 were venom mono positive and served as controls. Occurrence of CCD-sIgE was determined using bromelain, horseradish peroxidase (HRP) and MUXF³ on system A, and ascorbic acid oxidase (AAO), bromelain and HRP on system B. Further, a reference standard for CCD-sIgE evaluation was created: CCD positivity was assumed when at least 4 of the 6 test results were positive. **Results:** According to the defined reference standard, 45/65 venom double positive patients exhibited CCD-sIgE. Using system A, comparison with the reference standard revealed sensitivity and specificity values of 96 and 97%, respectively, for MUXF³, 100 and 100%, respectively, for bromelain, and 96 and 97%, respectively, for

HRP. Using system B, sensitivity and specificity was 98 and 97%, respectively, for AAO, 62 and 95%, respectively, for bromelain, and 96 and 69%, respectively, for HRP. Results of the 3 test allergens obtained with system A showed strong correlations ($r = 0.932$ – 0.976), whereas results with system B showed lower correlations ($r = 0.714$ – 0.898). **Conclusions:** All 3 test allergens used with system A are suitable for CCD-sIgE detection in hymenoptera venom allergy. With system B, only AAO seems to be a reliable tool.

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Introduction

Allergic reactions due to hymenoptera stings comprise a wide variety of clinical symptoms, including large local (>10 cm in diameter) and generalized skin reactions as well as systemic reactions of varying severity [1]. The only successful, long-lasting treatment of hymenoptera venom allergy is specific immunotherapy (SIT) with the responsible hymenoptera venom(s). Therefore, identification of the culprit insect, most commonly wasps (*Vespula* spp.) or honeybees (*Apis mellifera*), is an important aim [2–5]. Usually the culprit insect can be determined by a combination of patients' history, skin tests and the detection of

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Correspondence to: Dr. Melanie Mertens
Department of Dermatology, University of Münster
Von-Esmarch-Strasse 58, DE-48149 Münster (Germany)
Tel. +49 251 83 56589, Fax +49 251 83 58534
E-Mail allergologie@med.net.uni-muenster.de

venom-specific IgE (sIgE) antibodies in the patients' sera [6]. One major problem in diagnosing hymenoptera venom allergy is the fact that up to 60% of patients' sera show in vitro reactivity to both yellow jacket venom (YJV) and honeybee venom (HBV) [5, 7–10]. Causes for this phenomenon may be true, independent co-sensitizations or cross-reactions between YJV and HBV allergens, either on the basis of sequence homology, for example between HB- and YJ-hyaluronidases [11, 12], or, predominantly, due to the presence of sIgE directed against cross-reactive carbohydrate determinants (CCDs) [5, 8, 13, 14]. Whereas true double sensitization would be treated by immunotherapy with both venoms, in the case of double positivity due to cross-reactions, SIT with the primarily responsible venom alone would be sufficient [3].

CCDs are carbohydrate structures which are widely distributed on glycoproteins from plants and lower animals, and occur in hymenoptera venoms as well as in natural rubber latex, pollen, vegetables and fruits, but not in mammals. About 20% of pollen-allergic and 37% of hymenoptera venom-allergic patients possess CCD-sIgE [5, 15]. In venom double-positive patients the prevalence of CCD-sIgE varies from 56 to 80.5% [5, 8, 13]. Whether or not CCD-sIgE antibodies are able to induce allergic symptoms is still a matter of debate, but they seem to be clinically irrelevant – at least in the majority of CCD-positive patients [5, 16–18]. To date, for detection of CCD-sIgE different glycoprotein-containing extracts and single glycoproteins are used. The most commonly used ones are oilseed rape and natural rubber latex extracts, ascorbic acid oxidase (AAO), bromelain, and horseradish peroxidase (HRP) [5, 8, 13, 14, 16, 18, 19]. Recently, isolated *N*-glycan chains from bromelain (structure MUXF³; fig. 1) were also introduced for detection of CCD-sIgE [5, 14, 18]. Information about carbohydrate structures and number of carbohydrate chains of the glycoproteins analyzed is given in figure 1.

In order to identify the most suitable marker for CCD-sIgE detection in hymenoptera venom-allergic patients, prevalence, sensitivity and specificity of AAO, bromelain, MUXF³ as well as HRP were determined. Moreover, the outcome of two different test systems was compared.

Patients and Methods

Patients

One hundred eleven patients, who had been admitted to our outpatient clinic from 2001 to 2004 due to allergic reactions after a hymenoptera sting and were treated with venom SIT after diagnosis of hymenoptera venom allergy, were included in the study.

Allergy had been confirmed by determination of venom IgE levels and intradermal skin tests with YJV and HBV according to current guidelines [6]. Since CCD-sIgE antibodies are more likely in patients with higher IgE levels, only those patients with a venom-sIgE ≥ 0.7 kU/l were further analyzed ($n = 87$). Sixty-five patients investigated had sIgE antibodies against both YJV and HBV, the remaining 22 patients displayed sIgE only against one venom and served as controls. The sera analyzed were obtained in most cases ($n = 79$) before starting immunotherapy, blood of 8 patients (5 venom double positive, 3 controls) was taken within immunotherapy treatment. However, all sIgE determinations (venom- and CCD-sIgE) were performed with serum of the same blood withdrawal.

Skin Tests

Skin sensitivity to YJV and HBV was determined by intradermal skin testing with serial 10-fold dilutions, ranging from 0.0001 to 1 μ g/ml. A wheal reaction half of the size of the histamine wheal with surrounding erythema was considered as positive. The skin test was stopped at the first venom concentration where a positive result could be achieved. Venoms for skin testing were obtained from ALK-Scherax (Wedel, Germany).

Determination of Specific IgE

For detection of allergen-sIgE two different test systems, namely the UniCAP[®]100 (Phadia, Freiburg, Germany) and the Immulite[®] 2000 system (Siemens Medical Solutions Diagnostic GmbH, Bad Nauheim, Germany) were used according to the manufacturers' instructions. YJV (i3)- and HBV (i1)-sIgE was determined with UniCAP100. Measurement of CCD-sIgE levels, which was done retrospectively for routine diagnosis in mid-2007, was performed with both test systems using AAO (k226), bromelain (k202), HRP (k225/o400) and MUXF³ (o214). With one test system (in the following named system A), allergen-sIgE antibodies against MUXF³, bromelain and HRP were determined. Furthermore, allergen-sIgE antibodies against bromelain and HRP were also measured using the second test system (in the following named system B). Since to our knowledge MUXF³ was not available in mid-2007 for system B, sIgE against AAO (which was not available for system A) was measured additionally. All patient sera were analyzed consecutively and all 3 allergens investigated were measured with system A or B within the same run. The detection limit for both test systems was 0.1 kU/l, values ≥ 0.35 kU/l were considered positive.

Statistical Analyses

All statistical calculations were performed using SPSS version 15.0 (SPSS Inc., Chicago, Ill., USA). Prevalence of CCD-sIgE determined with the different glycoproteins or MUXF³ within double positive and control hymenoptera venom-allergic patients was confirmed by χ^2 test. Differences were considered as significant at a p value ≤ 0.05 .

For estimation of sensitivity and specificity of the CCD-containing allergens investigated, a reference standard for occurrence of CCD-sIgE was defined: when at least 4 of the 6 possible test results obtained with the glycoproteins or MUXF³ were positive, patients were assumed to be CCD-sIgE positive. Double positive patients with less than 3 concordant positive results and control patients were considered CCD-sIgE negative. Since double positive patients with only 3 of 6 concordant positive test results

Glycoprotein (origin)	Glycan structure(s)	No. of glycan chains	Reference
Hymenoptera-venom glycoproteins			
	MMF³F⁶ Manα1₆ - 4GlcNAcβ1 - 4GlcNAcβ1 - Asn Manα1₃ - 4GlcNAcβ1 - 4GlcNAcβ1 - Asn Fucα1	1–4 (depending on the glycoprotein)	Wilson, 2002 [20] Hemmer, 2008 [21]
	M(An⁴F³)F³F⁶ Manα1₆ - 4GlcNAcβ1 - 4GlcNAcβ1 - Asn Manα1₃ - 4GlcNAcβ1 - 4GlcNAcβ1 - Asn Fucα1 GalNAcβ1 - 4GlcNAcβ1 - 2Manα1₃ Fucα1		
Ascorbic acid oxidase (AAO) (zucchini, <i>Cucurbita pepo medullosa</i>)			
	MMX Manα1₆ - 4GlcNAcβ1 - 4GlcNAcβ1 - Asn Manα1₃ - 4GlcNAcβ1 - 4GlcNAcβ1 - Asn Xylβ1	4	Altmann, 1998 [22]
	MMXF³ Manα1₆ - 4GlcNAcβ1 - 4GlcNAcβ1 - Asn Manα1₃ - 4GlcNAcβ1 - 4GlcNAcβ1 - Asn Xylβ1 Fucα1		
Bromelain (pineapple, <i>Ananas comosus</i>)			
	MUXF³ Manα1₆ - 4GlcNAcβ1 - 4GlcNAcβ1 - Asn Manα1₃ - 4GlcNAcβ1 - 4GlcNAcβ1 - Asn Xylβ1 Fucα1	1	Ishihara et al., 1979 [23]
Horseradish peroxidase (HRP) (horseradish, <i>Armoracia rusticana</i>)			
	MMXF³ Manα1₆ - 4GlcNAcβ1 - 4GlcNAcβ1 - Asn Manα1₃ - 4GlcNAcβ1 - 4GlcNAcβ1 - Asn Xylβ1 Fucα1	7–8	Kurosaka et al., 1991 [24] Wuhrer et al., 2005 [25]
MUXF³ (N-glycan chains from bromelain)			
	MUXF³ Manα1₆ - 4GlcNAcβ1 - 4GlcNAcβ1 - Asn Manα1₃ - 4GlcNAcβ1 - 4GlcNAcβ1 - Asn Xylβ1 Fucα1	>1	

Fig. 1. Glycan structures as found on hymenoptera venom glycoproteins and on the test allergens investigated. The structure codes comply with the proglycan system (www.proglycan.com), that is, central *core* mannoses are not counted (compare MMXF³ and MUXF³). U = Unoccupied; Asn = asparagine; Fuc/F = fucose; GalNAc/An = N-acetylgalactosamin; GlcNAc = N-acetylglucosamine; Man/M = mannose; Xyl/X = xylose.

(n = 3) can neither be regarded as CCD-sIgE positive nor CCD-sIgE negative, these patients were excluded. Significance was verified by χ^2 test.

Correlation between results of different test allergens using one test system or between the same test allergen but different detection systems was examined using the Spearman rank correlation test. For these analyses, values <0.1 kU/l were assigned the value 0.1 kU/l.

Results

Characterization of Patients

Eighty-seven patients with confirmed hymenoptera venom allergy and venom-sIgE levels ≥ 0.7 kU/l were included in this study. Sixty-five of these patients (27 female, 38 male) possessed sIgE against both YJV and HBV.

Table 1. Clinical characterization of all hymenoptera venom-allergic patients investigated

	sIgE double positive patients (n = 65)	sIgE mono positive patients (n = 22)
Female	27	15
Male	38	7
History (culprit insect)		
Yellow jacket	60	21
Honeybee	5	1
Allergic reactions		
Grade 1	8	10
Grade 2	32	4
Grade 3	13	4
Grade 4	12	4
Specific IgE		
YJV	65	21
Median, kU/l	7.61 (0.91 to >100)	3.55 (0.7–37.5) ¹
HBV	65	1
Median, kU/l	2.96 (0.74 to >100)	2.28
Skin test		
YJV	43	21
HBV	4	1
YJV and HBV	18	–

Allergic reactions in grades were defined as follows: grade 1, generalized skin reactions; grade 2, symptoms of grade 1 and/or subjective symptoms such as anxiety, dizziness, drowsiness, dyspnoea, headache, nausea, palpitations, and stomach ache; grade 3, symptoms of grade 1 and 2 and/or objective symptoms such as cyanosis, diarrhoea, fall in blood pressure, incontinence, and vomiting; grade 4, loss of consciousness and/or collapse. Figures in parentheses are ranges.

¹ Included in the calculations are only positive responses (sIgE values ≥ 0.35 kU/l).

YJV-sIgE values ranged from 0.91 to >100 kU/l with a median of 7.61 kU/l, HBV-sIgE values ranged from 0.74 to >100 kU/l with a median of 2.96 kU/l (table 1). Sixty of sixty-five patients reported allergic reactions due to yellow jacket stings, the remaining 5 to honeybee stings; severity of reactions is given in table 1. Skin tests revealed that 43/65 patients were mono positive with YJV, 4/65 were mono positive with HBV and 18/65 patients were skin test double positive (table 1). Within this patient group, sIgE determination with CCD-containing test allergens revealed that 45/65 patients (69%) also showed sIgE reactivity to at least 4 of the 6 tested allergens (reference standard; data not shown). Thus, they were defined as true CCD-sIgE positive.

The remaining 22/87 hymenoptera venom-allergic patients investigated (15 female, 7 male) were either mono

positive with YJV (21/22) or HBV (1/22) in sIgE determinations and also mono positive in the skin test (YJV: 21/22, HBV: 1/22; table 1). Furthermore, 1 patient reported allergic reactions due to a honeybee sting, all other patients due to yellow jacket stings. Severity of symptoms is also given in table 1. The honeybee venom-allergic patient had an HBV-sIgE value of 2.28 kU/l, YJV-sIgE in the control group ranged from 0.7 to 37.5 kU/l with a median of 3.55 kU/l (table 1). Specific IgE-mono positive patients served as controls in the study.

All patients investigated received venom immunotherapy; 11 patients with both venoms, all other patients with one venom (data not shown).

Determination of CCD-sIgE with System A

For in vitro double positive patients, sIgE determination with system A revealed that 44/65 patients possessed sIgE against MUXF³, 47/65 patients against bromelain and 46/65 patients against HRP. The median of positive responses was 1.09 kU/l, 1.32 kU/l and 1.515 kU/l, respectively (table 2). Forty-one of sixty-five patients showed concordant positive, 16/65 patients concordant negative results with all three CCD-containing allergens investigated (data not shown). None of the control patients revealed positive results. Estimation of sensitivity and specificity for MUXF³, bromelain and HRP using the reference standard of ≥ 4 concordant-positive sIgE results for verified CCD-sIgE positivity resulted in 96 and 97%, respectively, for MUXF³, 100 and 100%, respectively, for bromelain, and 96 and 97%, respectively, for HRP (table 2). Analyses of the correlation confirmed the results for sensitivity and specificity of all three test allergens investigated: correlation varied between 0.976 (MUXF³ vs. bromelain) and 0.932 (MUXF³ vs. HRP) (fig. 2a), values were highly significant. Importantly, also the amount of sIgE values in double positive patients was very close; the regression line in all three plots was nearly 45 degrees (fig. 2a).

Determination of CCD-sIgE with System B

CCD-sIgE analyses using system B revealed that 45/65 venom double-positive patients additionally showed sIgE reactivity to AAO, 31/65 patients to bromelain and 54/65 patients to HRP. The median values of those patients with positive sIgE against AAO, bromelain and HRP were 1.9 kU/l, 0.945 kU/l and 4.2 kU/l, respectively (table 3). Twenty-six of sixty-five patients showed concordant positive, 9/65 concordant negative results to all three glycoproteins investigated (data not shown). Among control patients, 1/22 displayed sIgE against AAO, 0/22 against bromelain

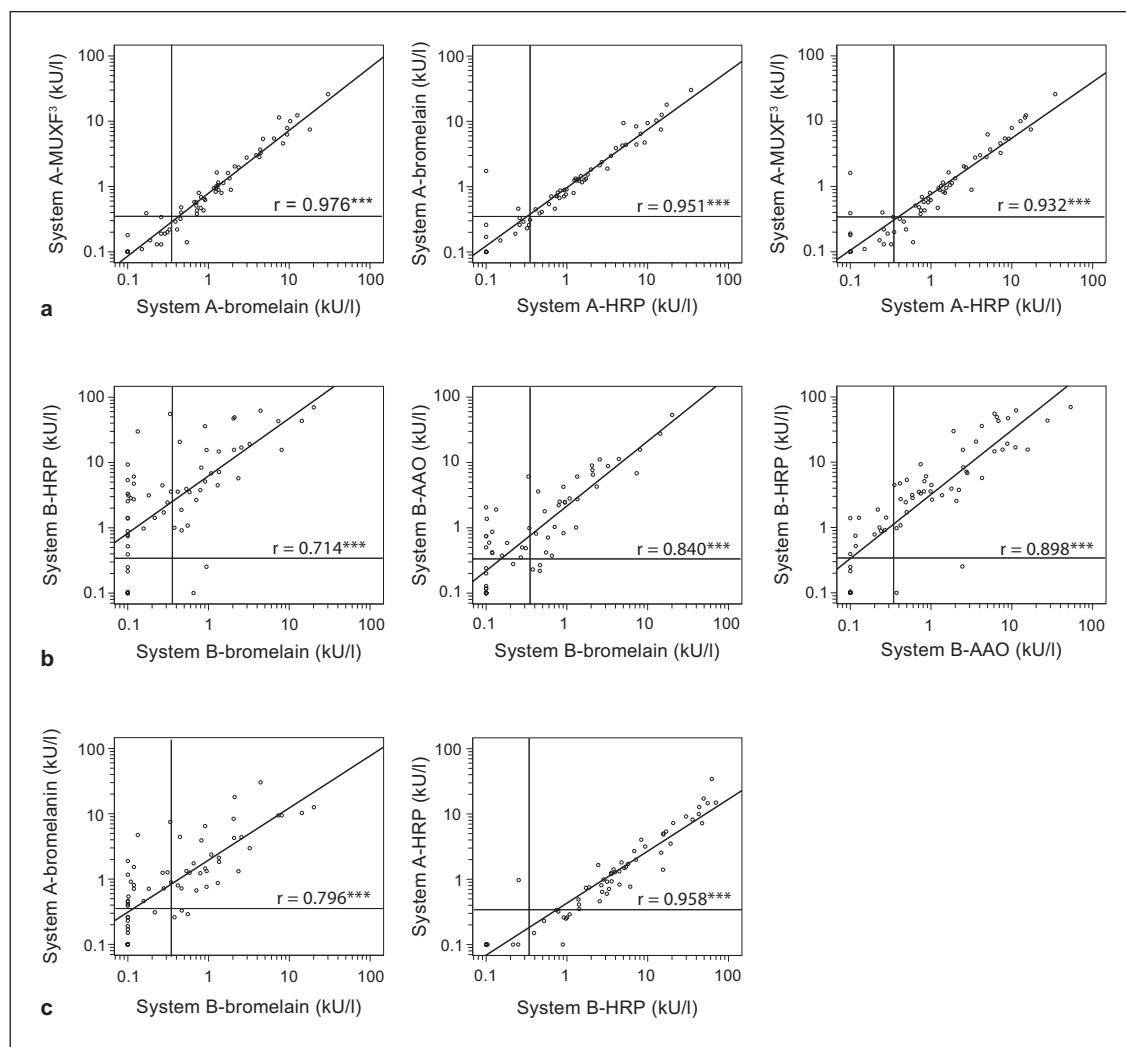


Fig. 2. Correlation between IgE concentrations obtained with the test allergens investigated. **a** Results obtained with system A. **b** Results obtained with system B. **c** Correlation between the same glycoprotein detected with both test systems. Values < 0.1 kU/l were assigned the value 0.1 kU/l. Horizontal and vertical lines indicate the threshold for sIgE positivity at 0.35 kU/l. *** $p < 0.0001$.

Table 2. sIgE determination with system A in patients with venom double and mono positive sIgE results

Allergen	Double positive (n = 65)			Mono positive (n = 22)	p	sensi- tivity	speci- ficity	p
	prevalence	median, kU/l	range, kU/l	prevalence				
MUXF ³	44	1.09	0.38–25.9	0	<0.0001	96%	97%	<0.0001
Bromelain	47	1.32	0.39–30.3	0	<0.0001	100%	100%	<0.0001
HRP	46	1.515	0.35–34.4	0	<0.0001	96%	97%	<0.0001

Only positive responses are included in the calculations (sIgE values ≥ 0.35 kU/l). Sensitivity and specificity were determined by means of the defined reference standard for verified CCD-sIgE occurrence. Statistics were calculated using χ^2 test.

Table 3. sIgE determination with system B in patients with venom double and mono positive sIgE results

Allergen	Double positive (n = 65)			Mono positive (n = 22)			p	Sensitivity	Specificity	p
	prevalence	median, kU/l	range, kU/l	prevalence	median, kU/l	range, kU/l				
AAO	45	1.9	0.35–53.5	1	0.9	–	<0.0001	98%	97%	<0.0001
Bromelain	31	0.945	0.38–20.1	0	–	–	<0.0001	62%	95%	<0.0001
HRP	54	4.2	0.39–69.9	4	0.4125	0.36–0.56	<0.0001	96%	69%	<0.0001

Only positive responses are included in the calculations (sIgE values ≥ 0.35 kU/l). Sensitivity and specificity were determined by means of the defined reference standard for verified CCD-sIgE occurrence. Statistics were calculated using χ^2 test.

and 4/22 against HRP. Estimation of sensitivity and specificity for each of the glycoproteins using the defined reference standard for verified CCD-sIgE positivity yielded in 98 and 97%, respectively, for AAO, 62 and 95%, respectively, for bromelain, and 96 and 69%, respectively, for HRP (table 3). sIgE results obtained with system B showed poorer correlations of $r = 0.714$ (HRP vs. bromelain), 0.840 (AAO vs. bromelain) and 0.898 (HRP vs. AAO) (fig. 2b). Interestingly, sIgE values for HRP were significantly higher compared to those of bromelain and AAO (fig. 2b).

Comparison of System A and B

Comparison of sIgE results achieved with the two different detection systems revealed that correlation of HRP was very high ($r = 0.958$), although HRP-sIgE values detected with system B were significantly higher than those detected with system A (fig. 2c). Correlation of data obtained with bromelain on system A and B was significantly lower ($r = 0.796$).

Discussion

In up to 60% of patients with confirmed hymenoptera venom allergy, sIgE reactivity to both YJV and HBV can be observed [5, 7–10]. Several studies have shown that the major cause for this phenomenon is most likely the presence of sIgE directed against carbohydrate epitopes (CCDs) [5, 8, 14]. Therefore, determination of CCD-sIgE levels in patients with venom double-positive sIgE should be performed.

In the present study the most commonly used glycoproteins for detection of CCD-sIgE in pollen and hymenoptera venom-allergic patients (AAO, bromelain and HRP) and MUXF³ (isolated N-glycan chains from bromelain; recently introduced for detection of CCD-sIgE)

were investigated to identify the most suitable marker for CCD-sIgE determination. Furthermore, the outcome of the applied test allergens on different detection systems in respect to the diagnosis of CCD-sIgE was evaluated.

Using the previously defined reference standard for existence of CCD-sIgE, 45/65 (69%) of the investigated in vitro venom double positive patients possessed CCD-sIgE. This correlates with earlier studies which showed a prevalence of 56–80.5% [5, 8, 13]. Interestingly, Erzen et al. [14] found a lower prevalence of about 40% for CCD-sIgE.

Analyses performed with system A revealed concordant positive results with MUXF³, bromelain and HRP in 41/65 double-positive patients, which is in good accordance with the reference standard. Moreover, sensitivity and specificity values for system A with all CCD-containing allergens tested were very similar. Additionally, also the correlation data fitted well. Thus, both applied glycoproteins as well as MUXF³ on system A are suitable for detection of CCD-sIgE in hymenoptera venom allergy.

For system B only 26/65 in vitro double positive patients showed concordant positive results with all three glycoproteins investigated, resulting in a difference of 19 patients compared to the reference standard. The major reason for this finding is most likely the divergent outcome obtained with HRP and bromelain: while the sIgE determination with HRP seems to have a lower specificity (69%, also reflected in 4/22 positive control patients), determination with bromelain seems to have a lower sensitivity (62%). Values obtained with AAO matched best to the results obtained with the test allergens applied on system A and the reference standard.

Interestingly, correlation between HRP-sIgE results obtained with the two different test systems was very strong. Nevertheless, the scatter plot in figure 2 underlines that all values obtained for HRP-sIgE with system B

were too high, resulting not only in false-positive results in the venom double-positive group but also in the control group. Due to the lower prevalence of bromelain-sIgE achieved with system B compared to system A, correlation of data was poor. Because results obtained with system A were very similar, whereas those obtained with system B were heterogeneous (HRP too high, bromelain too low, AAO in the same range), differences in the detection methods of system A and B (bound allergen versus liquid allergen) seem to have no influence on the results.

As shown in figure 1, the glycoproteins investigated vary in their glycan structures as well as in their number of glycan chains. Since determination with system A showed nearly the same prevalences with both glycoproteins and MUXF³, and correlation of sIgE values was also close, both parameters (glycan structure and number) seem to have no influence on either the number of detected CCD-positive patients or the amount of CCD-sIgE. In contrast to hymenoptera venom glycoproteins which possess N-glycans with only immunogenic fucose residues (equivalent to CCD epitopes), plant-specific glycoproteins possess immunogenic fucose and xylose residues (also equivalent to CCD epitopes) which can result in production of sIgE antibodies against both fucose- and xylose-containing epitopes in sensitized pollen- or food-allergic patients [17]. Thus, a similar study with pollen-allergic subjects may have a different outcome. Differences between results obtained with system B appear to be more a problem of the evaluation (such as wrong cutoff) than glycoprotein based.

In addition to the presence of CCD-sIgE in patient sera, in vitro venom double positivity can also be based on partial sequence identity of venom (glyco)proteins, for example, hyaluronidases or dipeptidyl peptidases [11, 12, 26], or true independent co-sensitizations to YJV and HBV. Importantly, the presence of CCD-sIgE in patients with double positive sIgE does not exclude true co-sensitization which would necessitate SIT with both venoms, whereas in the case of double positivity due to cross-reactions alone (CCD and/or peptide dependent), SIT with the primarily responsible venom would be sufficient [3].

Today, the problem with interfering CCD-sIgE antibodies cannot only be solved by detection of CCD-sIgE via glycoprotein-containing extracts or single glycoproteins (as discussed here) but also by circumvention of CCD-epitopes on the test allergens using recombinant, *Escherichia coli*-expressed, nonglycosylated allergens, like Api m 1, Api m 2 and Ves v 5 for hymenoptera venom allergy [5, 27]. This application allows detection of only protein- (and not carbohydrate-) sIgE antibodies. More-

over, it is discussed that sIgE antibodies directed against species-specific, recombinant, nonglycosylated hymenoptera venom allergens indicate true co-sensitizations [5]. However, current observations lead to the assumption that determination of venom-sIgE with commercially available recombinant hymenoptera venom allergens is less sensitive compared with whole venom extracts. Additionally, since it remains possible that in some patients CCD-sIgE may have clinical relevance [8], those patients would not benefit from diagnosis using recombinant, nonglycosylated allergens.

To the best of our knowledge, this is the first study that compared the most common test allergens used for CCD-sIgE detection. Our data revealed that using system B bromelain was less sensitive and HRP less specific compared to all other test allergens and to the other test system evaluated. Application of AAO gave the best results for CCD-sIgE detection with system B. Furthermore, these results fitted best to the results obtained with system A and to the created reference standard. Results obtained using system A showed strong correlation to the reference standard and to each other. Moreover, sensitivity and specificity were good overall. Thus, MUXF³, bromelain and HRP in combination with system A are all suitable test allergens for CCD-sIgE detection in hymenoptera venom-allergic patients. Using system B, only AAO is appropriate for CCD-sIgE determination.

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References

- 1 Bilò BM, Bonifazi F: Epidemiology of insect-venom anaphylaxis. *Curr Opin Allergy Clin Immunol* 2008;8:330–337.
- 2 Brehler R, Wolf H, Kütting B, Schnitker J, Luger T: Safety of a two-day ultrarush insect venom immunotherapy protocol in comparison with protocols of longer duration and involving a larger number of injections. *J Allergy Clin Immunol* 2000;105:1231–1235.
- 3 Bonifazi F, Jutel M, Bilò BM, Birnbaum J, Muller U: Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. *Allergy* 2005;60:1459–1470.
- 4 Bilò BM, Bonifazi F: Advances in hymenoptera venom immunotherapy. *Curr Opin Allergy Clin Immunol* 2007;7:567–573.

- 5 Müller UR, Johansen N, Petersen AB, Fromberg-Nielsen J, Haeberli G: Hymenoptera venom allergy: analysis of double positivity to honey bee and *Vespula* venom by estimation of IgE antibodies to species-specific major allergens Api m1 and Ves v5. *Allergy* 2009;64:543–548.
- 6 Biló BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JNG: Diagnosis of hymenoptera venom allergy. *Allergy* 2005;60:1339–1349.
- 7 Ebo DG, Hagendorens MM, Bridts CH, De Clerck LS, Stevens WJ: Hymenoptera venom allergy: taking the sting out of difficult cases. *J Investig Allergol Clin Immunol* 2007;17:357–360.
- 8 Jappe U, Raulf-Heimsoth M, Hoffmann M, Burow G, Hübsch-Müller C, Enk A: In vitro hymenoptera venom allergy diagnosis: improved by screening for cross-reactive carbohydrate determinants and reciprocal inhibition. *Allergy* 2006;61:1220–1229.
- 9 Egner W, Ward C, Brown DL, Ewan PW: The frequency and clinical significance of specific IgE to both wasp (*Vespula*) and honeybee (*Apis*) venoms in the same patient. *Clin Exp Allergy* 1998;28:26–34.
- 10 Hoffman DR, Miller JS, Sutton JL: Hymenoptera venom allergy: a geographic study. *Ann Allergy* 1980;45:276–279.
- 11 Jin C, Focke M, Léonard R, Jarisch R, Altmann F, Hemmer W: Reassessing the role of hyaluronidase in yellow jacket venom allergy. *J Allergy Clin Immunol* 2010;125:184–190.
- 12 Seismann H, Blank S, Braren I, Greunke K, Cifuentes L, Grunwald T, Bredehorst R, Ollert M, Spillner E: Dissecting cross-reactivity in hymenoptera venom allergy by circumvention of alpha-1,3-core fucosylation. *Mol Immunol* 2010;47:799–808.
- 13 Hemmer W, Focke M, Kolarich D, Wilson IB, Altmann F, Wöhl S, Götz M, Jarisch R: Antibody binding to venom carbohydrates is a frequent cause for double positivity to honeybee and yellow jacket venom in patients with stinging-insect allergy. *J Allergy Clin Immunol* 2001;108:1045–1052.
- 14 Erzen R, Korosec P, Silar M, Music E, Kosnik M: Carbohydrate epitopes as a cause of cross-reactivity in patients allergic to Hymenoptera venom. *Wien Klin Wochenschr* 2009;121:349–352.
- 15 Mari A: IgE to cross-reactive carbohydrate determinants: analysis of the distribution and appraisal of the in vivo and in vitro reactivity. *Int Arch Allergy Immunol* 2002;129:286–295.
- 16 Mari A, Ooievaar-de Heer P, Scala E, Giani M, Pirrotta L, Zuidmeer L, Bethell D, van Ree R: Evaluation by double-blind placebo-controlled oral challenge of the clinical relevance of IgE antibodies against plant glycans. *Allergy* 2008;63:891–896.
- 17 Altmann F: The role of protein glycosylation in allergy. *Int Arch Allergy Immunol* 2007;142:99–115.
- 18 Mertens M, Amler S, Moerschbacher BM, Brehler R: Cross-reactive carbohydrate determinants strongly affect the results of the basophil activation test in hymenoptera-venom allergy. *Clin Exp Allergy* 2010;40:1333–1345.
- 19 Guilloux L, Morisset M, Codreanu F, Parisot L, Moneret-Vautrin DA: Peanut allergy diagnosis in the context of grass pollen sensitization for 125 patients: roles of peanut and cross-reactive carbohydrate determinants specific IgE. *Int Arch Allergy Immunol* 2009;149:91–97.
- 20 Wilson IBH: Glycosylation of proteins in plants and invertebrates. *Curr Opin Struct Biol* 2002;12:569–577.
- 21 Hemmer W: Cross-reactivity to honeybee and wasp venom. *Hautarzt* 2008;59:194–199.
- 22 Altmann F: Structures of the N-linked carbohydrate of ascorbic acid oxidase from zucchini. *Glycoconj J* 1998;15:79–82.
- 23 Ishihara H, Takahashi N, Oguri S, Tejima S: Complete structure of the carbohydrate moiety of stem bromelain. An application of the almond glycopeptidase for structural studies of glycopeptides. *J Biol Chem* 1979;254:10715–10719.
- 24 Kurosaka A, Yano A, Itoh N, Kuroda Y, Nakagawa T, Kawasaki T: The structure of a neural specific carbohydrate epitope of horseradish peroxidase recognized by anti-horseradish peroxidase antiserum. *J Biol Chem* 1991;266:4168–4172.
- 25 Wuhrer M, Balog CIA, Koeleman CAM, Deelder AM, Hokke CH: New features of site-specific horseradish peroxidase (HRP) glycosylation uncovered by nano-LC-MS with repeated ion-isolation/fragmentation cycles. *Biochim Biophys Acta* 2005;1723:229–239.
- 26 Blank S, Seismann H, Bockisch B, Braren I, Cifuentes L, McIntyre M, Rühl D, Ring J, Bredehorst R, Ollert MW, Grunwald T, Spillner E: Identification, recombinant expression, and characterization of the 100 kDa high molecular weight Hymenoptera venom allergens Api m 5 and Ves v 3. *J Immunol* 2010;184:5403–5413.
- 27 Mittermann I, Zidarn M, Silar M, Markovic-Housley Z, Aberer W, Korosec P, Kosnik M, Valenta RJ: Recombinant allergen-based IgE testing to distinguish bee and wasp allergy. *Allergy Clin Immunol* 2010;125:1300–1307.