

Minor Interference of Cross-Reactive Carbohydrates with the Diagnosis of Respiratory Allergy in Standard Clinical Conditions

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Key Words

Glycoproteins · Carbohydrate epitopes · Immunoglobulin E · Hymenoptera venom

Abstract

Background: Immunoglobulin E (IgE) to N-glycans from plant and invertebrate glycoproteins induces extensive in vitro cross-reactivity. This study investigates the prevalence and diagnostic relevance of IgE to these N-glycans [cross-reactive carbohydrate determinants (CCDs)] in patients with suspicion of respiratory allergy. **Methods:** A total of 1,025 adult subjects with symptoms of rhinitis and/or asthma from a reference allergy clinic were studied. Determinations included a structured questionnaire, skin prick tests (SPT), total IgE, a multiallergen IgE test and specific IgE (sIgE) to bromelain, MUXF (the bromelain-type N-glycan) and honeybee phospholipase-A2. Inhibition studies with CCDs were performed in selected cases. **Results:** The prevalence of CCD sensitization (MUXF sIgE and/or bromelain-sIgE ≥ 0.1 kU_A/l) was 18.0%. Male sex and atopy (SPT positivity) were associated with CCD sensitization. Sensitization was more frequent in patients sensitized to pollens than in those sensitized to mites, the most common inhalant allergens in the area. A history of Hymenoptera stings was associated with CCD sensi-

tization and multiallergen IgE test positivity. CCD sensitization was not significantly associated with age, rural residence, alcohol consumption or smoking. Only 58 patients (5.6%) showed CCD-sIgE levels ≥ 0.35 kU_A/l. CCD-induced inhibition of pollen-sIgE or mite-sIgE in patients with respective positive SPT was minimal or absent in most cases. **Conclusions:** In this population of predominantly mite-allergic patients, CCD sensitization is relatively rare and CCD-sIgE levels are low. Thus, CCDs do not represent a major obstacle for the diagnosis of respiratory allergy in a specialized setting. Hymenoptera stings are associated with CCD sensitization.

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Introduction

Immunoglobulin E (IgE) to N-glycans from plant and invertebrate glycoproteins induces in vitro cross-reactivity that extends beyond the usual protein boundaries [1–3]. Hence, these N-glycans are commonly known as cross-reactive carbohydrate determinants (CCDs). Plant and invertebrate N-glycans have a backbone of two N-acetyl-glucosamines and a mannose [1–3]. Common additions to this backbone include MMXF (mannose, man-

nose, xylose, fucose), MUXF (mannose, unsubstituted, xylose, fucose) and MMF (mannose, mannose, fucose) [1–3]. Xylosylation and, in particular, fucosylation of the innermost N-acetylglucosamine are crucial to the immunogenicity of N-glycans [1–3]. According to current evidence, these CCDs have poor activity in vivo and therefore CCD sensitization is considered clinically irrelevant [1–4]. Recently, mammalian N-glycans with an alpha-gal structure have been reported to induce systemic allergic reactions in IgE-sensitized individuals [5]. From a diagnostic standpoint, widespread cross-reactivity induced by classic CCDs may interfere with determinations of IgE to plant and invertebrate allergens [1–3]. Allergy screening tests that use several allergens in a single IgE assay are particularly prone to CCD interference [3].

The prevalence of CCD sensitization varies by population. In the general adult population, the prevalence of IgE (≥ 0.35 kU_A/l) to a CCD marker (MUXF, the bromelain-type N-glycan) ranges from 2.1% in an urban population in Denmark [6] to 5.6% in a mostly rural population in Spain [7]. In an allergy clinic in Italy, the prevalence of IgE (>0.4 kU_A/l) to bromelain reached 23% [8]. Risk factors for CCD sensitization include atopy, particularly pollen sensitization [6–10]. The prevalence of CCD sensitization is highest among patients with a Hymenoptera venom allergy, particularly in patients with double (honeybee and wasp) IgE reactivity [11–15]. From the initial CCD description in 1984 [16], Hymenoptera stings have been suspected as a source of CCD sensitization [15]. Additional factors for sensitization may include alcohol consumption, which increases total IgE levels [17–21] and is consistently associated with CCD sensitization [6, 7, 22, 23]. This study aimed to investigate the prevalence of IgE-mediated CCD sensitization in patients who attended an allergy clinic due to suspicion of respiratory allergy. In addition, we investigated factors associated with CCD sensitization, including demographics, lifestyle factors and Hymenoptera stings.

Subjects and Methods

Study Population

This study consecutively enrolled adult individuals who attended for the first time an allergy clinic at a reference university hospital in Northwest Spain from February 2008 to June 2009. Patients were submitted from either primary care or hospital departments for study of symptoms of potential respiratory allergy (rhinitis and/or asthma). The hospital covers an area of approximately 500,000 people; nearly 90,000 live in the city of Santiago de Compostela, and the remainder live in primarily rural areas. The weather is predominately warm and humid, and mites are the

most relevant inhalant allergens [24]. From a total of 1,073 eligible individuals, 3 patients refused to participate and 45 who accepted did not complete the required laboratory studies. Thus, the study included 1,025 individuals (35.2% male) with a median age of 32 years (range: 18–79). The female predominance reflects the gender proportion in the clinic studied. The study was approved by the institutional review board and conformed to the Helsinki declaration. Written informed consent was documented for all individuals.

Main Determinations

Questionnaire

In addition to the routine clinical history, all patients underwent a physician-administered, structured questionnaire, which included:

- Residence, classified as urban when living in the city of Santiago de Compostela and rural otherwise.
- Smoking status, classified as never smokers, ex-smokers and current smokers; consumers of at least 1 cigarette per day were considered smokers.
- Alcohol consumption, registered as the weekly number of drinking units (a glass of wine, a bottle of beer or a drink of spirits), approximately equivalent to 10 g of alcohol [25].
- History of Hymenoptera stings, evaluated with the yes/no question: ‘Have you ever been stung by a honeybee or a wasp?’ In affirmative cases, the time since the last sting was recorded. The questionnaire did not include the number of stings and did not try to differentiate between honeybee and wasp stings.

Skin Prick Tests

The standard panel of skin prick tests (SPTs) included mites (*Dermatophagoides pteronyssinus*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*), pollens and pollen-related molecules (*Phleum pratense*, *Cynodon dactylon*, *Phragmites communis*, *Anthoxanthum odoratum*, *Plantago lanceolata*, *Parietaria judaica*, *Betula alba*, *Alnus glutinosa*, *Olea europaea*, *Cupressus sempervirens*, *Platanus acerifolia*, *Quercus robur*, profilin, polcalcin and peach lipid transfer protein), animal danders (cat and dog) and molds (*Alternaria alternata* and *Aspergillus* spp.; ALK-Abelló, Spain). Histamine and normal saline were used as positive and negative controls, respectively. Wheals >3 mm were considered positive. Patients with any positive SPT ($n = 745$, 72.7%) were considered atopic [26]. Among atopics, the most commonly positive SPT were mites (615, 82.5%), followed by pollens (433, 58.1%) and animal dander (177, 23.7%).

Serum Total IgE

Total IgE was measured by latex-enhanced nephelometry in a BN-II analyzer (Siemens, Marburg, Germany). Levels >170 IU/ml were considered abnormal [20].

Serum Specific IgE

Allergen-specific IgE (sIgE) was measured in the ImmunoCAP-250 analyzer (Phadia, Uppsala, Sweden). The study included the systematic determination of sIgE to:

- Two CCD markers, bromelain and MUXF. Bromelain is a glycoprotein from pineapple (*Ananas comosus*, k202) which contains a single MUXF-type glycan. The bromelain protein induces allergic sensitization very rarely and is therefore consid-

ered as a marker of CCD sensitization [1–4]. MUXF (Ro214) is the isolated glycan part from bromelain.

- Honeybee phospholipase-A2 (PLA2, k203), which mainly contains MMF-type glycans and protein epitopes that are able to induce allergic sensitization. Thus, the results of PLA2-sIgE were analyzed separately.

The reportable range for this method is 0.02–100 kU_A/l, although values <0.1 kU_A/l are extrapolated. The measuring range is 0.1–100 kU_A/l and the manufacturer considers values ≥0.1 kU_A/l as positive. Thus, patients with bromelain-sIgE and/or MUXF-sIgE ≥0.1 kU_A/l were considered as having CCD sensitization, similar to previous epidemiological studies [6, 22].

In addition, a PhadiatopTM test was performed in all patients. This multiallergen IgE screening test for respiratory allergy [27] consists of a single assay involving a mixture of relevant inhalant allergens. Following the manufacturer's instructions, results in serum samples are dichotomous (positive/negative).

Inhibition Studies

The ability of CCDs to inhibit IgE reactivity to *D. pteronyssinus* and timothy grass (*P. pratense*), the most representative mite and pollen in the area, respectively, was performed in selected individuals with high CCD-sIgE level (>1 kU_A/l) and available serum sample. As previously described [7], the inhibitor was a neoglycoprotein consisting of bromelain glycopeptides with a MUXF structure conjugated to bovine serum albumin (BSA), provided by Dr. F. Altmann, Institute of Chemistry, Universitaet fuer Bodenkultur (Vienna, Austria). Briefly, 150 µl of serum was pre-incubated with 150 µl of the PBS-buffered MUXF-BSA solution (100 µg/ml). The latter was chosen after testing several concentrations (4, 20 and 100 µg/ml) because it was capable of inhibiting 100% of MUXF-sIgE reactivity from positive sera. After overnight coinubation at 4°C, the mixture was assayed in the ImmunoCAP-250 analyzer. The degree of inhibition was expressed as a percentage of the whole.

Statistical Analyses

The χ^2 test was used to compare proportions. The Mann-Whitney test was used to compare numerical variables between groups. The Spearman rank test was used to assess correlation. Only positive cases with positive sIgE were included in correlation studies. Logistic regression was employed for multivariate analyses.

Results

Prevalence of CCD Sensitization

The prevalence of CCD sensitization [IgE ≥0.1 kU_A/l to any CCD marker (bromelain and/or MUXF)] was 18.0% (95% CI: 15.7–20.4; fig. 1). Titers of CCD-sIgE were generally low. Only 58 patients (5.6%) showed MUXF-sIgE and/or bromelain-sIgE class 1 or higher (≥0.35 kU_A/l) and only 8 patients (0.8%) showed sIgE class 3 or higher (≥3.5 kU_A/l).

There was a correlation between MUXF-sIgE and bromelain-sIgE (Rho 0.822, $p < 0.001$), which was particularly evident with high values (fig. 1). There was also a

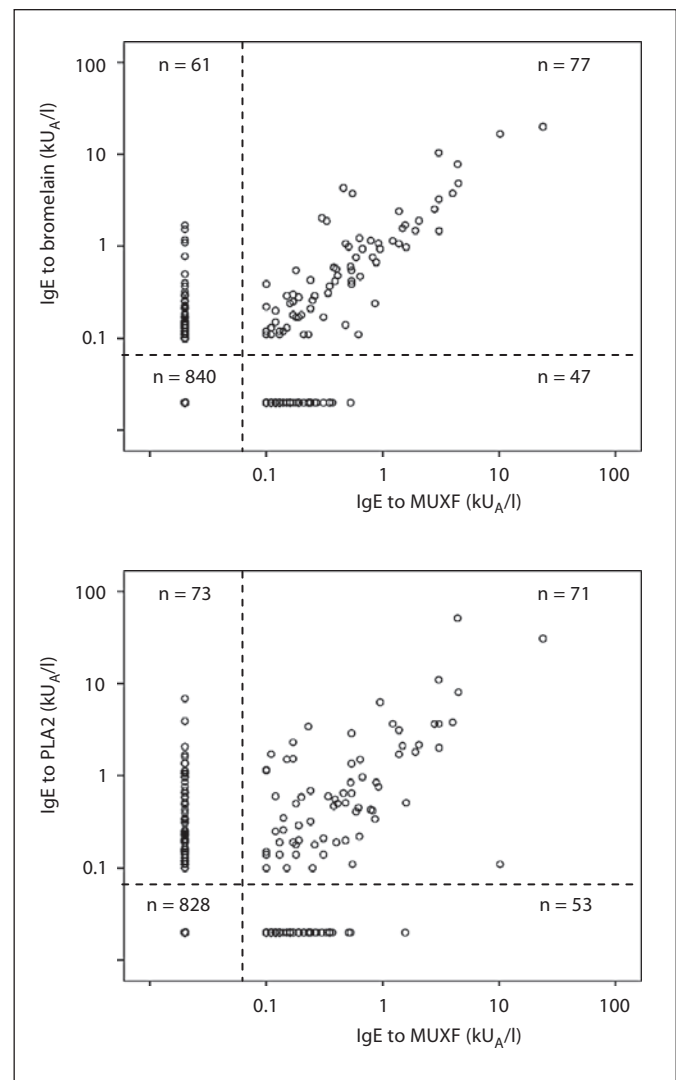


Fig. 1. Correlation between IgE to MUXF and IgE to bromelain and IgE to honeybee PLA2. Dotted lines represent the detection level.

significant correlation between PLA2-sIgE and bromelain-sIgE (Rho 0.468, $p < 0.001$), as well as between PLA2-sIgE and MUXF-sIgE (Rho 0.547, $p < 0.001$; fig. 1).

Association of CCD Sensitization with Atopy

The prevalence of CCD sensitization was higher in atopics than in nonatopics (169/745, 22.7%, vs. 16/280, 5.7%, respectively, $p < 0.001$). Among atopics, the prevalence of CCD sensitization was higher in those sensitized to pollens than in those sensitized to mites (table 1). The prevalence of CCD sensitization was similar in patients monosensitized to mites and in those cosensitized to

Table 1. Prevalence of positivity of CCD markers in patients stratified by SPT results

Positive SPT	IgE ≥ 0.1 kU _A /l to MUXF, % (n)	IgE ≥ 0.1 kU _A /l to bromelain, % (n)	IgE ≥ 0.1 kU _A /l to MUXF and/or bromelain, % (n)	IgE ≥ 0.1 kU _A /l to PLA2, % (n)
None (nonatopic)	3.9 (11/280)**	4.3 (12/280)*	5.7 (16/280)***	8.2 (23/280)
Mites only (ref.)	9.9 (25/253)	8.7 (22/253)	14.2 (36/253)	13.4 (34/253)
Mites plus animal dander	9.6 (5/52)	13.5 (7/52)	19.2 (10/52)	13.5 (7/52)
Pollens only	18.9 (21/111)*	19.8 (22/111)**	26.1 (29/111)**	12.6 (14/111)
Pollens plus any other	18.9 (61/322)**	23.3 (75/322)***	28.9 (93/322)***	20.5 (66/322)*

Patients exclusively sensitized to animal dander (n = 6) or to moulds (n = 1) were not included. * p < 0.05; ** p < 0.01; *** p < 0.001 with respect to the reference category (ref., individuals exclusively sensitized to mites).

Table 2. Inhibition studies of IgE reactivity to *D. pteronyssinus* and timothy grass (*P. pratense*), the most representative mite and pollen in the area, respectively, in selected cases with high levels of IgE to CCDs

Patient No.	Age years/sex	Respiratory disease	SPT to DP	SPT to TG	Additional positive SPTs	IgE to MUXF kU _A /l	IgE to DP kU _A /l	IgE to TG kU _A /l	Inhibition of IgE to DP with MUXF-BSA, %	Inhibition of IgE to TG with MUXF-BSA, %
1	27/F	R	positive	positive	AO, Po, Pe	2.05	3.59	66.0	<10	<10
2	18/M	R, A	positive	positive	AO, PC, CD, PL, Po	3.03	1.43	98.0	<10	<10
3	22/F	R, A	positive	positive	AO, PC, CD, PL, PJ, AG, OE, QR, CS, PA, Po, LTP, D, C, LD, TP	1.56	1.42	78.3	<10	<10
4	24/F	R	positive	positive	AO, PC, CD, PL, PJ, BA, AG, OE, QR, CS, PA, Po, LTP, D	3.05	1.03	93.0	<10	<10
5	39/F	R, A	negative	positive	AO, PC, CD, PL, PJ	3.99	0.08	94.1	<10	<10
6	37/M	R, A	negative	positive	AO, PC, CD, PL, BA, AG, QR, Pe, D, C	1.38	0.16	34.4	<10	<10
7	32/M	R	negative	positive	AO	4.47	0.68	8.36	91	95
8	37/M	R	positive	negative	LD, TP	1.48	73.2	6.05	<10	<10
9	68/F	R	negative	negative	LD, TP	1.38	1.89	1.16	<10	78
10	43/F	R	negative	negative	PJ, AG, OE, QR, LD	1.22	0.18	1.77	44	94
Positive control of inhibition	50/F	none	negative	negative	none	13.7	1.45	24.0	93	98

The positive control of inhibition corresponds to an alcoholic patient with CCD reactivity alien to the present study [7]. DP = *Dermatophagoides pteronyssinus*; TG = timothy grass (*Phleum pratense*) pollen; MUXF-BSA = bromelain-type glycans coupled to BSA; R = rhinitis; A = asthma; AO = *Anthoxanthum odoratum*; PC = *Phragmites communis*; CD = *Cynodon*

dactylon; PL = *Plantago lanceolata*; PJ = *Parietaria judaica*; BA = *Betula alba*; OE = *Olea europaea*; CS = *Cupressus sempervirens*; AG = *Alnus glutinosa*; PA = *Platanus acerifolia*; QR = *Quercus robur*; Po = polcalcin; Pe = peach; LTP = lipid transfer protein; LD = *Lepidoglyphus destructor*; TP = *Tyrophagus putrescentiae*; C = cat; D = dog.

mites and animal dander (table 1). With few exceptions, preincubation with CCDs (MUXF-BSA) did not inhibit IgE reactivity to allergens when SPTs to the respective allergens were positive, even in cases with the highest levels of CCD-sIgE (table 2), thus indicating that these cases were also sensitized to protein epitopes in the allergen source. The association of atopy with CCD sensitization was independent of potential confounders (table 3). Serum levels of total IgE correlated with those of bromelain-

sIgE (Rho 0.534, p < 0.001), MUXF-sIgE (Rho 0.441, p < 0.001) and PLA2-sIgE (Rho 0.406, p < 0.001).

Association of CCD Sensitization with Demographic and Lifestyle Factors

Male sex tended to be independently associated with CCD sensitization (table 3). CCD sensitization was not significantly associated with age, rural residence or smoking. Only 10 patients (0.9%) were alcohol abusers

Table 3. Factors associated with positivity of IgE to MUXF, bromelain and PLA2

	IgE ≥ 0.1 kU _A /l to MUXF		IgE ≥ 0.1 kU _A /l to bromelain		IgE ≥ 0.1 kU _A /l to MUXF and/or bromelain		IgE ≥ 0.1 kU _A /l to PLA2	
	% (n)	adjusted OR (95% CI)	% (n)	adjusted OR (95% CI)	% (n)	adjusted OR (95% CI)	% (n)	adjusted OR (95% CI)
Sex								
Female (ref.)	9.6 (64/664)	1	11.0 (73/664)	1	15.4 (102/664)	1	10.2 (68/664)	1
Male	16.6 (60/361)**	1.54 (1.01–2.35)*	18.0 (65/361)**	1.51 (1.00–2.26)*	23.0 (83/361)**	1.38 (0.96–1.99)	21.1 (76/361)***	1.96 (1.31–2.92)***
Age								
<45 years (ref.)	12.8 (105/822)	1	13.6 (112/822)	1	18.7 (154/822)	1	13.9 (114/822)	1
≥ 45 years	9.4 (19/203)	1.17 (0.67–2.06)	12.8 (26/203)	1.57 (0.94–2.62)	15.3 (31/203)	1.35 (0.84–2.16)	14.8 (30/203)	1.56 (0.95–2.55)
Residence								
Rural (ref.)	12.6 (89/704)	1	13.9 (98/704)	1	18.6 (131/704)	1	15.2 (107/704)	1
Urban	10.9 (35/321)	0.86 (0.56–1.32)	12.5 (40/321)	0.89 (0.59–1.34)	16.8 (54/321)	0.90 (0.63–1.30)	11.5 (37/321)	0.73 (0.48–1.10)
Smoking status								
Never smokers (ref.)	11.6 (86/742)	1	13.5 (100/742)	1	17.8 (132/742)	1	14.0 (104/742)	1
Ex-smokers	14.3 (7/49)	1.05 (0.44–2.49)	16.3 (8/49)	0.97 (0.42–2.24)	20.4 (10/49)	0.99 (0.46–2.12)	18.4 (9/49)	0.90 (0.40–1.99)
Smokers	13.3 (31/233)	1.15 (0.72–1.84)	12.9 (30/233)	0.96 (0.60–1.53)	18.5 (43/233)	1.06 (0.71–1.60)	13.3 (31/233)	0.94 (0.59–1.49)
Alcohol consumption								
Non-drinkers (ref.)	0.2 (49/479)	1	12.7 (61/479)	1	16.7 (80/479)	1	12.9 (62/479)	1
1–7 units/week	13.5 (55/407)	1.16 (0.75–1.79)	12.8 (52/407)	0.86 (0.56–1.31)	18.7 (76/407)	0.99 (0.69–1.44)	12.5 (51/407)	0.77 (0.51–1.18)
>7 units/week	14.5 (20/138)	0.97 (0.52–1.82)	18.1 (25/138)	1.02 (0.57–1.82)	21.0 (29/138)	0.91 (0.53–1.56)	22.5 (31/138)**	1.18 (0.68–2.05)
History of Hymenoptera stings								
No (ref.)	9.3 (40/428)	1	10.3 (44/428)	1	14.0 (60/428)	1	8.6 (37/428)	1
Yes	14.0 (83/594)*	1.42 (0.94–2.15)	15.7 (93/594)*	1.49 (1.00–2.21)*	20.9 (124/594)**	1.51 (1.06–2.15)*	17.8 (106/594)***	2.03 (1.35–3.06)***
Atopy								
No (ref.)	3.9 (11/280)	1	4.2 (12/280)	1	5.7 (16/280)	1	8.2 (23/280)	1
Yes	15.1 (113/745)***	4.26 (2.20–8.25)***	16.9 (126/745)***	4.92 (2.60–9.32)***	22.6 (169/745)***	5.04 (2.89–8.81)***	16.2 (121/745)**	2.22 (1.33–3.69)**

Complete data were available for 1,022 individuals. OR were adjusted for all listed variables. ref. = Reference category; MUXF = the N-glycan from bromelain.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ with respect to the reference category.

(consumers of ≥ 28 units/week). Alcohol consumption (>7 units/week) was independently associated with atopy and with high levels of serum total IgE (table 4). Alcohol consumption was associated with a statistically nonsignificant trend toward a high prevalence of CCD sensitization (table 3). In the nonatopic group, alcohol consumption (>7 units/week) was associated with positivity of PLA2-sIgE after adjusting for age, sex, residence, smoking status and the history of Hymenoptera stings (OR: 7.71, 95% CI: 1.81–32.7, $p = 0.006$).

Association of CCD Sensitization with Hymenoptera Stings

The history of Hymenoptera stings was independently associated with CCD sensitization and PLA2-sIgE positivity (table 3). The relationship between the time elapsed since the most recent sting and the prevalence of sIgE positivity showed a similar pattern for both CCD mark-

ers and PLA2 (fig. 2). Hymenoptera stings and atopy exhibited an independent effect in relation to CCD sensitization (fig. 3).

The history of Hymenoptera stings was independently associated with positivity of the multiallergen IgE test (table 4). Positivity of the multiallergen IgE test was associated with CCD sensitization in both atopic and nonatopic patients (fig. 4).

Discussion

Currently, it is understood that sensitization to plant or invertebrate CCDs can frequently interfere with determinations of sIgE [1–4]. However, the precise frequency of occurrence for this problem in the standard clinical conditions is not fully known. In our study, the observed prevalence of CCD sensitization in patients submitted for

Table 4. Factors associated with atopy (SPT positivity) and atopy traits (increased serum total IgE and positive results of multiallergen IgE test)

	Atopy (SPT positivity)		Total IgE >170 IU/ml		Positive multiallergen IgE test (Phadiatop)	
	% (n)	adjusted OR (95% CI)	% (n)	adjusted OR (95% CI)	% (n)	adjusted OR (95% CI)
<i>Sex</i>						
Female (ref.)	68.7 (456/664)	1	31.1 (206/662)	1	64.0 (424/663)	1
Male	80.1 (289/361)***	1.56 (1.10–2.20)*	39.3 (142/361)**	1.11 (0.81–1.51)	78.6 (283/360)***	1.75 (0.99–3.09)
<i>Age</i>						
<45 years (ref.)	79.7 (655/822)	1	35.2 (289/820)	1	75.9 (622/820)	1
≥45 years	44.3 (90/203)***	0.19 (0.13–0.27)***	29.1 (59/203)	1.35 (0.90–2.00)	41.9 (85/203)***	0.52 (0.28–0.94)*
<i>Residence</i>						
Rural (ref.)	73.7 (519/704)	1	34.9 (245/702)	1	70.3 (494/703)	1
Urban	70.4 (226/321)	0.89 (0.65–1.22)	32.1 (103/321)	0.91 (0.67–1.23)	66.6 (213/320)	0.94 (0.56–1.57)
<i>Smoking status</i>						
Never smokers (ref.)	72.9 (541/742)	1	32.8 (243/740)	1	69.9 (517/740)	1
Ex-smokers	69.4 (34/49)	0.75 (0.38–1.49)	32.7 (16/49)	0.87 (0.44–1.71)	69.4 (34/49)	1.04 (0.32–3.29)
Smokers	72.5 (169/233)	0.76 (0.53–1.09)	37.8 (88/233)	1.17 (0.83–1.64)	66.5 (155/233)	0.58 (0.33–1.03)
<i>Alcohol consumption</i>						
Non-drinkers (ref.)	69.9 (335/479)	1	28.5 (136/478)	1	66.1 (316/478)	1
1–7 units/week	74.2 (302/407)	1.16 (0.84–1.61)	35.9 (146/407)*	1.32 (0.97–1.79)	70.4 (286/406)	1.02 (0.60–1.72)
>7 units/week	77.5 (107/138)	1.82 (1.08–3.06)*	47.4 (65/137)***	2.02 (1.28–3.17)**	75.4 (104/138)*	1.50 (0.64–3.54)
<i>History of Hymenoptera stings</i>						
No (ref.)	70.6 (302/428)	1	33.9 (145/428)	1	64.5 (276/428)	1
Yes	74.1 (440/594)	1.02 (0.76–1.38)	34.1 (202/592)	0.90 (0.68–1.20)	72.3 (428/592)**	1.80 (1.10–2.95)***
<i>Atopy</i>						
No (ref.)	NA	NA	10.4 (29/280)	1	7.1 (20/279)	1
Yes	NA	NA	42.9 (319/743)***	7.04 (4.55–10.8)***	92.5 (687/743)***	154.3 (88.0–270.5)***

Complete data were available for 1,020 individuals. OR were adjusted for all listed variables. ref. = Reference category; NA = not applicable.
* p < 0.05; ** p < 0.01; *** p < 0.001 with respect to the reference category.

study of respiratory allergy to a specialized clinic was relatively low. Moreover, CCD-sIgE titers were low. Given that the correlation of CAP titers of CCD-sIgE and plant allergen-sIgE is nearly 1:1 in SPT-negative individuals [7], the spurious increase in allergen-sIgE induced by CCD-sIgE would be minimal in most cases. Furthermore, inhibition studies showed that, with few exceptions, CCDs did not inhibit IgE reactivity to allergen sources (timothy grass) when SPTs were positive, even in cases with the highest levels of CCD-sIgE. Taken together, these results indicate that the diagnostic problem induced by CCDs is minor in this type of patient in a setting with available SPTs, which are the most useful tests for demonstrating an IgE-mediated mechanism underlying clinical symptoms [28]. In addition, component-resolved diagnosis using recombinant, nonglycosylated allergens may help to minimize CCD-sIgE interference [22].

Most previous studies of the prevalence of CCD sensitization were performed in areas with a high prevalence of pollen allergy [8] and in selected samples of patients allergic to pollens [9, 10], vegetable foods [9, 10] or Hymenoptera venoms [11–15]. The largest of these studies included 1,831 adult and pediatric individuals with suspicion of respiratory allergy in Southern Italy [8], and reported a 23% prevalence of CCD sensitization (bromelain-sIgE >0.4 kU_A/l) compared with 4.7% in our series (data not shown) from an area of predominantly mite-allergic patients. Furthermore, we observed that the prevalence of CCD sensitization was higher in patients with positive SPTs to pollens than in patients with isolated positivity of SPTs to mites. This is not surprising since mite glycoproteins are not supposed to contain these xylosylated/fucosylated N-glycans [29]. However, some inhibition of IgE reactivity to *D. pteronyssinus* was

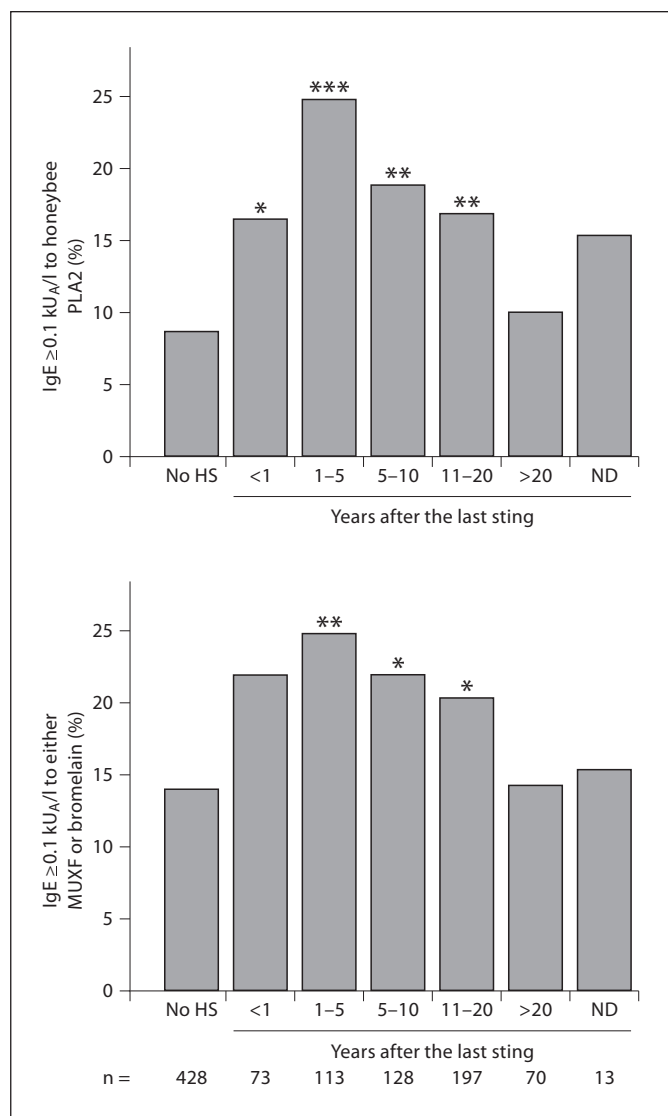


Fig. 2. IgE to CCD markers (bromelain and/or MUXF) and honeybee PLA2 in relation to the time elapsed since the last Hymenoptera (honeybee or wasp) sting (HS). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ with respect to patients with no history of Hymenoptera sting. ND = No data (undetermined time since the last sting).

observed after CCD coincubation in cases with negative SPTs to mites, confirming previous reports [30]. Overall, the prevalence of CCD sensitization was higher in atopic (SPT-positive) than in nonatopic individuals [6–8]. This could indicate that the predisposition to develop IgE to environmental substances (or atopy) involves not only protein determinants but also carbohydrates. Indeed, the level of CCD-sIgE significantly correlated with total IgE levels, as previously reported [31].

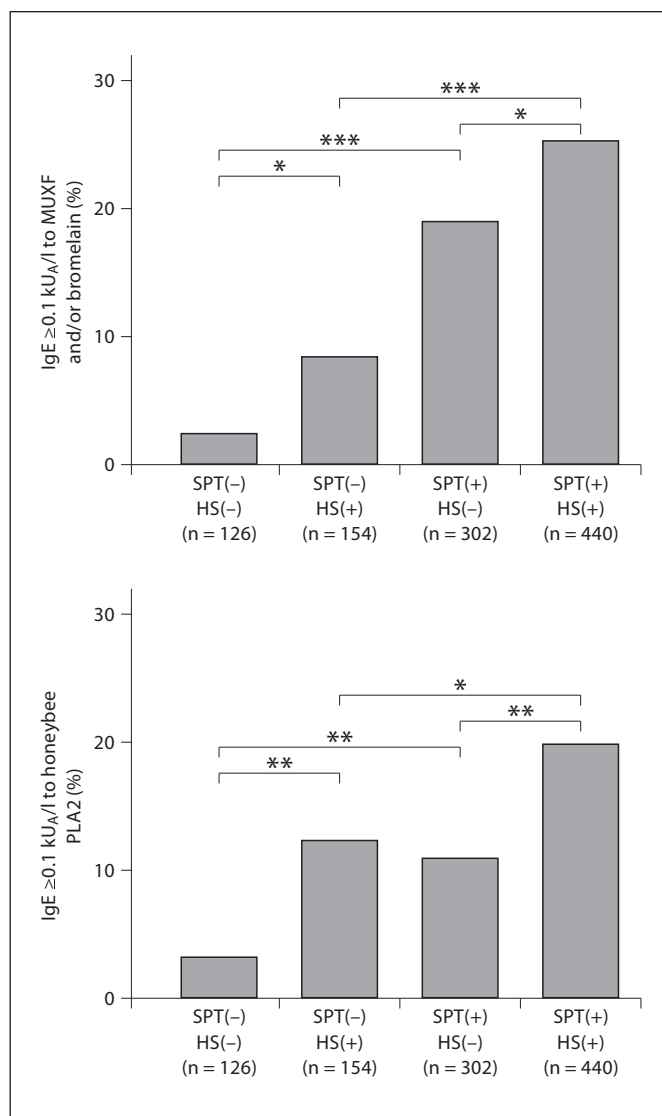


Fig. 3. IgE to CCD markers (bromelain and/or MUXF) and honeybee PLA2 in relation to SPT results and the lifetime history of Hymenoptera sting (HS). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (+) = Present or positive; (-) = absent or negative.

The prevalence of CCD sensitization was similar or even lower than that observed in the general adult population from the same area [7]. In a previous study of a random sample of 457 adults (26% of them atopics), we found a prevalence of MUXF-sIgE (≥ 0.35 kU_A/l) of 5.6% [7], compared with 4.3% in the present series. Additional differences between the study populations included age (median: 54 vs. 32 years), rate of rural residence (100 vs. 68%) and prevalence of heavy (>280 g/week) drinking (10

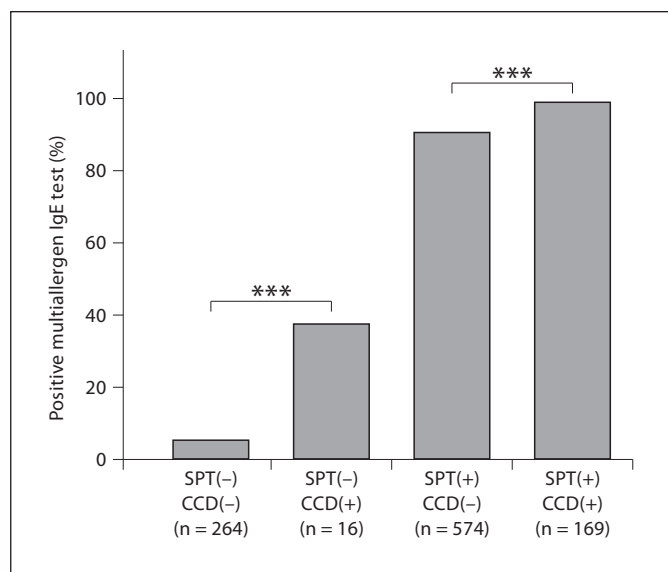


Fig. 4. Multiallergen IgE test (Phadiatop) results in relation to SPT results and positivity of IgE to CCD markers (≥ 0.1 kU_A/l to bromelain and/or MUXF). (+) = Present or positive; (-) = absent or negative. *** $p < 0.001$.

vs. $<1\%$) in the general population [7] and the allergy clinic, respectively. Interestingly, the prevalence of CCD sensitization was lower in an urban adult population in the Copenhagen area, where 39% were atopics, the median age was 39 years and 5% were heavy drinkers [6]. In that population, 6.2% of individuals showed a MUXF-sIgE ≥ 0.1 kU_A/l and 2.1% showed an IgE level ≥ 0.35 kU_A/l [6].

Alcohol consumption is consistently associated with increased levels of serum total IgE in selected samples of alcohol abusers [17, 19] in the general population [18, 20] and in allergy clinics [21], as is evident in the present study. Previous reports showed that heavy drinking is associated with CCD sensitization [6, 7, 22, 23]. This association could not be confirmed in this study population, which included a small number of heavy drinkers.

Living in a rural environment could promote CCD sensitization due to increased exposure to pollens or Hymenoptera stings. However, the present study did not find such an association after adjusting for confounders. The lifetime history of Hymenoptera stings was associated with CCD sensitization in patients without a Hymenoptera venom allergy. After serial determinations in 3 patients with a Hymenoptera venom allergy, Kochuyt et al. [15] reported that negative or very low CCD-sIgE levels prior to a sting increased in parallel along with the culprit venom-sIgE after the sting. In our study, the temporal

relationship between the last Hymenoptera sting and honeybee PLA2 reactivity paralleled that of CCD reactivity, which peaked 1–5 years after the last sting. Unfortunately, the questionnaire was not capable of distinguishing between honeybee and wasp stings, and the lifetime number of stings was not recorded. However, the history of Hymenoptera stings would be a simple question to add to the routine clinical history in patients not allergic to venoms. A positive history may be a clue for CCD sensitization and for the interpretation of discordant sIgE results in settings with unavailable SPTs, or when multiallergen IgE tests are used for screening. In our experience, a history of Hymenoptera exposure is an independent risk factor for positivity of multiallergen IgE tests. These screening tests, which use several inhalant allergens in a single assay, are prone to interference by CCD-sIgE [3, 6, 32].

Some methodological issues need to be considered. We used a standard method (CAP) for sIgE determination and included the two CCD markers (bromelain and its glycan portion, MUXF) that are currently available for the system. Glycan reporters or CCD markers are xylosylated/fucosylated N-glycan molecules without the protein part or linked to a protein that rarely induces IgE sensitization. Additional CCD markers (such as ascorbate oxidase or horseradish peroxidase) that are available in different platforms were not tested in the present study. In general, CCD markers are considered to be interchangeable.

As expected, we observed a significant correlation between MUXF-sIgE and bromelain-sIgE, although it was particularly evident at high titers. An ancillary conclusion of the study is that some discrepancies in supposedly similar CCD markers can be observed at low titers. Following the manufacturer's instructions, we considered 0.1 kU_A/l to be the threshold for positivity instead of the classic 0.35 kU_A/l. The 0.1-kU_A/l level may not be relevant from the clinical standpoint, but it aids the identification of positive cases for epidemiological purposes. The results of inhibition assays should be interpreted with caution since only a small subset of CCD-sIgE-positive cases were used. Caution is particularly needed for interpretation of inhibition of low-level IgE results. Finally, it should be noted that the cross-sectional design limits the inference of causality.

In summary, IgE-mediated sensitization to CCDs is rare and CCD-sIgE levels are low in this population. Thus, CCD sensitization is not a relevant problem for the diagnosis of patients with suspicion of respiratory allergy in an allergy clinic from our location. The problem may

be higher when only IgE to natural (glycosylated) allergens is used instead of SPTs. The diagnostic interference induced by CCDs may be also more relevant in populations with a higher rate of heavy alcohol consumption [6, 7, 22, 23], a higher rate of pollen sensitization [8–10] or a higher rate of Hymenoptera venom allergy [11–15]. Hymenoptera stings, even in individuals not allergic to their venoms, are factors for CCD sensitization that may induce positivity of allergy screening tests.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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