

Carbohydrate epitopes as a cause of cross-reactivity in patients allergic to Hymenoptera venom

Renato Eržen, Peter Korošec, Mira Šilar, Ema Mušič, Mitja Košnik

Hospital Golnik, Golnik, Slovenia

Summary. *Background:* Among patients with allergy to insect stings, double positivity in tests for IgE antibodies specific to honey bee and wasp venoms is a frequent diagnostic problem. True double sensitization and possible cross-reactivity of venom hyaluronidases and with carbohydrate determinants must be considered in such patients. We studied the frequency of sensitization to carbohydrate determinants and the role of these in double positivity in tests for specific IgE antibodies.

Materials and methods: A group of 66 patients (41 men, 25 women; 16–66 years) with double positivity for wasp and bee venoms were tested in the FEIA inhibition test in order to distinguish true double sensitization from cross-reactivity. Patients were tested for the presence of IgE antibodies specific to oilseed rape (OSR) pollen and MUXF3 allergens, both of which are rich in cross-reacting carbohydrate epitopes.

Results: Inhibition tests revealed true double sensitization in 37 patients (56.1%) and cross-reactivity in 29. Among those showing cross-reactivity, five were sensitized to honey bee venom and 24 to wasp venom. The median value of IgE specific for OSR pollen in patients sensitized to honey bee venom was 4.350 IU/ml, in patients sensitized to wasp venom 0.61 IU/ml, and in patients with double sensitization 0.25 IU/ml ($P = 0.028$, Kruskal–Wallis test). Findings for IgE specific for MUXF3 were similar. Discordance between OSR pollen positivity and MUXF3 positivity was found in 11.1% of the patients.

Conclusion: The values of IgE specific for OSR pollen and MUXF3 in patients with primary sensitization to either honey bee venom or wasp venom were significantly higher than in patients with double sensitization. These results confirm that IgE antibodies against carbohydrate epitopes are a frequent cause of double positivity in tests for anti-venom IgE antibodies.

Key words: Hymenoptera venom allergy, double positivity, carbohydrate epitopes.

Introduction

IgE antibodies specific to both honey bee venom and wasp venom are frequently found in patients with a his-

tory of allergic reaction after an insect sting. This double positivity causes diagnostic and therapeutic problems in patients who do not recognize the culprit insect and are skin-test negative. True double sensitization and also cross-reactivity must be considered as causes of the double positivity in this group of patients [1]. Cross-reactivity of venom hyaluronidases and with carbohydrate epitopes is known [2, 3], and distinguishing between double sensitization and cross-reactivity is crucial in the choice of allergen for specific immunotherapy in patients who did not recognize the culprit insect [4]. If double sensitization is proven, immunotherapy with both venoms is required; if cross-reactivity is the case, only the venom that caused sensitization should be used [1].

Cross-reacting carbohydrate determinants (CCDs) are present on glycoproteins and are widely distributed in plants (pollen, plant food) and in Hymenoptera venoms [5]. Anti-CCD IgE antibodies could therefore reflect sensitization to allergens in plants or in Hymenoptera venoms and are an important cause of in vitro double positivity. The clinical relevance of anti-CCD IgE antibodies remains controversial [6], although general opinion is that they have no clinical relevance. Van der Veen et al. studied patients with IgE antibodies to peanuts and confirmed that in patients without clinical allergy to peanuts a much higher concentration of peanut extract was needed to cause basophils degranulation than in patients who were clinically allergic [7]. Similarly, Mari et al. found that 82.7% of patients with IgE antibodies against plant glycans demonstrated IgE binding to transgenic lactoferrin but, in comparison with a major grass-pollen allergen, lactoferrin concentrations needed for biological activity of IgE were 5–6 orders of magnitude higher [8]. Nevertheless, the clinical importance of CCDs has been confirmed in other studies [9, 10]. Basophils of patients allergic to celery, tomato or zucchini degranulated in contact with bromelain. Deglycosylated tomato allergen Lyc e 2 lost its degranulating ability. A positive oral challenge test was observed with celery and zucchini in some patients, although in vitro testing indicated that these patients were sensitized only to CCDs in these foods.

In our study the frequency of IgE antibodies against CCDs was measured in patients with IgE antibodies against both wasp and honey bee venom. The role of CCDs in double positivity was evaluated.

Correspondence: Renato Eržen, MD, Hospital Golnik, 4204 Golnik, Slovenia, E-mail: renato.erzen@klinika-golnik.si

Patients, materials and methods

A total of 66 patients (42 men, 24 women; 16–66 years, average age 39) diagnosed between 2003 and 2007 with allergy to both wasp venom and honey-bee venom were included in the study. In UniCAP FEIA (Phadia, Uppsala, Sweden), all the patients had specific IgE antibodies >0.7 kIU/l against both the venoms. During this period we evaluated a total of 519 patients with double venom allergy; those with a clinical

Table 1. Number of patients with elevated and normal concentration of IgE antibodies specific for oilseed rape pollen (A) and MUXF3 (B) in patients with sensitization to honeybee venom, wasp venom and double sensitization (honeybee and wasp)

A			
Sensitization	No specific IgE to OSR pollen	Positive for specific IgE to OSR pollen	ALL
Honeybee venom	1 (20%)	4 (80%)	5
Wasp venom	11 (46%)	13 (54%)	24
Honeybee and wasp	22 (60%)	15 (40%)	37
ALL	34 (52%)	32 (48%)	66

B			
Sensitization	No specific IgE to MUXF3	Positive for specific IgE to MUXF3	ALL
Honeybee venom	1 (20%)	4 (80%)	5
Wasp venom	11 (48%)	12 (52%)	23
Honeybee and wasp	24 (69%)	11 (31%)	35
ALL	36 (57%)	27 (43%)	63

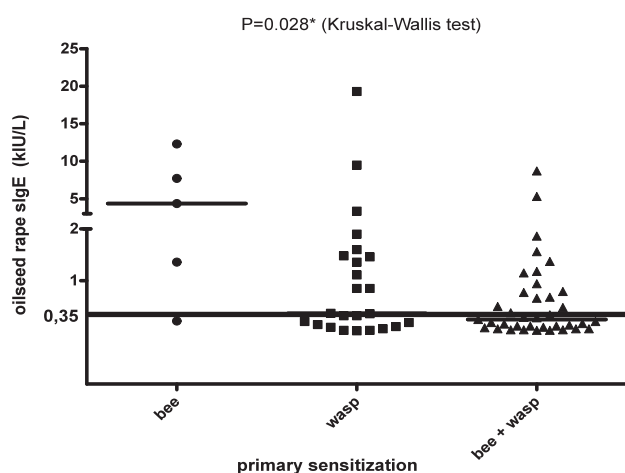


Fig. 1. Values of IgE antibodies specific for oilseed rape pollen in patients with sensitization to honeybee venom, wasp venom and double sensitization to both venoms

indication for inhibition tests were included in the present study.

All patients had a convincing history of severe anaphylactic reaction Mueller grades II–IV (median grade IV). The culprit insect was unknown in 28 patients (42.4%), honeybee in 12 patients (18.2%), wasp in 15 patients (22.7%), both honeybee and wasp in four (6.1%) and hornet in seven (10.6%).

Atopy was assessed in 27 patients (40.1%) in the skin prick test (Allergopharma Ltd, Reibek, Germany) in accordance with reported guidelines: we found atopy in 10 patients (37.0%), eight (29.6%) of whom were allergic to pollen allergens and two (7.4%) to house dust mite.

Inhibition tests were used for discrimination between double sensitization and cross-reactivity. Patients' sera were incubated at room temperature for 1 h with saline, honey bee venom and wasp venom (HAL Allergy, Düsseldorf, Germany) at a final venom concentration of 600 µg/ml. The concentration of remaining specific IgE was measured in FEIA tests and the percentage of inhibition calculated. Inhibition >80% was regarded as complete inhibition [1].

Among many allergens rich in CCDs we chose as representatives oilseed rape (OSR, *Brassica napus*) pollen and MUXF3 (N-glycan chain composed of 2 mannose, 1 xylose, 1 fucose and 2 N-acetylglucosamine molecules). IgE antibodies against OSR pollen were determined in all 66 patients and against MUXF3 in 63 of 66.

Statistical analysis

The distribution of data was analyzed using the Shapiro–Wilk test, $P < 0.0001$. The concentrations of specific IgE in different groups of patients were compared in Kruskal–Wallis and Mann–Whitney tests. Spearman's rank correlation was used for comparison of the two methods of measuring CCD-specific IgE. Fischer's exact test was used for evaluation of the probability of presence of CCD-specific IgE antibodies in patients with double sensitization and in patients showing cross-reactivity.

Results

Inhibition tests revealed true double sensitization in 37 patients (56.1%) and cross-reactivity in 29 (43.9%). Among those showing cross-reactivity, five were sensitized to honey bee venom and 24 to wasp venom.

Among patients with double positivity for anti-venom IgE, antibodies specific for OSR pollen were detected in 32 (48.5%) and MUXF3 in 27 (43%) (Table 1). The probability of the presence of CCD-specific IgE antibodies was the same in patients with double sensitization and in those showing cross-reactivity ($P = 0.21$, Fischer's exact test).

The concentration of IgE antibody specific for OSR pollen was significantly higher in patients who tested double positive and were sensitized to bee venom than in patients sensitized to wasp venom (Fig. 1). The mean concentration of IgE antibody specific for OSR pollen was significantly higher in patients showing cross-reactivity than in patients with double sensitization ($P = 0.029$, Mann–Whitney test) (Fig. 2). Similarly, MUXF3-specific IgE antibodies were significantly higher in patients showing cross-reactivity than in patients with double sensitization ($P = 0.027$, Mann–Whitney test) (Fig. 3). In 11.1% of test results there was discordance between IgE antibodies specific for OSR pollen and MUXF3: in OSR positive sera, the majority were MUXF3 negative (Table 2).

There was significant correlation between the two methods of measuring CCD-specific IgE antibodies ($r = 0.92$, $P < 0.0001$, Spearman's rank correlation) (Fig. 4).

Discussion

In this study we have shown that values for CCD-specific IgE antibodies in a group of patients with true double sensitization to bee and wasp venoms were significantly lower than in a group showing cross-reactivity.

A limitation of the study is that patients' sera were investigated indirectly for the presence of anti-CCD antibodies. We measured IgE antibodies specific for OSR pollen, a plant allergen with high content of CCD. Jappe

et al. suggest that other CCD allergens such as horseradish peroxidase might be used as supplementary screening for cross-reactivity in patients with allergy to Hymenoptera venom [11]. We used purified MUXF3 allergen for a more specific evaluation of CCD-specific IgE antibodies. MUXF3, an N-glycan chain, occurs frequently in plant glycoproteins.

We found significantly higher concentrations of specific IgE antibodies against OSR pollen and MUXF3 in patients showing cross-reactivity than in patients with double sensitization. The correlation between the two tests was excellent (Table 2). In a majority of patients with discordant results for IgE specific for OSR pollen and MUXF3, the OSR result was positive and MUXF3 negative, therefore we can speculate that some patients might be sensitized to a protein part of OSR pollen and not to a carbohydrate epitope.

The concentration of specific IgE against OSR pollen was significantly higher in patients who tested double positive and who were sensitized to bee venom (Fig. 1). The possible explanation for this finding is extensive glycosylation of phospholipase A2, a major allergen of honeybee and bumblebee venom [12].

In some patients with proven cross-reactivity, measurement of anti-CCD IgE antibodies was negative or only slightly elevated (Fig. 2). We speculate that protein epitopes were the cause of cross-reactivity in these patients.

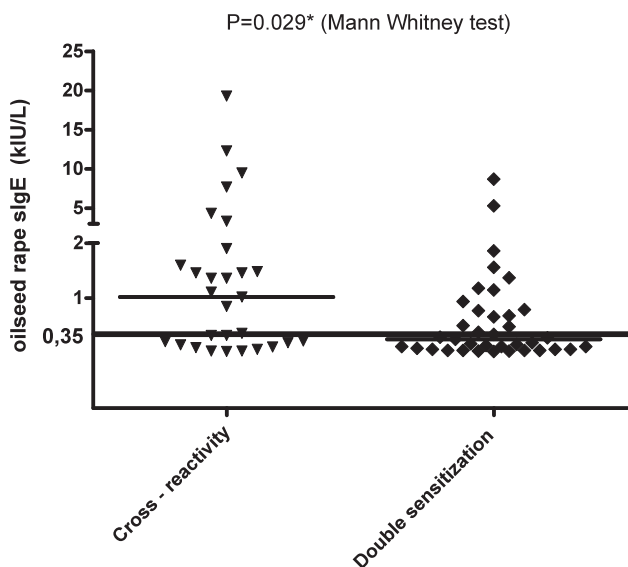


Fig. 2. Values of IgE antibodies specific for oilseed rape pollen in patients with proven cross-reactivity and double venom sensitization

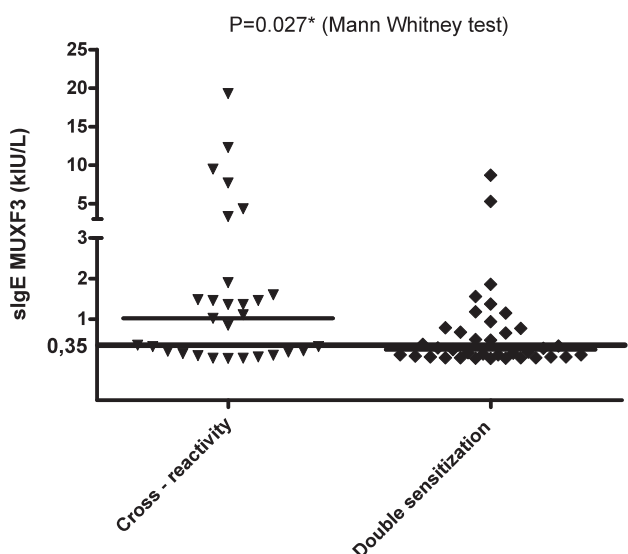


Fig. 3. Values of IgE antibodies specific for MUXF3 in patients with proven cross-reactivity and double venom sensitization

Table 2. Discordance between presence of anti-OSR and anti-MUXF3 IgE antibodies in the sera of patients with double positive tests for Hymenoptera venom allergy

	anti-OSR IgE present (%)	anti-OSR IgE absent (%)	ALL
anti-MUXF3 IgE present	25 (40)	2 (3)	27 (43)
anti-MUXF3 IgE absent	5 (8)	31 (49)	36 (57)
ALL	30 (48)	33 (52)	63 (100)

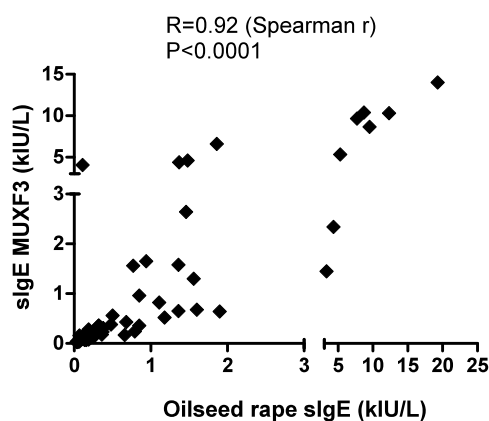


Fig. 4. Positive correlation of concentrations of IgE antibodies specific for oilseed rape pollen and MUXF3 in the sera of patients with allergy to Hymenoptera venom and double positive anti-venom IgE tests

Anti-CCD IgE antibodies could reflect sensitization to plant allergens or allergens in Hymenoptera venoms, therefore test results should be interpreted with caution. It has been proven that anti-CCD IgE antibody in a patient allergic to Hymenoptera stings could cause a false positive result in a pollen test [13], and vice versa. For that reason specific IgE against Hymenoptera venoms should be evaluated with caution in patients with pollen allergy, particularly when there is only a weak clinical suspicion of venom allergy. Further studies in an appropriate number of patients showing double positive anti-venom IgE tests with and without concomitant pollen allergy are needed for elucidation of this problem.

It should be stressed that CCD-specific IgE antibodies are detected only *in vitro* and do not elicit positive skin prick tests. However, negative skin prick tests with venoms do not rule out venom allergy as venoms are used at low concentration in these tests. At a high concentration of venom, the toxic activity of the allergen may produce a false positive result [14].

When inhibition tests cannot be performed, measurement of anti-CCD IgE antibody could have a clinical implication in patients who test double positive with low concentrations of anti-venom IgE antibody. In the inhibition test a patient's serum is mixed with equal volumes of either saline, honey bee venom or wasp venom, and the test requires a specific IgE concentration >0.7 kIU/l. Measurement of anti-CCD IgE antibody could be helpful in determining the reason for double positivity and in selection of allergen for immunotherapy in those patients. In testing for anti-CCD IgE, a negative result or only a slightly elevated concentration of antibody in these patients could be an indicator that double sensitization is the case, whereas markedly elevated concentrations of antibody indicates probable cross-reactivity.

In summary, this study suggests that concentrations of anti-CCD IgE antibodies in patients showing cross-reactivity are significantly higher than in patients with true double sensitization to bee and wasp venoms. These findings could be helpful in determining the reason for a double positive anti-venom IgE test. The two methods used for measurement of anti-CCD IgE antibodies seem comparable. The discordance of 11.1% in results for specific IgE antibodies against OSR pollen and MUXF3 is probably the result of true sensitization to OSR pollen in some patients. Our results support the importance of continued investigation of anti-CCD IgE antibody in patients with allergy to Hymenoptera venom and double positive anti-venom IgE antibody tests.

Conflict of Interest

The authors declare that there is no conflict of interest.

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