CASE REPORTS

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Triple IgE-positivity to hornet, wasp and bee venom in the patient with anaphylaxis: diagnostic and therapeutic approach

Trostruka IgE-pozitivnost na venome stršljena, ose i pčele kod pacijenta sa anafilaksom: dijagnostički i terapijski pristup

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Abstract

Introduction. Triple-positivity (TP) or double-positivity (DP) for serum-specific immunoglobulin E (sIgE) antibodies against hornet venom (HV), wasp venom (WV) and/or honeybee venom (BV) causes significant problem in a selection of appropriate venom immunotherapy. However, DP/TP can be caused by cross-reactions resulting either from partial sequence identity of protein allergens in the venoms, or may be related to cross-reacting carbohydrate determinants (CCDs). **Case report.** A 60-year-old man was stung by a wasp and two days later by hornet. In both cases, within 15 minutes he developed hypotension and generalized urticaria and he was successfully treated with epinephrine, corticosteroids and fluids. After eight weeks, the examination revealed the negative skin prick test for all three venoms, but the sIgE-determination (ELISA, Biopharm) showed triple sensitization to native BV (0.55 IU/mL), WV (3.35 IU/mL) and HV (0.37 IU/mL). He was receiving the venom immunotherapy with venom mixtures for one year. In order to distinguish true multiple sensitization from cross-reactivity, the molecular-allergy testing by ImmunCAP with the CCD-free recombinant major allergens was performed. A high sensitization to Antigen 5-rVes v5 of WV (31.4 kU/L) was demonstrated while sIgE to phospholipase A2-rApi m1 of BV (0.15 kU/L) was negative; sIgE to CCD-MUXF3-bromelain (0.75 kU/L) explained the sIgE-positivity for native BV. After these findings, a venom immunotherapy only with WV was initiated. **Conclusion.** In our patient, triple-IgE-positivity to native venoms detected by the ELISA was caused by cross-reactivity to CCDs. We recommend the molecular-allergy testing with the nonglycosylated recombinant allergens before starting the venom immunotherapy in patients with multiple-sIgE-positivity to native Hymenoptera venoms.

Key words:
anaphylaxis; bites and stings; cross reactions; desensitization, immunologic; hymenoptera; venoms.

Apstrakt

Uvod. Trostruka pozitivnost (TP) ili dvostruka pozitivnost (DP) serum-specifičnih imunoglobulin E (sIgE) antitela na venom stršljena (SV), venom ose (OV) i/ili venom pčele (PV) izaziva značajan problem u izboru odgovarajućeg venoma za venom imunoterapiju. Međutim, TP/DP može biti prouzrokovana unakrsnim reakcijma koje potiču od delimične identičnosti sekvenci proteinskih alergena ili venomi mogu biti povezani sa unakrsnom reaktivnošću na ugljenhidratne determinante (UHD). **Prikaz bolesnika.** Muškarca starog 60 godina ubola je osa i dva dana kasnije stršljen. U oba slučaja je tokom 15 minuta razvio hipotenziju i generalizovanu urtikariju i bio je uspešno lečen adrenalinom, kortikosteroidima i tečnošću. Posle osam nedelja, pregledom je utvrđeno da je kožni prik test bio negativan na sva tri venoma, ali sIgE određivanje (ELISA, R-Biopharm) je pokazalo trostruku senzibilizaciju na prirodni PV (0.55 IU/mL), OV (3.35 IU/mL) and SV (0.37 IU/mL). On je dobijao venom imunoterapiju sa mešavinom venoma. Da bi se razlikovala prava višestruka preosetljivost od unakrsne reaktivnosti sprovedeno je molekularno-alergološko testiranje sa ImmunoCAP sa alergenima oslobođenim od UHD. Utvrđena je visoka senzibilizacija na Antigen 5-rVes v5 OV (31.4 kU/L), dok je sIgE na phospholipase A2-rApi m1 PV (0.15 kU/L) bio negativan; sIgE na UHD MUXF3-bromelain (0.75 kU/L) je objasnio sIgE-pozitivnost na PV. Nakon ovih nalaza započeta je venom imunoterapijom samo sa OV. **Zaključak.** Trostruka-IgE-pozitivnost na prirodne venome detektovana ELISA testom kod našeg pacijenta je bila prouzrokovana unakrsnom reaktivnošću na UHD. Preporučujemo molekularno-alergološko testiranje sa

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neglikoliziranim rekombinantnim alergenima, pre započinjanja venom imunoterapije kod pacijenata sa višestrukom sIgE-pozitivnošću na prirodne Hymenoptera venome.

Ključne reči:
anafilaksija; ujedi i ubodi; unakrsne reakcije; desenzibilizacija, imunološka; hymenoptera; otrovi.

Introduction

Hymenoptera venom allergy (HVA) is responsible for more than 10% of all cases of anaphylaxis [1]. Identification of the culprit insect from the Hymenoptera venoms in the patients with HVA is often difficult from history because majority of them was unable to identify the insect. The positive testing results for serum-specific immunoglobulin E antibodies (sIgE) to the native Hymenoptera venoms (HVs) components do not always reflect a clinically relevant sensitization. In clinical practice, about 50% of patients with systemic allergic reactions to insect stings show double (DP) or even triple positivity (TP) for HVs, although most of them report the allergic reactions only to one sting. The multiple positive test results can be caused by cross-reactions resulting either from partial sequence identity of protein allergens in the venoms, or may be related to the cross-reacting carbohydrate determinants (CCDs). In the premolecular era, this often led to unnecessary venom immunotherapy (VIT) with more than one venom resulting in the higher costs, increased risk of side effects, unsatisfactory effects of VIT and *de novo* sensitization [1–3]. Nowadays, the molecular allergy testing with the CCD-free recombinant allergens can improve diagnostic precision in the patients with the HVA history, particularly in the patients with multiple sensitization.

Case report

A 60-year-old man presented with the generalized urticaria and hypotension (blood pressure 80/40 mmHg) 15 minutes after having been stung by a wasp in the neck. He was successfully treated at the emergency department with epinephrine, corticosteroids and intravenous fluids and followed up for one day. Two days later, only 5 minutes after a hornet sting, he developed the generalized urticaria, lip swelling and hypotension. Again, he received emergency treatment for anaphylaxis. Then, it took a few days to fully recover after the treatment.

Eight weeks later the skin prick tests (SPT) were performed with the native honey bee venom (BV), wasp venom (WV) and hornet venom (HV) (the Institute of Virology, Vaccines and Sera "Torlak", Belgrade, Serbia), which resulted in the wheal reactions of 1 to 2 mm in diameter for each of the venoms (positive histamine control and negative saline *control* test were included with wheal of 6 mm and 2 mm, respectively). Thus, the SPT were considered negative according to the recommendations [4].

The laboratory testing (ELISA, R-Biopharm, Germany) revealed positive reactions to all three native BV, WV and HV (Table 1). Basal serum tryptase of 4.27 mcg/L was not increased (normal value ≤ 11 mcg/L) (ImmunoCAP, Phadia, Uppsala, Sweden). Thereafter, subcutaneous VIT with the mixture of BV, WV and HV (Institute of Virology, Vaccines and Sera "Torlak", Belgrade, Serbia) was initiated and continued during one year. In order to distinguish the true double/triple sensitization to the HVs from a cross-reactivity, we performed additional testing using the retrieved frozen serum patient sample.

Therefore, we determined sIgE (FEIA, ImmunoCAP, Phadia, Uppsala, Sweden) to recombinant species-specific major allergens (rSSMA) to BV phospholipase A2 (rApi m1), to WV antigen 5 (rVes v5) and to CCDs, MUXF3-CCD-bromelain (Table 1).

The presence of sIgE to antigen 5, but not to phospholipase A_2 ruled out a true double sensitization. Moreover, we found cross-reacting sIgE to CCDs. According to the obtained results we decided to continue the long-term VIT with only WV.

Discussion

HVA carries a high risk of anaphylactic reactions with potentially fatal outcome. It has been shown that 9.2% to 28.7% of the adult population are sensitized to the Hymenoptera venoms and the prevalence of systemic sting reactions ranges between 0.3% and 7.5% [1]. In addition, there is a higher prevalence of more severe systemic reactions to HVs in the patients with the mast cell disorders [5]. Recent studies have demonstrated that the basal serum tryptase levels were elevated in approximately 10% of venom-allergic patients and these increased the levels correlated significantly with severity of Hymenoptera sting and age [6]. The normal basal serum tryptase level in our patient ruled out this possibility.

Table 1

Laboratory diagnostics in the patient stung by the wasp and hornet, two days consecutively

Native allergens	Test values [sIgE (Elisa), IU/mL]	Recombinant major allergens	Test values [sIgE (Immuno CAP-FEIA), kU/L]
Bee venom	0.55	rApi m 1	0.15
Wasp venom	3.35	rVes v 5	31.4
Hornet venom	0.37	Cross reacting carbohydrate determinants (CCDs)	0.75

Normal values: < 0.35, equal for both tests.
FEIA – fluorescence enzyme immunoassay; rApi m 1 rVes v 5 – recombinant phospholipase A_2 and recombinant antigen 5, consecutively.

The most common cause of an insect allergic reaction in Central Europe are stings from honeybee (*Apis mellifera*) and wasp, in the USA called yellow jackets (*Vespula vulgaris*) [3]. The diagnosis of HVA is based on the history of a systemic allergic reaction to insect sting, a positive SPT with the venoms and the presence of sIgE antibodies to the venom. SPT with venoms are the most sensitive for diagnosis of HVA, but can be false negative in less than 2% of patients due to a refractory period of 3–6 weeks, or previous treatment with antihistamines [4, 7]. Still, there are patients with a convincing history of anaphylaxis to Hymenoptera sting, but the negative diagnostic tests to the respected venoms. On the other hand, up to 50% of patients show the positive test results to several venoms [1]. Our patient had a convincing history of anaphylaxis to wasp and hornet stings, the negative SPT results and triple-sIgE-positivity to native BV, WV and HV detected by the ELISA. He was treated for one year by subcutaneous VIT using the mixture of BV, WV and HV. VIT is the most effective treatment for the patients suffering from HVA to avoid life-threatening anaphylaxis. VIT is very effective in inducing tolerance with a protection rate ranging from 75% to 98%. An unnecessary treatment with more than one or even with the wrong venom can lead to *de novo* sensitization and increased risk of side effects [1, 8].

However, a large proportion of patients with allergic reaction to bee, or wasp, or hornet stings have sIgE to the all three venoms. Such multiple positivity causes significant problems in the selection of venoms for immunotherapy. Diagnostic tests sometimes reflect genuine multiple sensitization indicating potential systemic allergic reactions to the next sting by any of mentioned insects. Nevertheless, more often we detect false DP/TP which is clinically irrelevant and can be caused by cross-reactions. The majority of cross-reactivity can be attributed to IgE antibodies that are directed to CCDs which are frequently present in allergens of insects and plants and are present in several allergens of HVs. In insects, the relevant carbohydrate epitope is defined by a α-1, 3-linked fucose residue of the N-glycan. [9, 10]. Another cause of multiple sensitization may be based on the recognition of common protein epitopes of homologous allergens, present in HVs as described for hyaluronidases (Api m 2 and Ves v 2), dipeptidyl peptidases (Api m 5 and Ves v 3) and vitellogenins (Api m 12 and Ves v 6) sharing around 50% sequence identity. The detection of sIgE to CCDs does not allow the exclusion of sensitization to protein epitopes of multiple venoms [10–12].

New molecular-allergy testing based on the detection of IgE antibodies against individual nonglycosylated major Hymenoptera allergens may help to distinguish between the cross-reactivity and genuine multiple sensitization [2, 3, 7]. Many patients with true double sensitization may be identified by means of the rSSMA: rApi m 1 (phospholipase A2) of BV, rVes v 1 (phospholipase A1) and rVesp v 5 (Antigen 5) of WV, that are available for routine *in vivo* diagnostics [13, 14]. We determined sIgE antibodies to major allergens from honey bee (rApi m 1) and the wasp (rVes v 5) venom which are structurally not related. We found sIgE-positivity only for rVes v 5 (Table 1). Elevated sIgE to CCDs explain the finding of increased sIgE levels against the native bee, wasp and hornet venoms (Table 1) detected by the ELISA [2, 3, 7, 15]. A high percentage, even up to 93% of subjects sensitized to the hornet venom have elevated sIgE to rVes v5 [16–18]. Cross-reactivity that occurs between the venoms of different Vespidae (*Vespula vulgaris*, *Vespa* crabo – European hornet) is strong, due to the similarities of venom composition and structure of allergens. Antigen 5 (rVes v 5) is the most potent allergen in the hornet and wasp venom, and the rVes v5 should be helpful for the serological confirmation of hornet and wasp venom sensitization [19]. Highly elevated sIgE to rVes v5 explain systemic reaction provoked by the wasp and hornet sting in our patient. According to our results (Table 1) we changed VIT from the commercial mixture of HVs to a single wasp venom therapy, having in mind that VIT with the wasp venom provides effective protection against both hornet and wasp venom allergy [20, 21].

Conclusion

We recommend molecular-allergy testing to recombinant the major allergens and cross-reactive carbohydrate determinants before the introduction of a long-term allergen-specific immunotherapy for the patients with inconclusive skin tests and/or multiple positive tests for sIgE against native Hymenoptera venoms. The treatment with the primarily sensitizing venom provides more efficient protection and reduces the risks comparing to immunotherapy with venom mixtures.

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