

Limited ability of recombinant Hymenoptera venom allergens to resolve IgE double sensitization

Julij Šelb, MD, PhD^a, Urška Bidovec Stojković, PhD^a, Nissera Bajrović, MD^b, Peter Kopač, MD^b, Renato Eržen, MD, PhD^b, Mihaela Zidarn, MD, PhD^b, Mitja Košnik, MD, PhD^b, and Peter Korošec, PhD^a



Clinical Implications

- The paper reveals the limited clinical usefulness of the current routinely available recombinant panel in patients who are *in vitro* double sensitized to Hymenoptera venom extracts.

TO THE EDITOR:

IgE antibodies against cross-reacting carbohydrate determinants (CCDs) are speculated to be one of the major causes (in addition to dual sensitization to and/or cross-reactivity between homologous venom proteins) of the *in vitro* double sensitization observed between honeybee (HB) and yellow-jacket (YJ) venom extracts.¹ Furthermore, because IgEs against CCDs lack biological activity, they were thought to be responsible for the lack of *in vitro* IgE discrimination between clinically apparent allergy and clinically irrelevant sensitization (the majority of venom extract double-sensitized [DS] patients have a clinically apparent allergy to only one venom).²

The advent of CCD-free recombinant venom components was therefore hoped to have solved the issue of clinically irrelevant double IgE sensitization. However, after the first observations of excellent sensitivity/specificity of single (rApi m 1/rVes v 5) recombinant venom components,^{3,4} signs of inadequate sensitivity (as low as 57%)^{5,6} of single recombinants began to emerge. Because of the limited sensitivity of single components, additional recombinants (rVes v 1, rApi m 2, 3, 5, 10) were added to the battery of routinely used recombinants to be tested in a Hymenoptera venom-allergic individual. This resulted in increased sensitivity of the recombinants to the panel,⁷ a fact that was still observed to be inadequate by a recent publication.⁸ On the other hand, it seems that adding further components decreases the specificity of the recombinant panel because it has been shown by Eberlein et al⁹ that 95% (21 of 22) of patients who are *in vitro* DS with venom extracts are also DS if tested with the extended spectrum of recombinant components.

To establish the utility of the routinely available component-resolved diagnosis in patients who are *in vitro* DS to venom extracts, we included all subjects with positive IgE antibodies against HB and YJ venom extracts who were evaluated for Hymenoptera venom allergy (systemic reaction after HB and/or YJ/Horner sting) at our university clinic in 2015. All included patients were tested with rApi m 1 and rVes v 5, and if either of the components was negative, additional routinely available recombinants were tested (ie, rVes v 1 and/or rApi m 2, 3, 5, 10; in 69 [70.4%] patients, additional recombinants had to be measured). In all patients, skin tests (skin prick [SPT] and/or intradermal [IDT]) with HB and YJ venom extracts (10/100 µg/mL for SPT and 0.1/1 µg/mL for IDT)

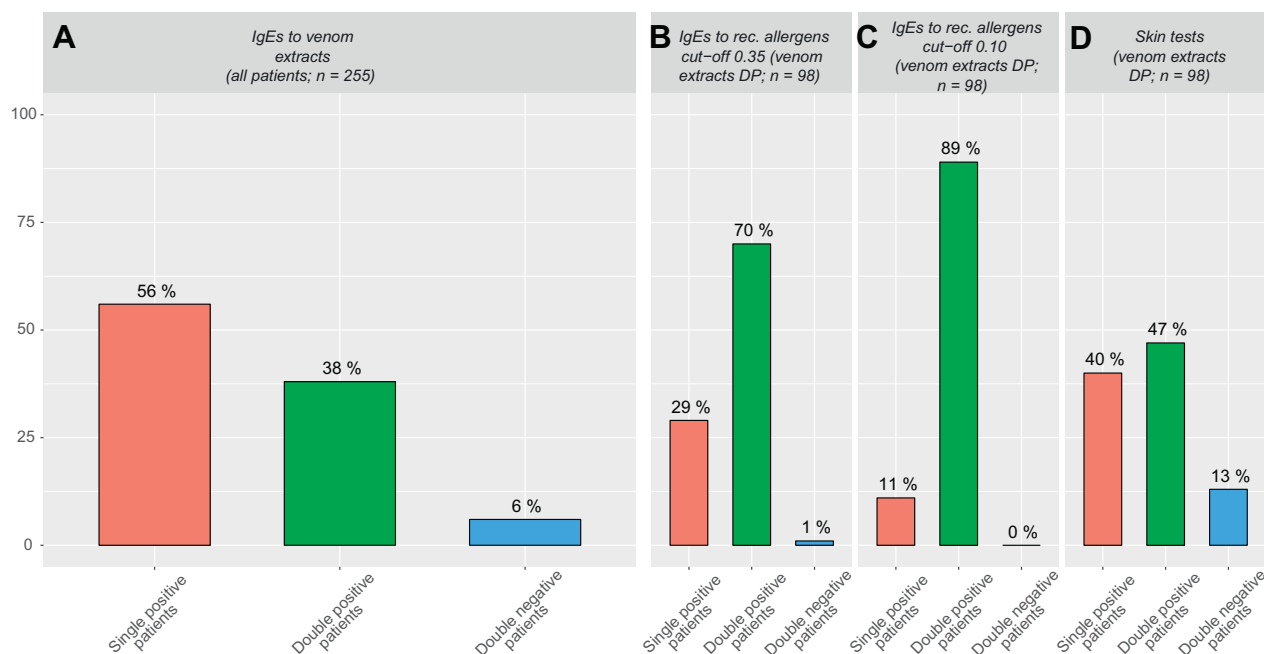


FIGURE 1. A, The percentage of patients single positive/double positive/double negative to venom extracts (all included patients). B-D, The percentage of patients single positive/double positive/double negative to recombinant panel components (B, 0.35 kU/L and C, 0.10 kU/L cutoff)/D, skin tests in venom extract double positive patients. DP, Double positive.

were performed. Furthermore, in all but 2 patients, IgE antibodies against MUXF3 (pure CCD-containing Ana c 2.0101 carbohydrate epitope) were measured.

IgE antibodies against venom extracts/venom components were measured via ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden) and IgEs against MUXF3 via the Immulite (Siemens, Tarrytown, NY) assay. The results of IgE antibodies were considered positive if they were ≥ 0.35 kU/L. Furthermore, the results of SPT were considered positive if the weal size was at least 3 mm bigger than the negative control, and erythema was present. The results of IDT were positive if the weal diameter after 20 minutes was at least 3 mm larger than the initial bleb (3–5 mm), and erythema was present.² These criteria, which are employed at our center, are based on British Society for Allergy and Clinical Immunology guidelines² and differ from the 5 mm cutoff accepted for positivity in IDT to venoms used in other reports.¹⁰

Of 255 (57.3% males, median age = 49 years old, interquartile range = 20.5 years) patients, 98 (38.3%) were *in vitro* DS with HB/YJ venom extracts. Of the 98 patients who were *in vitro* venom extract DS, 69 (70.4%; 95% confidence interval [CI] = 60.3% to 79.2%) were also DS with at least one recombinant venom component. Furthermore, if the cutoff for positivity was set to ≥ 0.10 kU/L, the rate of patients DS with at least one recombinant venom component rose to 88.8% (87 of 98; 95% CI = 80.8% to 94.3%). This high rate of recombinant double sensitization was apparent, even though we only measured the routinely available recombinant allergens and did not achieve allergenic coverage present in the venom extracts. At least 3 (Ves v 2, 3, 6) proteins from YJ venom extract and at least 7 (Api m 4, 6–9, 11, 12) from HB venom extract to which patients could have been allergic were missed, because they are lacking in the panel. The data are therefore contrary to the assumption that *in vitro* venom extract double sensitization is mostly due to IgE antibodies against CCDs. In our DS cohort, 55% (53 of 96) of patients had IgEs against MUXF3. However, 70% (69 of 98) of patients were double positive to CCD-free recombinant venom components.

With the use of 7 recombinant venom components, we were able to serologically dissect a relevant sensitizer venom in less than 30% of *in vitro* venom extract DS patients (Figure 1). Our data are consistent with the results of a recent study where 74.6% (94 of 126) of extract DS patients were also double positive with recombinant venom components.¹¹ The task of serological dissection was further complicated because none of the cross-reactive recombinant pairs (rApi m 2/rVes v 2, rApi m 5/rVes v 3, and rApi m 12/rVes v 6) are routinely (commercially) available as a pair (only one component per pair is available in most cases), and therefore, we were not able to infer a primary sensitizer. The current routinely available recombinant repertoire is therefore insufficient, not only because one cannot infer a primary sensitizer in a case of a positive cross-reactive recombinant component but also because it lacks adequate allergenic coverage (as shown by the patient who was *in vitro* DS with venom extracts, but was negative in all 7 tested recombinant components). For that reason, further routinely available components are probably needed. However, testing additional recombinant components will most likely also add to the pool of patients DS with the recombinant components (even higher than the already high 70%).

Overall, when used for Hymenoptera venom allergy diagnosis, the routinely available recombinant panel does not perform as

expected. It adds very little to the elucidation of a clinically relevant culprit in patients who are *in vitro* DS to venom extracts, a fact that is further evidenced by the phenomenon that the monosensitization rate with skin tests seems to be higher than the monosensitization rate with the recombinants (40% vs 29%, $P = .1$). However, with skin testing criteria² used in our study, we could have underestimated true double sensitization, as suggested by the quite high rate (13%) of skin test double negative patients in the *in vitro* DS cohort.

One possible explanation for the poor performance of the panel could lie in the fact that the panel does not contain the cross-reactive components from both venoms, and therefore one cannot infer a primary sensitizer in cases of sensitization to those components. The fact of the matter is that in 33 (48%) patients the component from the HB venom to which the patients were sensitized was either rApi m 2 or rApi m 5. Furthermore, in recombinant venom DS patients, there was almost a significant difference ($P = .08$) in the sensitization to species-specific components of both venoms between patients with double (62.5%) versus single (36.4%) positive skin tests. However, because of the lack of the appropriate cross-reactive component from the YJ venom, the relevancy of the sensitization could not be established.

The other possible explanation for such a result could be in the pathophysiology of the disease itself. Because the rate of asymptomatic sensitization in Hymenoptera venom allergy, a currently unexplained aspect of the disease, is quite high and clinical double reactivity to Apidae and Vespidae is rare,² perhaps measuring the sensitization profile is not a sufficient tool to infer the clinical relevancy of IgE venom extract double sensitization. Further research in the field is obviously needed.

^aLaboratory for Clinical Immunology and Molecular Genetics, University Clinic of Respiratory and Allergic Diseases Golnik, Golnik, Slovenia

^bClinical Ward 300, University Clinic of Respiratory and Allergic Diseases Golnik, Golnik, Slovenia

This study was a part of research program P3-0360 financed by Slovenian Research Agency.

Conflicts of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication January 2, 2018; revised April 13, 2018; accepted for publication April 29, 2018.

Available online May 23, 2018.

Corresponding author: Julij Šelb, MD, PhD, University Clinic of Respiratory and Allergic Diseases Golnik, Golnik 36, 4204 Golnik, Slovenia. E-mail: julij.selb@klinika-golnik.si.

2213-2198

© 2018 American Academy of Allergy, Asthma & Immunology

<https://doi.org/10.1016/j.jaip.2018.04.045>

REFERENCES

1. Hemmer W, Focke M, Kolarich D, Wilson IBH, Altmann F, Wöhrl S, et al. 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;108:1045-52.
2. Krishna MT, Ewan PW, Diwakar L, Durham SR, Frew AJ, Leech SC, et al. Diagnosis and management of Hymenoptera venom allergy: British Society for Allergy and Clinical Immunology (BSACI) guidelines. *Clin Exp Allergy* 2011; 41:1201-20.
3. Mittermann I, Zidarn M, Silar M, Markovic-Housley Z, Aberer W, Korosec P, et al. Recombinant allergen-based IgE testing to distinguish bee and wasp allergy. *J Allergy Clin Immunol* 2010;125:1300-1307.e3.
4. Muller UR, Johansen N, Petersen AB, Fromberg-Nielsen J, Haeberli G. Hymenoptera venom allergy: analysis of double positivity to honey bee and *Vespa* venom by estimation of IgE antibodies to species-specific major allergens Api m1 and Ves v5. *Allergy* 2009;64:543-8.

5. Korosec P, Valenta R, Mittermann I, Celesnik N, Erzen R, Zidarn M, et al. Low sensitivity of commercially available rApi m 1 for diagnosis of honeybee venom allergy. *J Allergy Clin Immunol* 2011;128:671-3.
6. Selb J, Kogovsek R, Silar M, Kosnik M, Korosec P. Improved recombinant Api m 1- and Ves v 5-based IgE testing to dissect bee and yellow jacket allergy and their correlation with the severity of the sting reaction. *Clin Exp Allergy* 2016;46:621-30.
7. Kohler J, Blank S, Muller S, Bantleon F, Frick M, Huss-Marp J, et al. Component resolution reveals additional major allergens in patients with honeybee venom allergy. *J Allergy Clin Immunol* 2014;133:1383-9. 1389.e1-6.
8. Arzt L, Bokanovic D, Schrautzer C, Schwarz I, Laipold K, Aberer W, et al. Questionable diagnostic benefit of the commercially available panel of bee venom components. *Allergy* 2017;72:1419-22.
9. Eberlein B, Krischan L, Darsow U, Ollert M, Ring J. Double positivity to bee and wasp venom: improved diagnostic procedure by recombinant allergen-based IgE testing and basophil activation test including data about cross-reactive carbohydrate determinants. *J Allergy Clin Immunol* 2012;130:155-61.
10. Golden DBK, Demain J, Freeman T, Graft D, Tankersley M, Tracy J, et al. Stinging insect hypersensitivity: a practice parameter update 2016. *Ann Allergy Asthma Immunol* 2017;118:28-54.
11. Frick M, Müller S, Bantleon F, Huss-Marp J, Lidholm J, Spillner E, et al. rApi m 3 and rApi m 10 improve detection of honey bee sensitization in Hymenoptera venom-allergic patients with double sensitization to honey bee and yellow jacket venom. *Allergy* 2015;70:1665-8.