

Letter to the Editor

Spiking venom with rVes v 5 improves sensitivity of IgE detection in patients with allergy to *Vespula* venom

To the Editor:

Diagnosis of yellow jacket (*Vespula* spp) venom allergy is based on a history of anaphylactic sting reactions, positive skin test responses, and/or detection of specific IgE to yellow jacket venom (YJV).¹ However, positive skin tests or serologic results obtained with conventional venom extracts do not always reflect a primary sensitization but may be caused by antibodies cross-reactive to homologous peptide sequences or to cross-reactive carbohydrate determinants. Additional value of IgE detection to cross-reactive carbohydrate determinant-free, species-specific recombinant (r) Hymenoptera venom allergens, such as rApi m 1, rVes v 1, and rVes v 5, has recently been reported in several studies.²⁻⁸ In patients with allergy to YJV, the diagnostic sensitivity of a combination of the currently available YJV allergens rVes v 5 and rVes v 1 has been reported to be as high as 92% to 96%,^{6,7} whereas the frequency of sensitization to rApi m 1 in patients with allergy to honeybee venom (HBV) appears to be lower, ranging from 58% to 80%, depending on the patient population studied.^{4-6,8,9}

Because a proportion of patients with allergy to insect venom, in particular those allergic to HBV, are missed by the currently

available recombinant venom allergens, the use of venom extracts is still recommended as the first line of laboratory investigation. For patients in which the causative insect is uncertain, and/or double-positive results are obtained with conventional venom extracts, the second-line analysis of IgE to available recombinant allergens has been found to be helpful in the identification of the relevant sensitization.^{2,3,4,7}

During the routine use of these allergens in the diagnosis of *Vespula* venom allergy, we frequently observed that IgE reactivity was higher to the major YJV allergen Ves v 5 than to YJV extract and that, in some cases, IgE reactivity was only detectable by rVes v 5 ImmunoCAP,⁴ suggesting a shortage of Ves v 5 reactivity in the conventional YJV ImmunoCAP (i3). Here, we address this issue in a large population of patients with allergy to YJV with the use of a YJV ImmunoCAP that has been enhanced by spiking with rVes v 5 (rVes v 5-spiked YJV ImmunoCAP).

Patients with systemic anaphylactic reaction to YJ stings (n = 308; 52% male) were recruited consecutively in 2 German allergy centers. Diagnosis of YJV allergy was based on the patient's history of an anaphylactic reaction to a YJ sting (identification of YJ as the culprit insect by the patient), a positive skin test to YJV and/or detection of IgE to YJV extracts, and, in some patients, on basophil activation or basophil histamine release tests. The skin tests were performed as titrated prick test with the use of 1, 10, and 100 µg/mL YJV extract. In case the prick test was negative, we

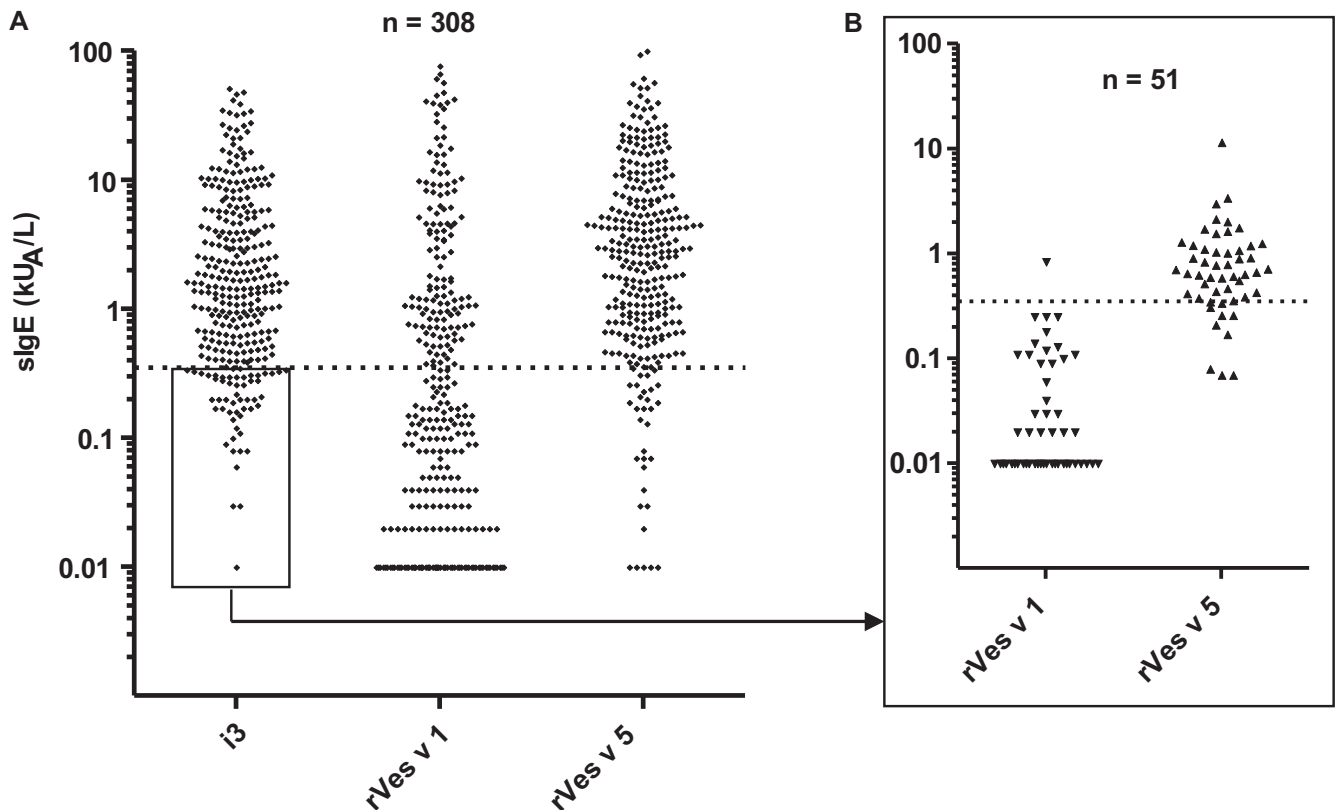


FIG 1. IgE antibody levels in patients with allergy to YJ sting (n = 308). **A**, IgE reactivity to YJV extract (ImmunoCAP i3), rVes v 1 (i211), and rVes v 5 (i209) in the entire population. **B**, IgE reactivity to rVes v 1 (i211) and rVes v 5 (i209) in the YJV extract (i3)-negative population.

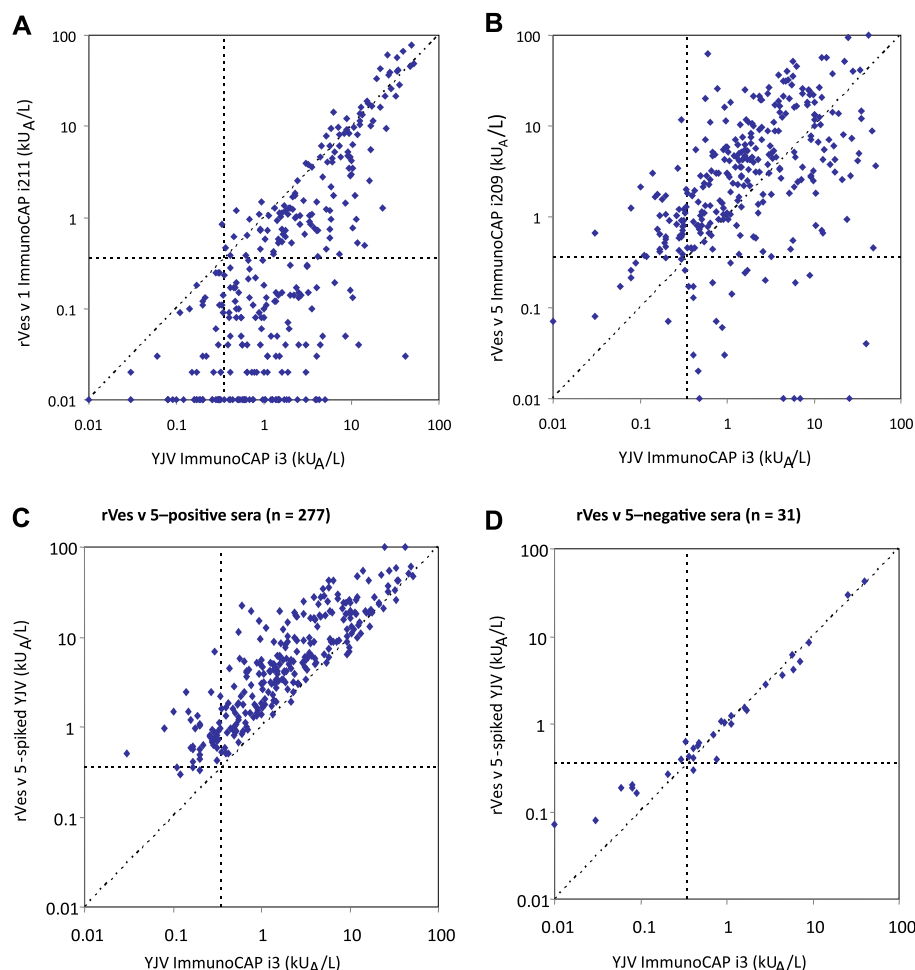


FIG 2. Comparison of IgE reactivity to conventional YJV extract, recombinant YJ allergens Ves v 1 and Ves v 5, and to Ves v 5-spiked YJV extract in patients with YJV allergy. **A**, Comparison of IgE levels with YJV ImmunoCAP (i3) and rVes v 1 (i211), all patients (n = 308). **B**, Comparison of IgE levels with YJV ImmunoCAP (i3) and rVes v 5 (i209), all patients (n = 308). **C**, Comparison of IgE levels with YJV ImmunoCAP (i3) and rVes v 5-spiked YJV extract in rVes v 5-positive patients (n = 277). **D**, Comparison of IgE levels with YJV ImmunoCAP (i3) and rVes v 5-spiked YJV extract in rVes v 5-negative patients (n = 31). *Hatched horizontal and vertical lines indicate the cutoff of 0.35 kU_A/L, and the hatched diagonal line represents a 1:1 ratio.*

additionally performed an intradermal test with the use of YJV extract at a test concentration of 1 μ g/mL. In 235 patients the diagnosis was based on history, positive skin test, and positive specific IgE (sIgE) to YJV extract (i3), in 22 patients on history and positive sIgE to YJV extract (skin test not done, n = 7; skin test negative, n = 15), in 47 patients on history and positive skin test (negative sIgE to YJV extract i3), in 1 patient on history and on basophile activation test (negative skin test, negative sIgE to YJV extract i3) and in 3 patients the diagnosis was based on history only. These patients had provided a convincing history of an anaphylactic reaction after a YJ sting but displayed a negative skin test, a negative sIgE to YJV extract, and cellular tests were not performed. Sixty percent of the entire patient group (n = 308) were monosensitized to YJV, whereas the remaining showed concomitant sensitisation to HBV extract (n = 122). IgE reactivity was analyzed to YJV extract (ImmunoCAP, i3), rVes v 1 (i211), rVes v 5 (i209), and to a newly developed ImmunoCAP in which the representation of Ves v 5 has been enhanced by spiking with rVes v 5 (rVes v 5-spiked YJV ImmunoCAP; Phadia AB, Uppsala, Sweden).

Sensitization (≥ 0.35 kU_A/L) to the conventional YJV extract ImmunoCAP (i3) was detected in 83.4%, to rVes v 1 in 44.2%, to rVes v 5 in 89.9%, and to either rVes v 1 or rVes v 5 or to both in 96.1% of the 308 study subjects (Fig 1, A). Among the i3-negative patients, only 1 patient was sensitized to rVes v 1, whereas 84.4% (42/51) tested positive to rVes v 5 (Fig 1, B). In most patients, the level of IgE was lower to Ves v 1 than to YJV, indicating that a significant part of the IgE reactivity to YJV is directed against determinants other than Ves v 1 (Fig 2, A). In contrast, the measured levels of IgE to rVes v 5 were substantially higher than level to YJV (Fig 2, B), with a median rVes v 5/YJV ratio of 2.4 among the Ves v 5-positive patients (n = 277), suggesting that Ves v 5 was underrepresented in the immobilized YJV extract. A number of mechanisms could theoretically explain a reduced Ves v 5 immunoreactivity in the extract compared with the recombinant protein, such as 1) a true shortage of Ves v 5 protein in the YJV extract, 2) inefficient coupling of Ves v 5 to the assay's solid phase, and 3) steric shielding of Ves v 5 epitopes by endogenous ligands of Ves v 5 or its attachment to the solid phase.

To examine whether this limitation in detection of Ves v 5 reactive IgE antibodies can be overcome by complementation of natural YJV with rVes v 5, we analysed IgE reactivity to a rVes v 5-spiked YJV ImmunoCAP. Compared with the conventional YJV ImmunoCAP i3, the rVes v 5-spiked YJV ImmunoCAP produced substantially higher IgE values in Ves v 5-positive sera ($n = 277$) (Fig 2, C), whereas no relevant difference in reactivity was observed in Ves v 5-negative sera ($n = 31$) (Fig 2, D). On analysis of all 308 subjects with allergy to YJV with this test, positive results were obtained in 298, compared with 257 with the conventional YJV ImmunoCAP i3. This increase in sensitivity from 83.4% to 96.8% was not accompanied by a change in specificity, as indicated by the analysis of IgE reactivity in 51 consecutive patients with confirmed HBV allergy and no history of YJV allergy (see Fig E1 in this article's Online Repository at www.jacionline.org). Twenty-four of these patients were mono-sensitized to HBV (i3 negative), and 27 patients displayed double sensitization to both HBV and YJV extract, 13 of whom displayed IgE reactivity to rVes v 5. Comparison of IgE reactivity to the conventional YJV and the rVes v 5-spiked YJV ImmunoCAP showed an excellent agreement among these patients (Fig E1).

Skin testing was performed in 301 of the 308 patients with allergy to YJV. Titrated skin prick test identified 48.8% (147/301), and a combination of prick and intradermal test identified 93.7% (282/301) of the patients. In skin test-negative patients ($n = 19$) positive IgE reactivity to Ves v 5-spiked YJV was detected in 18/19. A combination of skin test and IgE detection to Ves v 5-spiked YJV identified 99.7% (300/301) of the patients.

In conclusion, the conventional YJV ImmunoCAP, which is widely used in the diagnosis of insect venom allergy, displayed in our patient population a limited sensitivity of 83.4%, apparently because of incomplete capture of Ves v 5-reactive IgE antibodies. Spiking of the natural YJV extract with rVes v 5 substantially improved the sensitivity to 96.8%. Thus, the rVes v 5-spiked YJV ImmunoCAP will allow a more reliable detection of YJV sensitization in patients with insect venom allergy.

We thank Andrea Komann, Heike Neumann, and Michaela Rolle for excellent technical assistance and Dr Jonas Lidholm for providing the rVes v 5-spiked YJV ImmunoCAPs.

Byrthe Vos^{b,*}
Julian Köhler^{a,*}

Sabine Müller, MD^a
Eva Stretz^b
Franziska Ruëff, MD^b
Thilo Jakob, MD^a

From ^athe Allergy Research Group, Department of Dermatology, University Medical Center, Freiburg, Germany; and ^bthe Allergy Unit, Department of Dermatology, Ludwig-Maximilians-Universität, Munich, Germany. E-mail: thilo.jakob@uniklinik-freiburg.de.

*These authors contributed equally to this work.

Disclosure of potential conflict of interest: T. Jakob has received research support from the German Research Council, Landesstiftung Baden-Württemberg, COLIPA, Birken GmbH, Allergopharma, and Phadia/Thermo Fischer and is a board member for the European Immunodermatology Society and the German Society of Allergy & Clinical Immunology. F. Ruëff has received grants from Thermo Fisher and ALK-Abelló; has consultant arrangements with ALK-Abelló, HAL, and Thermo Fisher; has participated in studies for ALK-Abelló, Allergopharma, and HAL; and is chair of the Insect Venom Allergy Interest Group for the European Academy of Allergy and Clinical Immunology. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Biló BM, Ruëff F, Mosbech H, Bonifazi F, Oude-Elberink JN. Diagnosis of Hymenoptera venom allergy. *Allergy* 2005;60:1339-49.
2. 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 m 1 and Ves v 5. *Allergy* 2009;64:543-8.
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;125:1300-7.
4. Hofmann SC, Pfender N, Weckesser S, Huss-Marp J, Jakob T. Added value of IgE detection to rApi m 1 and rVes v 5 in patients with Hymenoptera venom allergy. *J Allergy Clin Immunol* 2011;127:265-7.
5. Sturm GJ, Hemmer W, Hawranek T, Lang R, Ollert M, Spillner E, et al. Detection of IgE to recombinant Api m 1 and rVes v 5 is valuable but not sufficient to distinguish bee from wasp venom allergy. *J Allergy Clin Immunol* 2011;128:247-8.
6. Hofmann SC, Pfender N, Weckesser S, Blank S, Huss-Marp J, Spillner E, et al. Reply. *J Allergy Clin Immunol* 2011;128:248.
7. Korosec P, Valenta R, Mittermann I, Celesnik N, Silar M, Zidarn M, et al. High sensitivity of CAP-FEIA rVes v 5 and rVes v 1 for diagnosis of Vespula venom allergy. *J Allergy Clin Immunol* 2012;129:1406-8.
8. 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.
9. Jakob T, Köhler J, Blank S, Huss-Marp J, Spillner E, Lidholm J. Comparable IgE reactivity to nApi m 1 and rApi m 1 in CCD negative bee venom allergic patients. *J Allergy Clin Immunol* 2012;130:276-8.

<http://dx.doi.org/10.1016/j.jaci.2012.07.041>

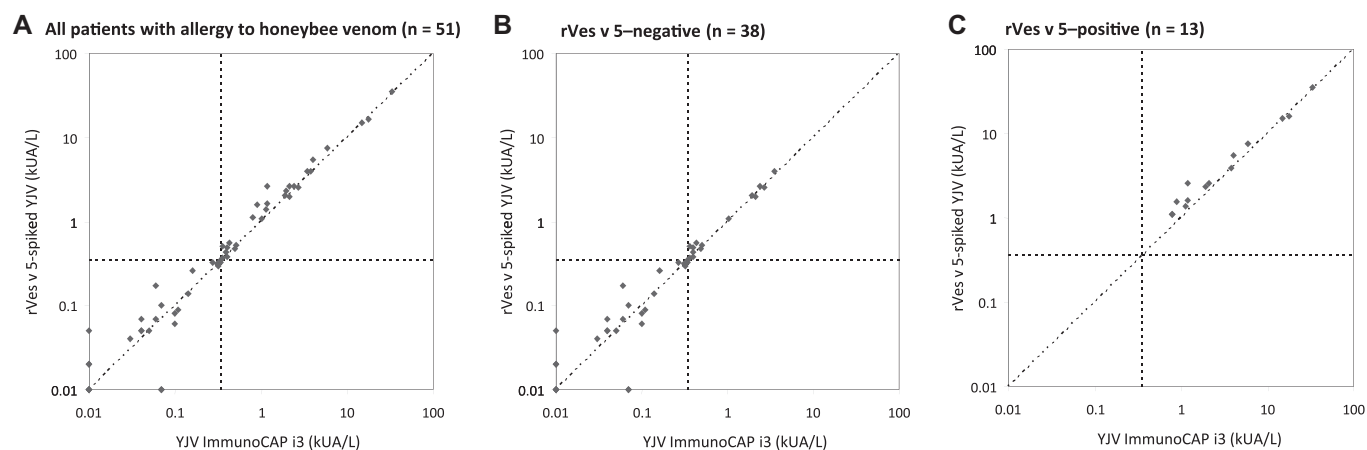


FIG E1. Comparison of IgE levels with YJV ImmunoCAP (i3) and rVes v 5-spiked YJV extract in patients with allergy to honeybee venom. **A**, Entire population (n = 51). **B**, rVes v 5-negative patients (n = 38). **C**, rVes v 5-positive patients (n = 13). *Hatched horizontal and vertical lines indicate the cutoff of 0.35 kUA/L, and the hatched diagonal line represents a 1:1 ratio.*