

### Routine clinical utility of honeybee venom allergen components

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#### Clinical Implications

- Using a complete panel of 5 honeybee venom components, we missed almost 10% of established honeybee-allergic patients. These findings imply that whole venom testing whether by skin test or by serology remains preferred to component testing.

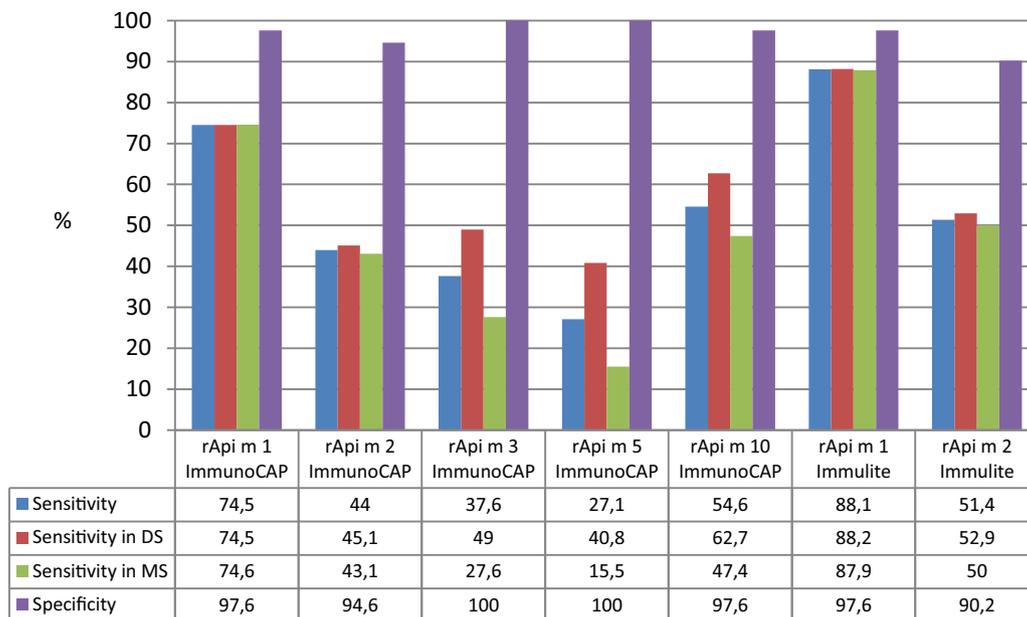
#### TO THE EDITOR:

Since 2010 to 2014, the ImmunoCAP rApi m 1 was the only honeybee venom (HBV) allergen component commercially available for component-resolved diagnostics (CRD) of honeybee venom allergy. Its clinical utility was limited by insufficient sensitivity (ranging from 57% to 82%)<sup>1-4</sup> and led to the search for additional HBV components to improve the overall diagnostic sensitivity of CRD in HBV-allergic patients.<sup>5</sup> Finally, a broader spectrum of HBV allergen components has become recently commercially available. Since entering the market, only a limited number of studies tested the clinical utility of these new components, and often with inconsistent results.<sup>6-8</sup> Therefore, these new components are currently questioned, and according to some, discredited for use in routine clinical practice.<sup>8</sup> For that reason, we evaluated the diagnostic utility of these components in our HBV-allergic patients, using 2 major currently available immunoassays ImmunoCAP (Phadia, Uppsala, Sweden) and Immulite (Siemens Healthcare GmbH, Erlangen, Germany). We aimed to calculate the sensitivity and specificity of each available component and component combinations for both test systems. In our multicenter study, we included 110 HBV-allergic patients (77 from Pilsen, Czech Republic, and 33 from Golnik, Slovenia; 66% males; median age, 49 years; range, 5-72 years). All patients had a history of systemic honeybee sting reaction (12 [10.9%] grade I, 20 [18.2%] grade II, 46 [41.8%] grade III, and 32 [29.1%] Mueller grade IV reaction), and in all patients, the sensitization was confirmed by whole HBV skin and/or IgE testing. Almost all patients (107 of 110 [97.3%]) are indicated for HBV immunotherapy. The control group consisted of 41 asymptomatic subjects (56% males; median age, 37 years; range, 13-73 years) with a history of Hymenoptera insect stings, but with no allergic reactions following the stings. In all HBV-allergic patients, specific IgE levels to commercially available recombinant HBV components using ImmunoCAP (rApi m 1, 2, 3, 5, and 10) and Immulite (rApi m 1 and 2) were assessed from samples taken during the initial diagnostic evaluation at least 2 weeks after the sting reaction. The value of 0.35 kIU/L was considered the standard threshold for positivity. Sensitivities of each component and component combinations were calculated in the whole group

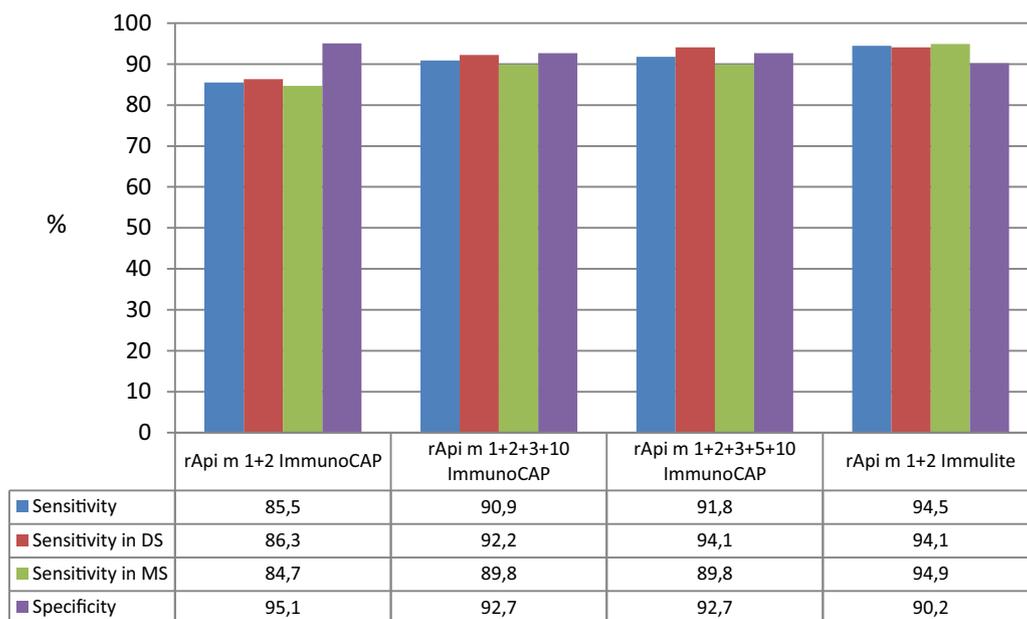
of 110 patients, and in 2 subgroups, in 59 mono-sensitized patients (only to HBV) and 51 double-sensitized (to HBV and yellow jacket venom [YJV]) patients. For the calculation of specificities, we used 41 control subjects.

Analysis of IgE to single HBV components (Figure 1) confirmed the importance of rApi m 1 as the major HBV component, with the highest sensitivity obtained by both test systems, with 74.5% for ImmunoCAP and 88.1% for Immulite. Although the sensitivity was significantly higher for Immulite ( $P < .001$ ), specificity, 97.6%, was the same using both test systems. A similar finding was observed for rApi m 2, that is, significantly higher sensitivity of rApi m 2 using Immulite (51.4%) compared with ImmunoCAP (44.0%;  $P = .023$ ), with similar specificity (90.2% and 94.6%, respectively). ImmunoCAP and Immulite quantitative data for both rApi m 1 and rApi m 2 correlate very well; the Spearman correlation coefficient is 0.934 for rApi m 1 and 0.932 for rApi m 2 (see Figure E1 in this article's Online Repository at [www.jaci-inpractice.org](http://www.jaci-inpractice.org)). Other available ImmunoCAP components showed sensitivity and specificity of 54.6% and 97.6% for rApi m 10, 37.6% and 100% for rApi m 3, and 27.1% and 100% for rApi m 5. There were no significant differences between mono-sensitized and double-sensitized HBV-allergic patients both for rApi m 1 and for rApi m 2 (with both test systems) and also for rApi m 10 (ImmunoCAP). However, significant differences were demonstrated for rApi m 3 ( $P = .029$ ) and especially for the potentially cross-reactive rApi 5 ( $P = .004$ ), with a very low sensitivity of only 15.5% in mono-sensitized patients. Analysis of component combinations revealed the higher sensitivity of 94.5% for rApi m 1 and rApi m 2 determination by using Immulite in comparison to only 85.5% by using ImmunoCAP. The addition of HBV-specific rApi m 3 and 10 increased the sensitivity up to 90.9%. However, the addition of rApi m 5 further increased the sensitivity by only 0.9%. Thus, the sensitivity for the whole ImmunoCAP panel of 5 components was 91.8%, which was still 2.7% lower than the Immulite rApi m 1 and m 2 testing (94.5%) (Figure 2). Notably, the reason for this difference was mono-sensitized patients (ImmunoCAP vs Immulite, 89.8% vs 94.9%, respectively) and not double-sensitized patients. Specificities of whole IgE panels were 92.7% and 90.2%, respectively, that is, 2.5% higher for ImmunoCAP. To summarize, the use of Immulite rApi m 2 improved the sensitivity by 6.4% (from 88.1% to 94.5%) in contrast to using rApi m 1 alone ( $P = .016$ ), and similarly, the use of a whole ImmunoCAP honeybee panel (addition of rApi m 2, 3, 5, 10) led to the increase in sensitivity by 17.3% in contrast to using rApi m 1 alone, from 74.5% up to 91.8% ( $P < .001$ ).

In addition, we showed that 3 (7.3%) control subjects were mono-sensitized to HBV, 8 (19.5%) to YJV, and 6 (14.6%) were double-sensitized (to HBV and YJV). This is consistent with other reports of the high level of asymptomatic Hymenoptera venom sensitization.<sup>9</sup> However, we further demonstrated that 2 HBV mono-sensitized and 2 double-sensitized control subjects were positive for HBV components (rApi m 1, 2, and/or 10; both systems) and even 7 YJV mono-sensitized and 4 double-sensitized control subjects were



**FIGURE 1.** Sensitivity and specificity of IgE to available bee venom components using ImmunoCAP and Immulite. *DS*, Double-sensitized patients to bee and yellow jacket venoms; *MS*, mono-sensitized patients to bee venom.



**FIGURE 2.** Sensitivity and specificity of IgE to bee venom component combinations using ImmunoCAP and Immulite. *DS*, Double-sensitized patients to bee and yellow jacket venoms; *MS*, mono-sensitized patients to bee venom.

positive for YJV components (rVes v 5 and/or rVes v 1; ImmunoCAP).

In conclusion, our study confirmed the dominant role of rApi m 1 as a key HBV component for the HBV allergy. Furthermore, we demonstrated that additional routinely available HBV components improve the overall diagnostic sensitivity of CRD in HBV-allergic patients, in case of both ImmunoCAP and Immulite testing. However, considering differently available

spectrum and significantly varying clinical significance of HBV components for different immunoassays, the clinicians should be aware of which method is being used. Thus, in the case of using Immulite, the assessment of rApi m 1 should be the first step, followed by rApi m 2 testing. In case of using ImmunoCAP, because of low diagnostic sensitivity of rApi m 1, the assessment should start with almost a whole panel with inclusion of rApi m 1, 2, 3, and 10 components; the addition of rApi m 5 seems to

have a very limited value as in our study it assisted for recognition of only 1 patient. However, even a complete ImmunoCAP panel of 5 components missed almost 10% of established HBV-allergic patients with a positive skin test result and/or HBV IgE. These results suggest that the addition of novel HBV components has still not corrected limited diagnostic sensitivity of CRD testing. Therefore, whole HBV testing whether by skin test or by serology remains preferred to CRD testing. Besides unsolved sensitivity issue, a higher cost of CRD testing (5 components) limits its use as a general strategy for routine evaluation of HBV-allergic patients in clinical practice.

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

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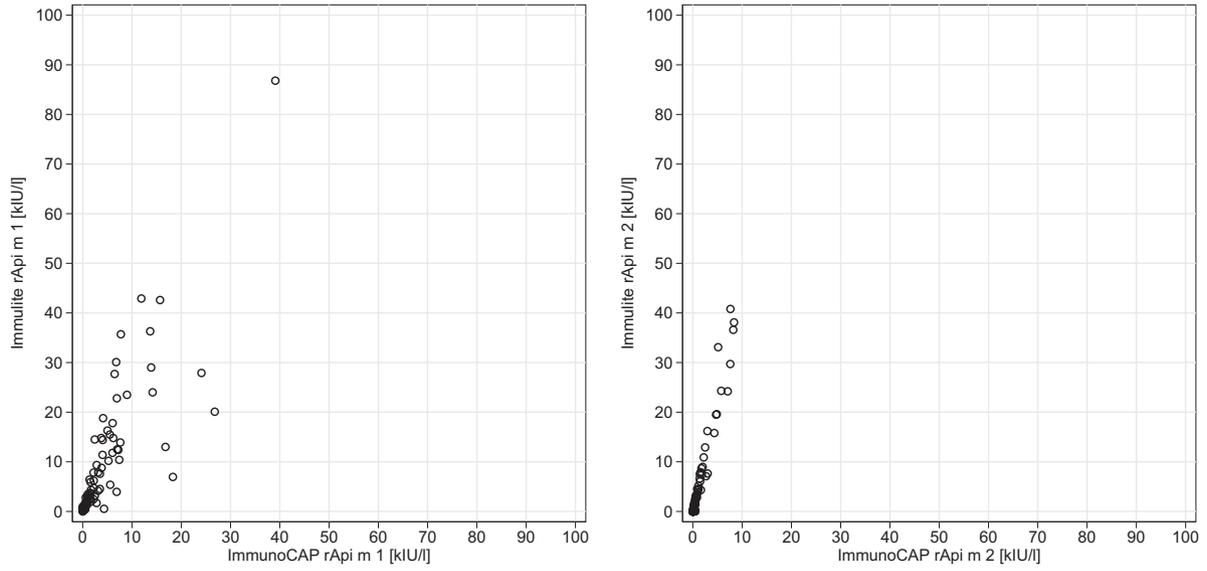
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**FIGURE E1.** Correlation plots for rApi m 1 and for rApi m 2 with the quantitative ImmunoCap data on the X axis and Immulite data on the Y axis.