

Correspondence

Comparing sensitivity of Hymenoptera allergen components on different diagnostic assay systems: Comparing apples and oranges?

To the Editor:

In a recent letter to the editor published in the *Journal*, Schrautzer et al.¹ reported the comparison of 2 different diagnostic assays, namely ImmunoCAP (Thermo Fisher Scientific, Waltham, Mass) and Immulite (Siemens, Tarrytown, NY), for the detection of specific IgE (sIgE) responses to allergen components in a population of patients with Hymenoptera venom allergy. The study reports a higher rate of positive test results (≥ 0.35 kU_A/L) on the Immulite compared with the ImmunoCAP system for sIgE to rApi m 1 in patients with bee venom allergy and for sIgE to rVes v 5 in patients with vespid venom allergy and concludes that the Immulite system displays a higher sensitivity for the detection of sIgE to these Hymenoptera venom allergens. Similar observations were reported in another recent publication by Selb et al.² In particular, the sensitivity of rApi m 1 has been a matter of debate.²⁻⁴ Schrautzer et al.¹ suggest that the rApi m 1 used in the ImmunoCAP has a reduced IgE-binding capacity and speculate that this might be due to altered protein folding without reference to a previous report in the same journal that had demonstrated equivalent IgE antibody binding of this particular recombinant protein and purified natural Api m 1.⁴

A test system with increased sensitivity and comparable specificity would certainly be of benefit for the diagnosis and management of patients with Hymenoptera venom allergy; however, a few issues need to be raised that have not adequately been considered by Schrautzer et al.¹ Systematic differences in assay characteristics of the 2 test systems have been reported before.⁵⁻⁷ Both systems use a calibration concept based on total IgE (tIgE) for determination of sIgE levels. Mouse-human chimeric IgE antibodies with defined allergen monospecificity and concentration have been used to evaluate the accuracy of sIgE measurements in different assay systems.⁵⁻⁷ In this approach IgE antibody of a single specificity comprises the entire amount of IgE present in the sample, allowing assessment of the system's calibration for sIgE by comparing the sIgE and tIgE measurements it delivers. Although equivalent sIgE and tIgE results were obtained with the ImmunoCAP system, the Immulite system reported sIgE concentrations that were on average 2- to 3-fold higher than the tIgE concentrations in the same sample. Considering that the tIgE measurements agreed well between systems and with the predefined tIgE concentration of the samples, the findings demonstrated that Immulite delivers overestimated sIgE levels. This will inevitably lead to a greater number of test results exceeding any given cutoff value but does not represent a higher sensitivity of the assay. Because a similar sIgE/tIgE discordance has already been reported for 5 different allergens (Bet v 1, Can f 1, Der p 2, Fel d 1, and Gal d 1),⁵⁻⁷ it is likely to apply also to the Hymenoptera venom allergens discussed here and explain the seemingly more sensitive detection of sIgE to rApi m 1 and rVes v 5 reported by Schrautzer et al.¹ In line with this is the observation by Selb et al.² that positive values of rApi m 1 and rVes v 5 were, on average, 2.7 and 2.3 times higher

with the Immulite than with the ImmunoCAP system.² Unfortunately, Schrautzer et al.¹ did not provide data on the actual sIgE levels obtained, preventing a corresponding evaluation of their results.

Finally, the question of whether rApi m 1 with a mutated glycosylation site that is used in the ImmunoCAP has a reduced sIgE-binding capacity has already been addressed in the past.⁴ Cross-reactive carbohydrate determinant-negative sera from patients with honeybee venom allergy displayed equivalent IgE reactivity to the natural purified Api m 1 and the rApi m 1 used in the ImmunoCAP. In addition, there was no relevant difference in the IgE reactivity to insect cell-expressed rApi m 1 with or without the point mutation in the glycosylation site.⁴

In conclusion, we think that the observed differences have little to do with the quality of the allergens but are rather a result of a systemic difference in the calibration of both systems. Thus we would like to conclude with the words of Wood et al: "Just because 2 systems present their results in the same units does not mean that the results are necessarily correct or interchangeable."⁵

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