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Reply

To the Editor:

We thank Rafei-Shamsabadi et al[1] for their interest in our article.[2] Our article proves that Hymenoptera venom–allergic patients negative to standard *in vitro* venom extract specific IgE (sIgE) detection are identified by *in vitro* analysis of sIgE to recombinant allergens. sIgE levels were measured by using UniCAP250 (Thermo Fisher), Immulite2000 (Siemens Healthcare Diagnostics, Eschborn, Germany), or ELISA.[2] Unexpectedly, Rafei-Shamsabadi et al found that almost all subjects negative to the newest standard venom extract–based measurements were negative to all recombinant allergens *in vitro*. This discrepancy may be the result of a different study sample, different period of sample collection, and/or a different sensitivity of the *in vitro* research prototype allergen used to detect sIgE against recombinant allergens.

Although our cohort included patients with a positive intradermal test result and a history of anaphylaxis above grade II, Rafei-Shamsabadi et al included subjects with a history of grade I anaphylaxis and/or subjects in whom the sIgE-mediated reaction is not fully demonstrated. For example, 25% of the subjects are negative to skin prick test, intracutaneous test, and *in vitro* sIgE to venom extract. About 65.7% of the remaining subjects are negative to prick test and positive at the single highest dose of 1 μg/mL of venom to intracutaneous test.

Because it has been previously reported that the selection criteria of patients affect test results even using the same test platform[3-5] and that the sensitivity of sIgE detection may vary according to the *in vitro* test performed,[6] we compared the sensitivity of the ImmunoCAP 250 and the Immulite 2000 systems. Through measuring sIgE to recombinant (r) Api m 1 in honeybee venom–allergic patients (Fig 1, *A*), we found that the Immulite2000 platform detected higher Api m 1 sIgE levels than did ImmunoCAP250. Furthermore, the Immulite2000 was about 18% more sensitive than the corresponding ImmunoCAP250 system (Fig 1, *A*).

To confirm that recombinant allergens improve the diagnosis of Hymenoptera venom–allergic patients, we studied 6 yellow jacket venom–allergic patients with a convincing history of anaphylaxis and negative or unclear serology to sIgE against venom extracts (see Table E1 in this article's Online Repository at www.jacionline.org). We measured sIgE reactivity toward recombinant allergens using both the ImmunoCAP250 and the Immulite2000 platforms. In agreement with Rafei-Shamsabadi et al,[1] patients with negative serology to the new ImmunoCAP yellow jacket venom extract spiked with rVes v 5 were also negative to rVes v 1 and rVes v 5 tested on the same platform (Fig 1, *B*). However, these patients were positive to venom extracts and rVes v 5, when tested by using the Immulite2000 platform. This platform was found to be more sensitive than the ImmunoCAP250 in this analysis (Fig 1, *B*). Moreover, patients negative to venom extracts in Immulite2000 were positive to rVes v 1 and/or rVes v 5 when tested with the Immulite2000 platform (Fig 1, *B*).

In line with previous reports,[6] our data suggest differential sensitivities of sIgE testing results with extracts and recombinant allergens when using Immulite2000 and ImmunoCAP250 immunoassay platforms. Furthermore, consistent with our previous report, it confirms that recombinant allergens improve the detection of extract-negative venom-allergic patients tested by using the current technologies.[2]

These new results together with our originally published data partially explain the discrepancy between our findings and the findings of Rafei-Shamsabadi et al. Furthermore, they highlight the need for harmonized multicenter studies to standardize the use of recombinant allergens for *in vitro* detection of Hymenoptera venom–allergic patients.

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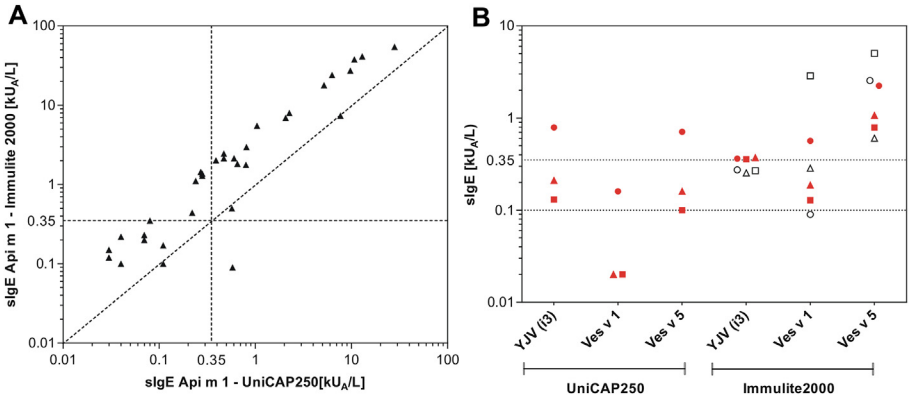

FIG 1. Improved detection of Hymenoptera venom–allergic patients by recombinant allergens tested in Immulite2000 but not in ImmunoCAP250. **A,** Comparison of sIgE antibody levels to rApi m 1 (i208) on ImmunoCAP250 and on Immulite2000 (using a research protopype of rApi m 1) immunoassay platforms. Hatched horizontal and vertical lines indicate the 0.35 kU_A/L cutoff, and the hatched diagonal line represents a 1:1 ratio. **B,** sIgE antibody responses to YJV extracts, rVes v 1, and rVes v 5 measured by using ImmunoCAP250 and Immulite2000 platforms. YJV extract spiked with rVes v 5 was used to measure IgE on ImmunoCAP250. *YJV,* Yellow jacket venom.

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Correction

With regard to the article in the April 2014 issue entitled "A common single nucleotide polymorphism impairs B-cell activating factor receptor's multimerization, contributing to common variable immunodeficiency" (J Allergy Clin Immunol 2014;133:1222-5), co-author Gina Fiala's affiliation was listed incorrectly. The correct affiliation is the Spemann Graduate School of Biology and Medicine (SGBM), Albert Ludwigs University, Freiburg, Freiburg, Germany. In addition, the acknowledgement is amended to include the following: "This work was supported by the German Research Foundation (DFG) through the Excellence Initiative GSC-4 (Spemann Graduate School SGBM)." The authors regret the error.

TABLE E1. Patients' characteristics

Patient no.	Culprit insect	Sting reaction grade	sIgE HBV (kU_A/L) IL2000	sIgE YJV (kU_A/L) IL2000	sIgE rVesv 1 (kU_A/L) IL2000	sIgE rVes v 5 (kU_A/L) IL2000	sIgE YJV (kU_A/L) IC250	sIgE rVesv 1 (kU_A/L) IC250	sIgE rVes v 5 (kU_A/L) IC250
1	YJ	III	<0.1	0.274	0.090	2.550	ND	ND	ND
2	YJ	III	<0.1	0.268	2.870	5.040	ND	ND	ND
3	YJ	III	<0.1	0.372	0.187	1.070	0.210	0.020	0.160
4	YJ	I	<0.1	0.363	0.565	2.240	0.790	0.160	0.710
5	YJ	II	<0.1	0.357	0.128	0.792	0.130	0.020	0.100
6	YJ	III	<0.1	0.254	0.285	0.605	ND	ND	ND

Culprit insect as identified by the patient, grade of clinical reaction (according to Ring J, Messmer K. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. Lancet 1977;1:466-9), total IgE, sIgE to honeybee venom (HBV), yellow jacket venom (YJV), rVes v 1 and rVes v 5 tested by using Immulite2000 (IL2000) or ImmunoCAP250 (IC250) platforms are shown. sIgE YJV IC250 refers to the ImmunoCAP250 i3 spiked with rVes v 5 technology.
ND, Not done; *YJ*, yellow jacket.