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Original article

Improved sensitivity to venom specific-immunoglobulin E by spiking with the allergen component in Japanese patients suspected of Hymenoptera venom allergy

Naruo Yoshida [a, 1], Hirokuni Hirata [a, *, 1], Mineaki Watanabe [a], Kumiya Sugiyama [c], Masafumi Arima [b], Yasutsugu Fukushima [c], Yoshiki Ishii [a]

[a] Department of Pulmonary Medicine and Clinical Immunology, Dokkyo Medical University, Tochigi, Japan
[b] Department of Developmental Genetics, Chiba University Graduate School of Medicine, Chiba, Japan
[c] Department of Pulmonary Medicine, Dokkyo Medical University Koshigaya Hospital, Saitama, Japan

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ABSTRACT

Background: Ves v 5 and Pol d 5, which constitute antigen 5, are recognized as the major, most potent allergens of family Vespidae. Several studies have reported the diagnostic sensitivity of the novel recombinant (r)Ves v 5 and rPol d 5 allergens in routine clinical laboratory settings by analyzing a group of *Vespula* and *Polistes* venom-allergic patients. In this study, we analyzed the sensitivity to venom specific (s)IgE by spiking with rVes v 5 and rPol d 5 in Japanese patients suspected of Hymenoptera venom allergy.

Methods: Subjects were 41 patients who had experienced systemic reactions to hornet and/or paper wasp stings. Levels of serum sIgE against hornet and paper wasp venom by spiking with rVes v 5 and rPold d 5, respectively, as improvement testing, compared with hornet and paper wasp venom, as conventional testing, were measured by ImmunoCAP.

Results: Of the 41 patients, 33 (80.5%) were positive (≥0.35 UA/ml) for hornet and/or paper wasp venom in conventional sIgE testing. sIgE levels correlated significantly ($P < 0.01$) between hornet ($R = 0.92$) or paper wasp venom ($R = 0.78$) in improvement testing and conventional testing. To determine specificity, 20 volunteers who had never experienced a Hymenoptera sting were all negative for sIgE against these venoms in both improvement and conventional testing. Improved sensitivity was seen in 8 patients negative for sIgE against both venoms in conventional testing, while improvement testing revealed sIgE against hornet or paper wasp venom in 5 (total 38 (92.7%)) patients.

Conclusions: The measurement of sIgE following spiking of rVes v 5 and rPol d 5 by conventional testing in Japanese subjects with sIgE against hornet and paper wasp venom, respectively, improved the sensitivity for detecting Hymenoptera venom allergy. Improvement testing for measuring sIgE levels against hornet and paper wasp venom has potential for serologically elucidating Hymenoptera allergy in Japan.

Introduction

Ves v 5 and Pol d 5 are 23-kDa proteins found in hornet (*Vespula* spp.) venom and paper wasp (*Polistes dominulus*) venom, respectively, of family Vespidae. Both proteins are known as antigen 5, which is recognized as the major and most potent Vespidae allergen.[1,2] Antigen 5 is not found in honey bee venom,[3] but has been found in the venom of various ant species, and great variation in protein sequence similarity to homologous proteins of various Vespidae species has been demonstrated.[4,5] Recently, several studies reported the diagnostic sensitivity of the novel recombinant (r)Ves v 5 and rPol d 5 allergens in a routine clinical laboratory setting by analyzing a group of *Vespula* and *Polistes* venom-allergic patients.[6–8] The rVes v 5 and rPol d 5 allergen components, which are expressed in insect cells, were developed in 2010 for use in the

* Corresponding author. Department of Pulmonary Medicine and Clinical Immunology, Dokkyo Medical University, Mibu-machi, Shimotsuga-gun, Tochigi 321-0293, Japan.
E-mail address: hirokuni@dokkyomed.ac.jp (H. Hirata).
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[1] These authors contributed equally to this work.

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ImmunoCAP solid-phase immunoglobulin (Ig)E assay (capsulated hydrophilic carrier polymer (CAP)-fluoro-enzyme immunoassay (FEIA); Phadia, Uppsala, Sweden). In particular, in patients sensitive to yellow jacket venom and paper wasp venom, the diagnostic sensitivity of the currently available rVes v 5 and rPol d 5 has been reported to be as high as 92–96% and 87.5%, respectively.[6,9–11] And about 10–20% of tests produced false negative results for specific-IgE (sIgE) in patients with Hymenoptera allergy.[12–15] Consequently, the low sensitivity makes this test unsuitable for use as a diagnostic tool. In addition, the diagnostic sensitivity of Hymenoptera allergy in Japan have not been revealed.

In this study, to assess improvements in sensitivity, we analyzed sIgE levels against hornet and paper wasp venom by spiking with rVes v 5 and rPol d 5 in Japanese patients suspected of having Hymenoptera venom allergy.

Methods

Participants

Subjects were 41 patients (28 men, 13 women; mean age, 62.3 ± 11.7 years; range, 39–86 years) who had experienced systemic reactions to hornet and/or paper wasp stings, but not honey bee stings, and who had visited Dokkyo Medical University between March and December 2013. Of the subjects, 14, 5, 6, and 16 had experienced anaphylaxis of Mueller grade[16] I, II, III, and IV, respectively, in the most severe systemic reactions, but toxic systemic reactions due to multiple Hymenoptera stings were excluded. None of the subjects had received allergen immunotherapy for Hymenoptera venom. Twenty volunteers (18 men, 2 women; mean age, 32.6 ± 4.5 years; range, 25–51 years) who had never experienced a Hymenoptera sting were enrolled as controls. All participants completed a medical examination involving an interview with an allergist and underwent peripheral blood tests. This study was approved by the Dokkyo Medical University Research Ethics Committee, and each participant provided written informed consent prior to study enrolment.

Blood testing

A 10-ml peripheral blood sample was drawn from each participant. The intervals between the last sting and peripheral blood collection differed among the subjects. Serum was extracted and stored at −80 °C until used for analysis. Levels of serum sIgE against (1) hornet venom and paper wasp venom, as conventional testing, (2) rVes v 5 and rPol d 5, as the allergen components, and (3) spiking of rVes v 5 for hornet venom sensitivity and rPold d 5 for paper wasp venom sensitivity, as conventional testing, were measured by Phadia Co. (Tokyo, Japan) to determine improved sensitivity. Detection of sIgE by FEIA using the CAP system was expressed in quantitative units (UA/ml) or as a conventional spectrum of 7 semi-quantitative classes ranging from class 0 (<0.35 UA/ml) to class 6 (≥100 UA/ml). In this study, sIgE-positive results were defined as an sIgE level ≥0.35 UA/ml (i.e., ≥class 1).

Statistical analysis

Data are presented as the means ± SD. **Single** regression analysis was used to assess the significance of the correlations among the sIgE values. A P value <0.05 was considered significant. Statistical analysis was performed using SPSS software version 15.0 for Windows.

Results

Correlations of sIgE levels between conventional testing and allergen components, between allergen components, and between conventional tests for hornet and paper wasp venom

In the 41 subjects, the levels of sIgE by improvement testing, compared to those by conventional testing, significantly increased in hornet (2.5 ± 5.3 UA/ml vs 9.2 ± 17.9 UA/ml, P< 0.01) and paper wasp (4.3 ± 8.6 UA/ml vs 15.3 ± 25.3 UA/ml, P < 0.01). Of the 41 subjects, 33 were positive for sIgE by conventional testing for hornet venom (3.1 ± 5.8 UA/ml; range, <0.35–25.8 UA/ml) and/or paper wasp venom (5.4 ± 9.3 UA/ml; range, <0.35–43.6 UA/ml). The remaining 8 subjects and 20 controls were negative for sIgE against hornet and paper wasp venom by conventional sIgE testing. No correlations were observed between the severity of systemic reactions according to Mueller grade and sIgE levels in conventional testing for hornet and paper wasp venom. In the 33 positive subjects, we analyzed by conventional testing the correlations of sIgE levels against hornet or paper wasp venom with those of rVes v 5 or rPol d 5, respectively, between rVes v 5 and hornet venom, and between rPol d 5 and paper wasp venom (Fig. 1). The mean levels of sIgE against rVes v 5 and rPol d 5 were 15.1 ± 26.3 UA/ml (range, <0.35–≥100 UA/ml) and 20.8 ± 29.4 UA/ml (range, <0.35–≥100 UA/ml), respectively.

Good correlations in sIgE levels were observed between conventional testing for hornet venom ($R = 0.84$, $P < 0.01$) or paper wasp venom ($R = 0.71$, $P < 0.01$) and rVes v 5 or rPol d 5, respectively and in the sIgE levels between rVes v 5 ($R = 0.74$, $P < 0.01$) or conventional testing for hornet venom ($R = 0.65$, $P < 0.01$) and rPol d 5 or conventional testing for paper wasp venom, respectively. There were no significantly correlations between the severities of systemic reactions and sIgE levels against rVes v 1 or rVes v 5. These results indicate that antigen 5 is a potent major allergen in hornet and paper wasp venom.

Correlations of sIgE levels between improvement testing for each venom and allergen component and between improvement testing for hornet and paper wasp venom

Next, in the 33 positive subjects, we analyzed by improvement testing the correlations of sIgE levels between hornet or paper wasp venom and rVes v 5 or rPol d 5, respectively and between hornet and paper wasp venom. The mean sIgE levels against hornet and paper wasp venom by improvement testing were 11.3 ± 19.4 UA/ml (range, <0.35–85.1 UA/ml) and 18.8 ± 27.1 UA/ml (range, 0.42–≥100 UA/ml), respectively. In contrast, all controls were negative for sIgE by conventional testing to both hornet and paper wasp venom and rVes v 5. One control was slightly positive for sIgE against rPol d 5; however, this result was considered a non-specific reaction of sIgE with rPol d 5. Significant correlations of sIgE levels were observed by improvement testing between hornet ($R = 0.98$, $P < 0.01$) or paper wasp venom ($R = 0.98$, $P < 0.01$) and rVes v 5 or rPol d 5, respectively by improvement testing between hornet and paper wasp venom ($R = 0.62$, $P < 0.01$) (Fig. 2). There were no significantly correlations between the severities of systemic reactions and sIgE levels by improvement testing for hornet and paper wasp venom.

Correlations of sIgE levels in hornet and paper wasp venom between conventional testing and improvement testing

In the 33 positive subjects, we analyzed the correlations of sIgE levels in hornet and paper wasp venom between conventional testing and improvement testing (Fig. 3). Significant correlations in

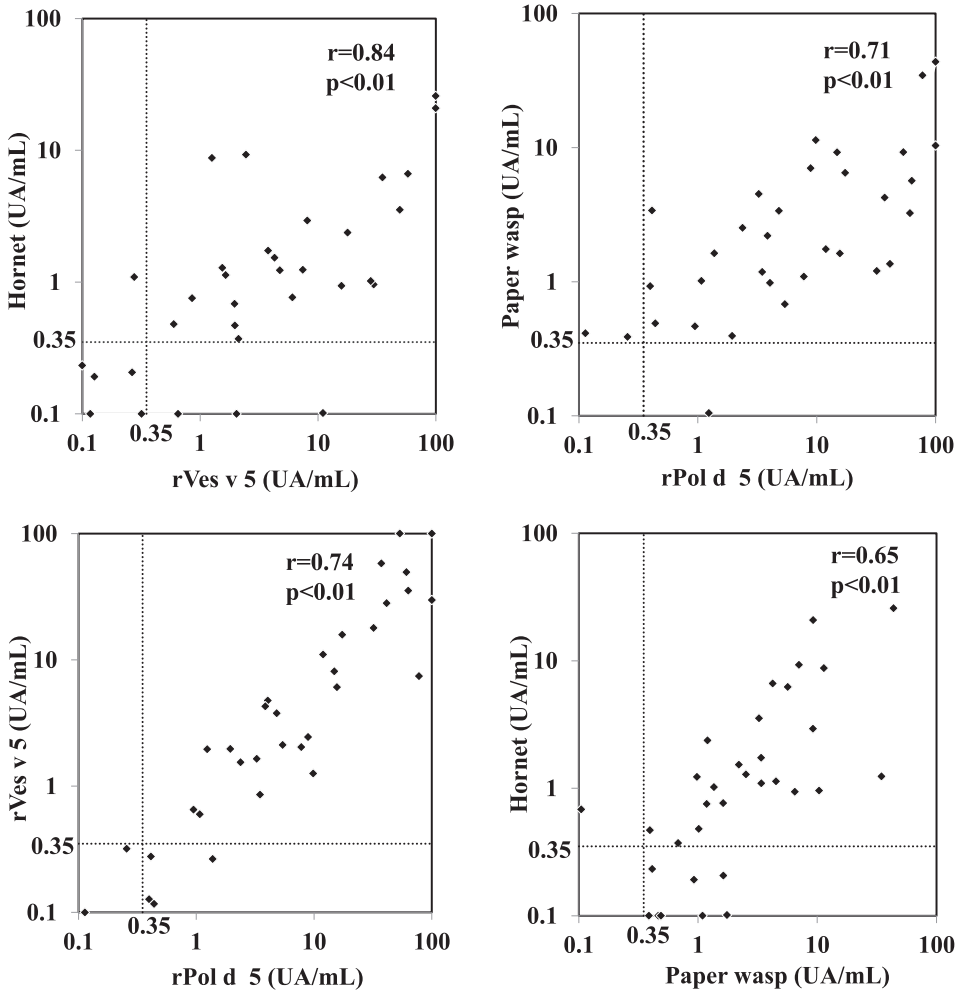

Fig 1. Correlations of specific immunoglobulin E (sIgE) levels between conventional testing and the allergen component, between each allergen component, and between conventional testing of hornet and paper wasp venom in patients (n = 33) with Hymenoptera allergy.

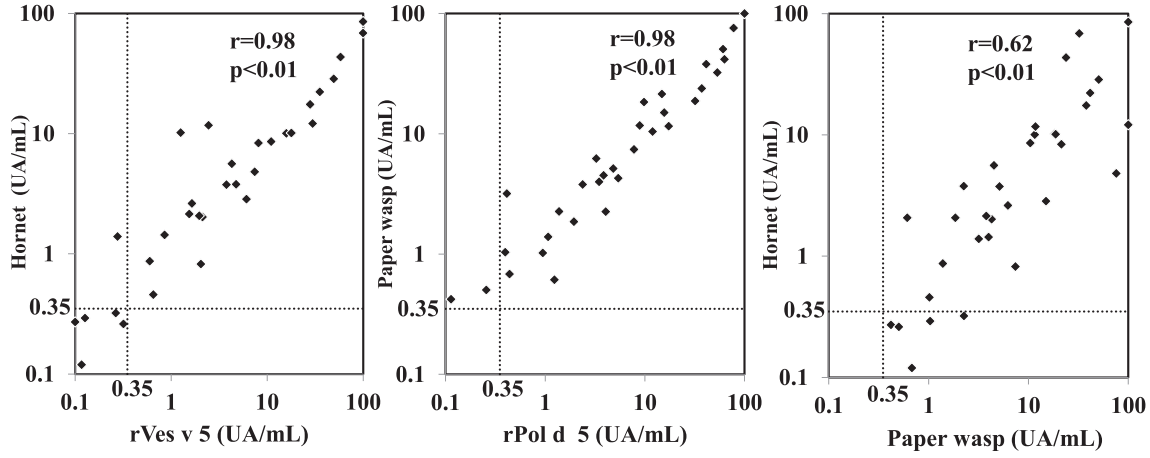

Fig 2. Correlations of sIgE levels between improvement testing of each venom and allergen component and between improvement testing of hornet and paper wasp venom in patients (n = 33) with Hymenoptera allergy.

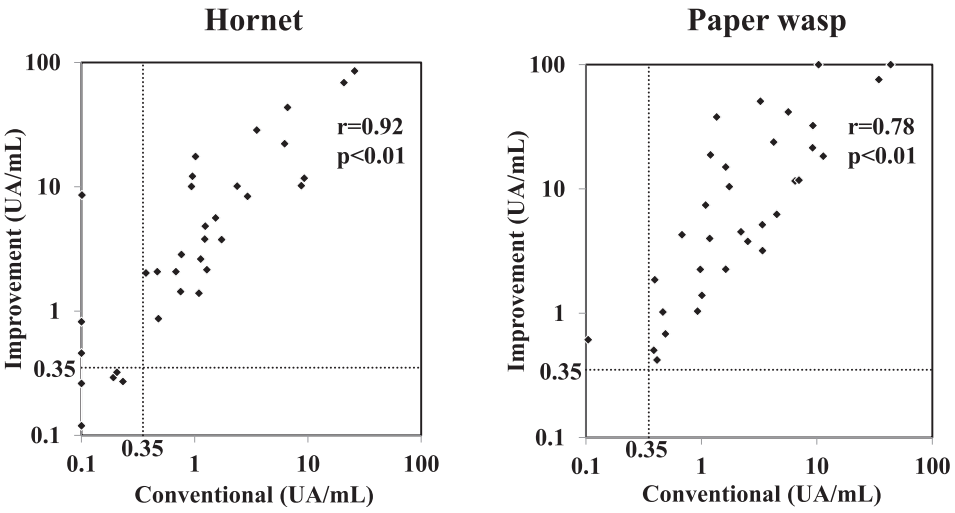

Fig 3. Correlations of sIgE levels in hornet and paper wasp venom between conventional testing and improvement testing. To determine agreement, correlations of the levels of sIgE against hornet and paper wasp venom between conventional testing and improvement testing in patients (n = 33) with Hymenoptera allergy were measured.

hornet (R = 0.92, $P < 0.01$) and paper wasp venom (R = 0.78, $P < 0.01$) were observed between conventional testing and improvement testing.

Correlations of sIgE levels between allergen components and between improvement testing of each venom in patients negative for sIgE in conventional testing to both hornet and paper wasp venom

To assess the improved sensitivity, we measured the correlations between the levels of sIgE against rVes v 5 and those against rPol d 5 in patients (n = 8) negative for sIgE by conventional testing and between the levels of sIgE against hornet venom and those against paper wasp venom by improvement testing (Table 1). Of the 8 subjects, 4 (50%) were positive by improvement testing for sIgE against rVes v 5 or rPol d 5, and 5 (62.5%) were positive by improvement testing for hornet or paper wasp venom. Conventional testing showed that spiking of rVes v 5 and r Pol d 5 in patients with sIgE against hornet and paper wasp venom, respectively, improved the sensitivity of IgE detection from 80.5% to 92.7%. In 4 patients with negative sIgE against both rVes v 5 and rPol d 5, 1 patient with sIgE against paper wasp venom exhibited improved positive results following spiking of rPol d 5.

Discussion

The present results revealed good correlations in sIgE levels between hornet venom and rVes v 5 and between paper wasp venom and rPol d 5 in patients (n = 33) sIgE-positive for Hymenoptera venom by conventional testing. Of the 33 patients, 31 (93.9%) were positive for sIgE against rVes v 5 and/or rPol d 5. These results indicate that Ves v 5 and Pol d 5 are potent major antigens for hornet and paper wasp venom, but not honey bee venom. In addition, sIgE in response to spiking of rVes v 5 and rPol d 5 in improvement testing correlated significantly with each component. With regard to specificity, all controls who had never experienced Hymenoptera stings were sIgE negative to spiking of both rVes v 5 and rPol d 5 in improvement testing. With regard to accuracy, strong correlations in sIgE levels against hornet and paper wasp venom were observed between conventional testing and improvement testing. With respect to advances in sensitivity, of the 8 patients negative for sIgE against both hornet venom and paper wasp venom by conventional testing, 5 (62.5%) were positive for sIgE in response to spiking of rVes v 5 and rPol d 5 in improvement testing.

The sensitivity and specificity of serum sIgE in Hymenoptera venom allergy has been reported at 76–91.2% and 85–100%, respectively.[12–15] In our study, of the 41 patients who had experienced systemic reactions to Hymenoptera (excluding honey bee) stings, 33 (80.5%) were positive for sIgE against hornet or paper wasp venom by conventional testing. As expected, the 20 controls were all negative for sIgE against both hornet and paper wasp venom by conventional testing. These results are similar to those of several studies on the sensitivity and specificity of sIgE to Hymenoptera venom. In addition, antigen 5 is a potent major allergen in hornet and paper wasp venom. Peter et al.[6] reported a rate of sIgE against rVes v 5 of 84.5% in 200 *Vespula* venom-allergic subject, while in a study of 308 subjects, ImmunoCAP revealed rates of sensitization (≥0.35 UA/ml) of 83.4% for conventional yellow jacket venom and 89.9% for rVes v 5.[9] In particular, in patients sensitive to yellow jacket venom, the diagnostic sensitivity of a combination of the currently available rVes v 5 and rVes v 1 has been reported to be as high as nearly 90%,[6,9,10] which is similar to not only rVes v 5 but also rPol d 5 in the present study. In addition, good correlations in sIgE levels were observed between rVes v 5 and rPol d 5, possibly due to cross reactivity between rVes v 5 and rPol d 5, which are both the same antigen 5, despite being produced by different Hymenoptera species. However, the negative sIgE against rPol d 5 and rVes v 5 in patients positive by conventional testing for sIgE against hornet and/or paper wasp venom was considered to be due to other allergens, such as phospholipase and hyaluronidase. In addition,

Table 1
Improved sensitivity to sIgE by spiking with the allergen component in patients negative for sIgE by conventional testing.

Subjects	Hornet	Paper wasp	rVes v 5	rPol d 5
1	<0.35	<0.35	<0.35	<0.35
2	6.71	3.80	13.8	8.31
3	<0.35	<0.35	<0.35	<0.35
4	<0.35	0.36	<0.35	<0.35
5	<0.35	1.24	<0.35	1.25
6	<0.35	<0.35	<0.35	<0.35
7	0.83	<0.35	0.87	<0.35
8	<0.35	1.45	<0.35	1.16

The table is shown sIgE levels (UA/ml) against rVes v 5 and rPol d 5, and those against hornet venom and paper wasp venom by improvement testing in patients (n = 8) negative for sIgE by conventional testing.

our results were demonstrated the strong correlations between the positive antibodies for hornet venom and paper wasp venom using the conventional method. The results are suggested the experiences of both hornet and paper wasp stings, and/or cross reactivity to the same antigen 5 in case of either hornet or paper wasp stings. Actually, it is difficult that the patients ascertain between hornet and paper wasp stings except honey bee stings. Namely, the measurement of sIgE against both paper wasp venom and hornet venom play an importance for the diagnosis of Hymenoptera allergy.

A recent study reported that spiking of rVes v 5 to determine hornet venom sensitivity by conventional testing improves the sensitivity of IgE detection in patients allergic to *Vespula* venom.[9] We demonstrated significant correlations in sIgE levels between hornet or paper wasp venom and each allergen component by improvement testing. Furthermore, we confirmed significant correlations in the levels of sIgE against hornet and paper wasp venom between conventional testing and improvement testing, and the specificity of sIgE against hornet and paper wasp venom by improvement testing was 100% in the controls. These results indicate that measurement of sIgE against hornet and paper wasp venom by improvement testing is helpful for the diagnosis of Hymenoptera venom allergy.

To assess improvements in sensitivity, we measured the levels of sIgE against hornet and paper wasp venom by improvement testing in patients suspected of having Hymenoptera allergy with negative sIgE by conventional testing. Conventional testing showed that spiking of rVes v 5 and r Pol d 5 in patients with sIgE against hornet and paper wasp venom, respectively, improved the sensitivity of IgE detection from 80.5% to 92.7%, indicating that the antigen 5 concentrations that conventional testing relies on might be too low for detecting sIgE. However, there were no significant ($P = 0.097$) differences in improvement of the sensitivity. The result is concluded small number of the subjects with negative sIgE levels in conventional testing for the statistical analysis. As the other analysis, in the 41 subjects, the levels of sIgE in improvement testing, compared to those in conventional testing, significantly increased in both hornet venom and paper wasp venom. The novel measurement plays an important analysis method for improvement of the sensitivity. Furthermore, interestingly in 4 patients with negative sIgE against both rVes v 5 and rPol d 5, 1 patient with sIgE against paper wasp venom exhibited improved positive results following spiking of rPol d 5, indicating the importance of measuring sIgE following spiking of the antigen in patients with sIgE against hornet venom and paper wasp venom to include the possible effect of other major allergens such as phospholipase and hyaluronidase. Many people find it difficult to morphologically distinguish a hornet from a paper wasp, but not from a honey bee. Therefore, measurement of sIgE levels following spiking of rVes v 5 and rPol d 5 will provide a more reliable method for detecting hornet and paper wasp venom sensitization in patients with Hymenoptera, but not honey bee allergy.

In conclusion, measurement of sIgE following spiking of rVes v 5 and rPol d 5 by conventional testing in Japanese subjects with sIgE against hornet and paper wasp venom, respectively, improved the sensitivity for detecting Hymenoptera venom allergy. Our findings may be useful for determining improvements in sensitization to Hymenoptera venom immediately and a long time after Hymenoptera stings. Taken together, measuring sIgE against both hornet venom and paper wasp venom by improvement testing is helpful for serologically elucidating Hymenoptera venom allergy in Japan.

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Conflict of interest

The authors have no conflict of interest to declare.

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