

The frequency and clinical significance of specific IgE to both wasp (*Vespula*) and honey-bee (*Apis*) venoms in the same patient

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Summary

Background Changeover from Phadebas RAST to Pharmacia AutoCAP increased double-positivity to both honey-bee and common wasp (*vespula*) venom in our patients.

Objective We examined the frequency of IgE double-positivity, its clinical relevance and utility in investigating potentially allergic patients.

Methods One hundred and eighty-two patients with hymenoptera allergy were tested using RAST ($n = 51$) and AutoCAP ($n = 131$) assays over 4 years. Patients had a history of reactions to vespulae (22), honey-bee (10) and unidentified hymenoptera (vespinae) (7).

Results After changing from RAST to AutoCAP double-positivity increased from 10 (5/51) to 30% (39/131) ($P < 0.01$). RAST and CAP assays gave similar median class results (vespula = 3, honey-bee = 2). Thirty-six CAP patients had systemic reactions of Mueller grade II and above. In vespula-allergic double-positive subjects, high CAP classes ($\geq$ class 3) to honey-bee were common (30%). In 25% the CAP classes were equal. In honey-bee-allergic subjects, all vespula venom CAP IgE was low titre (class 1 or 2) and 20% were equal for both venoms. In 43% of vespinae-allergic patients the CAP class was equal to both (class 2 and 3). In contrast, intradermal skin test double-positivity was uncommon. Double-negative skin test results were common in the CAP double-positive population (22% of honey-bee-allergic, 13% of vespula-allergic and 43% of vespinae-allergic patients). Vespula allergic patients have higher bee-venom IgE than *vice versa*. Twenty-seven per cent of CAP double-positive patients (representing 8% of all venom allergic patients tested over this period) had equal class IgE to both venoms which was not helpful in diagnosis. Combination of skin testing and CAP is unhelpful in only 5/37 (14%) of patients with double-positive serology.

Conclusion If used in isolation CAP may be misleading, especially if only one venom is tested. Identification of the causative venom must utilize both clinical history and skin testing in these double-positive patients, and challenge testing if indicated.

Keywords: Vespula, wasp, honey-bee, venom, allergy, CAP, RAST, skin test, diagnosis, challenge

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Introduction

Measurement of specific IgE to venom by radioallergo-sorbent assay (RAST) testing [1,2] may be useful for

identifying the species involved in allergic reactions to hymenoptera venom. Levels are often reported in six semi-quantitative 'classes'. Recently, automated assays using non-radioactive enzyme-labelled anti-IgE offer apparently increased sensitivity, precision and ease of use. This may lead to increasing use for the provision of clinical advice and choice of venom for hyposensitization immunotherapy.

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Crossreactivity between *vespula* and honey-bee venoms has been described using RAST and other methods, but the frequency and clinical significance in venom allergic patients in CAP assays is not clear. We therefore examined the number of double-positive patients over 4 years in 182 consecutive patients with suspected hypersensitivity to insect venom, using the CAP and RAST techniques employed in the Clinical Immunology laboratory at these times. We then compared these results with the clinical details and skin testing results.

Materials and methods

Patients

Results of all specific IgE assays to vespula and honey-bee venom over 16 months prior to (51 patients) and 30 months after (131 patients) introduction of the AutoCAP system were included for analysis. Rates of referral remained similar over this period (3.2 tests per month for RAST, 4.4 for CAP analysis). All patients had IgE to both venoms measured routinely at presentation. The details of double-positive patients are summarized in Table 1. The clinical details of all patients testing double-positive were obtained from the hospital notes and the diagnosis was established on the basis of the clinical history. Clinical severity of reactions was graded according to the scale of Mueller where grade 0 = local reaction only, grade I = urticaria/anxiety/malaise, grade II = angioedema/chest/tightness/nausea/vomiting/diarrhoea/abdominal pain/dizziness, grade III = shortness of breath/wheeze/stridor/dysphonia/confusion and grade IV = hypotension/loss of consciousness/incontinence (Table 2).

It should be remembered that these results apply only to the patient subgroup with double-positive CAP serology and refer to confirmation of the venom to which clinical allergy has been diagnosed, based primarily on the clinical history.

They do not apply to sting allergic patients as a whole, most of whom (70% of all the patients seen over a 4-year period) do not have specific IgE to both venoms. Eighty-two per cent of our patients could positively identify the insect, the remaining 18% were uncertain but were clinically most likely to have been stung by a vespinae.

Thirteen of the vespula allergic patients were candidates for desensitization with reactions of grade III or IV. Nine out of 13 vespula allergic patients were desensitized to vespula venom, five of nine were challenged, all were positive, the remaining four were desensitized without challenge. Three out of 13 were candidates but refused desensitization and were not challenged, one of 13 was a candidate but desensitization and challenge were contraindicated due to ischaemic heart disease. Of the remaining patients, two were challenged, both were negative.

All 10 of the honey-bee-allergic patients were candidates for desensitization. Eight out of 10 were challenged, seven out of eight were positive and were desensitized, one was negative and was not. The remaining two patients were high-risk honey-bee keepers and were desensitized without challenge.

Five out of seven of the probable vespinae-allergic patients were candidates for desensitization. Three were challenged, and the single positive was desensitized. The remaining two refused desensitization and were therefore not challenged.

Patients who were not candidates for desensitization or who refused desensitization were not routinely challenged with venom in view of the potential risk of increasing sensitization.

Specific IgE measurements

RAST (Phadebas Phadezyme RAST) and CAP (Pharmacia AutoCAP) assays for specific IgE measurements were performed according to the manufacturers operating instructions (Pharmacia, Kabi Diagnostics, Uppsala, Sweden).

Table 1. Characteristics of the hymenoptera-allergic population with double-positive specific IgE serology

Allergy diagnosis	Median age (years)	Age range (years)	Male:Female ratio
Vespula (CAP, <i>n</i> = 22)	39	9–61	12:10
Vespula (RAST, <i>n</i> = 4)	36	14–61	4:0
Honey-bee (CAP, <i>n</i> = 10)	50	13–69	4:6
Honey-bee (RAST, <i>n</i> = 1)	53	–	0:1
Vespinae (CAP, <i>n</i> = 7)	47	13–66	5:2

These data include three hymenoptera-allergic patients with local reactions only).

Table 2. Severity of reactions in patients with double-positive specific IgE serology

Specific IgE assay	Reaction grade (Mueller scale)	No. of honey-bee allergic patients (<i>n</i> = 10)	No. of <i>Vespula</i> allergic patients (<i>n</i> = 22)	No. of <i>Vespinæ</i> allergic patients (<i>n</i> = 7)
AutoCAP	0	0	2	0
	I	1	0	0
	II	2	7	2
	III	4	5	1
	IV	3	8	4
		No. of honey-bee allergic patients (<i>n</i> = 1)	No. of <i>Vespula</i> allergic patients (<i>n</i> = 4)	
RAST	0	0	1	–
	I	0	1	–
	II	0	1	–
	III	0	0	–
	IV	1	1	–

Internal and external quality control procedures were performed in each assay run and throughout the period of the study the laboratory has participated in the NEQAS quality assurance scheme with good results.

The CAP assay was calibrated in kU/L against a total IgE calibrator serum, which itself had been calibrated against the second WHO International reference preparation for IgE 75/502. The RAST system was calibrated in PRU (Phadebas RAST units) using the manufacturer's calibrator serum pool (containing specific IgE to birch pollen). Thus, the quoted units (PRU/mL and kU/L) are not directly comparable.

Intradermal skin tests

These were performed in a standardized manner developed within the department. Fifty microlitre volume intradermal injections of honey-bee venom or vespuia venom (equivalent to $\approx 2 \mu\text{g}$ pure venom) and diluent (as a negative control) were placed on the forearm $\approx 5 \text{ cm}$ apart. Weal-and-flare diameters were measured in two dimensions at right angles to each other after 15 min.

There is no agreed standardization of intradermal skin testing techniques. Some authors use $20 \mu\text{L}$ volumes but we find $50 \mu\text{L}$ easier to administer accurately, and the volume needed will be affected by potency of the venom used and the criteria used to determine positivity. Although some authors use venom preparations with lower apparent venom content for skin testing (especially to vespuia venom), there is no international standard preparation and each venom preparation may vary in potency and relative content of major

allergens. Our venoms are pure vespuia venom or honey-bee venom collected in the field by electrical stimulation, and are standardized *in vitro* on the basis of content of major antigens (Hyaluronidase and Phospholipase, and Mellitin in the case of bee venom) and *in vivo* by end-point titration skin-prick tests in venom allergic patients [3]. Positive reactions were deemed present if there was at least a 5 mm increment in mean weal diameter over the base, surrounded by erythema of at least 20 mm mean diameter. Flare (erythema) reactions alone were not considered significant since a proportion of diluent controls (4/24) also gave flare reactions larger than 20 mm in the absence of a significant weal.

Statistical methods

The chi-squared test (with Yates correction) was used to determine the significance in the change of frequency of double positivity. IgE classes were compared using the unpaired student's *t*-test. Log transformed data (base 10) were used for assessing the statistical difference in IgE titres.

Results

Most patients tested had specific IgE to a single venom. After introduction of the AutoCAP double-positivity increased to 39/131 (30%) from 5/51 (10%) ($P < 0.01$). Ninety-two per cent (36/39) of the CAP double-positive population had experienced a systemic reaction to a single

Table 3. RAST and AutoCAP double-positivity in patients with hymenoptera venom systemic reactions (grade II+)

Diagnosis	Honey-bee Class Median (Range)	Honey-bee IgE Median (Range)	Vespula Class Median (Range)	Vespula IgE Median (Range)
Honey-bee (CAP, <i>n</i> = 9)	4 (2–6)	22.7 (1.45–100)	2 (1–2)	1.02 (0.35–3.1)
Honey-bee (RAST, <i>n</i> = 1)	4 (4)	25 (25)	1 (1)	0.36 (0.36)
Vespula (CAP, <i>n</i> = 20)	2 (1–4)	1.16 (0.40–32.1)	3 (2–6)	10.8 (1.36–100)
Vespula (RAST, <i>n</i> = 3)	2 (1–2)	0.9 (0.38–3.1)	3 (2–4)	6.8 (2.4–20.5)
Vespiniae (CAP, <i>n</i> = 7)	2 (1–3)	1.6 (0.47–17.3)	3 (2–4)	5.8 (1.22–22.5)

hymenoptera species of $\geq$ Muller grade II (51% were sensitive to vespula, 26% to honey-bee, and 18% to an unidentified vespiniae, probably a vespula). Five per cent (2/40) had only a local reaction of grade 0 and these patients were excluded from further analysis, which refers only to patients with systemic reactions (Table 3, Fig. 1.) The differences between the CAP titres to honey-bee vs. vespula venom in the same patient (in both honey-bee-allergic and vespula-allergic patient groups) were significant ($P < 0.001$), but 5/20 (25%) vespula-, 2/10 (20%) honey-bee- and 3/7 (43%) vespiniae-allergic patients had CAP classes of equal positivity to each venom. There was no significant difference in the class or IgE titres between the subgroups of patients who gave a positive history of previous stings from other hymenoptera species, when compared with a sting history from unidentified hymenoptera or those without a previous sting history of any kind.

Thirty-two out of 37 double-positive CAP patients had intradermal skin tests to both venoms performed. Of the five patients who did not have skin tests, four were children and one adult refused. The relative levels and distribution of specific IgE to both venoms are shown in Fig. 1 and Table 3. Skin testing results are summarized in Fig. 2 and Table 4.

In those patients who had both skin tests and serology performed 8/32 (25%) of CAP results were not-diagnostic (equal positive) in this subgroup of patients, and 1/32 (3%) was false-positive. Seven of 32 (22%) skin tests were double-negative and one of 32 (3%) was double-positive.

Vespula-allergic patients with systemic reactions and double-positive CAP serology

Five of 20 (25%) double-positive patients with a clinical

diagnosis of vespula allergy, had equal (non-diagnostic) class IgE against both honey-bee and vespula venoms (Fig. 1) which does not discriminate the likely causative venom. Only one of 20 vespula-allergic patients had a higher IgE level to honey-bee than vespula (class 3 vs 2) which straddled the border between the classes.

Seven of 20 (35%) vespula-allergic patients had no history of a honey-bee sting, although two (10%) had a previous unidentified hymenoptera sting. Thus, 5/20 (25%) had no history of any other sting which could explain the presence of antibody to honey-bee venom. Six of 20 (30%) of vespula allergic patients had very high honey-bee specific IgE greater or equal to class 3 in the CAP system, whereas no patient had a honey-bee IgE titre of $>$ class 2 using the RAST system.

Sixteen of 20 vespula-allergic patients were skin tested. Two of 16 were negative to both venoms. In one of these (6%) neither test was helpful in elucidating the cause of the reactions (equal positive IgE + double-negative skin test), this patient nevertheless had a positive provocation test to vespula venom and was desensitized. Another patient was double-positive with a borderline positive honey-bee skin test, but a strongly positive vespula skin test and was desensitized to vespula venom.

Honey-bee-allergic patients with systemic reactions and double-positive CAP serology

In contrast to vespula allergic patients, in the patients allergic to honey-bee no patient had a vespula specific IgE titre of $>$ class 2 (Figs 1 and 2). Two out of 10 (20%) of the honey-bee double-positive patients had similar titres of IgE to both venoms (both class 2) (non-diagnostic) but had severe clinical reactions to honey-bee venom of Mueller grades III and IV. No honey-bee-allergic patient had a false

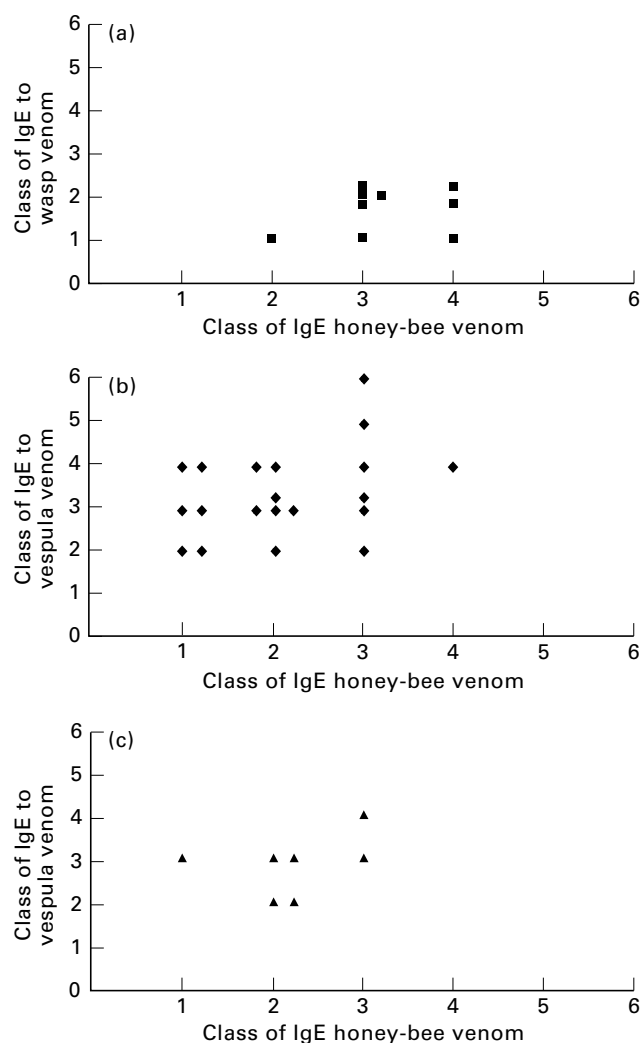


Fig. 1 High titre specific IgE to honey-bee venom in the AutoCAP assay system is found more commonly in vespula-allergic patients than vice versa. The same is true for the vespinae-allergic group whose double-positive serology is more like that of the vespula-allergic group. The reason for this is unknown, but is unlikely to reflect sting history, since honey-bee stings are less common than wasp stings. Positivity of equal class to each venom is mainly a problem in the vespid-allergic patients.

positive CAP result with vespula IgE class higher than the honey-bee class.

There was no previous history of vespula stings in five of 10 (50%) of the honey-bee-allergic patients and no other unidentified stings in 10/10. Thus, five of 10 (50%) had no remembered history of any other sting to explain the presence of IgE to vespula venom.

Nine of 10 of the double-positive honey-bee-allergic patients were skin tested to both venoms. The other (a child) had a positive honey-bee skin test and venom challenge (Fig. 2).

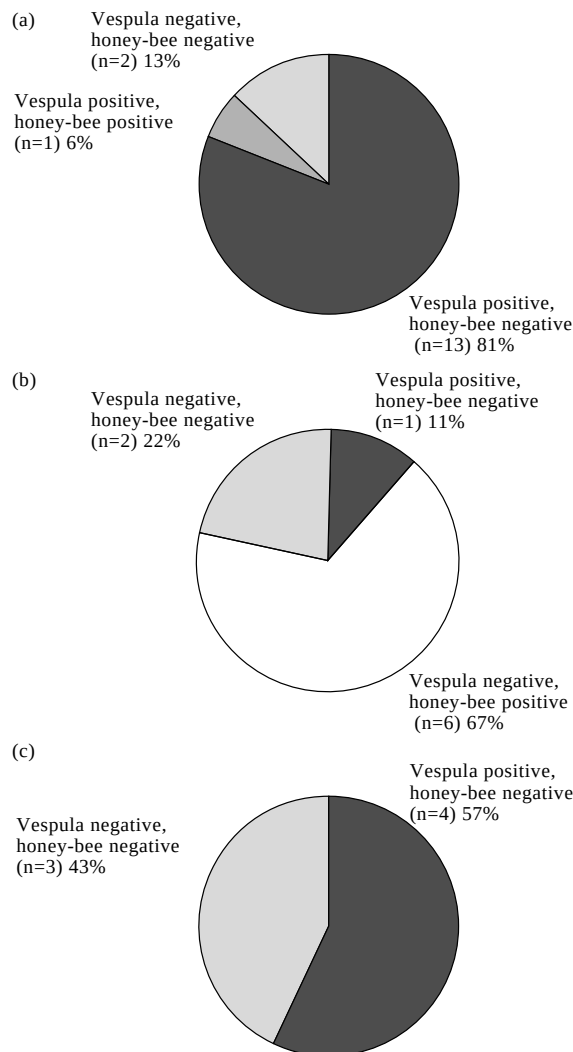


Fig. 2 Positive skin tests to the other venom are unusual in the CAP double-positive population using our intradermal skin-testing technique. Double-negative skin tests are more frequent. In the majority of patients the skin-testing results accorded with the clinical diagnosis from the patient's history. (a) skin test results in vespula allergic patients with double-positive CAP serology ($n = 16$); (b) skin test results in honey-bee allergic patients with double-positive CAP serology ($n = 9$); (c) skin test results in vespinae-allergic patients with double-positive CAP serology ($n = 7$).

Six out of nine (66%) of the double-positive honey-bee-allergic patients had single positive skin tests to honey-bee venom only, five out of six had positive venom challenges and were desensitized, one out of six had a negative challenge and was not desensitized. No honey-bee-allergic patients had positive skin tests to both venoms. However, one honey-beekeeper had a false-positive vespula venom skin test but negative honey-bee skin test despite having no history of vespula stings and a specific CAP IgE clearly

Table 4. Intradermal skin test results in patients double-positive in the CAP assay

Patient Group	Diluent Mean Weal (mm)	Diluent Mean Flare (mm)	Honey-bee Mean Weal (mm)	Honey-bee Mean Flare (mm)	Vespula Mean Weal (mm)	Vespula Mean Flare (mm)
Vespula allergic (n = 16)	10.2	3.1	10.5	15.1	19.8	48.3
Honey-bee allergic (n = 9)	9	0	21.4	39.7	10.1	34.4
Vespinae allergic (n = 7)	9.9	3.2	10.3	11.6	16.9	48.4

more positive to honey-bee venom (class 5 vs class 1). In addition, two out of nine (22%) of the CAP double-positive patients were negative to both venoms on skin testing, both of whom merited desensitization. One had a positive venom challenge the other proceeded to desensitization without challenge.

Vespinae-allergic patients with systemic reactions and double-positive CAP serology

This subgroup of patients could not positively identify the hymenoptera species causing the reaction, but most thought that a vespula sting was likely. These are therefore probable vespinae stings (including both *vespula* and *vespa* [hornet] species). This was also by far the most likely clinical diagnosis from the circumstances of the sting. *Polistes* (paper wasp) and *Vespa* (hornet) stings are extremely uncommon in our practice. None had a positive honey-bee-venom skin test. Three out of seven had a double negative skin test. Five out of seven had clinical reactions of Mueller grade III or IV, and were candidates for desensitization. Only one of five had a non-diagnostic CAP result of equal positivity. In contrast, four of five skin tests were positive to vespula. In four of five desensitization candidates the skin tests and CAP results were concordant and confirmed that a vespula was the most likely cause. Two out of four refused desensitization and were therefore not challenged, of the remainder one was positive the other negative to vespula venom challenge. The patient with a positive challenge proceeded to desensitization. Of the two remaining patients with Muller grade II reactions, both had double-negative skin tests and non-diagnostic CAP serology. One was negative on venom challenge.

Six of seven (86%) patients had previous vespula stings, and one of seven (14%) had previous honey-bee stings, one

of seven (14%) had previous unidentified stings. Therefore only two of seven (29%) had remembered a previous sting to explain the presence of honey-bee-specific IgE.

Utility of both skin testing and CAP serology in the CAP double-positive population

Test results were combined as described below in the 16 vespula-allergic, nine honey-bee-allergic, and seven vespinae-allergic CAP double-positive patients for whom skin-test results were available. Despite the use of both tests, we were unable to confirm the insect species in one of 16 (6%) vespula-allergic, one of nine (11%) honey-bee-allergic and three of seven (43%) vespinae-allergic patients. Both of these vespula and honey-bee sensitive patients had positive challenges and were desensitized, and two of three of the suspected vespinae-allergic patients who were eligible for desensitization had negative challenges to the suspected venom. The third vespinae allergic patient was not a desensitization candidate and was therefore not challenged.

Other allergy

Many double-positive patients (46%) had a history of at least one other allergy, but this was not obviously more prevalent than the general population. Nine of 21 vespula-allergic CAP patients had raised IgE with a median value of 654 IU/mL (range 189 to >2000). Only two patients had values greater than 1000 IU/mL. Total IgE was raised in five of nine honey-bee allergic CAP patients with a median value of 230 IU/mL (range 181–425).

Discussion

We noticed an apparent increase in the number of

patients with double-positive specific IgE results after changing to the AutoCAP system. We therefore examined the clinical details of patients assayed using the RAST and CAP system in the routine regional immunology laboratory over a 4-year-period to determine the significance of double-positivity and the characteristics of the double-positive population. Previous studies demonstrate a good correlation between RAST and CAP results [2,4], particularly for inhalant allergens. CAP is consistently more sensitive but may also be less specific [5–7]. Most immunology laboratories calibrate RAST using the manufacturer's Birch-specific serum in PRU/mL. AutoCAP, in contrast, is calibrated against the WHO total IgE international reference preparation. Low level CAP positivity for insect venom (up to class 2) is reported to be equivalent to a RAST titre anywhere between 0 and (2) [7]. It is not generally appreciated that CAP and RAST classes are not strictly equivalent. Class reporting can also disguise clinically useful differences in titres.

Change-over to AutoCAP appeared to increase double-positive IgE results (30% vs 10%, $P < 0.01$). Twenty-five per cent of vespula-allergic, 50% of honey-bee-allergic and 72% of Vespinae-allergic IgE double-positive patients had no clear history of previous exposure to the other venom. Many patients therefore appear to have crossreacting antibodies. In contrast, in another study of high-titre cross-reactive IgE detected by RAST, 92% (24/25) had a history of previous stings, mostly unidentified [8].

Serology and skin testing reflect exposure, not clinical sensitivity. There is no clear proof in this series or in the published literature on RAST that the incidental presence of IgE to another insect species represents a significant risk of clinical hypersensitivity. IgE to venom detectable by RAST is found in 6% of populations without reactions [9,10] and up to 60% of people have been stung in the past [11]. This may be higher if CAP is more sensitive [2,7]. Asymptomatic skin test positivity may be more common, since some skin prick tests are reported to be even more sensitive than CAP [4,12–16].

Several investigators have demonstrated double-positivity in RAST testing of healthy controls at frequencies of 6–29% for honey-bee and 0–7% for vespula venom [17]. Furthermore, RAST IgE to the other venom ($\geq$ class 2) was found in 18% of honey-bee allergic and 34% of vespula allergic patients [17], similar to the frequencies described in this study. Intradermal skin tests however, showed the opposite pattern, with more positive vespula skin tests in honey-bee allergic subjects (34%) than honey-bee positivity in vespula-allergic patients (17%) [17]. Our results show that in the double-positive subpopulation, intradermal testing was more likely to be double-negative (13% of vespula-, 22% of honey-bee-, and 43% of vespinae-allergic patients), and only one of our vespula-allergic patients and one honey-bee-allergic

patient was positive to the other venom. This difference may be explained by differences in the properties of each skin test methodology, or the patient populations.

Positivity to both venoms is sometimes taken as an indication for immunotherapy with both [8,18] but documented clinical reactions to both species are rare [17,19,20]. We did not make a diagnosis of clinical hypersensitivity to a second venom in any patient, and no one has had a reaction to the other venom over the period of the study. Even with a clear history of a symptomatic reaction to a sting and positive confirmatory tests to one venom only, the risk is not predictable and some investigators favour a controlled sting challenge to select patients for desensitization immunotherapy [21–23], although this is still controversial [24]. Indeed after immunotherapy, there is clinical non-reactivity but persisting serology and/or skin test positivity in most patients [3,25]. However, reactions are rare in patients negative in both tests, but cannot be absolutely excluded since a few have no detectable venom-specific IgE at presentation [20,26]. The reason for this is not clear. In two of our patients with both unhelpful serology and negative skin tests a venom challenge was positive, but there were also two patients with unhelpful tests who had a negative venom challenge.

The level of IgE to venom does not reliably reflect the severity of the last reaction [3,23,27,28] and this is confirmed in our study of double-positive patients, where the same proportion (50%) of vespula-allergic patients with specific IgE of class 2 and below, or class 3 and above, were candidates for desensitization therapy. Whether the level of venom-specific IgE or IgG is a predictor of subsequent reactivity is still controversial [1,3,29–34], but there is little evidence in this cohort of frequent clinical sensitivity to both venoms in double-positive patients.

Possible explanations for double-positivity include crossreactive components of both venoms (e.g. phospholipase or hyaluronidase, Sol I 1), other environmental antigens (dietary carbohydrate antigens), or previous sting exposure to both venoms [35,36]. It is not clear why immunization with vespula venom appears to produce higher titres of crossreactive antibodies to honey-bee venom than vice versa, as confirmed by this study. A few patients have been reported with definite clinical allergy to both venoms but their specific IgE antibodies were of distinct specificity for each venom and were not cross-inhibitable [19,20,37] suggesting separate sensitization against each species. In some of these patients prior immunotherapy with whole body may confound interpretation [19,37]. Crossreactive RAST IgE was found in 17/25 (68%) patients selected for double-positive RAST serology [8] but none had a clear clinical history of allergy to both venoms. All 17 had truly crossreactive antibodies in RAST inhibition studies, and most had

systemic reactions, but all were selected for high-titre RAST positivity, whereas our study includes allergic patients with low titre positivity in the CAP assay. Interestingly, the remaining seven patients (28%) in that study had non-crossreactive antibodies which most probably arose from exposure to both venoms, since 92% had a history of previous exposure to other stings.

Testing for specific IgE should be used selectively and interpreted with regard to the clinical information. It should not be interpreted as evidence of hypersensitivity in isolation. Both honey-bee and vespula IgE should be assayed upon initial investigation if skin testing cannot be used initially. The majority of our patients clearly identified the causative insect and the diagnosis is not in doubt. In the 19% for whom a positive visual identification was not available a vespinae sting was likely and was confirmed by using additional skin testing in four of seven (57%). Skin testing using our technique is rarely double-positive in serologically double-positive patients (3%), although double-negativity was seen in 19%. CAP double-positivity is common in our vespula-allergic patients, and is often of similar high titre to both venoms. In most cases this does not appear to be clinically important but could be misleading. Thirty per cent of the AutoCAP results and 25% of the skin tests were non-diagnostic. However, using both tests supported the clinical diagnosis in 84% of our double-positive population. Some of the non-diagnostic results after combining both tests (5/32, 16%) may represent patients who are no longer sensitive due to attrition of IgE titres with time. Four of these were challenged because they were candidates for desensitization on clinical assessment and two of four were positive. Unfortunately there is no test (even challenge) which can incontrovertibly distinguish these patients who are no longer sensitive.

Although CAP double-positivity is common, CAP IgE or intradermal skin test are rarely uninformative, especially when used in combination. Challenge testing may be necessary in a few difficult patients. If CAP is to be used routinely in the diagnosis of allergic reactions to insect venom, IgE to both venoms should be measured.

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References

- 1 Ewan PW. Allergy to insect stings: a review. *J R Soc Med* 1985; 78:234–9.
- 2 Ewan PW, Coote D. Evaluation of a capsulated hydrophilic carrier polymer (the ImmunoCAP) for measurement of specific IgE antibodies. *Allergy* 1990; 45:22–9.
- 3 Ewan PW, Deighton J, Wilson AB, Lachmann PJ. Venom-specific IgG antibodies in bee and wasp allergy: lack of correlation with protection from stings. *Clin Exp Allergy* 1993; 23:647–60.
- 4 Eiken P, Mosbech H, Jacobsen L et al. Comparison between two different assays for measurements of allergen-specific IgE and skin prick test in the diagnosis of insect venom allergy. *Allergy* 1992; 47:495–7.
- 5 Leimgruber A, Lantin J-P, Frei PC. Comparison of two in vitro assays, RAST and CAP, when applied to the diagnosis of anaphylactic reactions to honeybee or yellow jacket venoms: correlation with history and skin tests. *Allergy* 1993; 48: 415–20.
- 6 Leimgruber A, Mosimann B, Claeys M et al. Clinical evaluation of a new in-vitro assay for specific IgE, the immuno CAP system. *Clin Exp Allergy* 1991; 21:127–31.
- 7 Jeep S, Kirchoff E, O'Connor A, Kunkel G. Comparison of the Phadebas RAST with the Pharmacia CAP system for insect venom. *Allergy* 1992; 47:212–7.
- 8 Reisman RE, Wypych JJ, Lazell MI. Further Studies of Patients with Both Honeybee- and Yellow-Jacket-Venom-Specific IgE. *Int Arch Allergy Appl Immunol* 1987; 82:190–4.
- 9 Charpin D, Vervloet D, Haddi E et al. Prevalence of allergy to Hymenoptera stings. *Allergy Proc* 1990; 11:29–32.
- 10 Charpin D, Birnbaum J, Vervloet D. Epidemiology of hymenoptera allergy. *Clin Exp Allergy* 1994; 24:1010–5.
- 11 Charpin D, Birnbaum J, Lanteaume A, Vervloet D. Prevalence of allergy to hymenoptera stings in different samples of the general population. *J Allergy Clin Immunol* 1992; 90:331–4.
- 12 Golden DB, Marsh DG, Kagey-Sobotka A et al. Epidemiology of insect venom sensitivity. *JAMA* 1989; 262:240–4.
- 13 Harries MG, Kemeny DM, Youlten LJ, Mills MM, Lessof MH. Skin and radioallergosorbent tests in patients with sensitivity to bee and wasp venom. *Clin Allergy* 1984; 14:407–12.
- 14 Li JT, Yunginger JW. Management of insect sting hypersensitivity. *Mayo Clin Proc* 1992; 67:188–94.
- 15 Settignano GA, Boyd GK. Natural history of insect sting allergy: the Rhode Island experience. *Allergy Proc* 1989; 10:109–13.
- 16 Green-Graif Y, Ewan PW. Diagnostic value of the skin-prick test and RAST assay in insect sting allergy. *Clin Allergy* 1987; 17:431–8.
- 17 Mueller UR. *Insect Sting Allergy: Clinical picture, diagnosis and treatment*, 1st Edn. Stuttgart: Gustav Fischer Verlag, 1990: 75–91.
- 18 Reisman RE. Venom hypersensitivity. *J Allergy Clin Immunol* 1994; 94 (4):651–8.
- 19 Reisman RE, Muller UR, Wypych JJ, Lazell MI. Studies of coexisting honeybee and vespidae-venom sensitivity. *J Allergy Clin Immunol* 1984; 73:246–52.
- 20 Reisman RE, Osur SL. Allergic reactions following first insect sting exposure. *Ann Allergy* 1987; 59:429–32.
- 21 van der Linden PW, Hack CE, Struyvenberg A, van der Zwan JK. Insect-sting challenge in 324 subjects with a previous

- anaphylactic reaction: current criteria for insect-venom hypersensitivity do not predict the occurrence and the severity of anaphylaxis. *J Allergy Clin Immunol* 1994; 94:151–9.
- 22 Goldberg A, Confino-Cohen R, Mekori YA. Deliberate hymenoptera sting challenge as a diagnostic tool in highly selected venom-allergic patients. *Ann Allergy Asthma Immunol* 1995; 75:30–2.
 - 23 Muller U, Helbling A, Bischof M. Predictive value of venom-specific IgE, IgG and IgG subclass antibodies in patients on immunotherapy with honey bee venom. *Allergy* 1989; 44:412–8.
 - 24 Franken HH, Dubois AE, Minkema HJ, van der Heide S, de Monchy JG. Lack of reproducibility of a single negative sting challenge response in the assessment of anaphylactic risk in patients with suspected yellow jacket hypersensitivity. *J Allergy Clin Immunol* 1994; 93:431–6.
 - 25 Haugaard L, Norregaard OF, Dahl R. In-hospital sting challenge in insect venom-allergic patients after stopping venom immunotherapy. *J Allergy Clin Immunol* 1991; 87:699–702.
 - 26 Clayton WF, Georgitis JW, Reisman RE. Insect sting anaphylaxis in patients without detectable serum venom-specific IgE. *Clin Allergy* 1985; 15:329–33.
 - 27 Miller SD, Munemasa KH, Ber D, Klein DE, Boyd GK. Follow-up of hymenoptera-sensitive patients without active immunotherapy. *Allergy Proc* 1994; 15:65–8.
 - 28 Savliwala MN, Reisman RE. Studies of the natural history of stinging-insect allergy: long-term follow-up of patients without immunotherapy. *J Allergy Clin Immunol* 1987; 80:741–5.
 - 29 Day JH, Buckeridge DL, Welsh AC. Risk assessment in determining systemic reactivity to honeybee stings in sting-threatened individuals. *J Allergy Clin Immunol* 1994; 93:691–705.
 - 30 Heinig JH, Engel T, Weeke ER. Allergy to venom from bee or wasp: the relation between clinical and immunological reactions to insect stings. *Clin Allergy* 1988; 18:71–8.
 - 31 Mosbech H. Insect allergy. A comparative study including case histories and immunological parameters. *Allergy* 1984; 39:543–9.
 - 32 Reisman RE, DeMasi JM. Relationship of serum venom-specific IgE titers to clinical aspects of stinging insect allergy. *Int Arch Allergy Appl Immunol* 1989; 89:67–70.
 - 33 Wilson AB, Deighton J, Lachmann PJ, Ewan PW. A comparative study of IgG subclass antibodies in patients allergic to wasp or bee venom. *Allergy* 1994; 49:272–80.
 - 34 Golden DB, Addison BI, Gadde J, Kagey-Sobotka A, Valentine MD, Lichtenstein LM. Prospective observations on stopping prolonged venom immunotherapy. *J Allergy Clin Immunol* 1989; 84:162–7.
 - 35 Aalberse RC, Koshte V, Clemens JGJ. Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and Hymenoptera venom. *J Allergy Clin Immunol* 1981; 68:356–64.
 - 36 Hoffman DR, Dove DE, Moffitt JE, Stafford CT. Allergens in Hymenoptera venom. XXI. Cross-reactivity and multiple reactivity between fire ant venom and bee and wasp venoms. *J Allergy Clin Immunol* 1988; 82:828–34.
 - 37 Muller U, Roth A, Yman L, Patrizzi R. Use of RAST Technique in Wasp Sting Hypersensitivity. *Allergy* 1978; 33:197–202.