

Diagnostic value of the skin-prick test and RAST assay in insect sting allergy

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Summary

The results of skin-prick tests to four concentrations of venom (0.1, 1, 10 and 1000 $\mu\text{g/ml}$) carried out on two occasions were analysed in relation to the history of adverse reactions to stings and to the level of venom-specific IgE antibody in serum, in forty-two subjects allergic to insect stings (sixteen to bee and twenty-six to wasp). Fifty control subjects (some of whom had never been stung by bee or wasp) with no history of adverse reaction to stings were also studied. No subject gave a positive skin-test reaction to 0.1 $\mu\text{g/ml}$, and small numbers reacted to either 1 or 10 $\mu\text{g/ml}$. The lowest concentration of venom to which most subjects had a positive skin test was 100 $\mu\text{g/ml}$. Our data suggest that in wasp-allergic patients a positive skin test to 100 $\mu\text{g/ml}$ is normally significant (reflecting the presence of specific IgE), whereas in bee-allergic patients a skin test reaction to 100 $\mu\text{g/ml}$ is usually non-specific for the following reasons.

(i) In the allergic patients when skin tests were repeated, a reaction to 100 $\mu\text{g/ml}$ bee venom often became negative (in six of eight), whereas for wasp venom the reaction became more positive (at 10 or 1 $\mu\text{g/ml}$) in seven of eight patients. Whilst this might reflect lack of reproducibility, the consistent direction of change for either bee or wasp venom suggests responses to this concentration of these venoms may have different interpretations.

(ii) In bee-allergic patients, where positive skin tests to 100 $\mu\text{g/ml}$ wasp venom were found they were repeatable in all patients, and occurred only in patients who had been stung by a wasp. In wasp-allergic patients, skin test reactions to 100 $\mu\text{g/ml}$ bee venom were not reproducible between studies in any patient and often occurred in subjects never stung by a bee.

(iii) A better correlation between skin test and RAST occurred for wasp venom when a skin-test reaction to 100 $\mu\text{g/ml}$ was included as a positive (rather than reactions to 1 or 10 $\mu\text{g/ml}$ only). For bee venom the correlation did not improve when skin-test reactions to 100 $\mu\text{g/ml}$ were included.

(iv) In the control group, skin-test reactions to 100 $\mu\text{g/ml}$ bee venom were often false positives (seven of eight had never been stung by a bee). Four out of fifty controls reacted to wasp venom 100 $\mu\text{g/ml}$, but three of these had received a wasp sting.

These findings suggest that in routine clinical practice skin tests should be carried out over the range 1–10 $\mu\text{g/ml}$ in bee-allergic subjects but in wasp-allergic patients 100 $\mu\text{g/ml}$ should also be included. Extracts of 0.1 $\mu\text{g/ml}$ need not be used.

Introduction

The diagnosis of allergy to bee and wasp stings is made largely from the patient's history. Skin-prick tests and/or RAST tests are used to confirm the history [1–4] and may also be of value when the insect has not been identified. It is not clear whether skin-prick tests to venom or RAST tests for venom IgE antibody are more useful diagnostically. Skin tests have the advantage of being simple, quick and inexpensive. In addition to the diagnostic role of the skin test, it has been claimed that skin tests are of value in identifying patients at risk of reacting to the next sting [5]. This is controversial and there is compelling evidence, particularly from sting challenge in skin-test positive subjects with a history of allergic reaction, to suggest that the skin test has no predictive value [6,7].

The interpretation of skin tests to venom, unlike skin tests to inhalant allergens, is difficult and presents special problems. It is usual to skin test with a series of concentrations of venom (0.1, 1, 10 and 100 $\mu\text{g/ml}$). Weals are small and, if higher concentrations of venom are used, normal (non-allergic) subjects may produce weals, attributed to 'toxic' (non-specific) reactions [2,5]. These false-positive reactions are not due to the presence of specific IgE antibody.

Despite the problems outlined above, skin tests are in routine practice clinically. Because it is not clear how these should be interpreted, inappropriate conclusions are likely to be drawn. The aim of this study is to determine how best to interpret the results of skin tests and to establish their clinical significance. We have examined responses to bee venom and to wasp venom separately. Previous studies have assumed these are similar and have not distinguished bee from wasp, when reporting results of skin tests [5,8]. Our clinical studies [9] have demonstrated differences between bee and wasp allergy: fewer stings are required to sensitize to wasp than to bee and it is notable that the majority of bee-allergic subjects are beekeepers, their relatives or neighbours with a high risk and incidence of stings, whereas wasp allergy seems to be a random event. The results of skin tests to different concentrations of venom in bee- and wasp-allergic patients have been analysed and skin-test responses studied in relation to the history of allergic reactions and the venom-specific IgE levels in serum. The results were also considered in the light of skin tests in subjects with no previous history of sting or adverse reaction to sting to determine which concentrations of bee and wasp venom give true positive (IgE-mediated) weals, and which are likely to cause non-specific reactions. By true positive, we mean a weal due to the presence of specific IgE antibodies on the mast cell, as opposed to a toxic or non-specific reaction. This reflects sensitization but does not indicate whether a sting will cause a clinical allergic reaction.

Subjects and methods

Patients

Forty-two patients, twenty-four females and eighteen males, age range 17–76 years, who had been referred to the Allergy Clinic because of allergic reactions to bee or wasp stings, were studied. Allergic reactions consisted of either large local reactions (LR) (extensive oedema around the site of sting persisting for at least 72 hr) or generalized reactions (GR), which included at least one of the following: urticaria, angioedema, erythema, pruritus, asthma, laryngeal oedema, faintness, loss of consciousness, or gastro-intestinal symptoms including vomiting and abdominal pain. A GR that occurred after multiple (thirty) bee stings simultaneously, in a patient who otherwise had isolated LRs to single stings, was not included as a true GR. Twenty-six patients were allergic to wasp stings and two of these had isolated LRs only. Sixteen patients were allergic to bee stings, eleven having GR $\pm$ LR and five having LR only. Of sixteen subjects, nine were beekeepers, five were relatives of beekeepers and only two had no connection with beekeeping. The mean number of stings received by bee-allergic subjects was thirty-two and by wasp-allergic subjects was five.

In addition to the full assessment made when the patients were originally referred to the clinic, each patient was studied on two further occasions (study 1 and study 2) separated by a 3-month interval. On each occasion a detailed history was taken, skin-prick tests were performed and total serum IgE and bee and wasp venom IgE levels were measured. Two of the bee-allergic subjects were stung between study 1 and study 2. No patients had received immunotherapy with pure venom extracts but two had been treated with whole body extracts (known to be ineffective), stopping more than 2 years before study 1.

Controls

Fifty control subjects were studied (twenty-seven females and twenty-three males, age range 19–59 years) in whom there was no history of adverse reactions to either bee or wasp sting. Sixteen of these subjects had never been stung by either a bee or a wasp. In this group, a history was taken and skin tests carried out as in the patients. Radio-allergo-sorbent tests were not performed.

Skin-prick tests

Skin-prick tests were performed using Morrow Brown needles and Pharmalgen bee (*Apis mellifera*) venom and wasp (*Vespula* species) venom (Pharmacia Diagnostics AB, Uppsala, Sweden). These freeze-dried extracts were reconstituted in human serum albumin. Each venom was tested in four concentrations 0.1, 1, 10 and 100 μ g/ml, in duplicate on the volar surface of the same forearm in both studies. Human serum albumin (diluent) and histamine 1 mg/ml were included as negative and positive controls. The site on the forearm for the various venom concentrations was chosen so that there was a standard distance between duplicates. All skin tests were performed and interpreted by the same individual.

The weal size was measured after 15 min. The weal was outlined in ink and a permanent record obtained by transferring onto Sellotape. The mean of the largest diameter and the diameter perpendicular to this (measured to 1 mm) were calculated. The mean diameter of duplicates was then calculated. A positive result was taken as a weal of 2 mm or greater.

Total and specific serum IgE

Bee and wasp venom IgE were measured by radio-allergo-sorbent test (RAST, Pharmacia Diagnostics AB, Uppsala, Sweden). Results are obtained from a standard curve, on which the percentage of total activity bound to the discs is plotted. Results were expressed as a RAST class (0-4).

Results*Patients*

Five of sixteen bee-allergic patients had experienced localized reactions (LR) only, while eleven had had at least one episode of generalized reaction (GR). Only two of twenty-six wasp-allergic patients had experienced LR on its own. The results of skin tests to different concentrations of venom in study 1 and study 2 are shown in Table 1. No patients gave positive reactions to 0.1 $\mu\text{g/ml}$. The mean time from the last sting is shown and responses to higher concentrations did not occur because of a longer interval from the test sting.

The change in results from study 1 to study 2 for each patient is shown in Table 2. The lowest concentration giving a positive reaction changed in twelve of twenty-five wasp patients and in nine of fourteen bee patients. There was no change in the two bee

Table 1. Results of skin tests: number reacting at different concentrations of venom (expressed as the lowest concentration of venom to give a positive reaction)

Venom concentration ($\mu\text{g/ml}$)	Bee patients			Wasp patients		
	Skin test with bee venom		Time* (year)	Skin test with wasp venom		Time* (year)
	Study 1	Study 2		Study 1	Study 2	
0.1	0	0	—	0	0	—
1.0	1	1	8.0	1	2	0.5
10.0	3	3	1.5	9	7	3.5
100.0	6	6	2.5	12	13	4.5
Negative	6	4	0.5	4	3	3.5
Total	16	14		26	25	

* Mean time from last sting to study 1.

Table 2. Change of result of skin test (study 1 and 2) in patients who reacted to 100 $\mu\text{g/ml}$ in either study

Skin-test response*	Bee patients (n = 8)	Wasp patients (n = 8)
100 to negative	6	1
100 to 10	1	6
100 to 1	1	1

* Venom concentration ($\mu\text{g/ml}$) giving weal ≥ 2 mm.

Table 3. Correlation between skin test and RAST

	RAST*			
	Study 1		Study 2	
Skin test†	Negative	Positive	Negative	Positive
Bee patients tested with bee venom				
Negative	6	2	5	5
Positive	2	6	1	3
<i>n</i> (total)	16		14	
Wasp patients tested with wasp venom				
Negative	6	10	5	11
Positive	6	4	3	6
<i>n</i> (total)	26		25	

* RAST results: negative = class 0-1, positive = class 2-4.

† Skin test results: negative = no reaction or weal at 100 µg/ml, positive = weal at 1 or 10 µg/ml.

Table 4. Correlation between skin test and RAST, depending on definition of a positive skin test

Allergy	Definition of positive	Correlate	Not correlate
Bee patients tested with bee venom			
Bee	ST 1-10*	8	6
	RAST 2-4†		
	ST 1-100*	8	6
	RAST 2-4		
Wasp patients tested with wasp venom			
Wasp	ST 1-10	11	14
	RAST 2-4		
	ST 1-100	18	7
	RAST 2-4		

* Skin test results: 1-10 = weal at 1 or 10 µg/ml, 1-100 = weal at 1, 10 or 100 µg/ml.

† RAST results: positive = class 2-4.

patients who were stung between study 1 and study 2. There was no trend in change of concentration from study 1 to study 2. Eight bee patients had positive skin tests to 100 µg/ml (in either study) and six changed to a negative response in the other study. In contrast, of eight wasp patients with positive skin tests at 100 µg/ml, seven reacted to a higher dilution (six at 10 µg/ml and one at 1 µg/ml) in the other study. Thus, the range over which the skin tests changed differed for bee and wasp patients. In bee patients, a

positive skin test to 100 $\mu\text{g/ml}$ was usually negative when repeated, whereas in wasp patients skin tests positive at 100 $\mu\text{g/ml}$ usually became more positive.

Skin tests to wasp venom in the bee-allergic patients showed, in study 1, two of sixteen were positive at 100 $\mu\text{g/ml}$ and one was positive at 10 $\mu\text{g/ml}$. In study 2, four of fourteen were positive at 100 $\mu\text{g/ml}$; three had been stung by a wasp and one had probably been stung. Three of these had also been positive in the first study and two of the three had a history of small local reactions to wasp sting. Thus, a skin-test reaction to 100 $\mu\text{g/ml}$ wasp venom was (i) consistently positive, (ii) occurred in patients with a history of wasp sting and (iii) often associated with an allergic reaction to wasp sting. When the wasp-allergic patients, were skin tested with bee venom, two of twenty-six were positive at 10 $\mu\text{g/ml}$ and one was positive at 100 $\mu\text{g/ml}$ in study 1. In study 2, four were positive at 100 $\mu\text{g/ml}$ and they were all different patients from those who reacted in study 1. Of the seven patients positive in either study, four had never been stung by a bee and only one had a history of localized allergic reaction to a bee sting.

Venom skin test and specific IgE serum antibody levels were compared in both studies, and results for both bee- and wasp-allergic patients are shown in Table 3. By taking RAST class 2–4 and skin-test reactions to 0.1, 1 or 10 $\mu\text{g/ml}$ as positive results, any weak (or doubtfully) positive results were excluded from each test. A good correlation between skin test and RAST was shown for bee venom. For wasp venom the RAST was more often positive than the skin test. Two different definitions of a positive skin test (a weal at 1–10 $\mu\text{g/ml}$ or 1–100 $\mu\text{g/ml}$) were then compared with a single definition of a positive RAST, and the correlation between skin test and RAST compared. Table 4 shows this data, taking class 2–4 as a positive RAST. When the skin test to 100 $\mu\text{g/ml}$ was included, there was no change in correlation for bee patients, but for wasp patients a better correlation was obtained. Similar results were obtained if a positive RAST was taken as class 1–4 and compared with the two definitions of a positive skin test (data not shown).

Control group

In the control group, sixteen of fifty subjects did not recall being stung by either a bee or a wasp; twenty-nine (58%) had been stung by a wasp, twenty (40%) had been stung by a bee and fifteen had been stung by both insects.

Eight (16%) of the fifty subjects had positive skin tests to bee venom at 100 $\mu\text{g/ml}$; seven of the eight had never been stung by a bee. One person had a positive skin test to 1 $\mu\text{g/ml}$ and another had a positive to 10 $\mu\text{g/ml}$ of bee venom, and both of these subjects had been stung by a bee. Only four (8%) of the control group had positive skin tests to wasp venom at 100 $\mu\text{g/ml}$, and three of these had been stung by a wasp. In summary, testing over the range 0.1–100 $\mu\text{g/ml}$, ten of fifty (20%) had positive skin tests to bee venom but only three of these (30%) had been stung. Four of fifty (8%) had positive skin tests to wasp venom, and three of these (75%) had been stung. Considering results to 100 $\mu\text{g/ml}$ only, eight subjects were positive to bee venom, but seven of these had never been stung; four were positive to wasp venom, and three of these had been stung.

Discussion

This study attempts to define the value of skin tests to bee or wasp venom, in particular, to establish how skin-test results should be interpreted to distinguish between normal (non-venom IgE producing) subjects and potentially allergic (venom IgE positive) subjects.

There have been two previous studies where skin-prick tests to venom were performed over a range of concentrations, in subjects with no history of adverse reactions to bee or wasp stings. Harries *et al.* [5] found that eight of forty-six subjects (17%) had positive skin tests to 100 $\mu\text{g/ml}$ and Bjorkander & Belin [8] found twelve of fifty (24%) reacted to 100 $\mu\text{g/ml}$. Neither of these studies distinguished results of skin tests for bee and wasp venom.

In this study we report differences in responses to bee venom and to wasp venom. In our control subjects, with no history of adverse reaction to sting, ten of fifty (20%) had positive skin tests to bee venom, eight at 100 $\mu\text{g/ml}$, seven of whom had never been stung by a bee. This suggests that at least seven out of eight reactions to 100 $\mu\text{g/ml}$ bee venom were not due to bee venom IgE antibody to cutaneous mast cells, and may have been 'toxic' reactions. In contrast, only four of fifty controls had positive skin tests to wasp venom, all occurred at 100 $\mu\text{g/ml}$ and three of these four subjects had been stung by a wasp. This suggests that, in the case of wasp venom, the skin tests at 100 $\mu\text{g/ml}$ are more likely to be detecting IgE antibody and may be of some value. While it is possible that the history of previous stings may not be accurate, this consideration should not account for the difference we have observed between the two groups.

Skin tests to wasp venom in bee-allergic patients gave reactions mainly in patients who had been stung by wasps (four of fourteen patients were positive: three of these four had definitely, and one had probably, been stung by a wasp). Furthermore, half of these patients had had LR_s to wasp stings. Again this suggests the wasp venom skin test at 100 $\mu\text{g/ml}$ is detecting specific IgE antibody. In contrast, skin test to bee venom in wasp-allergic patients gave no consistent results between study 1 and study 2, three patients were positive in study 1 and four in study 2, and of these seven, four had never been stung by a bee. Three of these four had a weal to 100 $\mu\text{g/ml}$ bee venom. The bee venom skin tests, particularly at 100 $\mu\text{g/ml}$, seem to be less reliable.

A positive skin test to bee or wasp venom at 100 $\mu\text{g/ml}$ is felt to be of doubtful value, although there is little data on this. In our patients, looking at the change in the venom concentration to give a positive skin test, between study 1 and study 2, many showed a change of concentration, most changing by only one step. This clearly may be due to lack of reproducibility since only two patients were stung between study 1 and study 2. However, the concentration range over which the change took place differed for bee and wasp patients. Six of eight bee patients had results that changed between negative and positive at 100 $\mu\text{g/ml}$. This suggests a weal only to 100 $\mu\text{g/ml}$ bee venom may be due to a toxic reaction (and not reflect specific IgE) and should be classified as a negative result. In contrast, of the wasp patients only one of eight had a skin test that changed from negative to positive at 100 $\mu\text{g/ml}$ between study 1 and study 2. Seven out of eight changed from positive at 100 $\mu\text{g/ml}$ to positive at 10 $\mu\text{g/ml}$ or 1 $\mu\text{g/ml}$. This suggests that a positive reaction to 100 $\mu\text{g/ml}$ of wasp venom is more likely to indicate a true (IgE-mediated) positive whereas for bee venom a reaction to 100 $\mu\text{g/ml}$ does not.

If the RAST test could be taken as objective evidence of the presence of venom IgE antibody, the correlation between RAST and skin test responses to different concentrations of venom might help to define a true-positive skin test. For wasp patients, a better correlation with RAST (positive defined as class 2-4) was obtained if the criterion for a positive skin test was a reaction in the range 0.1-100 $\mu\text{g/ml}$ than when a positive skin test was defined as a reaction to 0.1, 1 or 10 $\mu\text{g/ml}$ only. For bee patients, the correlation between skin and RAST did not improve when the criterion for a positive skin test was changed to include reactions to 100 $\mu\text{g/ml}$. This observation held

true, when we took RAST class 1-4 as positive. This is further evidence that wasp venom skin test to 100 $\mu\text{g}/\text{ml}$ is detecting specific IgE. A perfect correlation between skin test and RAST is not, of course, expected [3,4] and possible reasons for this include differences in amount of tissue-fixed circulating venom IgE, differences in the proportion of antibodies to the different venom proteins, or that some positive skin tests may depend on a non-IgE mechanism such as IgG homocytotropic antibody.

This paper suggests that skin-test responses to bee and wasp venom may not be the same and should be interpreted differently. A reaction to 100 $\mu\text{g}/\text{ml}$ wasp venom frequently indicates the presence of specific IgE antibody, whereas a reaction to the same concentration of bee venom is likely to be non-specific. While it is clear from this, and other studies, that the skin test is not a perfect and reliable test, it remains the most practicable test for diagnosis of insect sting allergy. It is quick, simple and readily available compared to RAST, and may be more sensitive than RAST. The skin test does not predict the reaction to the next sting and, although it has been suggested that a challenge sting is the only valid test for this, this assertion is questionable. Studies of natural history [9] show that responses to sequential stings can vary so that a positive reaction to a challenge sting does not necessarily imply a similar reaction to the next sting. In this context, it is important to remember that a positive skin test simply reflects the presence of venom-specific IgE antibody on the cutaneous mast cell, but gives no indication of the releasability of mast cells at different sites following a sting. At present we cannot reliably predict the reaction to the next sting. Further studies of natural history and sequential tests in relation to a series of stings (skin-prick tests, RAST and others) are required.

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