

Comparison of Skin Tests and RAST for the Diagnosis of Bee Sting Allergy

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Skin tests and estimation of specific IgE-antibodies by the RAST are evaluated as diagnostic procedures in bee sting hypersensitivity with the following results:

- (1) Tests with venom antigens are highly superior to tests with wholebody extracts.
- (2) With bee venom results of skin tests and RAST correspond in 82%, with bee wholebody extracts in 68%.
- (3) Both with skin tests and the RAST with bee venom a good discrimination between patients with bee sting hypersensitivity and non-allergic controls is observed. Skin tests with bee venom are somewhat more sensitive than bee venom-RAST.
- (4) With the careful skin test procedure chosen, no adverse reactions were observed.

Key words: bee sting allergy; bee venom, wholebody extract; skin tests, RAST.

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Skin tests with whole body extracts of bees, yellow jackets and wasps are still widely used in the diagnosis of *Hymenoptera* sting allergy. Their value is, however, controversial (1, 2, 7, 13). Mueller (7) claims that skin testing with whole body extracts (WBE) may also cause serious general reactions. Several recent studies making use of the RAST (radio-allergo-sorbent-test) (9, 12, 14) or of the histamine release assay (5), have demonstrated the superiority of venoms to whole body extracts for the *in vitro* diagnosis of *Hymenoptera* sting allergy. The same superiority has been suggested by some studies dealing with venom skin testing (4, 6, 15). These papers, however, either fail to compare venoms with WBE (4), or skin testing is not compared with routine *in vitro* procedures such as the RAST (4, 15), or there are no control groups (6).

This study therefore evaluates skin testing with venom and WBE in patients with bee

sting allergy and non-allergic controls and compares these results with those of the RAST.

MATERIAL AND METHODS

Patients and controls

Thirty-three patients with bee sting hypersensitivity with general allergic reactions of Type I were investigated (Table 1). The reaction time between sting and first allergic symptoms was less than 30 min in all patients. The severity of the allergic reaction was determined according to Mueller (8) (Table 1).

Twenty-eight patients were investigated within 6 months after their last sting, the remaining five within 3 years after the last exposure.

One control group consisted of 20 non-atopic persons without a history of general or severe local reactions to *Hymenoptera*

stings. Sixteen had been stung by bees before. A second control group comprised 15 atopic patients with pollinosis and without a history of general or severe local reactions to *Hymenoptera* stings. All of them had been stung by bees before.

Skin testing and blood collection were performed on the same day in all individual patients and controls. The sera were kept at -30°C until used for estimation of specific IgE antibodies against bee venom (BV) and bee wholebody extracts (BWBE).

Antigens

Lyophilized BV for skin testing was obtained from Fa Mack Nachf. Illertissen, Bayern, BRD. The venom was collected by electro-stimulation and contained approximately 50% mellitin, 12% phospholipase A, 3% hyaluronidase, 2% Apamin, 2% mast-cell degranulating peptide and 25% low molecular constituents. BWBE was obtained from Bencard Allergy Unit, England. Twelve per cent extracts (12 g of whole insect body extracted in 100 ml of Evan's solution) were

used for the preparation of discs, 6% extracts for prick testing and 0.1% and 1% extracts for i.c. tests.

Skin tests

Lyophilized BV was dissolved in 0.9% saline and sterilized by ultrafiltration. Then serial tenfold dilutions to concentrations from 10^{-1} up to 10^{-8} mg/ml were prepared in 0.9% saline. Dilutions were used within 1 month after their preparation.

Skin testing with bee venom was started by a prick test with the solution of 10^{-6} mg/ml. If this was negative, 0.01 ml of the solution of 10^{-8} mg/ml were given intracutaneously. If this test was negative the dose was increased by a factor 10 at intervals of 30 min (10^{-7} , 10^{-6} , 10^{-5} ... mg/ml) until the test became positive. BWBE for skin testing was obtained from Bencard Allergy Unit., Switzerland, and the test procedure performed according to the prescriptions of this firm. We started with a prick test with the 6% solution of BWBE. If this was negative, 0.01 ml of a 0.1% solution was

Table 1
Classification of allergic reactions to *Hymenoptera* stings (according to Mueller (1959))

Grade of reaction	No. of patients	Signs and symptoms
I Slight general reaction	3	Generalized urticaria, itching, malaise and anxiety
II General reaction	8	Any of the above plus two of following: angioneurotic oedema, dizziness, constriction of chest, abdominal pain, nausea, vomiting, diarrhoea
III Severe general reaction	10	Any of the above plus two of following: dyspnoea, dysphagia, hoarseness or thickened speech, confusion, feeling of impending disaster
IV Shock reaction	12	Any of the above plus two of following: cyanosis, fall in blood pressure, collapse, incontinence, unconsciousness

given intracutaneously. If this was still negative, 0.01 ml of a 1% solution was given i.c. Prick tests were considered to be positive, if a weal of 3 mm or more in diameter with erythema was observed; intracutaneous tests, if a weal of 5 mm or more in diameter with erythema resulted. The solvents served as a control in all prick and intracutaneous skin tests.

RAST (radio-allergo-sorbent-test)

IgE antibodies against bee venom and BWBE were determined by the Phadebas RAST® (Pharmacia Diagnostics AB, Uppsala, Sweden). Bee venom discs were those of the Phadebas RAST. The experimental WBE discs were prepared as described previously (11). Twelve per cent BWBE for preparation of the discs was obtained through courtesy of Mr. G. Green, Bencard Allergy Unit., England. All test results were evaluated according to the Phadebas RAST reference system.

Statistical analysis

For statistical analysis, the RAST-values were given in per cent of Reference C of the Phadebas RAST reference system and then transformed logarithmically. Mean values of skin tests and RAST results with BV and BWBE were compared in patients and controls by the t-test. Correlations between various diagnostic parameters including the

grade of severity of an allergic reaction were calculated in patients and controls separately.

RESULTS

Skin testing

Prick tests with BV at a concentration of 10^{-6} mg/ml were negative in all patients and controls. Table 2 shows the results of i.c. tests. At a BV-concentration of 10^{-4} mg/ml or less, 31 (94%) of the patients with bee sting hypersensitivity had a positive skin test, compared with only one (3%) of the controls. At higher BV concentrations positive skin tests were observed in several of the controls. On the basis of these results a skin test with BV was regarded as positive if a clearcut reaction was observed at a concentration of 10^{-4} mg/ml or less.

With BWBE, 22 (66%) of the patients, but also six (17%) of the controls had a positive skin test with at least one of the three concentrations used. With the 0.1% solution given i.c., 13 (40%) of the patients and two (6%) of the controls were skin test positive, while prick testing was only positive in four patients (Table 3, Fig. 1).

RAST (Table 3, Fig. 1)

Specific IgE antibodies against BV were detected in 73% of the patients and 6% of the

Table 2
I.c. skin tests with bee venom (BV) in patients with bee sting hypersensitivity and controls

Concentration of BV in mg/ml	Patients	Controls	
		Non-atopic	Atopic
10^{-1}	—	5	—
10^{-2}	—	12	8
10^{-3}	2*	2	7
10^{-4}	3	1	—
10^{-5}	8	—	—
10^{-6}	7	—	—
10^{-7}	7	—	—
10^{-8}	6	—	—
$\leq 10^{-4}$	31 (94%)	1 (5%)	—

*Skin tests performed 3 and 5 days after the last exposure to a bee sting.

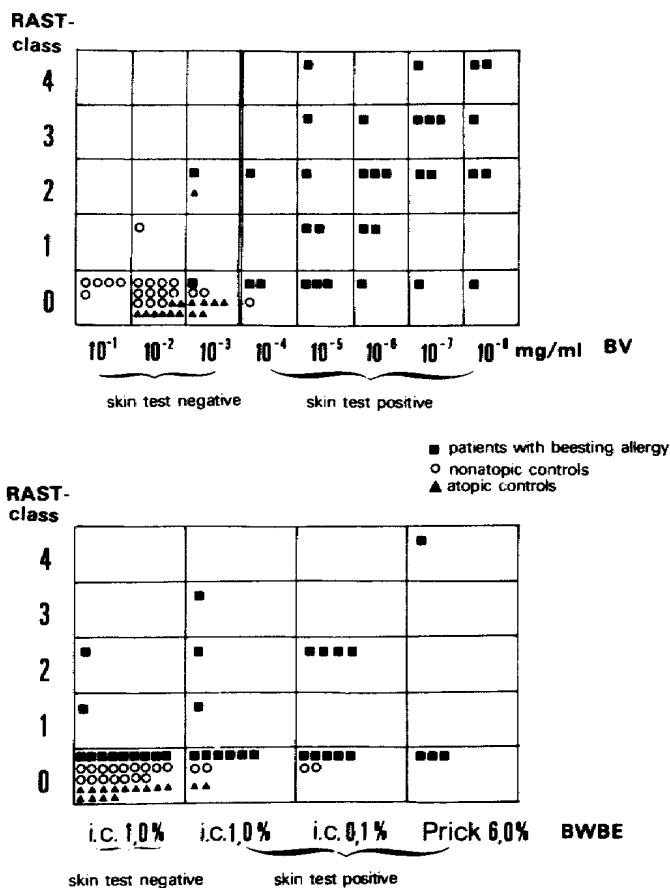


Fig. 1. Comparison of skin tests and RAST with bee venom (BV) and bee wholebody extract (BWBE) in patients with bee sting hypersensitivity and controls.

controls, against BWBE in 30% of the patients and none of the controls.

Comparison of skin tests and RAST (Fig. 1)

With BV 23 (70%) of the patients had positive, one patient (3%) and 32 controls (91%) had negative results with both tests. Thus in 82% there were corresponding results. Eight patients and one control had a positive skin test and a negative RAST, one patient and two controls a negative skin test and a positive RAST.

With BWBE eight patients (24%) had

positive, nine patients (27%) and 29 controls (83%) had negative results with both tests. Corresponding results were thus found in 68%. Fourteen patients and six controls were skin test positive and RAST negative and two patients were skin test negative and RAST positive.

Statistical analysis (Table 4)

With all four tests (skin tests and RAST with both BV and BWBE) a highly significant difference ($P < 0.01$) between patients and controls was found. Both tests with BV

resulted in a substantially better discrimination between patients and controls than those with BWBE, skin tests with BV giving the best results.

A positive correlation was found in patients but not in controls between BV-RAST and BV-skin tests, BV-skin tests and BWBE-skin tests, and BWBE-RAST and BWBE-skin tests.

Between BV-RAST and BWBE-RAST a positive correlation was observed in both patients and controls. The results of skin tests and RAST with either BV or BWBE did

not correlate significantly with the grade of the allergic reaction.

DISCUSSION

Our results indicate that intracutaneous skin testing with bee venom is a very reliable diagnostic procedure, giving positive results in 94% of the patients and negative in 97% of the controls (Table 3, Fig. 1). The two patients with negative skin tests were investigated on the 3rd and the 5th day after their last sting respectively. The negative

Table 3
Skin tests and RAST in bee-sting allergy

	<i>n</i>	Skin test				RAST			
		BV		BWBE		BV		BWBE	
		+	-	+	-	+	-	+	-
Patients with bee-sting allergy	33	31 (94%)	2	22 (66%)	11	24 (73%)	9	10 (30%)	23
Controls	35	1 (3%)	34	6 (17%)	29	2 (6%)	33	0	35
Atopic	15	0	15	2 (13%)	13	1 (7%)	14	0	15
Non-atopic	20	1 (5%)	19	4 (20%)	16	1 (5%)	19	0	20

Table 4
Results of statistical evaluation

- Comparison of mean values of skin test (ST) and RAST - results in patients and controls by the *t*-test:

	<i>t</i> -test	<i>P</i>
ST with BV	13.72	<0.01
ST with BWBE	4.75	<0.01
RAST with BV	7.07	<0.01
RAST with BWBE	4.89	<0.01

- Correlation between various diagnostic tests and the grade of an allergic reaction (GR) in 33 patients with bee sting allergy and 35 controls:

	Patients		Controls	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
RAST BV/ST BV	0.45	<0.01	0.3	>0.05
RAST BWBE/ST BWBE	0.38	<0.05	-0.01	>0.1
RAST BV/RAST BWBE	0.74	<0.001	0.5	<0.01
ST BV/ST BWBE	0.72	<0.001	-0.11	>0.1
ST BV/GR	0.21	>0.1	—	—
ST BWBE/GR	-0.16	>0.1	—	—
RAST BV/GR	-0.15	>0.1	—	—
RAST BWBE/GR	-0.07	>0.1	—	—

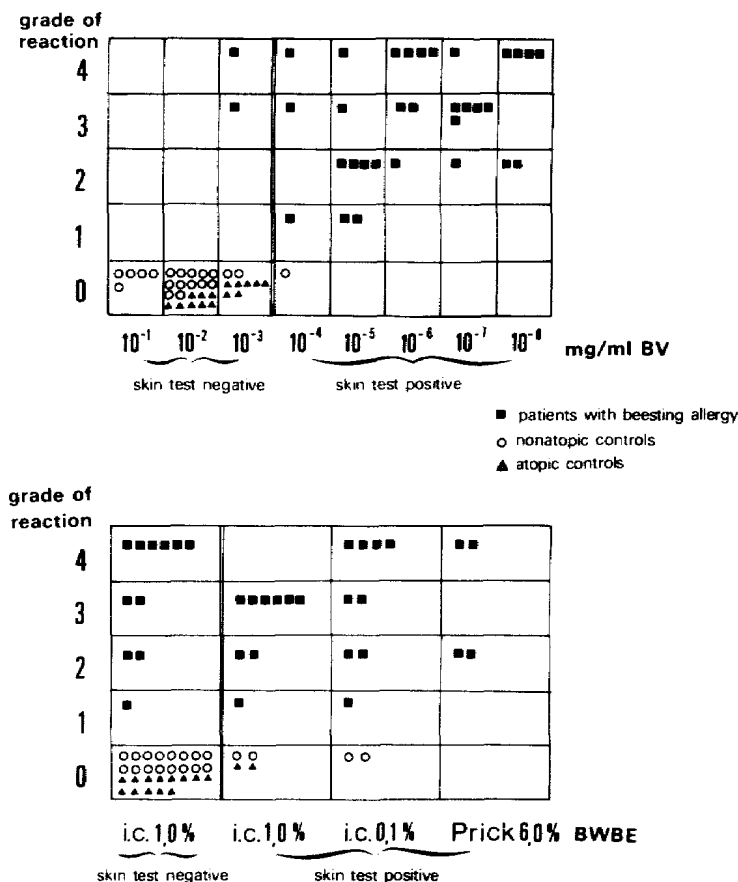


Fig. 2. Correlation between the grade of an allergic reaction and skin test results with bee venom (BV) and bee whole-body extract (BWBE) in patients with bee sting hypersensitivity and controls.

result thus may be due to a state of antibody consumption. The positive skin test to BV in one control could be explained by sensitization following his last bee sting. The results of venom skin testing thus compare well with those of the literature (4, 15). I.c. skin testing was preferred to prick testing in this study because the amount of allergen getting in the skin is thought to be more constant with the intracutaneous application.

False positive reactions, which occur more frequently in i.c. than in prick tests could be identified by using several dilutions of the

antigens in all patients and controls. With BV they occur at concentrations of 10^{-5} mg/ml, and more and are probably due to tissue toxicity of mellitin. The amount of BV corresponding to one bee sting lies considerably higher and is approximately 5×10^{-2} mg.

For clinical routine prick testing may also be used. We would suggest starting with a dilution of 10^{-6} mg/ml. False positive reactions are to be expected at concentrations of 10^{-1} mg/ml and more.

With the test procedure chosen, no general

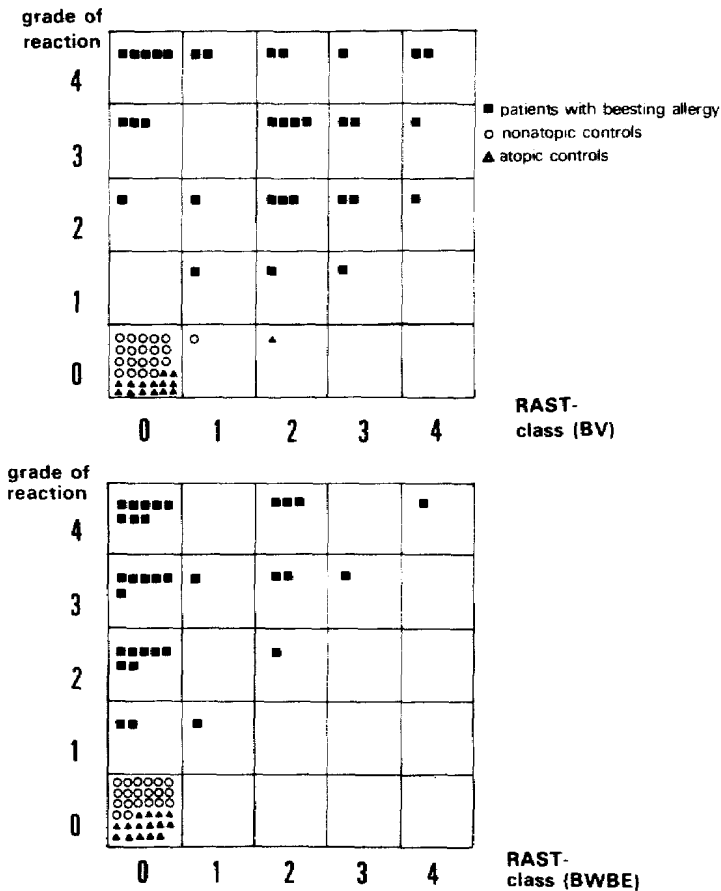


Fig. 3. Correlation between the grade of an allergic reaction and RAST-results with bee venom (BV) and bee whole-body extract (BWBE) in patients with bee sting hypersensitivity and controls.

or severe local reactions were observed. Regarding the frequency of slight general reactions during hyposensitization therapy with venoms (3) these are to be expected, if less careful test procedures are used.

Skin testing with BWBE resulted in a considerably less good discrimination between patients and controls, thus confirming the results of Light et al. (6) and Zeleznick et al. (15) that venoms are superior to WBE in skin testing. The results obtained with BWBE, however, correlated well with those

obtained with BV, distinguished significantly between patients and controls, and are thus substantially better than those reported by other authors (1, 2, 13). A different venom content of the various WBE used could account for this discrepancy. Skin test results with BV and BWBE only correlate in patients, while RAST results with the two antigens correlate in patients and controls. The lack of correlation in skin testing of controls is probably due to non-specific, toxic effects of higher concentrations of BV

or BWBE on the skin. The good correlation observed between RAST values with the two antigens suggests that also "non-allergic" exposed persons may have low levels of specific IgE against BV.

The RAST results with BV and BWBE are similar to those previously reported (9, 10, 11). BV-RAST results are somewhat less favourable than results of i.c. tests with BV, eight patients showing a positive skin test and a negative RAST, but only one patient a negative skin test and a positive RAST. The correlation between the two tests with BV ($r=0.45$ $P<0.01$) was good, however, (Fig. 1, Table 4), giving corresponding results in 82%. It is not surprising that skin tests in general are superior to the RAST in *Hymenoptera* sting allergy, where the allergen usually enters the body through the skin.

Regarding the high cost of hyposensitization therapy and the relative frequency of adverse reactions during this therapy with *Hymenoptera* venoms (3) we think that both diagnostic procedures – skin tests and RAST – should be performed in any patient with suspected *Hymenoptera* sting allergy, in order to: 1) verify the diagnosis, and 2) obtain an optimal choice of the venoms to be used for hyposensitization therapy.

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