

Lack of reproducibility of a single negative sting challenge response in the assessment of anaphylactic risk in patients with suspected yellow jacket hypersensitivity

Hilbrandt H. Franken, MD, A. E. J. Dubois, MD, PhD, H. J. Minkema, MD, Sicco van der Heide, MSc, and Jan G. R. de Monchy, MD, PhD
Groningen, The Netherlands

To investigate the reproducibility of a single negative response to sting challenge with a living insect, we rechallenged a group of 61 patients who showed no clinical response to a first sting challenge. All patients had previously had symptoms suggestive of anaphylaxis after a yellow jacket field sting. Thirteen patients (21%) had anaphylactic responses after the second sting challenge, and six of these patients had severe reactions including symptomatic hypotension requiring administration of Adrenalin. This rate was significantly lower than the response rate of the original patient group to a first sting challenge (39%). Thus although fewer positive responses were observed in patients who had had a previous negative challenge response, the number of anaphylactic reactions was considerable and included patients with potentially life-threatening symptoms. Consequently, a single sting challenge may not be used to select patients for venom immunotherapy. (J ALLERGY CLIN IMMUNOL 1994;93:431-6.)

Key words: Sting challenge, reproducibility

Since the first studies of venom hypersensitivity that utilized intentional sting challenge with a living insect were performed, a consistent and unexplained finding has been the lack of clinical reactivity in approximately 50% of patients who are sensitized to venom and have a history of a systemic reaction to a field sting (FS). This has led to the formulation of two opposing schools of thought. The first view is that although only one half of patients react to a sting challenge, both reactor and nonreactor groups retain a substantial risk for future anaphylaxis and should be considered for venom immunotherapy (VIT).¹ The opposing view holds that nonreactors are at low risk for repeat anaphylaxis, may safely be excluded from VIT, and indeed, should not be offered VIT because such treatment is unnecessary.² Consistent with this latter notion is the recommendation

Abbreviations used

FS: Field sting
SC1: First sting challenge
SC2: Second sting challenge
VIT: Venom immunotherapy

that a single diagnostic sting challenge be used to select patients for VIT.^{2, 3} This assumes that a single negative response to a sting challenge is an event with good reproducibility in individual patients. Studies of this problem have been limited and results have been conflicting. Parker et al.⁴ rechallenged four patients who had had a negative sting challenge response and a history of a systemic reaction after an FS and found no reactors. In a study by Kampelmacher et al.,² 11 patients with a negative sting challenge response were rechallenged and had no response.² Blaauw et al.⁵ reported 10 patients with a negative sting challenge response who subsequently tolerated an FS. In contrast to these data Forster et al.⁶ found four severe systemic reactions after the second challenge in patients who had a previous negative challenge response in a group of 30 children who underwent two bee sting challenges.

From the Department of Allergology, State University Hospital Groningen, The Netherlands.

Received for publication Jan. 5, 1993; accepted for publication June 18, 1993.

Reprint requests: J. G. R. de Monchy, MD, PhD, State University Hospital Groningen, Oostersingel 59, 9713 EZ Groningen, The Netherlands.

Copyright © 1994 by Mosby-Year Book, Inc.
0091-6749/94 \$3.00 + 0 1/1/49531

We therefore studied this problem directly by subjecting patients who showed no response to a first sting challenge (SC1) (and consequently received no VIT) to a second challenge.

METHODS

Selection of patients

Patients with a positive history of a systemic response after a yellow jacket FS and a positive skin test and/or RAST result to yellow jacket venom and who were not excluded because of abnormal electrocardiogram, clinical evidence of congestive heart failure, coronary disease, hypertension, viral infections at the time of sting challenge or β -blocker therapy were subjected to an in-hospital challenge with a living insect. Between 1980 and 1991, 228 patients were challenged. Ninety patients reacted systemically and were treated with VIT (39%). One hundred and thirty-eight patients exhibited no reaction. Of these, eight patients were excluded because they started β -blocker therapy or because of pregnancy or age. Of the remaining 130 patients, 104 could be reached and 61 agreed to a second sting challenge (SC2) and gave their informed consent. SC2s took place in 1991 or 1992.

Skin test and RAST assay

Intracutaneous skin tests, performed during the first outpatient clinic visit and interpreted according to international guidelines,^{7, 8} were applied in aliquots of 0.03 ml venom solution provided by Pharmacia Diagnostics (Uppsala, Sweden) in concentrations of 0.0001 μ g/ml, 0.001 μ g/ml, 0.01 μ g/ml, 0.1 μ g/ml, and 1 μ g/ml. Skin test results were considered positive when the orthogonal wheal diameter after 15 minutes was greater than 5 mm, and the lowest concentration producing a positive test result in each patient (threshold concentration) was used to calculate the median for the group. Serum-specific IgE was measured by RAST with the Phadezym RAST method. The values were expressed as Phadebas RAST units per milliliter.

The in-hospital challenge with a living insect

Patients underwent sting challenge under intensive care conditions. Intravenous access and cardiac monitoring were established, and blood pressure was recorded regularly. Predominantly two species of yellow jackets with highly cross-reactive venom⁹ were used. Fresh insects were obtained on the morning of the challenge. A single insect was applied to the volar side of each patient's forearm with a pair of tweezers until a clearly painful sting occurred. Patients remained under constant medical supervision and were treated with saline solution, intravenous antihistamine (clemastine), subcutaneous Adrenalin, inhaled bronchodilator (salbutamol), and intravenous or oral corticosteroids (prednisolone) as needed. Symptoms and medication were recorded.

Statistical analysis

Demographic data and potential risk factors for venom anaphylaxis were compared for the patients who were eligible for rechallenge in 1991 and who had had their first (negative) challenge between 1989 and 1991 ($n = 65$). Twenty-six of these patients were challenged a second time. Risk factors for those 26 rechallenged patients were compared with the remaining 39 who did not undergo SC2. A similar analysis was also carried out in all 61 patients undergoing SC2 in which the 13 reactors were compared with the 48 nonreactors. This was done with the Mann-Whitney U test, because the parameters were not normally distributed. To compare the percentage of reactors after SC1 with SC2, Fisher's exact test was used.

RESULTS

In the group of 61 patients who exhibited no reaction to a previous sting challenge, SC2 resulted in 13 systemic responses (21%). Six of these patients experienced hypotension and required subcutaneous administration of Adrenalin. Two complained of gastrointestinal symptoms such as nausea, abdominal cramping, and fecal incontinence. In one case urinary incontinence occurred after a few minutes (Table I).

One patient experienced a feeling of throat tightness a few minutes after being challenged. This feeling persisted for over 1 hour without worsening and eventually disappeared. No other symptoms occurred. The reaction was therefore considered to be negative.

Analysis of possible selection bias performed on the patients who were eligible for rechallenge in 1991 was done by comparing possible risk factors in the participating group of 26 patients with the remaining group of 39 patients who were also eligible but declined SC2 (Table II). There were no significant differences found with regard to age or sex distribution of the patients, severity of the FS reaction, time interval between FS and SC1, skin test threshold concentrations, or RAST score. Patients undergoing SC2 reported an original FS in the head and neck region significantly less often than the rest of the eligible patients ($p < 0.02$). Only one patient undergoing SC2 reported an FS between SC1 and SC2. Thus because risk factor distribution in this first group of eligible patients showed no significant differences between those undergoing SC2 and those refusing a further challenge, this analysis was not extended to the patients who were rechallenged in 1992.

The percentage of reactors (21%) in the rechall-

TABLE I. Age, gender, symptoms, and medication of reactors to SC2

Patient No.	Age (yr)	Gender	Reaction to SC2	Medication
1	30	M	A,I	C,P
2	37	F	A,H,GI	Adr,C,P
3	42	M	I,E*	C
4	45	M	U,A,T	C,P
5	31	F	U,A,GI,H	Adr,C,P
6	16	F	U,A	C,P
7	53	F	U,A,H	Adr,C,P
8	49	F	I,D	C
9	43	F	I,H	Adr,C,P
10	36	M	U,A	C,P
11	15	F	I,E,A	C,P
12	40	F	I,E,H	Adr,C,P
13	35	F	E,H	Adr,C,P

A, Angioedema; I, itching; C, clemastine; P, prednisolone; H, hypotension (and reactive tachycardia), GI, gastrointestinal symptoms; Adr, Adrenalin; E, erythema; U, urticaria; T, tachycardia; D, dyspnea.

*Patient's reaction included redness and swelling at site of SC1.

TABLE II. Comparison of demographic data and risk factors for venom anaphylaxis in eligible patients subjected to ($n = 26$) and not subjected to ($n = 39$) SC2 in 1991

	Patients subjected to SC2 ($n = 26$)	Patients not subjected to SC2 ($n = 39$)
Age (yr) (mean $\pm$ SD)	36.8 (+11.5)	38.7 (+12.2)
Gender (F:M)	13:13	24:15
Site of FS		
Head/neck:other	3:23	15:24*
Severity of FS reaction		
Skin:more than skin	11:15	18:21
Time between FS and SC1 (median in mo)	12	12
Skin test threshold concentration (μ g/ml) (median)	0.01	0.01
RAST (PRU/ml) (median)	1.0	1.0

PRU, Phadebas RAST units.

* $p < 0.02$ compared with patients subjected to SC2.

lenged group was compared with the percentage of reactors to SC1. Of the original 228 patients, 90 (39%) had positive responses. This difference was statistically significant ($p < 0.01$).

Analysis of risk factors was carried out by comparing reactors and nonreactors to SC2 (Table III). There were no significant differences found between the groups in any of the parameters studied.

DISCUSSION

The use of a diagnostic sting challenge for selection of patients for specific VIT is based on the observation that only 28% to 45% of patients who report systemic reactions after FSs and who

have demonstrable levels of venom-specific IgE show reactivity on subsequent sting challenge.^{2-5, 10} There are data demonstrating that venom anaphylaxis may be a unique event in some patients.¹¹ Despite this, the reproducibility of a single negative sting challenge response has never been firmly established.

In our patient group, 21% of patients who showed no response to SC1 exhibited systemic responses to SC2, and approximately one half of these patients had severe reactions. This response rate is considerably higher than that of the general population, which has been found to be 0.15% to 3.9%.^{12, 13}

Patients who do not react to sting challenge

TABLE III. Comparison of demographic data and risk factors for venom anaphylaxis in reactors ($n = 13$) and nonreactors ($n = 48$) to SC2

	Reactors to SC2 ($n = 13$)	Nonreactors to SC2 ($n = 39$)
Age (yr) (mean $\pm$ SD)	35.0 $\pm$ 11.4	35.4 $\pm$ 12.3
Gender (F:M)	4:9	28:20
Site of FS*		
Head/neck:other	3:9	11:33
Severity of FS reaction		
Skin:more than skin	6:7	13:35
Time between FS and SC1 (median in mo)	12	12
Skin test threshold concentration (μ g/ml) (median)	0.01	0.01
RAST (PRU/ml) (median)	0.50	0.34

None of the above differences between reactors and nonreactors were statistically significant.

PRU, Phadebas RAST units.

*Five patients (one reactor, four nonreactors) were unable to recall the site of the sting.

have not been found to differ from reactors either clinically or immunologically, and thus no parameters have been found to reliably predict reactivity to a sting challenge.^{2, 3, 5, 10} This is also confirmed in the present study, in which comparison of factors thought to confer risk for anaphylaxis to FSs between reactors and nonreactors revealed no significant differences (Table III). We nevertheless examined the possibility of selection bias in our study population by comparing risk factors in the first 26 patients subjected to SC2 (1991) (six of whom had systemic responses) with the remaining eligible patients who did not undergo SC2. Only the site of the sting was observed to differ significantly between the two groups (Table II), and this parameter has been shown not to confer risk in the setting of a sting challenge.² Hence, it is unlikely that selection could account for the high rate of reactivity observed in our study population.

Another possibility is that SC1 resulted in considerably more false-negative outcomes than SC2. In this study both SC1 and SC2 were performed in a technically uniform manner as described in the Methods section. In the cases of the first 26 patients undergoing SC2, both SCs were carried out and assessed by the same investigator with yellow jackets procured from the same source. Moreover, the rate of reactivity of SC1 (39%) is in keeping with response rates reported by others,^{4, 5} making a significant false-negative rate to SC1 unlikely.

The reactivity rate to SC2 (21%) was statistically significantly lower than the reactivity to SC1 (39%). This difference could be due to a loss of clinical reactivity over time.¹⁴ Also, a relative

increase in the number of patients who show no anaphylactic response to SCs and who were probably misdiagnosed with regard to the original FS reaction could be a contributing factor. Because it is not possible to identify such patients, they are likely to be present in treatment groups from centers that do not use a diagnostic sting challenge for selection of patients for VIT. Despite numerous theoretic objections to the unnecessary treatment with VIT of patients not requiring such treatment, including potential risk of sensitization,¹⁵ extensive experience with VIT suggests that the health risks of such overtreatment are small. Furthermore, our data show that this risk is clearly overshadowed by the risks of undertreatment resulting from the selection of patients for VIT by means of a single diagnostic sting challenge in that approximately 10% of patients undergoing SC2 had life-threatening reactions.

The intermittent reaction pattern in patients with known sensitization and exposure to insect venom shows parallels to food and drug reactions as previously pointed out by Reisman.¹ Interestingly, Reisman¹ described 90 patients who had anaphylactic responses to FSs and were not treated with VIT who went on to have repeated re-stings.¹¹ Of these patients, 20 had no reaction to the next FS that occurred, but went on to have systemic reactions to subsequent FSs. These patients, who show an "on-off-on" pattern of clinical reactivity, thus represent 22% of the patients who have repeated re-stings, a figure which is quite comparable to the percentage of reactors to SC2 (21%) in whom a similar pattern of clinical reactivity was seen. Both patient- and nonpatient-related factors could influence the outcome of a

sting challenge. To what extent quality and quantity of the venom injected by the insect influence the response is unknown. Schumacher et al.¹⁶ showed that honeybee (*Apis mellifera*) venom content was markedly different among insects caught in one area. With respect to yellow jacket venom this could be even more relevant because several species of yellow jackets are present. In the past extensive cross-reactivity among vespid species has been shown. This however, does not exclude the presence of unique allergenic determinants.¹⁷ In 1992, all insects used for sting challenge were analyzed by an entomologist. Thirty-three were shown to belong to the subspecies *Vespula germanica* and two to the subspecies *V. vulgaris*. Because insects for SC1 and SC2 were obtained from the same source, it is probable that both sting challenges were carried out predominantly with *V. germanica*. Thus these data do not support the hypothesis that subspecies-related variability was a major contributing factor to the lack of reproducibility of sting challenge responses observed in some patients.

Patient factors such as specific IgE, IgG (subtypes), and skin test threshold concentration have not proven to be predictive for the outcome of a sting challenge.^{2, 4, 5, 18} Other patient factors are partly speculative and have been discussed by others.^{2, 19} These include non-IgE-mediated responses, involvement of the complement system, spontaneous loss of hypersensitivity, and pharmacologic or toxic reactions. All of these factors may contribute to the lack of reproducibility of a single negative sting challenge response.

A diagnostic sting challenge may be useful in cases in which uncertainty exists with regard to the causative insect or the outcome of the reaction. A positive response will establish an indication for VIT. A single negative response must, however, be interpreted with caution. Moreover, it is apparent from our data and that of others^{2-5, 10} that sting challenges in untreated patients can result in serious reactions. This procedure may therefore only be deemed ethical if it results in a benefit for the patient. For most patients, the sting challenge provides no useful information and thus no benefit. Furthermore, a negative sting challenge response may give patients and physicians a false sense of security and result in delay or omission of appropriate preventive and therapeutic measures.

In summary, until a means of identifying patients at low risk for repeat anaphylaxis has been established, all patients with an appropriate clinical

history and demonstrable levels of venom-specific IgE should be considered for venom-specific VIT. A diagnostic sting challenge may not be used routinely to safely exclude patients from this treatment.

We thank Robert Terko Simon Thomas, entomologist, for identification of the insects in 1992.

REFERENCES

1. Reisman R. Stinging insect allergy: progress and problems. *J ALLERGY CLIN IMMUNOL* 1985;75:553-5.
2. Kampelmacher MJ, van der Zwan JC. Provocation test with a living insect as a diagnostic tool in systemic reactions to bee and wasp venom: a prospective study with emphasis on the clinical aspects. *Clin Allergy* 1987;17:317-27.
3. van der Linden PWG, Hack E, Poortman J, Vivie-Kipp YC, Struyvenberg A, van der Zwan JC. Insect-sting challenge in 138 patients: relation between clinical severity of anaphylaxis and mast cell activation. *J ALLERGY CLIN IMMUNOL* 1992;90:110-8.
4. Parker JL, Santrach PJ, Dahlberg MJE, Yunginger JW. Evaluation of Hymenoptera sting sensitivity with deliberate sting challenges: inadequacy of present diagnostic methods. *J ALLERGY CLIN IMMUNOL* 1982;69:200-7.
5. Blaauw PJ, Smithuis LOJM. The evaluation of the common diagnostic methods of hypersensitivity for bee and yellow jacket venom by means of an in-hospital insect sting. *J ALLERGY CLIN IMMUNOL* 1985;75:556-62.
6. Forster J, Hauck P, Urbanek R. Sequential bee sting challenges before hyposensitization therapy in children with bee venom allergy [Abstract]. *J ALLERGY CLIN IMMUNOL* 1991;87:238.
7. Norman PS. In vivo methods of study of allergy. Skin and mucosal tests, technique and interpretations. In: Middleton E Jr, Ellis EF, Reed CE, Arbesman CE. *Allergy, principles and practice*. St. Louis: CV Mosby, 1983:297.
8. The Committee on Skin Test Standardization of the Netherlands Society of Allergology. Report on skin test standardization. *Clin Allergy* 1988;18:305-10.
9. Wicher DM, Reisman RE, Wypych J, et al. Comparison of the venom immunogenicity of various species of yellow jackets (genus *Vespula*). *J ALLERGY CLIN IMMUNOL* 1980; 66:244-9.
10. Hunt KJ, Valentine MD, Sobotka AK, Benton AW, Amodio FJ, Lichtenstein LM. A controlled trial of immunotherapy in insect hypersensitivity. *N Engl J Med* 1978;299: 157-61.
11. Reisman RE. Natural history of insect sting allergy: relationship of severity of symptoms of initial sting anaphylaxis to re-sting reactions. *J ALLERGY CLIN IMMUNOL* 1992;90: 335-9.
12. Abrishami MA, Boyd GK, Settignano GA. Prevalence of bee sting allergy in 2,101 girl scouts. *Acta Allergol* 1971; 26:117-20.
13. Settignano GA, Newstead GJ, Boyd GK. Frequency of Hymenoptera allergy in an atopic and normal population. *J ALLERGY CLIN IMMUNOL* 1972;50:146-50.
14. Savliwala MN, Reisman RE. Studies of natural history of stinging-insect allergy: long-term follow-up of patients without immunotherapy. *J ALLERGY CLIN IMMUNOL* 1987; 80:741-5.

15. Lichtenstein LM, Valentine MD, Sobotka AK. Insect allergy: the state of the art. *J ALLERGY CLIN IMMUNOL* 1979;64:5-12.
16. Schumacher MJ, Schmidt JO, Egen NB, Dillon KA. Biochemical variability of venoms from individual European and Africanized honeybees. (*Apis mellifera*). *J ALLERGY CLIN IMMUNOL* 1992;90:59-65.
17. Hoffman DR. Allergens in Hymenoptera venom. XVI. Studies of the structures and cross-reactivities of vespid venom phospholipases. *J ALLERGY CLIN IMMUNOL* 1986;78:337-43.
18. Hoffman DR, Gillman SA, Cummins LH, Kozak PP Jr, Oswald A. Correlation of IgG and IgE antibody levels to honeybee venom allergens with protection to sting challenge. *Ann Allergy* 1981;46:17-23.
19. van der Linden PWG, Hack CE, Kerckhaert JAM, Struyvenberg A, van der Zwan JC. Preliminary report: complement activation in wasp-sting anaphylaxis. *Lancet* 1990;336:904-6.

Bound volumes available to subscribers

Bound volumes of THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY are available to subscribers (only) for the 1994 issues from the Publisher, at a cost of \$66.00 for domestic, \$88.62 for Canadian, and \$84.00 for international subscribers for Vol. 93 (January-June) and Vol. 94 (July-December). Shipping charges are included. Each bound volume contains a subject and author index, and all advertising is removed. Copies are shipped within 30 days after publication of the last issue in the volume. The binding is durable buckram with the journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby-Year Book, Inc., Subscription Services, 11830 Westline Industrial Dr., St. Louis, MO 63146-3318; phone 1 (800) 453-4351 or (314) 453-4351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular journal subscription.