
Reproducibility of skin testing and serum venom specific IgE in Hymenoptera venom allergy

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Background: The decision regarding an immunotherapy regimen for venom-allergic patients is based on the results of skin testing and serum venom specific IgE measurements. However, their reliability has been questioned, and their reproducibility has not been examined.

Objective: To evaluate the reproducibility and reliability of the results of skin testing and serum venom specific IgE measurement in venom-allergic patients.

Methods: Patients with a systemic reaction after an insect sting were evaluated twice, 2 to 6 weeks apart, by intradermal skin tests and by determination of serum venom specific IgE to Hymenoptera venoms.

Results: Thirty-five patients were evaluated 1 to 168 months (mean, 23 months) after the sting reaction. Reproducibility of skin test results for all venoms at the 2 sessions was found in 23 patients (66%). Reproducibility of venom specific IgE results for all venoms was found in 16 (59%) of 27 patients from whom 2 blood samples were available for evaluation. Concordance between skin test and venom specific IgE results for all venoms was found in 30 (51%) of 59 samples available for evaluation.

Conclusions: The reproducibility of venom skin test and serum venom specific IgE results is relatively poor. It is common practice for therapeutic decisions regarding venom immunotherapy to be based on a single diagnostic evaluation. Consequently, many patients are either overtreated or undertreated. Better diagnostic methods are required in venom allergy.

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INTRODUCTION

Skin testing (ST) is the basic procedure used in establishing the diagnosis of Hymenoptera venom allergy. Along with serum venom specific IgE determination, both measures, together with a positive history of a systemic reaction (SR) to a sting, constitute the basis for the diagnosis and the specific venom immunotherapy (VIT), which is the proven effective therapy for SRs inflicted by insect stings. This is why the reliability of these diagnostic methods is extremely important. A previous study¹ reported the low accuracy of venom ST in predicting the outcome of future stings. Systemic reactions in patients with negative ST responses also reflect the limited diagnostic sensitivity of this method.^{2,3} The reproducibility of venom prick ST results was suspected to be poor in one study,⁴ whereas another study,⁵ conducted in a small number of patients, found ST results to be reliable. When considering VIT, allergists want to avoid it in patients who would be reasonably safe without it, reserving this treatment for those with potential life-threatening SRs. Therefore, reliable diagnostic methods with optimal predictive values are highly desirable.

In the present study, we focus on the reproducibility of venom intradermal ST and serum venom specific IgE results. Patients who had experienced a SR after an insect sting

underwent ST on 2 occasions, 2 to 6 weeks apart, using honeybee, yellow jacket, and wasp venoms, which constitute the common Hymenoptera species in Israel. Serum venom specific IgE measurements were checked as well at both sessions using a commercial immunoassay kit. Evaluation of reproducibility was based solely on whether the therapeutic intervention, ie, the administration of any venom for immunotherapy, would have differed as a result of the repeated ST or venom specific IgE measurement.

METHODS

Patients

Consecutive adult patients who had experienced a SR inflicted by an insect sting were evaluated twice, 2 to 6 weeks apart. The first evaluation was performed at least 1 month after the SR. After signing an informed consent form, patients underwent ST and had blood samples collected for venom specific IgE determination.

Skin Testing

At both sessions, intradermal ST was performed by a single experienced nurse whose reproducibility of histamine and diluent injection technique had previously been shown to be highly satisfactory in 10 other individuals. Freshly prepared commercial preparations of honeybee, yellow jacket, and wasp (*Polistes* species) venoms (ALK-Abello, Horsholm, Denmark) diluted with albumin saline with 0.4% phenol (Hollister-Stier Laboratories, Spokane, WA) were used. The same batch of venom was used at both sessions. Venoms were kept at 4°C to 8°C at all times. Skin testing was performed at both sessions on the volar aspect of the same

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arm; 0.02 mL was injected intradermally. The wheal response was measured 15 minutes later by the same nurse. Skin testing began in both sessions with a venom concentration of 0.01 $\mu\text{g/mL}$. If the difference between the wheal diameters of the venom ST and the diluent control was smaller than 5 mm, the test was repeated using 0.1 $\mu\text{g/mL}$ and finally using 1 $\mu\text{g/mL}$. As recommended by the manufacturer, a positive reaction was defined as a wheal diameter of 5 mm or greater compared with the diluent control, together with an erythema diameter larger than 10 mm in the absence of a reaction to the diluent control and in the presence of a positive reaction to histamine phosphate, 0.1 mg/mL (Center Laboratories, Port Washington, NY) (injected volume of both: 0.02 mL).

Serum Venom Specific IgE Measurements

Serum venom specific IgE measurements were performed in duplicate using an enzyme-linked immunosorbent assay (ELISA) kit (AlaSTAT; Diagnostic Products Corp, Los Angeles, CA). This is an enzyme-labeled immunoassay based on liquid allergen complexes. The kit's standard curve uses 7 concentrations of the calibrator, ranging from 0 to 100 IU/mL. The typical coefficient ranges from 0.3% to 3.5%. As recommended by the manufacturer, a level greater than 0.7 IU/mL was regarded as positive. Serum samples were frozen and eventually were all analyzed on the same day.

Data Analysis

The reproducibility of ST or serum venom specific IgE results was defined as the existence of similar results for these variables, either positive or negative, for all 3 venoms at both sessions. For example, if ST reactions were positive at 0.01 $\mu\text{g/mL}$ at visit 1 and at 1 $\mu\text{g/mL}$ at visit 2, these results were considered to be reproducible. Individual reproducibility for each of the 3 venoms was also examined. Concordance between ST and serum venom specific IgE results was defined as the existence of similar results (either positive or negative) for the 2 methods for all 3 venoms at the same session.

Statistical differences were analyzed using the χ^2 test or the Fisher exact test. The reproducibility for each venom separately in the 2 sessions regarding ST or serum venom specific IgE results and the concordance between ST and serum venom specific IgE results were analyzed using paired tests—the McNemar and Wilcoxon rank sum tests, each when appropriate. Power was calculated using a statistical software program (SamplePower; SPSS Inc, Chicago, IL).

RESULTS

Patients and Samples

Thirty-eight patients were enrolled. One patient did not return for the second evaluation. Two other patients returned only 2 to 3 months after the initial evaluation. These 3 patients were excluded from the study. The mean age of the remaining 35 patients (33 men and 2 women) was 22.3 years (range, 18–37 years). They did not take any medications during the week before the sessions. The mean time elapsed between the SR

and the initial evaluation was 23 months (range, 1–168 months). None of the patients were stung during the interval between the first and second evaluations. The severity of the SRs according to the criteria of Mueller⁶ was as follows: 11 patients with grade 1, 17 with grade 2, 2 with grade 3, and 5 with grade 4. For several patients, blood samples were either lost or not collected. Therefore, serum venom specific IgE determinations at both sessions were accomplished in only 27 patients. For 5 more patients, blood samples were available at a single session only. On 2 occasions, specific IgE results for wasp venom were not determined owing to technical error.

Reproducibility

Positive ST results for the first session were found in 20, 26, and 16 patients for honeybee, yellow jacket, and wasp venom, respectively. At the second session, positive ST reactions to these venoms were found in 22, 26, and 16 (not necessarily the same) patients, respectively. None of the patients had negative ST results in both sessions.

Results of the reproducibility of ST and serum venom specific IgE results for all 3 venoms and for each of the venoms separately are given in Table 1. The reproducibility of each venom separately was higher compared with the reproducibility of the 3 venoms together. Lack of reproducibility of ST results for all 3 venoms at both sessions was found in 34% of the patients. The reproducibility for yellow jacket venom was significantly higher compared with that of honeybee venom, but there was no significant statistical dif-

Table 1. Lack of Reproducibility of Skin Testing and Serum Venom Specific IgE Results at Both Sessions

	Lack of reproducibility, % (No.)
Skin testing (n = 35)	
All 3 venoms	34 (12)
Honeybee venom	23 (8)*
Yellow jacket venom	0*
Wasp venom	11 (4)
Skin testing for all 3 venoms (n = 12)	
Positive reactions at 1 $\mu\text{g/mL}$ at 1 session	58 (7)
Positive reactions at 0.1 or 0.01 $\mu\text{g/mL}$ at 1 session	42 (5)
Skin testing for all 3 venoms (n = 35)	
Patients with mild-to-moderate systemic reactions (Mueller grade 1–2) (n = 28)	32 (9)
Patients with severe systemic reactions (Mueller grade 3–4) (n = 7)	43 (3)
Serum venom specific IgE (n = 27)	
All 3 venoms	41 (11)
Honeybee venom	11 (3)
Yellow jacket venom	22 (6)
Wasp venom	15 (4)
Serum venom specific IgE for all 3 venoms (n = 11)	
Mildly positive (0.7–3.49 IU/mL)	55 (6)
Highly positive (≥ 3.5 IU/mL)	45 (5)

* $P = .008$.

ference compared with wasp venom (power = 99%). No difference in ST reproducibility for all 3 venoms was found between patients who had experienced the initial SR with various degrees of severity according to the grading of Mueller. Lack of ST reproducibility was not related to the venom concentration that had induced the positive ST results. The time elapsed between the SR and the first evaluation did not affect the reproducibility of ST and venom specific IgE results (data not shown). The size of the wheal in response to histamine injection did not vary between the sessions, and neither could we detect a trend for either ST or serum IgE results for all 3 venoms in the first session to become more positive or more negative, as a group, in the next session. Similarly, the results of both assays varied independently, with no correlation between the change in the results in one assay to the change in the other assay.

In 5 patients (14%), positive ST results in the first session became negative in the second session. Negative ST results in the first session became positive in the second session in 7 patients (20%). Such changes in serum venom specific IgE results were found on 7 and 6 occasions, respectively.

Concordance

When the results of ST and serum venom specific IgE measurement in the same session were compared, lack of concordance for all 3 venoms was found in 29 (49%) of 59 occasions. The results for each of the 3 venoms are depicted in Table 2. Altogether, on 175 occasions, the results of ST and serum IgE measurement of a single venom could be compared. As seen in Table 2, lack of concordance was associated with the degree of ST reactivity. Thus, when the ST result was positive at 1 µg/mL, lack of concordance was significantly more common compared with the lack of concordance found at positive ST concentrations of 0.1 and 0.01 µg/mL. The time elapsed between the SR and the first evaluation did not affect the concordance, and neither was it affected when the concordance of vespid venoms (yellow jacket and wasp) was compared with that of honeybee venom (data not shown).

Table 2. Lack of Concordance Between Skin Testing and Serum Venom Specific IgE Results at the Same Session

	Lack of concordance, % (No.)
Skin testing and venom specific IgE (n = 59)	
All 3 venoms	49 (29)
Honeybee venom	25 (15)
Yellow jacket venom	17 (10)
Wasp venom	22 (13)
Skin testing and venom specific IgE for a single venom (n = 39)	
Skin test reaction positive at 1 µg/mL at 1 session	69 (27)*
Skin test reaction positive at 0.1 or 0.01 µg/mL at 1 session	31 (12)*

* P = .002.

On 22 (56%) of 39 occasions with lack of concordance between ST and venom specific IgE results, there was a combination of positive ST results and negative venom specific IgE results, whereas on 17 occasions (44%), negative ST results together with positive venom specific IgE results were found. To evaluate whether the lack of reproducibility of ST results was associated with the lack of concordance between ST and serum IgE results, we compared these variables at all single sessions in patients who had nonreproducible ST results and in whom serum IgE measurement was available at this session. In 11 (52%) of 21 sessions there was no concordance between serum IgE and ST results. The presence of concordance between ST and venom specific IgE results in the first session resulted in a mild, insignificantly improved reproducibility of ST results.

DISCUSSION

Venom immunotherapy is the treatment of choice in venom-allergic patients. Two conditions must be met before the initiation of VIT: a positive history of a SR inflicted by an insect sting and the demonstration of venom specific IgE by either ST or measurement of blood levels.⁷ The decision to treat and what venoms to use are based solely on these results. Therefore, the reliability of these diagnostic measures is extremely important. Reproducibility is one of the major criteria in assessing the reliability of every biological test. Allergen extracts like venoms, prepared from natural raw materials, contain, besides the relevant allergenic proteins, many nonallergenic proteins, carbohydrates, histamine, and immunosuppressive agents.⁸ Their quality is affected by various factors, including the quality of the preliminary raw material, the extraction procedure, the protease content, and the storage conditions.⁹ This is why the American Academy of Allergy, Asthma, and Immunology¹⁰ and the European Academy of Allergology and Clinical Immunology¹¹ strongly recommend the use of standardized extracts for the diagnosis of allergic conditions. In addition, the reproducibility of prick ST results has generally been shown to vary significantly according to the technique used,¹² the different devices used,¹³ the size of the reaction,¹² and the number of interpreters.¹⁴ Most studies addressing this issue focused on prick ST, whereas the reproducibility of intradermal ST results was studied only in specific allergens.¹⁵ Although intradermal ST is not associated with many of the previously mentioned pitfalls of prick ST, and although current available venom extracts are standardized, the reproducibility of intradermal venom ST results has not been examined. Previous studies^{2,3} have already provided indirect evidence of the poor reproducibility of venom ST results. Thus, Golden et al² advocated that venom ST should be repeated whenever the results are negative, along with a serologic anti-venom IgE test, and they concluded that better diagnostic ST reagents were urgently needed. The limitation of ST reagents has prompted the development of supposedly better reagents, such as recombinant allergens.^{9,16}

Two previous studies have evaluated directly, to some extent, the reproducibility of the diagnostic measures in venom allergy. However, in the first study,⁴ only prick STs were compared, and the number of patients in the second study⁵ was small. To our knowledge, the present study is the first to examine this issue systematically in a meaningful number of patients tested intradermally and the first to examine the reproducibility of ST and serum venom specific IgE results and to compare these 2 methods. In addition, by having the ST performed and the results measured by the same nurse at both sessions, we overcame the variation of the interpreters, which constitutes one of the major pitfalls in the interpretation of ST results.^{12,14} The question not addressed by this study was whether the poor reliability observed herein could be a result of the biological variability of the patients from one test day to the other. Having the ST performed on a single patient by 2 different technicians and tested on opposite sides of the body might have been indicated whether the variability was inherent in the patients or in the methods. Adhering to the manufacturer's guidelines for the interpretation of ST results, as we did in our study, might occasionally be problematic, especially when the diluent control is extremely large or when the histamine control is extremely small. Although we were not encountered by this dilemma in our population, this factor should also be considered in equivocal situations. Performing ST in duplicate might either add another confounding factor to the interpretation of the tests or improve their reproducibility. Because the aim of this study was to examine whether the common diagnostic methods in venom allergy could be trusted by physicians, we adhered to the routine diagnostic workup usually performed by practicing allergists. Therefore, the effect of duplicate ST was not determined.

The ELISA system used by us was a second-generation serologic assay. An editorial by Reisman¹⁷ emphasized that measurements of serum venom specific IgE should be performed at the Johns Hopkins University reference laboratory, where the radioallergosorbent test technology had been optimized. However, another, more recent survey,¹⁸ which compared the performance of various serologic assays for venom specific IgE, including the one used by us and that of the Johns Hopkins technique, was not conclusive regarding the superiority of any particular assay. Thus, an inherent problem in our assay that could have been the source of presumably incorrect results is probably unlikely.

In the present study, the reproducibility of ST results regarding all 3 venoms could not be accomplished in one third of the patients. Better results were observed for the reproducibility of each of the venoms separately. However, the practical meaning of lack of reproducibility of all 3 venoms literally means the number of patients who would have been treated differently rather than the number of non-reproducible ST or serum IgE results, which is less relevant for the clinician. Therefore, we preferred to refer mainly to the results of all 3 venoms together rather than to each venom separately. The reproducibility of yellow jacket venom was

significantly higher than that of honeybee venom. We found this result difficult to explain. The frequency of venom allergy among our patients is in the following order: honeybee, yellow jacket, and wasp. Thus, it is unlikely that primary sensitization was to yellow jacket, and sensitization to honeybee was secondary and therefore more evanescent.

Lack of reproducibility of serum venom specific IgE results regarding all 3 venoms was found in 41% of the patients. Lack of concordance for all 3 venoms between ST and venom specific IgE results was even higher, appearing in half of the measurements. The ELISA kit and the venoms used for the ST were produced by 2 different manufacturers. Lack of concordance between these assays has been shown in the past,¹⁹ and we speculate that it was partially due to failure to use the same venom preparation for both assays. In our population, lack of concordance for a single venom was significantly associated with a higher venom concentration (1 mg/mL) inducing the positive ST result compared with lower venom concentrations. Because eliminating the 1- μ g/mL concentration from the ST might have resulted in an unacceptable reduction of ST sensitivity, it is more likely to assume that higher serum IgE sensitivity is required to improve the concordance of these 2 assays.

Historically, intradermal ST has been the preferred diagnostic method because it has been considered biologically relevant and the most sensitive test for venom specific IgE antibody.²⁰ Reisman²¹ pointed out that for most people who experience insect sting reactions, ST is an accurate (and reliable) evaluation of their clinical sensitivity. Indeed, current guidelines rely on ST as the initial basic diagnostic procedure,²² whereas serum venom specific IgE measurement is usually reserved as a complementary method and for equivocal cases. No guidelines have recommended routine repetition of either ST or serum IgE measurement.^{11,22} Contrary to the methods used in the present study, in daily clinical practice, different batches of venoms are used at different points for STs that are occasionally performed not by a single, well-trained nurse. This may decrease the reliability of ST even further. In addition, the decision regarding what venoms should be included in the immunotherapy regimen is probably made by most allergists on the basis of a single set of ST or serum venom specific IgE results. Because the concordance between ST and serum IgE results at a single session was not associated with better reproducibility of ST results at the 2 sessions, the clinical decision made by us was to include in the VIT protocol every venom that had induced positive ST results at any of the 2 sessions. Had we performed only the first ST, one fifth of the patients would not have been treated with all the venoms to which they were sensitized. Had we decided to disregard positive nonreproducible ST results, 14% to 20% of the patients would have been left untreated with at least 1 venom.

Both ST and serum venom specific IgE results have limited predictability value for a future sting. Thus, even in patients who have highly reproducible ST and serum IgE results, a sting may or may not cause a SR. Therefore, the extent of

overtreatment or undertreatment cannot be determined based solely on these assays. Their poor reproducibility, as observed herein, may even increase the degree of overtreatment and undertreatment. Overtreatment and the administration of venoms that the patient is not allergic to may be associated with the inconvenience of unnecessary injections, occasional pain and swelling, and excessive costs. Undertreatment and failure to use all the venoms that the patient is allergic to may result in ineffective VIT. Better selection of venoms for immunotherapy on the basis of the patient's IgE cross-reactivity has been proposed previously.²³ This approach demands expensive and time-consuming laboratory work. As previously reported, only 28% to 61% of patients with a history of previous SRs and positive intradermal venom ST reactions developed a SR after a sting^{1,24,25} or a field challenge.²⁶ Although another study²⁷ showed that in 21% of patients with negative first sting challenge results a second challenge resulted in an SR, there still remained many patients in whom VIT was of uncertain necessity. Failure to treat was similarly present in patients with SRs to insect sting who repeatedly developed SRs after deliberate sting challenges, despite having negative ST and serum venom specific IgE results.²⁵ All in all, the rates of overtreatment and undertreatment cannot be determined based on our results. The present study solely addresses the reproducibility and concordance of the currently available diagnostic methods in venom allergy rather than their predictive value for clinical reactivity. Practically, in the unique setting of the study, we chose to treat the patients according to all positive ST reactions at both sessions. This may be the preferable general approach for all patients to avoid undertreatment. Nevertheless, this approach may be associated with overtreatment and is inevitably more inconvenient and expensive. Performing live sting challenges using the suspected insects is an alternative. However, besides the obvious disadvantages associated with this approach, such as increased costs, increased incidence of adverse reactions, and the difficulty of obtaining patients' compliance, this method has also been shown to lack reproducibility.²⁷

We are aware of the limited number of patients in this study. Nevertheless, the results demonstrate the relatively poor reproducibility of ST and serum venom specific IgE determinations. Previously collected data describing serial venom specific IgE assays did not show marked variability in most patients in the absence of venom exposure across several weeks to several months.^{19,28} However, the number of patients described in both works was limited to 9, in many of whom IgE determination was initially performed in the first month after the SR, with the following determination performed only several months later.²⁸ In addition, venom specific IgE assays in those works were not performed in duplicate using a commercial kit and international IgE units, as we did herein, but rather using a locally developed assay and a positive standard arbitrarily assigned a value of 100 U. Thus, considering the small number of patients, the different timetable for obtaining the serum samples, and the different,

nonstandardized assay of IgE determination, it does not seem appropriate to compare the results of these works with those of the present study.

Several steps in the ST technique, and possible variability in the human allergic response across time, might theoretically be responsible for the variability in ST reproducibility. Inconsistency of the allergenic content of the commercial venom preparation is unlikely when discussing standardized venom extract, especially when the same lot number was used for all ST and fresh extracts were prepared and kept in optimal conditions at all times. Neither can the single trained nurse who performed the ST be held responsible for this variability. Considering that ST was performed well after the sting reaction and in the absence of additional venom exposure between measurements, significant changes in specific IgE concentrations would be unlikely. Variability in histamine responsiveness of the skin is also unlikely considering the lack of such variability at the 2 sessions. Variability in the releasability of skin mast cells across time might be a possible explanation, which could have been evaluated by measuring the skin response to codeine, but this was not performed in the present study. We could not find in the literature any data addressing the reproducibility of other standardized allergens, such as cat or dust mites, which might explain our results. Thus, further work is needed to shed light on this subject. Still, it is important to stress that the clinical significance of our results has not been established. Enrolling a larger number of participants may better clarify whether other cutoff points of ST or venom specific IgE results may be more reliable in making the right clinical decisions. This approach was evaluated by changing the cutoff points in risk evaluation of sting-threatened individuals and may lead to unacceptably reduced sensitivity.²⁹ In summary, at this time, allergists should be aware of the inaccuracy occasionally associated with their decision-making process when it is based on a single determination of either ST or serum venom specific IgE.

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