

Variability of venom skin tests

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Purpose of review

Venom skin tests constitute the cornerstone in establishing the diagnosis of venom allergy. In spite of their fundamental role, data regarding their reproducibility and variability are rather sparse. This paper is an overview of our current knowledge on the extent of variability in venom skin testing, the possible causes for this phenomenon and its clinical implications. It points out certain clinical situations in which this possible variability should be taken into account and anticipates potential venues of expanding our understanding of this debatable subject.

Recent findings

A single recent study addressed the reproducibility of skin tests and serum venom-specific immunoglobulin E levels. Using a simple positive–negative or vice versa criterion for all three venoms examined on two different sessions, this study showed an overall 66% reproducibility of the skin test reactions and 59% reproducibility of the venom-specific immunoglobulin E assay results. According to an accompanying editorial, however, the validity of these results needs to be confirmed.

Summary

Determination of the real magnitude of venom skin test variability is required. At present, in specific clinical situations, repeated skin tests and measurement of serum venom-specific immunoglobulin E should be considered before the initiation of venom immunotherapy.

Keywords

reproducibility, skin tests, variability, venom allergy

Introduction

Allergy skin testing is the primary modality used in the diagnosis of venom allergy and guides specific treatment plans. Recent data have raised doubts about the reproducibility of these tests, resulting in concerns regarding the reliability of this fundamental diagnostic method.

The role of skin testing in venom allergy

Similar to other allergic situations, the diagnosis of venom allergy has been traditionally based on skin test results. In-vitro determination of venom-specific immunoglobulin E (IgE) is also used occasionally for the same purpose. Considering the time-consuming nature and the excessive cost associated with this procedure, however, its role has become more of a complementary one. Several other in-vitro methods that assess cellular activation in response to stimulation with the suspected venom have been used principally for research purposes. These include basophil or whole blood histamine release, peripheral blood mononuclear cell proliferation or cytokine [interleukin (IL)-4] production, basophil activation markers such as CD63, CD203c and the sulfideleukotriene-based leukocyte stimulation test [1]. A review of the various in-vitro methods for the diagnosis of Hymenoptera venom allergy was published previously [2] and the current issue provides an update on basophil activation tests in insect allergy [3]. Sting challenges have also been attempted in this regard and have been reviewed elsewhere [4]. Although the sting challenges are still the best available test for ascertaining clinical reactivity to insect venoms, at the present time they remain an adjunct diagnostic procedure for carefully selected patients with certain limitations that need to be taken into account upon interpreting their outcome. In practice, most allergists prefer the handy venom skin test as the sole diagnostic procedure in the vast majority of patients, leaving in-vitro determination of venom-specific IgE only to patients with negative skin test responses who would be candidates for immunotherapy. This approach was also supported by the recent practice parameter update on stinging insect hypersensitivity [5]. Thus, considering the fundamental role of skin tests in establishing the diagnosis of venom allergy and accordingly providing or renouncing treatment for a potentially life-threatening situation, their value cannot be overemphasized.

Skin-testing variability in general

In a survey published recently [6^{*}], significant diversity in skin testing practices was found among allergists. In skin testing for aeroallergens, a significant degree of variability

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Abbreviations

CCD cross-reactive carbohydrate determinant
Ig immunoglobulin

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was reported regarding the number of skin tests performed, extract concentrations, skin test devices, interpretation and documentation of results, and quality assurance procedures. Another study [7] demonstrated that even when the same photographed skin test results were reviewed by different allergists, interphysician variation in the scoring and interpretation of epicutaneous skin tests was apparent. Statistically significant differences were found in a head-to-head comparison of the performance of eight test devices for skin prick testing [8] and the body site where the tests were performed was an additional causative factor for the variability in their results. Patient's age [9], circadian rhythm [10] and total serum IgE [11] have also been shown to affect skin test results.

Potential causes for venom skin-testing variability

Most of the above-mentioned studies on skin test variability focused on prick skin tests. Data on intradermal skin test variability are sparse and refer to specific groups of patients [12] or to specific allergens [13]. Although skin prick testing is the initial step in the diagnostic work-up of venom allergy, they are not used by many allergists [5]. An accelerated method for performing venom skin testing has been described and has demonstrated that only a very small minority of patients with positive skin tests also had a positive skin prick test at 1 $\mu\text{g}/\text{ml}$ (one of 331, 0.3%) [14]. Even earlier, Reisman [15] suggested an almost exclusive usage of intradermal tests. As previously mentioned, however, data on the variability of intradermal skin testing are generally lacking with regard to venom intradermal tests.

Although Hymenoptera venom preparations are standardized, they are made from raw materials that contain, besides the relevant allergenic proteins, many nonallergenic proteins, carbohydrates, histamine, and immunosuppressive agents [16]. Thus, their quality may be affected by the quality of the preliminary raw materials, the extraction procedure, the protease content, and the storage conditions [17]. For this reason, the prescribing information issued by the pharmaceutical companies regarding venom immunotherapy advocates a 50% reduction of the administered dose upon changing the venom batch or upon introducing a new bottle. The practicing allergist has virtually no influence on most of the above-mentioned parameters other than freshness and storage conditions.

Another possible factor that may affect the interpretation of skin test results, which is unique for intradermal testing, is the volume of the preparation injected during the procedure. Although most studies advocate the injection of 0.02 ml, this is occasionally difficult to accomplish accurately even for an experienced technician. In

practice, the amount injected is usually enough to produce a visible, 2–3 mm sized bleb. There are no available data regarding the effect of slightly different injected volumes on intradermal skin tests results.

Cross reactivity among Hymenoptera venoms was recognized a long time ago and was recently reviewed by Hamilton [2] and Bilo *et al.* [18]. Cross-reactive carbohydrate determinants (CCDs) are glycol epitopes that share significant structural homologies beyond the limits of protein families. They have been shown to be an important cause of dual sensitivity between honeybee and yellow jacket venoms [19]. More interestingly, sensitization to CCDs caused by pollen (timothy grass and mugwort) as well as by Hymenoptera venom allergens may elicit false-positive IgE antibodies against latex and apple [20], whereas venom allergens induced specific IgE to grass, tree and weed pollen in another study [21]. All published studies on the role of CCDs based their conclusions, partially or entirely, on in-vitro determinations of specific IgE. Moreover, no data have been published about the opposite possibility, that is the induction of false-positive venom-specific IgE by pollen allergens through CCDs. Thus, the much more common sensitization to pollen allergens may have accounted for false-positive venom sensitization. Although it deserves further exploration, this is another venue in which variability in venom skin testing can arise.

The extent of variability in venom skin testing

Despite its ample role in the diagnosis of venom allergy, a relatively small body of evidence has been established regarding the variability and reproducibility of venom skin tests. Venom skin prick testing has proved to be nonreproducible in most patients studied at 3-month intervals (six of eight bee and seven of eight wasp venom allergic patients, respectively) [22]. Since this study demonstrated a consistent tendency for bee venom skin tests to become negative and for wasp venom to become positive upon repetition of skin testing, the authors suggested that these results may be interpreted differently. In contrast, Tsicopoulos *et al.* [23] performed intradermal skin tests at 6-week intervals in 10 venom-allergic patients and found that variation in skin test sensitivity was not significant. It should be noted that nine of their patients were allergic to *Vespula germanica*, which is a yellow jacket species. These results raise the question of whether this finding can be accepted as a general one or should be regarded as species specific.

Recently, Graif *et al.* [24**] examined the variability of intradermal venom skin tests in 35 patients. In an attempt to avoid some of the previously mentioned potential causes for skin test variability, they performed their skin tests twice, 2–6 weeks apart, in the absence of interval venom exposure and long after the insect sting. Skin tests

were performed and read by the same trained nurse who used freshly prepared venom dilutions from the same batch of venom. Matching positive or negative results, though not necessarily identical, on both sessions, for all three venoms that were examined (bee, yellow jacket and wasp), were considered reproducible skin test results. In only 66% of patients were skin tests reproducible for all three venoms on both sessions. Skin test reproducibility for each of the individual venoms was 77%, 89% and 100% for bee, wasp and yellow jacket venoms, respectively. Results for reproducibility of serum venom-specific IgE were even poorer, reaching 59% for all three venoms on both sessions. Reisman [25[•]], who was invited to deliver an editorial on this study, suggested that a much better examination of the technical quality of the diagnostic tests performed in this study was needed before its conclusions could be accepted. He concluded that for most suspected insect-allergic people, venom skin testing is a reliable diagnostic test. He based his argument on earlier works that serially determined serum venom specific IgE utilizing an empirical quantization method and showed little variability across varying periods [26,27]. Although the number of patients described in these studies was small, they were only illustrative examples of a large number of people evaluated over a long period of time [25[•]].

Reaching a comprehensive conclusion regarding venom skin test variability based on the above-mentioned, frequently contradicting studies is practically impossible. This is because some of the studies examined only serum venom-specific IgE and used noncommercial methods, different from those used nowadays [26,27]. A different study examined only venom skin prick tests [22] whereas another study concentrated on a small group of patients, most of which were only allergic to yellow jacket venom [23]. The most updated study [24^{••}] found a much lower degree of reproducibility than previously expected when the skin test results of all three venoms were compared for the two sessions. Indeed, the reproducibility of each of the individual venoms was greater compared with the reproducibility of all three venoms together, and for yellow jacket venom it even reached 100%, similar to a previous report [23]. The reasons for the difference between the venoms remain to be elucidated. Nevertheless, although this study was conducted in an attempt to overcome many of the already known factors contributing to skin test variability, it was composed of a relatively small number of patients, skin tests were not performed in duplicates and more satisfactory controls for various technical aspects of the tests were missing.

Clinical implications of venom skin test variability

The severity of venom-induced anaphylaxis is unrelated to venom-specific IgE titers or venom skin test reactivity

[28]. In 10% of the cases with sting-induced fatal anaphylaxis, serum venom-specific IgE could not be detected [29]. Nevertheless, skin test results still determine the composition of venoms administered to the patient throughout venom immunotherapy. Neglecting to administer all the required venoms may put the patient in danger of recurrent anaphylaxis. Administration of venoms to which the patient is not allergic may be associated with the inconvenience of unnecessary injections as well as excessive costs. Indeed, in Graif *et al.*'s work [24^{••}], the number of venoms administered for immunotherapy increased by 20% when all the venoms that the patients were found to be sensitized to were used for this treatment upon repetition of the skin tests. Single-venom therapy in patients with multiple venom sensitivities was advocated based on the known cross reactivity of allergens in different venoms [30]. This approach, however, still cannot be applied in all patients. Increased skin test reproducibility and decreased variability will undoubtedly assist in the appropriate selection of venoms for immunotherapy.

Do we need to worry about skin test variability? What is next?

Based on the prevailing data, it seems that there is no definite answer for this question. Moreover, it is probable that in everyday situations, unlike the academic setting used in a research protocol, the variability may be even bigger due to the performance and interpretation of the skin tests by various personnel who use different batches of venoms.

Although skin test results indicate sensitization to the venom rather than prediction for future stings, they still remain the cornerstone of evaluation of venom allergy, despite doubts about their variability. In the unique situation of negative skin test results in patients with histories of severe life-threatening systemic reaction to a sting, it has already been recommended that skin tests and serum-specific IgE be repeated [31]. Skin test variability may have partially accounted for the different skin tests results observed in these cases [32].

Should repetition of skin tests be recommended routinely for every patient with venom allergy? At the present time the answer is definitely not. When there is an obvious discrepancy between the history of the systemic reaction and the skin test results, however, they should be repeated and determination of serum venom-specific IgE should be considered as well. An example of such a unique case is the presence of a negative skin test for bee venom and positive skin tests for vespid venoms in a patient who developed a systemic reaction after being stung while collecting honey from a beehive. Another situation in which this approach may be considered is associated with the likelihood of the occurrence of

another sting reaction to be primarily related to the severity of the initial sting reaction [28]. Considering the possible variability of skin test results, repeated skin tests and obtaining serum IgE may be worthwhile when evaluating a patient with a severe, life-threatening sting reaction. This is in order to include all the venoms the patient may be allergic to upon repeating skin tests in the immunotherapy regimen.

Recombinant venom allergens will certainly improve the diagnosis of venom allergy in the near future [18]. Comprehensive, well controlled studies that may better define the various confounders of skin test variability will hopefully shed light on this issue.

Conclusion

Due to the uncertainty about venom skin test variability, a large, better controlled study that will address all the disputed issues is required. Until then, venom skin tests continue to be the best available diagnostic method for venom allergy. Occasionally, in specific clinical situations, repeating skin tests and determining serum venom-specific IgE should be considered before the initiation of venom immunotherapy.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 361).

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