

Diagnostic venom skin tests and venom specific IgE assays: do we need to worry?

The article by Graif et al¹ in this issue of the *Annals* concludes that the reproducibility of venom skin test and serum venom specific IgE assay results is poor, contrary to generally accepted views. This observation could have serious clinical implications regarding the diagnosis of venom allergy and the subsequent recommendations for immunotherapy. Graif et al¹ offer no explanation for this discrepancy and conclude that better diagnostic methods are needed. In my opinion, review of their methods, other related data, and general “experience” suggest that much better scrutiny of the technical quality of their diagnostic tests is needed before these conclusions can be accepted as realistic or worrisome.

Many aspects of the study design are appropriate. Venom skin tests were performed using the same batch of venom. All paired serum samples had venom specific IgE assays performed at the same time. The skin tests and serologic assays were performed twice, 2 to 6 weeks apart, in the absence of interval venom exposure and long after the insect sting. The overall results showed 66% reproducibility of the skin test reactions and 59% reproducibility of the venom specific IgE assay results (using a simple positive-negative or vice versa criterion). Of particular concern are 5 of 35 patients who had a positive venom skin test result at a concentration of 0.01 or 0.1 $\mu\text{g/mL}$ and a negative result on the other occasion, a 100- to 1,000-fold change in skin test reactivity. Five of 27 people had a highly positive serum venom specific IgE titer on 1 occasion and a negative titer on the other testing occasion. These are extraordinary changes during a 2- to 6-week period in the absence of venom exposure.

As Graif et al¹ point out, there is no obvious biological explanation for the discrepant results to explain a change in skin test reactivity or serum venom specific IgE titers during a 2- to 6-week period in the absence of venom exposure. Are these results then really credible?

Studies^{2,3} performed at the Allergy Research Laboratory of Buffalo General Hospital, Buffalo, NY, and referenced by Graif et al, early after purified venoms were available were designed to explore the immunologic basis of venom allergy. Measurements of serum venom specific IgE were performed for long periods after insect stings and immunotherapy. The extreme variability in the antibody titers described in this study was not found. Graif et al¹ dismissed these results as irrelevant because of the small number of patients described and an empirical quantitation method used to measure the antibody titers. In fact, the described patients were only

illustrative examples of many people evaluated for a long time. Venom specific IgE determinations were repeated frequently as serial serum samples were obtained, after either insect stings or immunotherapy. Other than the expected changes related to venom exposure, the results were reproducible and showed little variability across varying periods. The use of an empirical standard to quantitate the results proved to be reproducible. At that time, the in vitro assays were not precisely quantitated. Thus, I believe that these older data are certainly appropriate for comparison with the data of Graif et al¹ and do in fact show that in vitro assays are reproducible without the extreme changes reported in this study.

Graif et al¹ make reference to my comment regarding the measurement of serum venom specific IgE only at the Johns Hopkins University reference laboratory.⁴ This comment was only regarding evaluation of the rare patient who has had serious sting anaphylaxis and has a negative venom skin test result. In this setting, a more sensitive in vitro assay may be necessary, and it would seem that the Johns Hopkins assay, developed in a research laboratory, would fill that criterion. I did not imply that in vitro assays performed in other laboratories were not reproducible. I also have not found the extreme variability in intradermal venom skin test responses in large numbers of people who were tested on a regular basis, particularly while receiving venom immunotherapy, even using varying batches of venom.

The critical issue regarding the failure of Graif et al¹ to find reproducible results is the quality of the test assays, both the in vitro tests and the skin tests. The in vitro assays were performed using a commercial kit. We are not told where the tests were performed or by whom. It would have been easy for the authors to verify the in vitro results by repeating the assays on another day in a blinded manner. If the study patients' serum samples were not available, then other samples could have been provided to ensure the accuracy and reproducibility of the in vitro assay results.

The skin tests were performed and apparently read by 1 trained technician. Although this has potential advantages, the discrepancy in the results “cries out” for control testing evaluation by another trained person. It is not even clear whether the physician authors read the skin tests. Thus, until the technical aspects of the tests are properly controlled, the results cannot be accepted as valid.

The differences in the results of the in vitro assays and the skin tests, noted by Graif et al¹ and in other earlier articles, is in part due to different venom sources used for the 2 tests. Whenever 2 tests are compared, the same allergen source is necessary. In the case of venom, it is possible that the amount of individual venom fractions, such as antigen 5, may differ. In addition, the issue of the accuracy of the skin test and the IgE assay discussed previously herein may be pertinent to this comparison.

Several other aspects of the authors' discussion deserve comment. Shortly after the availability of purified venoms and the clinical application of diagnostic tests, the dichotomy between clinical sensitivity and immunologic reactivity became known. The severity of venom-induced anaphylaxis is unrelated to venom specific IgE titers or venom skin test reactivity. The likelihood of the occurrence of another sting reaction, in the absence of venom immunotherapy, also is not related to the skin test reaction or IgE titers but primarily to the severity of the initial sting reaction.⁵ The cross-reactivity of allergens in different venoms, particularly vespid venoms, has been well studied. The clinical application of this knowledge leads to successful single-venom therapy⁶ even in the presence of multiple positive venom skin test results. Individual in vitro inhibition tests are not needed to make this decision.

To summarize, this study seriously questions the accuracy of diagnostic venom skin tests and in vitro assays of serum venom specific IgE. I have major concerns regarding the lack of controls to ensure the quality of the technical assays. It is more regrettable that these concerns were raised early in the long review process of the manuscript and were not directly resolved by the authors. Certainly either the patient base for this study or other insect-allergic people could have been

evaluated to ensure the reproducibility of the skin test and in vitro assay results. The editorial board of the *Annals* accepted the manuscript for publication. I would have demanded the obvious test controls to ensure the validity of the results. At present, my opinion has not changed. For most suspected insect-allergic people, venom skin testing especially and, if needed, the venom specific IgE assay are reliable diagnostic tests.

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