

Fatal Reactions to Hymenoptera Stings

Donald R. Hoffman, Ph.D.

ABSTRACT

Previous studies measuring immunoglobulin E (IgE) antibodies in postmortem sera for determining the cause of fatal anaphylaxis have reported only single cases or small groups. Recently, more attention has been paid to reports of patients with venom allergies who are negative by venom skin testing, by in vitro testing or by both tests. The aim of this study was to determine the reliability of postmortem-specific IgE antibody testing in venom anaphylactic death and the range of antibody levels found. Radioallergosorbent testing was performed on sera from three groups: 51 anaphylactic deaths from insect stings, 20 anaphylactic/anaphylactoid deaths from food and drugs, and 31 control subjects. Results were analyzed by descriptive statistics, chi-squared test, and receiver operating characteristic curve. The sera in the sting death group were significantly different from the other two groups, which were not statistically different from each other. The maximal sensitivity of the radioallergosorbent test was 90% at 0.35 ng/mL, and the optimal sensitivity was 73% with a specificity of 86% at 0.54 ng/mL. IgE antibody could not be detected in 10% of the sting death sera and levels from 0.35 to 0.65 ng/mL were found in 24%. The level of specific IgE antibodies against venoms is not predictive of the severity of anaphylactic reaction. (Allergy and Asthma Proc 24:123–127, 2003)

From the Department of Pathology and Laboratory Medicine, Brody School of Medicine at East Carolina University, Greenville, North Carolina

Presented in part at the 58th annual meeting of the American Academy of Allergy, Asthma, and Immunology, New York, March 4, 2002

Address correspondence and reprint requests to Donald R. Hoffman, Ph.D., Department of Pathology and Laboratory Medicine, Brody School of Medicine at East Carolina University, Greenville, NC 27858

The purpose of venom vaccine therapy is to prevent fatal and other anaphylactic reactions from insect stings. In 1983 our laboratory published a study showing that specific immunoglobulin E (IgE) antibody against venoms could be measured in postmortem sera from nine victims of anaphylactic death from stings.¹ Since that report a number of small studies have confirmed these findings in venom-induced anaphylaxis and anaphylaxis from foods and drugs.^{2–7} Recent reevaluation of both *in vivo* skin testing and *in vitro* methods for confirming the diagnosis of hymenoptera venom allergy strongly suggest that these methods are less sensitive than previously thought.^{8,9} Recently, a number of investigators have presented evidence that some severe anaphylactic-type reactions to insect stings may not be IgE mediated in patients that have an underlying diagnosis of occult mastocytosis.^{10–12} These patients have an elevated baseline serum tryptase and lightly pigmented skin lesions, and some develop other signs and symptoms of systemic and cutaneous mastocytosis.^{11,12}

These newer findings prompted us to examine our population of sera from victims of anaphylactic death. The sera from insect sting deaths are compared with a group of food and drug allergic death sera as well as with a control population.

METHODS

Venom Allergic Sera

Sera from patients known or suspected to have died after reactions to insect stings were sent by medical examiners primarily from the eastern half of the United States. Specimen clinical and identifying information was handled in compliance with the medical examiner statutes. All serum specimens were separated and stored frozen at -20°C in a manual defrost freezer. Because of the limitations of postmortem collection, many of the specimens were in poor condition and some were collected in sodium fluoride preservative and were hemolyzed completely. Specimens were collected from 1979 to 2000 and were tested within 5 days of receipt.

Food and Drug Allergic Sera

Sera from subjects who experienced fatal anaphylactic reactions to foods or fatal anaphylactic or anaphylactoid reactions to drugs were provided by medical examiners. Venom testing was performed as part of specificity controls, because many of these cases involved litigation. All venom testing was performed in a single batch. These sera were received in similar conditions to those from venom death cases.

Control Sera

Control sera were obtained from surplus specimens used for functional complement testing. This population typically is ~85% patients with rheumatic disease and is found in eastern North Carolina. In compliance with the Institutional Review Board specimen conditions were identified only as to age and sex. The population was 26% men with an age range of 20–70 years. All venom testing of these sera was performed in a single batch.

Radioallergosorbent Test

The radioallergosorbent test (RAST) was performed using the standard method in our laboratory¹³ with vespid and imported fire ant venoms purchased from Vespa Laboratories, Inc. (Spring Mills, PA), and bee venom was purchased from Champlain Valley Apiaries (Middlebury, VT). The same preparation of anti-IgE was used to prepare radiolabeled detection reagent over the entire period. Although different lots of venom and of cyanogen bromide-activated disks were used, quality assurance parameters were consistent throughout the period of the investigation. No changes in methodology were made in the venom testing procedure for the study specimens. Nonspecific binding was defined as the mean binding of the control sera. Cutoff levels were calculated from receiver operating characteristic curve analysis.¹⁴

Statistical Analysis

Statistical analysis including descriptive statistics, chi-squared analysis using Yates' correction, Fisher's exact test, and receiver operating curve analysis¹⁴ were performed using the Vassar Stats web site.¹⁵

RESULTS

During the period of the study 56 specimens were submitted with a history of suspected death from anaphylaxis caused by insect stings. Five cases were excluded from the study; four cases had no specific sting history in the time preceding death and no findings consistent with anaphylactic death; the fifth case was a 4-year old boy who suffered more than 400 yellow jacket stings causing death because of cerebral edema that was probably a toxic reaction. None of the sera from these five cases contained detectable IgE antibodies to venoms. The demographics of the remaining 51 cases are shown in Table I. The first nine

TABLE I

Deaths from Sting Anaphylaxis	
Male subjects*	45
Female subjects	5
Ages# (years)	10–76
Mean (years)	52
Insect responsible	
Honeybee	9
Vespid	37
Imported fire ant	5

*Data not provided for one case.

#Data not provided for 11 cases, all adults.

TABLE II

Deaths from Food and Drug Reactions	
Male subjects	6
Female subjects	14
Ages (years)	0.5–65
Mean (years)	30
Causes	
Foods	(9)
Shrimp	(2)
Peanut	(2)
Egg	
Oyster	
Milk	
Walnut	
Brazil nut	
Drugs	(8)
Penicillin	(2)
Ampicillin	
Hydrocodone	
Clindamycin	
Vaccine	(2)
Methylprednisolone sodium succinate	
Anaphylactoid	(3)
Lisinopril	(2)
Thiamine	

of these cases have been described previously in detail.¹ The comparison anaphylactic death group included nine cases of anaphylaxis from foods, eight cases of anaphylaxis from drugs, and three cases of anaphylactoid reactions from drugs, as is shown in Table II. One of these cases has been described previously.⁶

The 31 control sera and 20 food and drug sera were tested for IgE antibodies against the six venoms. Normal ranges and statistics were calculated from the values for the 31 control sera. The RAST was calibrated with disks coupled with anti-IgE and sera with known amounts of total IgE and the values were converted to nanograms per milliliter using the graph shown in Fig. 1. Two results for imported fire ant venom in the control population were not used in the calculations because they were >10 SD above the mean of the

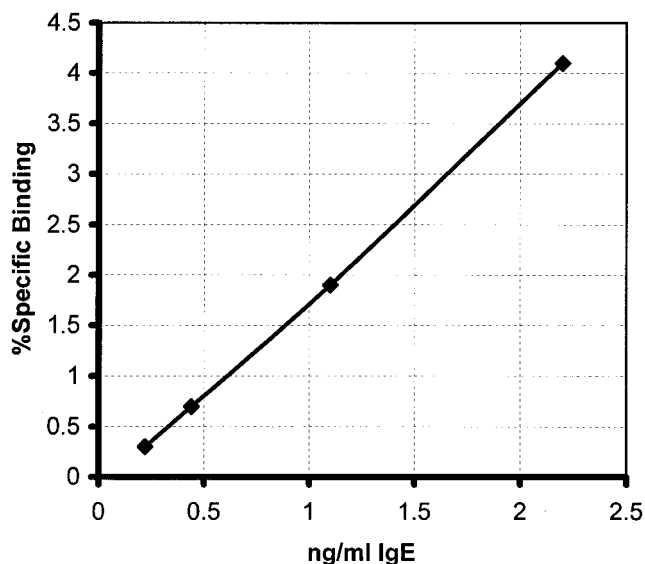


Figure 1. Calibration curve for the RAST using cyanogen bromide-activated disks coupled to anti-IgE antibodies and sera with known amounts of total IgE.

TABLE III

RAST Results for Control Populations

Venom	Controls			Food and Drug
	Mean* (% binding)	+2 SD (ng/mL)	+3 SD (ng/mL)	Mean (ng/mL)
Bee	0.86	0.3	0.44	0.12
Yellow jacket	0.88	0.24	0.35	0.08
Yellow hornet	0.87	0.2	0.3	0.12
White faced hornet	0.85	0.24	0.35	0.30
Paper wasp	1.20	0.24	0.35	0
Imported fire ant	0.70	0.3	0.44	0.16

*Defined as 0 ng/mL.

other 29 values. The finding of a high level of IgE against fire ant venom is expected in a significant fraction of sera obtained from subjects resident in an endemic area.¹⁶ The control results are shown in Table III. In the control group, sera from four patients showed binding >3 SD above the mean to one or more venoms. In the food and drug anaphylaxis group sera from six patients showed elevated binding to one or more venoms. Of the 51 sera from venom anaphylaxis deaths, 42 gave binding at least 3 SD above the mean for the appropriate venom. The range of IgE antibodies against venoms for this group is shown in Table IV. The control group and food and drug anaphylaxis group were not statistically different from each other ($p = 0.13$), but

TABLE IV

IgE Antibody Levels Against Venoms for 51 Sera Obtained from Victims of Fatal Venom Anaphylaxis

5 Sera (10%)	negative (<0.22 ng/mL)	
4 Sera (8%)	0.35–0.49 ng/mL	(+2–2.9 SD)
8 Sera (16%)	0.5–0.65 ng/mL	(+3–4 SD)
12 Sera (24%)	0.7–1.1 ng/mL	
22 Sera (43%)	>1.1 ng/mL	

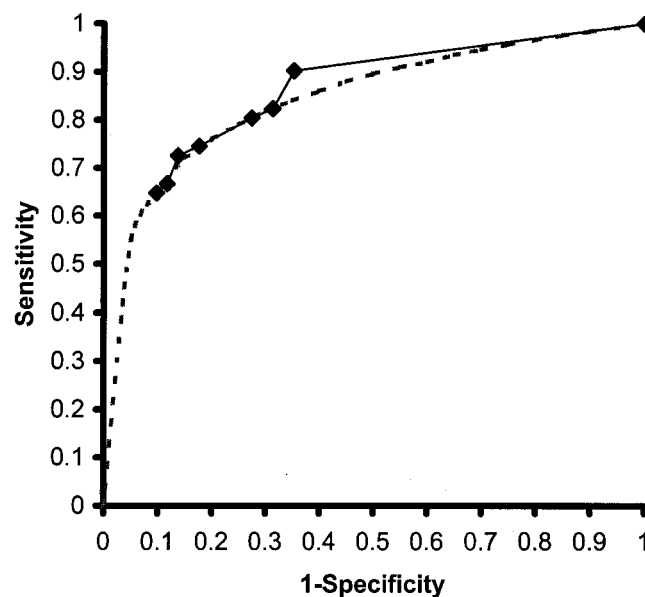


Figure 2. Receiver operating characteristic curve calculated from testing sera from subjects with fatal reactions from hymenoptera stings and the combined groups of controls and fatalities from food and drug reactions. The points are the experimental data and the dashed line is the calculated curve. The area under the curve is 0.85; the optimal point is at 0.47 ng/mL with 77.5% sensitivity and specificity. The maximum sum of sensitivity and specificity is at 0.54 ng/mL with 73% sensitivity and 86% specificity.

both were different with $p < 0.001$ from the venom anaphylaxis group.

Specific IgE antibodies to venom could not be detected in five sera from victims of fatal anaphylactic reactions to stings. Each of these cases had a convincing clinical history with corroboration by at least one witness. In four of these cases, the insects were clearly identified as yellow jackets, hornets, or paper wasps. Low levels of specific IgE antibodies (below 0.65 ng/mL) were found in 12 sera. Specific IgE antibody levels above 1.1 ng/mL were found in only 43% of the cases.

Receiver operating characteristic¹⁴ analysis was performed comparing the sting anaphylaxis group to the control and food and drug anaphylaxis groups combined. The plotted values and calculated curve are shown in Fig. 2. The area under the curve was 0.85. Specificity and sensitivity were equal at 77.5% with 0.47 ng/mL of specific IgE

antibody. The maximum sum of sensitivity and specificity was found at 0.54 ng/mL with 73% sensitivity and 86% specificity. The maximum sensitivity of 90% was found at a cutoff of 0.35 ng/mL with a specificity of 65%.

DISCUSSION

This study including sera from 51 victims of fatal venom-induced anaphylaxis suggests that previous smaller studies may not be representative. It was not possible to detect specific IgE antibodies in 10% of the sera and an additional 24% of the sera contained very low levels of specific IgE antibodies. High levels of specific antibodies were found in only 43% of the sera. It is most reliable to perform diagnostic studies after the anamnestic response that follows a sting reaction,^{8,9} although it is not uncommon for testing to be performed at an extended interval of years after a sting reaction. One limiting factor in this study may have been the condition of the postmortem specimens, because many were hemolyzed completely when received. However, very high levels of specific IgE antibodies were measured in the sera from all nine of the victims of fatal food anaphylaxis, some of which were received in a similar condition. Sampson has reported¹⁷ that symptomatic food allergy usually requires high levels of specific IgE antibody. Limited studies in our laboratory have found degradation of specific IgE levels on extended storage and freeze-thaw cycles. Degradation is most significant in sera with low levels of IgE.

Although skin testing was conventionally thought to be more sensitive than *in vitro* testing for hymenoptera venoms, significant evidence has been presented showing that patients with convincing histories of recent severe anaphylactic reactions to stings may be positive only to skin tests, only to *in vitro* tests, or negative to both.^{8,9,18,19} An incidence of 10% of patients negative by RAST at the maximal sensitivity is consistent with these observations. It is not known how many of the victims of fatal anaphylaxis might have shown positive skin tests to venom, because only 10 victims were reported to have experienced previous reactions to stings by history and only 1 victim had been seen by an allergist.

Complete medical histories are not available for most cases. Many of the victims of fatal sting anaphylaxis did have preexisting cardiovascular or pulmonary compromise by history or autopsy. One victim was taking a β -adrenergic blocker; none of the histories mentioned use of acetylcholinesterase inhibitors. The number of stings was usually one precipitating the fatal reaction. Histories from 10 cases indicated more than four stings. The fire ant cases had from one to multiple stings.

It is possible for the venom from the stings to inhibit partially the specific IgE binding. Studies of patients with toxic reactions from large numbers of stings from Africanized honeybees reported by Franca *et al.*²⁰ have shown that

a large fraction of the venom is present in the circulation for at least 2 days after the stings. Typically, 0.1–1 μ g of venom protein is required for 50% inhibition of a paper disk RAST.²¹ In an adult man with a plasma volume of $\sim$ 2500 mL, this would require 50–500 honeybee stings or 125–1250 yellow jacket stings.

A number of positive results of specific IgE antibodies to venoms were observed in both the food and the drug anaphylaxis group and the control group. This was expected, given the prevalence of nonsymptomatic sensitization in the population,²² especially in imported fire ant endemic areas¹⁶ where regular stings are common. The very high levels of IgE antibody against imported fire ant venom found in two of the control patients, who live in the endemic area, were not used in the calculation of normal ranges. A second cause for the presence of IgE antibodies reacting with venoms is the presence of IgE antibody against cross-reactive carbohydrate determinants.²³ Because only low levels of reactivity were found in the six venom-positive sera from food and drug anaphylactic deaths, testing for IgE against cross-reactive carbohydrate was not performed.

The results of this study show that the level of specific IgE antibody against venom is not predictive of the severity of the reaction. Only 14% of the sera contained high levels (>2.5 ng/mL) of venom-specific IgE antibody, and 57% of the sera contained <1.1 ng/mL. Fatal anaphylactic reactions to stings can occur in the presence of any amount of specific IgE antibody.

ACKNOWLEDGMENTS

This study would not have been possible without the cooperation of the many medical examiners who provided the specimens and accompanying data. Excellent technical assistance was provided by Marvin B. Alligood, Jr. The author especially thanks Dr. R. Page Hudson and Dr. Mary G.F. Gilliland for their helpful discussions.

REFERENCES

1. Hoffman DR, Wood CL, and Hudson P. Demonstration of IgE and IgG antibodies against venoms in the blood of victims of fatal sting anaphylaxis. *J Allergy Clin Immunol* 71:193–196, 1983.
2. Schwartz HJ, Yunginger JW, Teigland JD, et al. Sudden death due to stinging insect hypersensitivity: postmortem demonstration of IgE antivenom antibodies in a fatal case. *Am J Clin Pathol* 81:794–795, 1984.
3. Schwartz HJ, Squillace DL, Sher TH, et al. Studies in stinging insect hypersensitivity: postmortem demonstration of antivenom IgE antibody in possible sting-related sudden death. *Am J Clin Pathol* 85: 607–610, 1986.
4. Schwartz HJ, Sutheimer C, Gauerke MB, and Yunginger JW. Hymenoptera venom-specific IgE antibodies in post-mortem sera from victims of sudden, unexpected death. *Clin Allergy* 18:461–468, 1988.
5. Yunginger JW, Sweeney KG, Sturmer WQ, et al. Fatal food-induced anaphylaxis. *JAMA* 260:1450–1452, 1988.
6. Hoffman DR, Hudson P, Carlyle SJ, and Massello W III. Three cases of fatal anaphylaxis to antibiotics in patients with prior histories of allergy to the drug. *Ann Allergy* 62:91–93, 1989.

7. Yunginger JW, Nelson DR, Squillace DL, et al. Laboratory investigation of deaths due to anaphylaxis. *J Forensic Sci* 36:857–865, 1991.
8. Muller UR. *Insect Sting Allergy*. Chapt. 4 Diagnosis. Stuttgart: Gustav Fischer Verlag, 1990. pp 66–108.
9. Golden DBK, Kagey-Sobotka A, Norman PS, et al. Insect sting allergy with negative venom skin test response. *J Allergy Clin Immunol* 107:897–901, 2001.
10. Kors JW, van Doormaal JJ, and de Monchy JG. Anaphylactoid shock following hymenoptera sting as a presenting symptom of systemic mastocytosis. *J Intern Med* 233:255–258, 1993.
11. Ludolph-Hauser D, Rueff F, Fries C, et al. Constitutively raised serum concentrations of mast-cell tryptase and severe anaphylactic reactions to hymenoptera stings. *Lancet* 357:361–362, 2001.
12. Ludolph-Hauser D, Schopf P, Rueff F, and Przybilla B. Okkulte kutane Mastozytose. *Hautarzt* 52:390–393, 2001.
13. Hoffman DR. The use and interpretation of RAST to stinging insect venoms. *Ann Allergy* 42:224–230, 1979.
14. Metz CE. Basic principles of ROC analysis. *Sem Nucleic Med* 8:283–298, 1978.
15. Lowry R. <http://faculty.vassar.edu/lowry/VassarStats.html>.
16. Hoffman DR, Dove DE, Moffitt JE, and Stafford CT. Allergens in Hymenoptera venom XXI. Cross reactivity and multiple reactivity between fire ant venom and bee and wasp venoms. *J Allergy Clin Immunol* 82:828–834, 1988.
17. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 107:891–896, 2001.
18. Hoffman DR, Cummins LH, Kozak PP, and Gillman SA. Diagnosis of honeybee venom allergy. *Ann Allergy* 40:311–313, 1978.
19. Hoffman DR. Comparison of the radioallergosorbent test to intradermal skin testing in the diagnosis of stinging insect venom allergy. *Ann Allergy* 43:211–213, 1979.
20. Franca FO, Benvenuti LA, Fan HW, et al. Severe and fatal mass attacks by ‘killer’ bees (Africanized honey bees—*Apis mellifera scutella*) in Brazil: clinicopathological studies with measurement of serum venom concentrations. *Quart J Med* 87:269–282, 1994.
21. Hoffman DR. Allergens in hymenoptera venom. VI. Cross reactivity of human IgE antibodies to the three vespidae venoms and between vespidae and paper wasp venoms. *Ann Allergy* 46:304–309, 1981.
22. Golden DBK, Marsh DG, Kagey-Sobotka A, et al. Epidemiology of insect venom sensitivity. *JAMA* 262:240–244, 1989.
23. Hemmer W, Focke M, Kolarich D, et al. Antibody binding to venom carbohydrates is a frequent cause for double positivity to honeybee and yellow jacket venom in patients with stinging-insect allergy. *J Allergy Clin Immunol* 108:1045–1052, 2001. □

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.