

Utilization of Serum Tryptase and Immunoglobulin E Assay in the Postmortem Diagnosis of Anaphylaxis

Kevin D. Horn, MD, John F. Halsey, PhD, and Ross E. Zumwalt, MD

Abstract: The postmortem diagnosis of anaphylaxis is difficult. Serum concentrations of tryptase (a mast cell product released during anaphylaxis) have been used after death as an indicator of possible antemortem anaphylaxis. However, studies have indicated that tryptase may be elevated with increasing postmortem interval (PMI), or in nonanaphylactic deaths with significant atherosclerosis or chest trauma. Serum total IgE has been used by some to confirm anaphylaxis when tryptase is elevated. Serum levels of tryptase from 57 decedents with varying PMI, all dying of presumed nonanaphylactic causes, were determined. In cases with elevated levels (>11.4 ng/mL), an assay of total serum IgE was also performed. Both tryptase and IgE demonstrated significant elevations with increasing PMI. Decedents were categorized according to presence of cardiovascular disease, chest trauma, or both; many demonstrated elevation of 1 or both markers, without statistically significant differences between categories. Postulated mechanisms for nonanaphylactic elevations of these markers are reviewed. The possible utility of allergen-specific IgE or allergen panels is discussed.

Key Words: tryptase, IgE, anaphylaxis, postmortem, autopsy

(*Am J Forensic Med Pathol* 2004;25: 37–43)

The postmortem diagnosis of fatal anaphylaxis is often difficult, even when a reliable history of sensitivity and an exposure is available. Most cases occur with exposure to certain foods (such as peanuts and shellfish), medication, or other ingested substances. Amounts of medication causing reaction may be minimal and therefore undetectable by routine toxicologic testing. Winged stinging insects, particularly

bees and wasps, are the second most common cause of fatal anaphylaxis in the United States.¹ Such stings may not be readily apparent at autopsy or not recognized as an important factor in the death.

Pathologists have traditionally relied upon a history of atopy or prior anaphylaxis, a documented sensitivity to an allergen, and autopsy findings to confirm the diagnosis. Gross findings in such cases may include obvious sting sites with or without retained portions of the stinging apparatus, laryngeal or epiglottic edema, mucus plugging of airways, or the presence of ingested allergen in the gastrointestinal tract. The microscopic confirmation of mucosal edema and mast cell infiltration of edematous tissue further supports the diagnosis. Anatomic findings of tissue edema and/or increased numbers of tissue mast cells as indicators of fatal anaphylaxis have not proven reliable, due to their nonspecific and inconstant nature. Attempts to measure histamine in postmortem serum have been unsuccessful due to its short half life following an allergen challenge.^{1,2}

Serum mast cell tryptase is a reliable indicator of systemic mast cell activation (such as occurs in anaphylaxis) in the living patient and has been used in the postmortem diagnosis of anaphylaxis in recent years.^{3,4} A recent study found that evidence of mast cell activation (as determined by elevation of serum tryptase) could be detected in up to 13% of sudden unexpected deaths in adults.⁵ The authors suggested that many of these reactions may represent unrecognized anaphylaxis due to unwitnessed allergen exposures (food, insect stings) or unexpected allergic reactions to licit/illicit drugs and medications. In many of the cases with a known drug exposure, death may be erroneously attributed to the toxicologic effects of the drugs (even when determined to represent relatively small doses) rather than a possible fatal anaphylactic reaction.⁵ However, other studies have reported elevations of serum tryptase in individuals dying of documented causes other than anaphylaxis (such as crushing blunt force trauma, particularly of the thorax, and arteriosclerotic heart disease) without any known toxic or significant drug exposures.⁶ Accordingly, it has been suggested that combining results from the serum assay for tryptase with a more specific assay for allergen sensitivity, such as allergen-spe-

Manuscript received March 19, 2003; accepted August 11, 2003.

From the Maricopa County Forensic Science Center, Phoenix, Arizona (K.D.H.); IBT Reference Laboratory, Lenexa, Kansas (J.F.H.); and the Office of the Medical Investigator, UNM Health Sciences Center, Albuquerque, New Mexico (R.E.Z.).

Funding provided, in part, by a grant from the American Academy of Forensic Sciences.

Reprints: Kevin D. Horn, MD, Medical Examiner, Maricopa County Forensic Science Center, 701 W. Jefferson Street, Phoenix, AZ 85007. E-mail: khorn@mail.maricopa.gov.

Copyright © 2004 by Lippincott Williams & Wilkins

ISSN: 0195-7910/04/2501-0037

DOI: 10.1097/01.paf.0000113814.56572.de

cific serum IgE assays, may prove more conclusive. Use of the radioallergosorbent (RAST) technique (if the offending allergen is known or suspected) or a total serum IgE (if a candidate allergen is not known) may support a postmortem diagnosis of anaphylaxis.⁷⁻⁹

The concentrations of many serum enzymes and other substances rise in blood and serum with increasing postmortem interval (PMI). While previous studies have demonstrated elevation of serum tryptase in nonanaphylactic deaths, the effect of PMI on these assays has not been specifically addressed. If a progressive elevation of serum tryptase with increasing PMI can be demonstrated, utility of the tryptase assay may prove more reliable in the early postmortem period.

The use of assays for serum IgE elevation as a confirmatory test for anaphylaxis has not been thoroughly studied. Although IgE can be detected and measured in postmortem serum,⁷ its stability postmortem has not been studied.

MATERIALS AND METHODS

Sample Collection and Preparation

Samples of femoral blood were collected via a transcutaneous approach in 57 postmortem examinations, of which 54 subsequently underwent full autopsy. The PMI was known for all cases (with a range of error of $\pm$ approximately 30 minutes). All individuals were under direct medical care at the time of death or died immediately following a witnessed traumatic injury.

Immediately following collection, blood samples were placed in gel serum separator tubes, without preservative. Serum was derived from whole blood by centrifugation, decanted into plastic aliquot tubes and stored at -80°C . Samples were shipped on ice to the IBT Reference Laboratory (Lenexa, KS) for analysis. Information regarding the cause of death was not provided to the reference laboratory performing the assays.

Serum Tryptase Assay

Serum tryptase concentrations were determined utilizing a competitive immunofluorescent enzyme assay (UniCAP 100; Pharmacia and Upjohn Diagnostics, Peapack, NJ) using monoclonal mouse antihuman tryptase antibodies against both the α and β structural forms of tryptase. These antibodies were incubated with a serum aliquot; the sample was washed, and enzyme-labeled antitryptase was added, followed by incubation. The sample was washed a second time, developer was added, and the fluorescence in the aliquot was measured via the UniCAP 100 analyzer. The amount of fluorescence emitted by the sample was directly proportional to the concentration of tryptase in the sample. The assay quantitates collective totals of α and β tryptase forms and is thus a more sensitive indicator of systemic mast cell activity.

This allows a more reliable determination of concentration than in prior studies, which used a radioimmunoassay method, which only detected the β form of the tryptase molecule.

Serum Total IgE Assay

Serum total IgE was determined utilizing a competitive chemiluminescent immunometric assay. Five microliters of serum were incubated with beads coated with mouse antihuman IgE antibodies. Following a washing step, antimouse IgG antibodies labeled with a chemiluminescent tag were added, incubated, and the washing step repeated. Chemiluminescence was then measured using the Immulite Analyzer (Diagnostic Products Corporation, Los Angeles, CA).

The normal ranges of serum total IgE are age-dependent. For the purposes of this study, an elevation of serum total IgE was defined as that exceeding the highest upper limit for all age groups (greater than 96 IU/mL, age 15–19).

PMI

Causes of death, the PMI, and postmortem levels of serum tryptase (and, when indicated, serum total IgE) were the variables under consideration in the study. None of the decedents had a history of anaphylaxis or were suspected to represent an allergy-related death. Furthermore, none had a reported history of atopy.

PMI (defined as time interval from death to the time of collection of the sample) ranged from 1 to 126 hours. As the New Mexico Office of the Medical Investigator (based in Albuquerque) serves the entire state, transport times to the facility could vary widely. This allowed a wide selection of cases which reflected a broad spectrum of PMIs. Additionally, deaths at the University of New Mexico Hospital, adjacent to the Office of the Medical Investigator, could be included in the study within a very short interval (some as soon as 30 minutes) following death.

Causes of Death and Postmortem Serum Tryptase

Since decedents with pathologically defined conditions, notably chest trauma and atherosclerotic cardiovascular disease had been reported to display apparently nonanaphylactic elevations of serum tryptase in prior studies,^{6,10} groups of decedents in this study were classified on the basis of postmortem diagnoses into 4 categories. These included those without atherosclerotic cardiovascular disease (ASCVD) or chest trauma (CT; group 1), those with ASCVD but not CT (group 2), those with CT but not ASCVD (group 3), and those with both (group 4). Because of the low numbers, the effect of the PMI within each subgroup was not specifically addressed.

RESULTS

PMI

Thirty-five out of a total of 57 sera assayed (61.4%) displayed an elevated serum tryptase (defined as that beyond the accepted normal range of <11.4 ng/mL). Serum total IgE was measured using samples from those 35 individuals. Eleven (31.4%) of the 35 samples had elevated serum total IgE assayed for serum total IgE revealed elevation of this analyte (above 96 IU/mL).

Serum tryptase levels tended to increase proportionally with the PMI (Table 1 and Fig. 1). Average values of serum tryptase within 10-hour increments of PMI were calculated. Utilizing the Student *t* test for nonpaired data, average levels in the PMI range of greater than 50 hours after death were significantly greater ($P < 0.05$) than the defined normal range.

Table 2 and Figure 2 reveal similar trends for serum total IgE. Utilizing the Student *t* test for nonpaired data, average levels of serum total IgE in 10-hour increments were determined to be significantly greater ($P < 0.05$) than the highest upper reference value (96 IU/mL) in the PMI range of greater than 40 hours after death.

For purposes of direct comparison between serum tryptase and IgE with increasing PMI, values for each are rendered unitless by normalization (ie, each value is divided by the maximum value obtained for the analyte, to obtain a unitless ratio between 0 and 1.0). These normalized data are presented in Figure 3, and again reveal trends of proportional increase in serum levels for both analytes with increasing PMI.

Causes of Death and Postmortem Serum Tryptase

Analysis of serum tryptase in groups divided on the basis of postmortem diagnosis revealed elevation (above the reference interval limit of 11.4 ng/mL) in 12 of 22 (54.5%) of group 1, 13 of 22 (59%) of group 2, 6 of 7 (85.7%) of group 3, and 4 of 5 (80%) of group 4 (see Table 3). Comparison of

the average values of serum tryptase between these 4 groups was not statistically significant ($P > 0.05$).

Those samples from the above groups with elevation of serum tryptase beyond the defined normal range underwent quantification of serum total IgE (see Table 3). Analysis of serum total IgE in these groups reveals elevation (above the highest reference interval value of 96 IU/L) in 5 of 12 (41.7%) of group 1, 5 of 13 (38.5%) of group 2, 0 of 6 (0%) of group 3, and 1 of 4 (25%) of group 4. Again, comparison of the average values of total IgE between these 4 groups was not statistically significant ($P > 0.05$).

DISCUSSION

A search for a reliable serum indicator of anaphylaxis first focused upon histamine, a mast cell product which increases vascular permeability and is primarily responsible for the early tissue manifestations of an acute allergic reaction. However, histamine has a very short half-life in serum and is rarely detectable, even a few minutes following an anaphylactic episode; sample degradation following death further interferes with postmortem analysis.⁴ Furthermore, coagulation may induce release of basophil histamine by apparently nonimmunologic mechanisms, complicating interpretation of observed plasma levels.⁴ Postmortem histamine release from basophils has also been documented in cases of nonanaphylactic death.¹² The use of vitreous fluid (a nonclotting medium) has been proposed, but not studied, as a possible substrate for serum.¹³

Recent investigation has centered on elevated tryptase concentrations in serum as an indicator of anaphylaxis. Tryptase is a neutral protease with vasoactive properties, released almost exclusively from tissue mast cells. Only trace amounts are present in circulating basophils. It is produced in 2 structural forms, α and β . The α form is constitutively expressed by mast cells. The β form is stored in cytoplasmic granules in the mast cells and is primarily responsible for the anaphylactic response to allergen challenge.⁶ It is released in direct response to crosslinking of bound cell surface IgE antibody when complexed with specific circulating allergen. In the living patient, elevated serum β -tryptase has been detected up to several hours following an allergic episode.⁶ For this reason, serum mast cell tryptase has been shown to be a useful and reliable indicator of systemic mast cell activation in the clinical assessment of allergic conditions. Since tryptase does not undergo appreciable degradation through the early postmortem period, despite wide variation in environmental exposure, methods of specimen retrieval, and postretrieval storage conditions, considerable interest has been generated in its potential use in the postmortem diagnosis of anaphylaxis.^{3,4} However, in spite of its initial promise, elevations of serum β -tryptase have been reported in deaths due to causes other than anaphylaxis, such as crushing blunt force trauma and arteriosclerotic heart disease.⁶

TABLE 1. Mean serum tryptase values within 10-hour postmortem intervals (PMI)

PMI (10-hour intervals)	Mean serum tryptase (ng/mL)	Standard deviation
1	15.7 (N = 13)	17.59
2	18.99 (N = 19)	20.68
3	37.66 (N = 13)	52.33
4	33.2 (N = 4)	25.10
5	24.6 (N = 3)	1.65
6+	81.8 (N = 4)	76.20

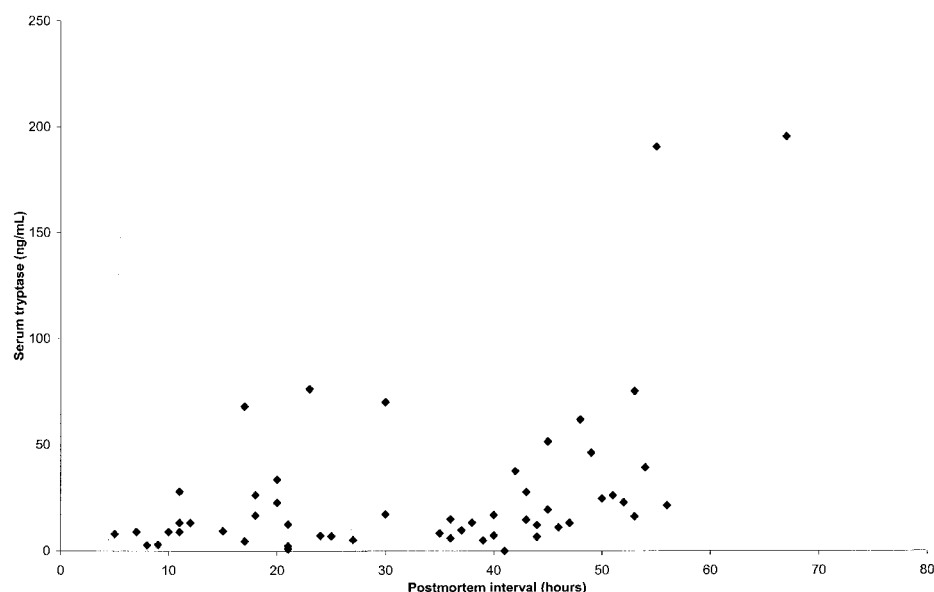


FIGURE 1. Serum tryptase (ng/ml) at increasing postmortem intervals (PMI).

Elevation of serum tryptase in fatal blunt force injuries of the chest may be related to the relatively large numbers of mast cells in lung and gastric tissue.¹⁴ These cells may react nonspecifically to mechanical stress of surrounding tissue by degranulating. Accordingly, a lower serum tryptase concentration would be anticipated in the blood collected from a peripheral source (ie, the femoral artery or vein) than that from the heart or cardiac vessels. Similarly, increased numbers of mast cells have been observed in and around atheromatous plaques (even from the earliest stages of plaque development) and have been shown to undergo reactive degranulation with plaque stress (superficial plaque tears, ruptures and supra- and intraplaque thrombosis).^{15,16} Atheromata are subjected to considerable and relatively constant mechanical stresses as they disrupt the usual laminar patterns of blood flow within a vessel. Particularly stressful situations, such as trauma or significant natural disease, may further promote such degranulation by inducing transient hyperten-

sion. Cardiopulmonary resuscitation with chest compressions may also promote plaque destabilization.

While we observed elevated concentrations of serum tryptase in fatalities from chest injury and from arteriosclerosis, the amount of elevation was similar in both groups. We could not evaluate whether the elevation came from traumatized pulmonary mast cells, atherosclerotic plaques, or some other source.

Combining results from the serum assay for tryptase with a more specific assay for allergen sensitivity, such as total or allergen-specific serum IgE assays, has been proposed as a means to allow confirmation of true antemortem anaphylactic episodes.⁷⁻⁹ According to this model, if death is due to a nonanaphylactic cause, serum total IgE should remain near its previous (antemortem) level, and one could conclude that the elevated tryptase result was spurious or nonspecific.⁶ Serum IgE has been demonstrated to be stable in properly collected and stored postmortem serum samples; even samples kept at room temperature for several weeks and refrigerated samples with marked gross hemolysis displayed only a slight overall decline in levels.⁹

However, the use of total serum IgE as a confirmatory postmortem test for anaphylaxis presents several problems. IgE levels normally demonstrate a slow rise throughout childhood, as exposures to candidate allergens increase. These levels generally plateau in the second decade of life. However, individual levels may vary widely. While total serum IgE may be elevated in many atopic individuals, this value waxes and wanes with the season, number and duration of allergen exposures, and the general state of health of the individual. Some people with atopy may not display any detectable elevation, as some allergens may not proceed

TABLE 2. Mean serum IgE values within 10-hour postmortem intervals (PMIs; for cases with elevated serum tryptase)

PMI (10-hour intervals)	Mean serum IgE (IU/L)	Standard deviation
1	20.73 (N = 3)	6.31
2	184.5 (N = 6)	373.85
3	60.42 (N = 5)	79.58
4	72.97 (N = 6)	58.63
5	167.78 (N = 8)	257.72
6+	227.99 (N = 7)	15.67

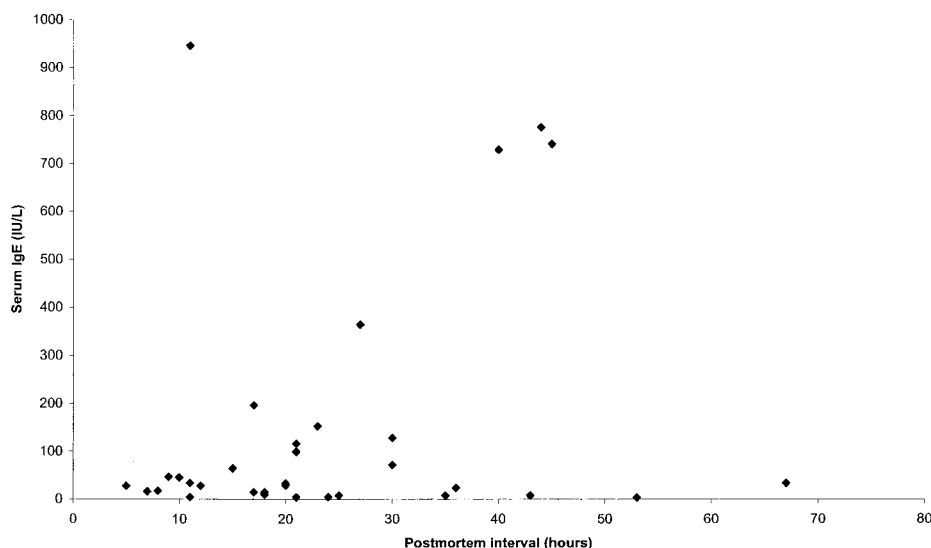


FIGURE 2. Serum IgE (UI/mL) at increasing postmortem intervals (PMI).

though an IgE pathway to activate a response. Also, relatively high titers of IgE specific for a given allergen may not cause a generalized IgE elevation beyond the recognized “normal” range. Such a specific IgE elevation can be elucidated if the offending allergen is known or suspected and a specific IgE assay such as RAST is used. Unfortunately, these deaths are often unwitnessed and the offending allergen is not known.^{17–20} A panel approach, utilizing an array of allergens known to be associated with anaphylactic reactions, tested against decedent sera, may be more conclusive.

Also, similar to serum tryptase, elevation of total serum IgE has been reported in association with trauma and other physiologic states/conditions with a significant inflammatory component (sepsis and disseminated malignancies), and levels

tend to rise with prolonged survival. Investigators have speculated that allergic mechanisms may be involved in the physiologic response to such stresses and that cells involved in the usual immune responses to these conditions may be activated by as-yet-undefined IgE-dependent mechanisms.^{21–23}

Total serum IgE may be elevated in other nonatopic conditions, including some parasitic and fungal infections, and the rare conditions of IgE myeloma and some heavy-chain class-switching disorders. Each of these possibilities should be considered when evaluating the results of these tests.

The cause of the many observed elevations of total serum IgE in our study is unclear; none of the individuals included in the study had a reported history of atopy or prior

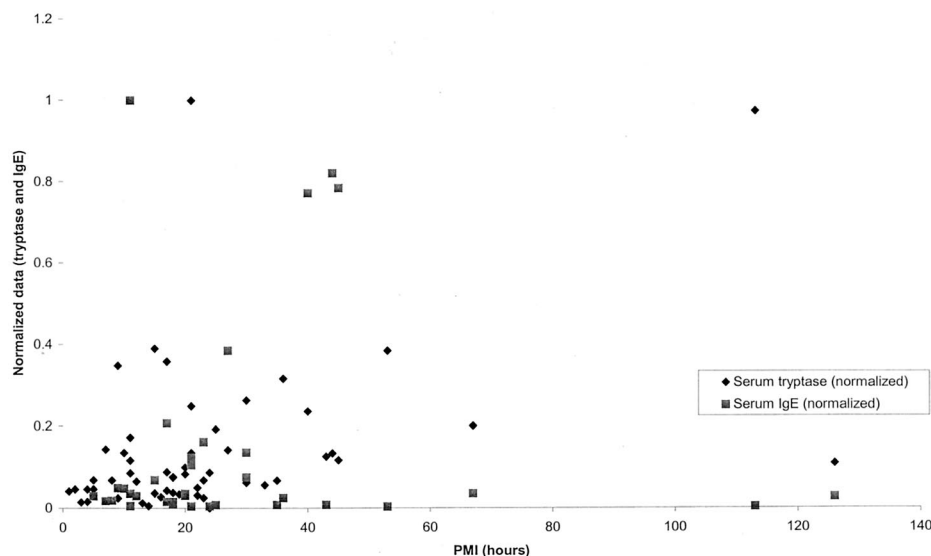


FIGURE 3. Serum tryptase and total IgE (normalized ratios) at increasing postmortem intervals (PMI).

TABLE 3. Mean serum tryptase and IgE values for cause of death categories (I–IV)

Diagnosis groups (I–IV)	Mean serum tryptase (ng/mL)	Standard deviation	Mean serum IgE (IU/L)*	Standard deviation
–ASCVD/–CT (I)	27.0 (N = 22)	41.95	115.08 (N = 12)	204.77
+ASCVD/–CT (II)	28.19 (N = 22)	40.82	248.35 (N = 13)	343.43
–ASCVD/+CT (III)	26.7 (N = 7)	22.57	14.55 (N = 6)	16.65
+ASCVD/+CT (IV)	28.2 (N = 5)	27.95	31.15 (N = 4)	23.30

*Performed in cases with tryptase elevation beyond normal limit (>11.4 ng/mL).

anaphylactic episodes. However, it suggests that serum IgE may be chronically or seasonally elevated in a large proportion of the general population, given the relative high prevalence of allergic conditions. In light of such a high prevalence of atopic disease in the United States and other industrialized countries, the entire concept of a “normal” range of serum IgE may be brought into question. These observations call into question the specificity and utility of nonspecific total IgE assays in the postmortem diagnosis of anaphylaxis.

If the identity of an allergen causing anaphylaxis is known or suspected, specific IgE assays (such as RAST) to assess reactivity for that allergen may be pursued, ostensibly providing a more specific means of diagnosing anaphylaxis. However, this method must be approached with some care. For example, individuals with documented allergy to honeybee venom may show moderate elevations of specific IgE to related but distinct allergen classes (such as wasp and ant venoms). Therefore, a single test for either of these antigens may well mislead the investigator to diagnose anaphylaxis on the basis of a moderate elevation of titer, when a panel would disclose a range of sensitivities and may reveal the true culprit. Also, limited availability of commercially prepared extracts for many specific antigens, such as in cases of many food and drug reactions, has prompted investigators to attempt to create their own panels of antigens utilizing samples of food or other substances recently consumed by the decedent. Such analyses may provide useful information but obviously must be pursued with diligent attention to quality control in sample preparation and preservation to avoid artifact and misinterpretation.^{9,24}

Unanswered questions about these phenomena remain compelling. Several nonimmunologic stimuli, such as various antibiotics, medications (metabisulfite, codeine), and controlled substances (morphine), may promote in vitro degranulation of mast cells in the absence of activating IgE, the so-called anaphylactoid immune reaction.^{25,26} Indeed, an unknown percentage of apparent drug intoxication deaths may represent anaphylactoid reactions via undefined pathways. Anaphylactoid reactions with elevated serum tryptase have also been reported in association with the clinical phenome-

non of “exercise-induced anaphylaxis.”^{27,28} Several of these factors may be involved in the large variation in postmortem tryptase levels noted in our study and warrant further investigation.

CONCLUSIONS

The postmortem diagnosis of anaphylaxis remains a problematic area. A diligent scene investigation, interview with family members and witnesses (if available), and a thorough review of the decedent’s medical records remain vitally important. Gross and microscopic indications of anaphylaxis are often absent but when present may provide the best evidence that such a reaction has occurred. The assay of serum tryptase, particularly in the early postmortem period, remains a helpful test but should not be relied upon as the sole criterion for diagnosis. The utility of a total serum IgE as a confirmatory test is questionable, given the apparent variation of this marker within a population with a high prevalence of atopic disease. Described elevations of total serum IgE in the setting of trauma, sepsis, and other nonatopic conditions further confound the interpretation of the assay. Specific IgE assays may provide helpful information, particularly if the offending allergen is known or suspected and if a commercially prepared specific allergen extract is available. Panels of candidate antigens may also provide an effective approach.

ACKNOWLEDGMENTS

The authors would like to thank Nicole Gabel for her technical assistance. Funding for this study was provided in part by a grant from the American Academy of Forensic Sciences.

REFERENCES

1. Delange C, Irely NS. Anaphylactic deaths: a clinicopathologic study of 43 cases. *J Forensic Sci.* 1972;17:525–540.
2. Ansari MQ, Zamora JL, Lipscomb MF. Postmortem diagnosis of acute anaphylaxis by serum tryptase analysis. *Am J Clin Pathol.* 1993;99:101–103.
3. Schartz LB, Metcalfe DD, Miller JS, et al. Tryptase levels as an indicator of mast-cell activation in systemic anaphylaxis and mastocytosis. *N Engl J Med.* 1987;316:1622–1626.
4. Schartz LB, Yunginger JW, Miller J, et al. Time course of appearance and disappearance of human mast cell tryptase in the circulation after anaphylaxis. *J Clin Invest.* 1989;83:1551–1555.

5. Schwartz HJ, Yunginger JW, Schwartz LB. Is unrecognized anaphylaxis a cause of sudden unexpected death? *Clin Exp Allergy*. 1995;25:866–870.
6. Randall B, Butts J, Halsey JF. Elevated postmortem tryptase in the absence of anaphylaxis. *J Forensic Sci*. 1995;40:208–211.
7. 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*. 1986;85:607–610.
8. Hoffman DR, Wood CL, Hudson P. Demonstration of IgE and IgG antibodies against venoms in the blood of victims of fatal sting anaphylaxis. *J Allergy Clin Immunol*. 1983;71:193–196.
9. Yunginger JW, Nelson DR, Squillace DL, et al. Laboratory investigation of deaths due to anaphylaxis. *J Forensic Sci*. 1991;36:857–865.
10. Ennis M, Sangmeister M, Neugebauer E, et al. Plasma histamine levels in polytraumatized patients. *Agents Actions*. 1990;30:271–273.
11. Deleted in proof.
12. Castells M, Irani AA, Schwartz LB. Evaluation of human peripheral blood leukocytes for mast cell tryptase. *J Immunol*. 1987;138:2814–2819.
13. Weedn VW. Anaphylactic deaths. *J Forensic Sci*. 1988;33:1108–1110.
14. Metcalfe D, Baram D, Mekori Y. Mast cells. *Physiol Rev*. 1997;77:1033–1079.
15. Jeziorska M, McCollum C, Woolley DE. Mast cell distribution, activation, and phenotype in atherosclerotic lesions of human carotid arteries. *J Pathol*. 1997;182:115–122.
16. Marone GM, de Crescenzo G, Florio G, et al. Immunological modulation of human cardiac mast cells. *Neurochem Res*. 1999;24:1195–1202.
17. Halpern GM. Markers of human allergic disease. *J Clin Immunoassay*. 1983;6:131–139.
18. Hamilton RG, Adkinson NF. Clinical laboratory methods in allergic disease. *Lab Management*. 1983;21:37–50.
19. Hornberger HA, Yunginger JW. Laboratory testing in the diagnosis and management of allergic diseases. *Clin Lab Annual*. 1983;2:351–388.
20. Mandy FF, Perelmutter L. Laboratory measurement of total human serum IgE. *J Clin Immunoassay*. 1983;6:140–148.
21. DiPiro JT, Hamilton RG, Howdieshell TR, et al. Total IgE in plasma is elevated after traumatic injury and is associated with sepsis syndrome. *Ann Surg*. 1992;215:460–466.
22. Beekman JF, Bosniak S, Canter HG. Eosinophilia and elevated IgE concentration in a serous pleural effusion following trauma. *Am Rev Respir Dis*. 1974;110:484–489.
23. DiPiro JT, Howdieshell TR, Hamilton RG, et al. Immunoglobulin E and eosinophil counts are increased after sepsis in trauma patients. *Crit Care Med*. 1998;26:465–469.
24. Yunginger JW, Sweeney KG, Sturmer WQ, et al. Fatal food-induced anaphylaxis. *JAMA*. 1988;10:1450–1452.
25. Veien M, Szlam F, Holden JT, et al. Mechanisms of nonimmunological histamine and tryptase release from human cutaneous mast cells. *Anesthesiology*. 2000;92:1074–1081.
26. Edston E, van Hage-Hamsten M. Anaphylactoid shock: a common cause of death in heroin addicts? *Allergy*. 1997;52:950–954.
27. Casale TB, Keahey TM, Kaliner M. Exercise-induced anaphylactic syndromes. *JAMA*. 1986;255:2049–2053.
28. Schartz HJ. Elevated serum tryptase in exercise-induced anaphylaxis. *J Allerg Clin Immunol*. 1995;95:917–919.