

Medicolegal Implications of Biphasic Anaphylaxis

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Abstract: Biphasic anaphylaxis is an uncommon IgE-mediated condition whose pathophysiological mechanisms, risk factors, and predictive signs are not properly understood. Fortunately, the lethality of biphasic anaphylaxis, although probably underestimated, is low. Preventive clinical measures for biphasic anaphylaxis are neither standardized nor commonly applied. Furthermore, there are no laboratory protocols or anaphylactic markers to help identify the onset of biphasic anaphylaxis in clinical settings. The aim of this review is to highlight the medicolegal difficulties facing coroners and forensic pathologists in terms of the diagnosis and assessment of harm for victims and survivors of biphasic anaphylaxis.

Key Words: biphasic anaphylaxis, postmortem diagnosis, forensic medicine, tryptase, biochemical marker

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Anaphylaxis is an acute and potentially life-threatening syndrome resulting from the unexpected IgE-mediated release of specific mediators from mast cells and basophils into the systemic circulation. This in turn results in mucocutaneous, cardiorespiratory, and gastrointestinal signs and symptoms.^{1–5}

The 3 main time courses of anaphylactic events are (1) *uniphasic* or *monophasic* when the patient has a single reaction, (2) *protracted* when the patient's anaphylactic symptoms take a long time to resolve despite relevant treatment, and (3) *biphasic* when the patient has a recurrence of symptoms after a symptom-free period.⁶

Biphasic, or rebound, anaphylaxis occurs when a patient is apparently successfully treated for a primary anaphylactic IgE-mediated reaction, but subsequently develops a second, or “biphasic,” reaction, in the absence of any further exposure to the trigger antigen.^{1–5,7,8}

An anaphylactic reaction, whether uniphasic, protracted, or biphasic, is regarded as a sudden and potentially fatal pathological IgE-mediated event.^{1,7}

The diagnostic criteria are provided by the US National Institute of Allergy and Infectious Diseases and the US Food Allergy and Anaphylaxis Network and state that any one of the following 3 criteria must be fulfilled during a period of minutes to several hours from the onset of the reaction which was brought about by exposure to a known allergen:

- 1) Acute onset of an illness affecting the skin and/or mucosal tissue, together with either respiratory symptoms and/or low systolic blood pressure.
- 2) Two or more of the following systems are affected: skin mucosal, respiratory, cardiovascular, gastrointestinal.
- 3) Low systolic blood pressure (less than 90 mm Hg, less than the age specific value or a decrease of at least 30% from the person's baseline).^{1,7}

Ring et al and other authors^{5,9–13} developed a grading system of the clinical severity of uniphasic and biphasic anaphylaxis on a scale of 1 to 4 based on the symptoms reported, summarized in Table 1.

The clinical presentation of anaphylactic IgE-mediated reactions can vary with respect to different triggers.^{5,9–12}

Pumphrey and Roberts¹³ reported that, in addition to mucocutaneous manifestations, respiratory symptoms are predominant in cases of anaphylactic reactions to food, whereas cardiovascular symptoms and shock are more frequent when there are reactions to venom or iatrogenic causes.

In terms of the cardiovascular system, anaphylactic IgE-mediated reactions, whether biphasic or uniphasic, may induce coronary constriction or narrowing.^{14–17}

This is through the interaction of histamine with H₁ vascular receptors, and also the rupture of plaques and platelet stimulation through the interaction with FcεRI and FcεRII activating receptors located on the surface of platelets.^{14–17}

Tryptase, as one of the most widely used biomarkers of anaphylaxis, may also induce, through different and not fully established enzymatic pathways, plaque disruption or rupture, and the activation of thrombin receptors.^{14–17}

These events may cause 3 pathophysiological variants which Kounis described as follows: type I, coronary spasm with or without cardiac enzyme/troponin release and myocardial infarction; type II, coronary spasm in the presence of preexisting atheroma with or without plaque rupture, cardiac enzyme/troponin release, and myocardial infarction.

Type III, thrombosis in subjects with a preexisting coronary stent, with or without cardiac enzyme/troponin release and myocardial infarction, whose aspirated specimens, when stained with hematoxylin-eosin and Giemsa reveal the presence of eosinophils and mast cells, respectively.^{14–17}

Episodes of biphasic anaphylaxis were first described in 1984 by Popa and Lerner,¹⁸ who reported 3 cases of the reappearance of anaphylactic IgE-mediated symptoms, and coined the term biphasic anaphylaxis.

The second reactions occurred some time later after the initial allergic reaction suffered by patients who were each exposed to different antigens (rabies vaccine, immunotherapy with rye grass extract and a yellow jacket sting).¹⁸ During the following decades, other cases were published but, as suggested by Brazil et al, these studies were not done using standard observational and descriptive approaches and lacked any thorough evaluation of frequency, time of latency, outcome, lethality, etiology, or the clinical and therapeutic aspects of this uncommon syndrome.^{1,19–23}

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TABLE 1. Severity Grading of Anaphylactic Reactions

Severity Grading	Skin	Cardiovascular System	Respiratory System	Gastrointestinal System	Nervous System
1	Itch, flush, urticaria, angioedema				
2	Itch, flush, urticaria, angioedema	Systemic pressure perturbations, tachycardia, arrhythmia	Hoarseness, dyspnea, wheezing	Nausea, vomiting, cramping, diarrhea	
3	Itch, flush, urticaria, angioedema	Cardiovascular collapse, profound hypotension, shock, arrhythmia	Laryngeal edema, bronchospasm, hypoxia	Incontinence	Confusion, reduced level of consciousness, sopor
4	Itch, flush, urticaria, angioedema	Cardiac/circulatory arrest	Respiratory failure	Incontinence	Coma

Therefore, biphasic anaphylaxis also fits Golden's²¹ description of uniphasic anaphylaxis as an entity, which is frequently “underrecognized, underreported, undertreated, and poorly understood.”

However, a peculiar clinical feature of biphasic anaphylaxis, highlighted by various authors, is that it often affects, in addition to the skin and mucosae, the same organ systems which had already been affected during the primary reaction. These are, frequently but not exclusively, the respiratory and gastrointestinal system, followed by the cardiovascular system.^{1,7,8,24–29}

The severity of biphasic anaphylactic reactions is highly variable and may range from mild to severe with occasional fatalities.^{2–4}

Furthermore, research studies and clinical reports on biphasic anaphylaxis often produce discordant results and probably underestimate the epidemiological magnitude of this uncommon condition, as this review article will attempt to show.

ALLERGENS CAUSING BIPHASIC ANAPHYLAXIS

Although needed to initiate an anaphylactic reaction the literature suggests that in 14% to 28% of cases the triggering allergens were not identified. For biphasic anaphylaxis, the triggers are commonly food, insect stings, and drugs.^{8,22,23,27–33}

The allergens most commonly involved in biphasic reactions are foods, such as peanuts (20–25%) and other types of nut, peaches and tangerines (10%) and shrimps (5%), hymenoptera venom (25%), and drugs, such as penicillin, nonsteroidal anti-inflammatory drug, or immunotherapy compounds (10–33%).^{8,22,23,27–33}

Pediatric cases were reported with similar frequencies involving foods (nut 16%, fish 16%), hymenoptera venom (33%), drugs (dicloxacillin, trimethoprim-sulfamethoxazole 33%), and unknown causes (17%).^{8,22}

On the other hand, some small molecules, such as contrast medium compounds, are also considered to be capable of: (a) causing non-IgE-mediated mast cell degranulation because of un-specific interactions on cellular membranes (this is typical of anaphylactoid reactions, namely, non-IgE-mediated reactions); (b) acting as haptens, which bind to proteins or peptide carriers linking IgE antibodies; and (c) acting as prohaptens which, after undergoing metabolic bioactivation, become haptens.^{34–37}

A significant frequency of biphasic reactions (23%) was reported in patients undergoing allergen immunotherapy who were administered aeroallergens (64%), hymenoptera venom (35%), or oral food challenge (10%).^{34–37}

PATHOPHYSIOLOGICAL MECHANISMS OF BIPHASIC ANAPHYLAXIS

The pathophysiological mechanisms responsible for biphasic anaphylaxis are still not fully understood. The following mechanisms

have been postulated by different authors; however, it cannot be excluded that some of these may act synergistically or triggered one by another:

- Temporary pharmacological suppression of systemic effects due to epinephrine administration, with persistent anaphylactic/inflammatory mediator release after the primary reaction. This would produce an apparent second reaction because the effects of the epinephrine wear off.^{2–4,18,19,25,26,38}
- Insufficient dose of epinephrine or the wrong injection site (ie, subcutaneous rather than intramuscular), which delays the time to maximum concentration provoking a second reaction.^{2–4,18,19,25,26,38}
- Delayed systemic release of stored antigens from one or more tissue compartments (especially from the gastrointestinal wall after food allergen ingestion) which produces a second wave of antigens resulting in a biphasic reaction.^{8,10–12,39–42}
- Individually high platelet activation factor (PAF) levels, causing abnormal release of mast cell tryptase, histamine, anaphylatoxins (C3a, C4a, C5a), cytokines (IL-2, IL-6, IL-10), due to low activity of PAF acetylhydrolase (PAF-AH) which normally degrades the PAF.^{11,12,43–45}
- Individually high PAF levels produced by abnormal activation of the PAF-positive feedback system: a consequent rapid increase in circulating PAF activates the nuclear factor- κ B pathway, which is considered to be a pivotal activator of some proinflammatory cytokines, such as tumor necrosis factor- α and interleukin-1, which both promote further synthesis of PAF.^{38,46} tumor necrosis factor- α also activates phospholipase A2 (PLA₂) which may lead to the synthesis of arachidonic acid metabolites such as prostaglandins, leukotrienes and adjunctive amounts of PAF.^{38,46}
- Low basic levels or a phenotypic dysfunction of serum angiotensin-converting enzyme (ACE), aminopeptidase P, and/or carboxypeptidase N, all of which are involved in the breakdown of different kinds of bradykinins, or use ACE inhibitor drugs (given an increased vulnerability to the hemodynamic effects of anaphylaxis in terms of blood pressure disturbances).^{47–49}
- Anomalous populations of mast cells and basophils, equipped with different numbers of IgE receptors (surface of mast cells ranges from 300,000 to 1,000,000 receptors per cell—surface of basophils ranges from 40,000 to 100,000 receptors per cell) allocated in different anatomical areas (ie, respiratory, cardiovascular, digestive systems) and able to produce a second wave of degranulated mediators responsible for biphasic anaphylaxis.^{14,15,50}
- Existence of uninvestigated phenotypic polymorphisms, causing secondary reactions, and involving: (a) epitopes (parts of the antigen to which an IgE antibody binds) or paratopes (parts of the IgE antibody which bind to the epitope), (b) mast cells and basophil IgE receptors.^{1,51–59}

FREQUENCY OF BIPHASIC ANAPHYLAXIS

The published material shows that biphasic anaphylaxis occurs in between 1% and 23% of all episodes of anaphylaxis, with an average frequency close to 20% in adults and 10% in children.^{2,3,8,22,23,25-27,30-32,60-62}

Brazil et al argued that the wide frequency ranges of biphasic anaphylaxis are probably due to the absence of standard observational and descriptive approaches in research studies and clinical reports.^{1,19-23}

Although biphasic anaphylaxis crises are fatal in only a minority of cases, some authors have claimed that the figure can be as high as 20% to 30%.^{39,63} Moreover, Sampson et al. reported a significant frequency of life-threatening events in children, whereas Lee et al^{30,32,39,60-62} noted that up to 50% of patients experiencing biphasic anaphylaxis required epinephrine administration and 8% to 14% were admitted to an intensive care unit.

Owing to a lack of published material, the overall frequency of biphasic anaphylaxis is probably underestimated. To establish the true incidence and prevalence of biphasic anaphylaxis, more standardized diagnostic criteria and reporting are needed.

FREQUENCY OF BIPHASIC ANAPHYLAXIS-RELATED DEATHS

Although biphasic anaphylaxis may have many of the characteristics of a life-threatening event, the actual number of fatal cases reported in the literature is fortunately very low, and our international author group has not yet reported a definitive case.

On the other hand, the reason why biphasic anaphylaxis is probably underreported is that forensic pathologists might not even be aware of this condition, and thus not report it.

Eleven cases of death due to biphasic anaphylaxis have been published in the past century (shown in more detail in Table 2):

- Franck³³ reported 1 case of an adult who died from multiple wasp stings (latency 48 hours, second reaction at home, died at home).
- Vidoni⁶⁴ reported 1 case of an adult who died after 1 or 2 wasp stings (latency 11 hours—second reaction at home—died in hospital).
- Sampson et al³⁹ reported 3 cases of children who died because of a reaction to foodstuffs (latency, 1–2 hours—second reaction at school/home—died in hospital).
- Pumphrey⁶³ reported 1 case of death (age not reported) due to 1 wasp sting (latency not reported—whereabouts of second reaction and death not mentioned).
- Bock et al¹⁹ reported 2 cases of a child and an adult who died after a reaction to foodstuffs (latency respectively 0.5 and 1 hour, respectively—second reaction at a video store and university cafeteria—place of death not mentioned).
- Cortellini et al⁶⁵ reported one case of an elderly man who died following multiple wasp stings (latency almost 1.5 hours—second reaction at home—died at home).
- Nagai et al⁶⁶ reported 1 case of a teen who died after the venous administration of nonionic contrast medium (latency 8 hours—second reaction at hospital—death in hospital).
- Gupta et al⁶⁷ reported 1 case of a child who died because of a surgically removed hydatid cyst (latency 12 hours—second reaction at hospital—death in hospital).

TIME OF ONSET OF BIPHASIC ANAPHYLAXIS

As reported by Lieberman et al, the latency time for the onset of new symptoms after the resolution of the first phase ranges

from 1 to 78 hours, but the second reaction seems to be most frequent between 8 and 24 hours after the first.^{2,3,8,13,24-27,30,32,62}

In cases of fatal biphasic anaphylaxis, the latency time for the onset of the second reaction could be 8, 12, or even 48 hours but appears to be most frequent after 1–2 hours: this would suggest that a close temporal proximity between the initial and second reaction might be an indicator for fatality.^{19,33,39,63-67}

RISK FACTORS AND PREDICTORS OF BIPHASIC ANAPHYLAXIS

Analysis of the published material does not allow us to identify signs, symptoms, or laboratory markers which might predict the occurrence of a second reaction with reasonable certainty. However, the following risk factors were mentioned as predictive in various different studies:

- High doses or more than 1 dose of epinephrine administered to treat the initial reaction, especially in children.^{2-4,22,23,38}
- Delay or inadequate administration of epinephrine to treat the initial reaction so that, after the effects of the initial treatment have worn off, a subsequent “rebound” of anaphylaxis mediators may occur.^{4,23}
- Administration of steroids or antihistamines, when epinephrine is not used even with the presence of cardiorespiratory symptoms, as first-line therapy.^{2-4,38}
- Delay of more than 30 minutes between the interaction with the offending antigen and the appearance of symptoms during the initial reaction.^{2-4,22,38}
- Admission to hospital more than 90 minutes after the onset of the initial reaction.⁶¹
- Presence of hypotension, respiratory symptoms (especially laryngeal edema), bradypnea, or abdominal pain and diarrhea during the first reaction.^{2-4,24,38,68}
- People who have a rapid and extreme reaction to the antigen and those who take longer to stabilize during the primary reaction.²⁷
- People with a previous history of anaphylactic IgE-mediated reactions, whether uniphasic or biphasic.^{1-9,24,38}
- Oral administration of the offending antigen.^{2-4,38,39}
- Unknown antigen responsible for the primary reaction.^{24,30}
- Initial reactions occurring in patients taking β blockers and/or ACE inhibitors or in elderly individuals with cardiovascular issues.^{5,48,49,69}
- Overuse of β_2 agonists for daily asthma treatment with possible desensitization of β_2 receptors to epinephrine.⁷⁰
- Children aged 6 to 9 years.⁶¹

OBSERVATION PERIOD FROM THE INITIAL REACTION UNTIL DISCHARGE FROM HOSPITAL

Although fatalities are rare in cases of biphasic anaphylaxis, it must be highlighted that when a patient is discharged after the primary reaction, he/she may return home or elsewhere, sometimes far enough from a hospital for a secondary reaction to be fatal. This is of critical importance in terms of the relevant clinical and medicolegal implications.^{1-9,23,31,38,45,61,71,72}

Simons et al^{69,73-75} maintain that 6 to 12 hours is appropriate for patients with respiratory symptoms, a period which can be extended up to 12 to 24 hours in cases of hemodynamic disturbance requesting significant doses of epinephrine during the first reaction.

However, in the absence of any clear guidelines, the duration of observation is best handled on a case by case basis, keeping under observation for up to 24 hours because of the risk of rebound especially in case of: (a) severe primary reaction with a slow onset, (b) primary reaction caused asthma or severe breathing difficulty,

TABLE 2. Demographic Data of Fatal Cases of Biphasic Anaphylaxis Reported in the Literature

Author	Study Design	Observational Period	No. Deceased	Age	Offending Antigen	Symptoms (From NIAID-FAAN Definitions)	Latency After Primary Reaction	Place of Onset	Place of Decease
Franck, 1930 ³³	Case report	—	1	Adult	Wasp venom (multiple stings)	Cutaneous angina pectoris Hypotension Vomiting	48 h	Home	Home
Vidoni, 1968 ⁶⁴	Case report	—	1	Adult	Wasp venom (1 or 2 stings)	Cutaneous dyspnea Hypotension Vomiting	11 h	Home	Hospital
Sampson et al, 1992 ³⁹	Retrospective study	14 mo	3	Children	Peanut, cashew	All suffered from respiratory gastrointestinal hypotension	1–2 h	School Home	Hospital
Pumphrey, 2000 ⁶³	Retrospective study	6 y	1	Not reported	Wasp venom 1 sting	Hypotension	Not reported	Not reported	Not reported but after leaving hospital
Bock et al, 2001 ¹⁹	Retrospective study	5 y	2	Children Adult	Peanut Other nuts	Respiratory	0.5 h 1 h	Video store University cafeteria	Not reported
Cortellini et al, 2005 ⁶⁵	Case report	—	1	Elderly man	Wasp venom Multiple stings	Cutaneous respiratory hypotension	1.5 h	Not reported	Home after departure of emergency doctor
Nagai et al, 2012 ⁶⁶	Case report	—	1	Teen	Contrast medium (iohexol)	Cutaneous dyspnea Hypotension Vomiting	8 h	Hospital	Hospital
Gupta et al, 2019 ⁶⁷	Case report	—	1	Child	Hydatid cyst	Respiratory hypotension	12 h	Hospital	Hospital

NIAID, National Institute of Allergy and Infectious Diseases; FAAN, Food Allergy and Anaphylaxis Network.

(c) offending antigen was ingested orally, (d) known history of biphasic reactions.^{2-4,71,72,75}

Cases when the first crisis was due to multiple hymenoptera stings could be prudentially kept under observation for up to 48 hours.³³

Patients experiencing an anaphylactic reaction should be provided with an emergency self-treatment kit (epinephrine autoinjector and a topical bronchodilatory aerosol spray). They should also understand and be able to execute the instruction on self-administration (when, where, and how to administer epinephrine and/or bronchodilators, antihistamines, and steroids) should they experience a rebound reaction. Patients should be educated on recognizing symptoms and to know how to alert the emergency services or access the nearest emergency unit so that he/she can be cared for by an expert allergist-immunologist.^{5,7-9,24,69-74}

Finally, in cases where the trigger antigen is known, the patient should be taught ways of avoiding any future contact.

MEDICOLEGAL IMPLICATIONS

Many aspects of biphasic anaphylaxis are unclear, with many reports and studies disagreeing on epidemiology, pathophysiology, risk factors, clinical treatment, postacute phase observation, and postdischarge therapies and precautions (the majority of fatal biphasic reactions have been reported as occurring in nonhospital environments). Because of the absence of standardized research protocols, the literature shows no agreement as to the frequency of biphasic reactions (from 1% to 23% of all anaphylactic cases), with only 11 published cases of fatalities available.^{2-4,6-8,19-27,30-33,38,39,44-49,60-68,75-80}

When a medicolegal evaluation is requested by the courts, a diagnosis of biphasic anaphylaxis in a nonclinical setting proves to be very difficult and can be impossible, whether the patient survived or not.⁷⁹

In cases where the patient survived, a forensic evaluation may be needed to ascertain whether the anaphylactic event was managed correctly and to evaluate the outcome, in the event of claims for compensation due to medical malpractice. In cases where death occurred, accurate forensic investigation is needed so as to be able to ascertain the causes and circumstances of death.⁷⁹

The medicolegal diagnosis of biphasic anaphylactic death should be done as follows:

- Identification of time of death—The time of death, normally evaluated at the external postmortem, and by examining circumstantial data and any medical documents and records available, is usually difficult to ascertain when the death did not occur in a hospital, and without medical assistance or witnesses.⁷⁹

- Identification of macroscopic and microscopic signs of anaphylaxis—Fatalities because of uniphasic or biphasic anaphylaxis may cause death due to the rapid onset of serious cardiorespiratory complications, such as asphyxia or shock, so there may be insufficient time for specific autopsy features to appear.⁷⁹

Pumphrey reported that the main indicators of possible anaphylactic death are: cutaneous erythema or angioedema (5%), upper-airway mucosal congestion and edema (8–49%), acute lung hyperinflation, and mucous plugging with bronchial mucosal congestion and hypereosinophilia (26%) (with airway remodelling, basement membrane thickening, mucinous metaplasia, and bronchial wall inflammatory cell infiltration also present in chronic asthmatic subjects), and asphyxial petechiae (17%).^{13,79,81-85} Pulmonary congestion and edema with splanchnic congestion (spleen, liver, kidneys) is also frequently detected (73%) as a result of cardiovascular decompensation and shock, and brain edema

resulting from subsequent cerebral hypoxia (26%).^{13,79,81-85} In addition to eosinophils, mast cells and basophils must also be histologically analyzed in lung, heart, and parenchymal tissues using conventional (hematoxylin-eosin, Giemsa) or immunohistochemical (CD117, anti-tryptase, anti-chymase) staining.^{13,79,83,85-87} All materials present inside the gastric or intestinal cavities must always be sampled and exhaustively examined in suspected or known food allergy (ie, vegetable or fish). Yunginger et al^{79,84} also suggested using the deceased's serum containing IgE antibodies to identify allergens in uneaten portions of food consumed shortly before the anaphylactic event.

Byard²⁰ reported that it is reasonable, in cases of an unexplained sudden death, to suspect an anaphylactic etiology, keeping in mind that when an anaphylactic death is uncertain, “while excluding other causes of death is always necessary, it is also important not to confuse an illness or condition that somebody may have simply died with, with something that they may have actually died from”. Therefore, the possibility that a death was caused by anaphylaxis (whether uniphasic or biphasic) should always be considered in cases of sudden death focusing on a scrupulous perusal of the death scene and clinical history, followed by the evaluation of autopsy and laboratory data.^{13,20,79}

- Identification of markers of anaphylactic death—The use of analytes, such as tryptase, total and specific IgE and histamine have several limitations in both clinical and postmortem diagnoses: (1) during emergency situations, for both uniphasic or biphasic anaphylaxis, the common laboratory procedures used to detect and quantify these analytes take a relatively long time and are not routinely performed in acute pre-mortem phases because they are of no help in diagnosis and/or ongoing treatment; (2) the analysis of histamine levels is not reliable at postmortem because its rapid metabolism; (3) total and specific IgE levels, although stable for weeks postmortem, may be absent in people suffering from anaphylaxis; (4) tryptase (upper limit for living subjects 11.4 µg/L) whose levels are generally stable for some days after death (postmortem cutoff of 23 µg/L was recently determined by Garland et al. in cases of nonanaphylactic death) may be within normal limits even in cases of fatal anaphylactic shock; (5) tryptase levels are affected by numerous factors (such as post-mortem interval, hemolysis, resuscitation procedures, trauma, acute cardiovascular disease, bacterial/viral infections, drug overdose, sudden infant death syndrome, mastocytosis).^{13,79,80,88-99}

Therefore, the levels of the analytes currently in use, such as tryptase and total/specific IgE, should always be carefully interpreted in the context of the wider clinical history, symptoms, and postmortem findings.^{13,79,80,88-99}

Studies of biphasic anaphylaxis make it clear that the second reaction, especially cases where death results, often appears around an hour after the primary reaction.^{2-4,6-8,19,23-27,30,32,33,39,63-67,73,74,77-80,86,90}

In 2016, the Working Group of the Resuscitation Council (UK) proposed the following sample timing protocol for the surveillance of tryptase: (1) initial sample as soon as possible after first aid or resuscitation has started; (2) second sample at 1 to 2 hours after the onset of symptoms because its peak concentration has been reported to be reached about an hour after the primary reaction and to disappear with a half-life 2 hours; (3) third sample 24 hours later, this protocol appears to be appropriate for the surveillance of all patients suffering from anaphylaxis, including those experiencing biphasic reaction (although supplementary samples may be acquired between 2 and 24 hours).^{7,72-74,77-80,86,90}

Increased levels of tryptase within 1 to 2 hours after the onset of symptoms may often but not exclusively suggest a “secondary

mast cell degranulation,” which, on the other hand, may occur within the following 24 hours. Therefore, the evaluation of tryptase levels from 1–2 to 24 hours (48 hours in case of multiple hymenoptera stings) after the appearance of an anaphylactic event could be useful both for clinical surveillance and postmortem investigations.^{7,33,72–74,77–80,86,90,99}

- Identification of the offending allergen—It is vital to attempt to ascertain the specific allergen which triggered the anaphylactic reaction, though it is not always possible in every case. Any available medical or anamnestic data on the victim's history of allergies must be evaluated; if the patient dies out of hospital, and without medical intervention or witnesses, it is only the evaluation of circumstantial data at the death scene that might be of any help in clarifying the underlying mechanism.⁷⁹ When hymenoptera venom is the suspected trigger, the body should be carefully examined for evidence of stings; if 1 or more bee stings are found (typically with a portion of the abdominal wall of the bee torn at the moment of release), they must be removed, photographed, and evaluated by an entomologist to identify the type of insect responsible for the allergic reaction.^{20,79,81}

The forensic pathologist has to be extremely careful during an examination of the victim's body because a sting can easily be overlooked or misinterpreted during an external examination.

Laboratory analysis of specific IgE for the suspected allergen may also be useful to confirm the trigger agent responsible for the anaphylactic reaction.⁷⁹

- Identification of the main terminal pathophysiological phases—As reported by Byard, death from anaphylaxis results from a synergy of respiratory effects (asphyxia due to upper airway obstruction by mucosal edema and/or bronchial smooth muscle constriction) and cardiovascular effects (shock due to acute cardiac failure, lung congestion and edema, vasodilation, and vascular permeability with a shift of intravascular fluids to extravascular space because up to 35% to 50% of intravascular fluid may move to the extravascular space when anaphylactic shock occurs); at autopsy, signs of the above multiorgan effects must be looked for.^{13,20,79,100}

Detectable myocardial damage caused by coronary artery constriction or plaque disruption and endothelial thrombosis induced by histamine (or leukotrienes, prostaglandins, and PAF) released by mast cells located in the heart may also be responsible for anaphylactic death.^{14–16,69,73,74,78} Kounis^{14,15} also reported that the anaphylaxis mediators, namely, histamine and tryptase with arachidonic acid products, proteases, cytokines and chemokines, and PAF, may act not only on the coronary vessels but also on cerebral and mesenteric arteries.

Inhalation of vomit may also be fatal in cases of food anaphylaxis involving a sudden gastric reaction.^{20,101}

- Evaluation of medical treatment and evidence of possible medical malpractice—As reported by Lindor et al,¹⁰² the main indicators of malpractice are: (1) failure to promptly recognize and or predict primary and secondary reactions; (2) Inappropriate timing and dosage of epinephrine administration; (3) administration, in a medical setting, of an allergen known to be a trigger for a particular patient (ie, contrast media, antibiotics, nonsteroidal anti-inflammatory drug) and which causes either a primary or secondary anaphylactic reaction.

Therefore, after anaphylactic events the following questions must be answered: (1) Were the primary and secondary reactions recognized promptly? Was the patient, after the primary reaction, identified as a subject at high risk of a biphasic reaction? If anamnestic data existed, were physicians informed of a patient's preexisting allergic problems in cases where a drug allergen was erroneously administered in hospital? (2) Was epinephrine given at the right time(s), in the right dose and in the correct site (intramuscular or intravenous)? Were the timing and method of all resuscitation procedures correct? Were steroids and antihistamines administered only as adjunctive therapy and not as first line or single therapy? (3) Was the patient properly supervised and monitored before discharge from hospital? (4) Was the patient correctly equipped with an epinephrine self-injector and told how to use it (intramuscular preferable to subcutaneous) in the event of a secondary reaction away from the hospital? (5) Was the site and skin-to-muscle depth of any emergency epinephrine injection identifiable at autopsy? (6) Were the dimensions and depth of the puncture hole comparable with needle caliber and length?^{2–4,24,30,31,60,71,103}

CONCLUDING REMARKS

A diagnosis of biphasic (or uniphasic) anaphylactic death may be difficult for the following reasons: lack of data or unclear data regarding a patient's history of allergy, the rapidity of death, remoteness of location (as is the case with farm workers, beekeepers, etc.), absence of witnesses and rescuers, lack of observation protocols and reliable diagnostic laboratory tests, and nonspecific macroscopic and microscopic findings at autopsy.^{1–9,19–23,32,38,47–49,75,79,80}

Forensic pathologists and coroners may be required by the courts to evaluate not only the survivors of a biphasic anaphylactic episode (in the event of medical malpractice claims) but also fatalities where it is necessary to ascertain:

- Time of death, macroscopic and microscopic signs of anaphylaxis, which are frequently unspecific or absent, and the main pathophysiological processes involved.
- Levels of anaphylactic markers currently in use (most common are total and specific IgE as well as tryptase).
- Offending allergen from an examination of the death scene, circumstantial data, medical history, medical records, and identification of specific IgE.
- The accuracy or appropriateness of the diagnosis, therapy, clinical monitoring, the time of discharge from hospital, and prescriptions and instructions given to the patient upon discharge.^{20,75,79,80,102}

Obviously, all data acquired (circumstantial, clinical, autopsic, and laboratorial) should be carefully evaluated and contextualized with the cooperation of allergologists, immunologists, and emergency, as well as intensive care physicians.^{20–23,30–74,76–79}

The current analytes in use, which are tryptase, total and specific IgE, and histamine, because of their limitations, are considered not fully reliable in clinical and postmortem diagnosis of anaphylaxis and must always carefully interpreted and contextualized.^{13,79,80,88–99}

Further research into the kinetics of other anaphylaxis biomarkers (such as chymase and carboxypeptidase A3), with stable high blood levels (both pre-mortem and post-mortem) and a half-life longer than tryptase, is needed. This may produce new emergency laboratory procedures and protocols for monitoring the onset of a biphasic reaction. New rapid-response analytical techniques, faster than common enzyme-linked immunosorbent assay and radioallergosorbent test procedures, are also

needed especially, but not only for use in emergency departments.^{2-4,6-8,24-27,30-32,62,69-74,77-80,88-90,99}

Lastly, further research should be undertaken to explore other potential anaphylaxis biomarkers and to improve current laboratory procedures both for clinical practice (such as developing innovative analyte sampling protocols and rapid-response analytical technologies for emergency applications) and for postmortem investigations (by identifying more stable, meaningful, and reliable analytes for testing).^{20,75,79,80,99,102}

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