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RETHINKING POST-MORTEM TRYPTASE: A CRITICAL ANALYSIS OF ITS ROLE IN DIAGNOSING ANAPHYLACTIC DEATHS

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SUMMARY

Post-mortem tryptase is used to support the diagnosis of anaphylaxis. However, despite its common use, the interpretation of elevated tryptase levels is still debated among experts. This review critically examines the use of post-mortem tryptase levels to identify fatalities resulting from anaphylaxis within forensic pathology. By synthesizing data from multiple studies, we aim to evaluate the reliability and consistency of post-mortem tryptase as a marker for anaphylactic deaths. Our methodical approach sheds light on the complexities and potential confounding factors in interpreting elevated tryptase levels. The findings suggest that the current understanding of post-mortem tryptase may require cautious interpretation and highlight the need for a more nuanced approach in forensic examinations. This review calls for a reassessment of existing methodologies and encourages further research to enhance our understanding in this crucial area of forensic science.

Key words

Tryptase; post-mortem levels; anaphylaxis; forensic pathology.

Impact statement

This study suggests that the current understanding of *post-mortem* tryptase may require cautious interpretation and encourages further research to better understand its role.

BACKGROUND

Tryptase: biological functions and significance

Tryptase is a trypsin-like protease, present mainly in the secretory granules of mast cells. Its release is typically related to mast cell activation, a phenomenon that can occur during allergic reactions, including anaphylaxis (1, 2). Tryptase is an essential enzyme of the human immune system, necessary for allergic reactions, inflammation and other immune defense processes (3, 4).

Anaphylaxis represents a serious and potentially life-threatening allergic response. It manifests itself with symptoms including difficulty breathing, decreased blood pressure and skin reactions. In the case of a living individual, the diagnosis of anaphylaxis is based primarily on clinical evaluation, supplemented by the presence of elevated serum tryptase concentrations. Serum mast cell tryptase (MCT) plays a critical role in facilitating

the diagnosis of anaphylaxis (5). However, when it comes to post-mortem analysis, the interpretation of tryptase levels presents substantial challenges, thus complicating the determination of death from anaphylaxis (6-8).
The prescribed clinical threshold for total tryptase appears insufficient in post-mortem scenarios, mainly due to largely unexplored factors leading to considerably elevated levels in such samples (5). As a result, there is currently a lack of a universally accepted threshold for diagnosing deaths caused by anaphylaxis.
In the context of post-mortem examinations, existing literature recommends rapid sampling of femoral blood for tryptase analysis immediately after death. Establishing a post-mortem tryptase threshold presents formidable challenges for several reasons (5, 9). The clinically approved 95th percentile value for tryptase, set at 11.4 mg/L, has limited relevance in the post-mortem setting, as various processes and conditions can exert an influence on tryptase levels (10, 11).
Instead, a proposed post-mortem threshold of approximately 43/44 μg/L serves as a benchmark for the biochemical diagnosis of anaphylaxis in several studies (9, 11). This differentiation has immense significance as numerous non-anaphylactic causes of death, and peri/post-mortem conditions have the potential to increase post-mortem tryptase levels making the adoption of tryptase for confident diagnosis questionable.

TRYPTASE IN POST-MORTEM ANALYSIS

Historical perspective and current practices

The historical progression of tryptase quantification in the field of forensic pathology has been a journey marked by noteworthy milestones and technological advancements. This evolution mirrors the increasing significance of biochemical markers in comprehending post-mortem occurrences and their implications in forensic inquiries (12).
The initial utilization of tryptase as a marker in forensic pathology can be traced back to the latter part of the 20th century. In its early stages, tryptase measurement relied on fundamental enzymatic assays. However, these assays were marred by limitations in terms of sensitivity and specificity, occasionally leading to ambiguous interpretations within the context of forensic pathology (12).
The emergence of more sophisticated techniques, such as enzyme-linked immunosorbent assay (ELISA), heralded a substantial enhancement in the quantification of tryptase. ELISA offered heightened sensitivity and specificity, facilitating more precise assessments of tryptase levels (13). Subsequently, advancements in mass spectrometry provided an even more precise methodology for quantifying tryptase, enabling the detection of minuscule quantities with remarkable accuracy (14, 15).
Contemporary forensic pathology places great emphasis on the timing of sample collection and the specific site of collection. Tryptase levels can exhibit significant variations based on these factors (16). Common sites for sample collection encompass cardiac blood, peripheral blood, and, on occasion, vitreous humor.
Presently, ELISA remains a widely adopted approach for tryptase measurement due to its equilibrium between accuracy, accessibility, and cost-effectiveness. Nevertheless, in intricate scenarios or when minute concentrations necessitate measurement, techniques such as liquid chromatography-mass spectrometry (LC-MS) are employed (14, 15).
Interpreting tryptase levels in a post-mortem context necessitates a nuanced comprehension of diverse factors, including decomposition, potential allergic reactions prior to death, and other underlying medical conditions (8). Modern forensic pathology integrates data from tryptase levels with other biochemical, histological, and circumstantial evidence to formulate comprehensive conclusions.
The utilization of tryptase measurement has evolved from being a novel biochemical marker to becoming a standard instrument in forensic pathology (17). Forensic pathologists, toxicologists, and medical researchers persist

in exploring the frontiers of tryptase measurement, including its role in distinguishing post-mortem intervals and its interactions with other post-mortem biochemical changes.

TRYPTASE: BIOCHEMISTRY AND PHYSIOLOGY

Molecular characteristics of tryptase

Tryptase possesses a distinctive characteristic among serine proteases due to its tetrameric structure, which plays a vital role in its enzymatic function (1, 17). This structure comprises four identical subunits, each contributing significantly to the enzyme's stability and specificity. In humans, the TPSAB1 gene encodes this enzyme, which can be categorized into two primary forms: alpha-tryptase and beta-tryptase.
Beta-tryptase, the form most relevant to anaphylaxis, is stored within the secretory granules of mast cells and is released upon the activation of these specialized mature blood cells, which have a distinct role in immediate hypersensitivity reactions (12, 18). However, mast cells also play a role in various other inflammatory-related processes, including conditions such as arthritis and multiple sclerosis.
Mast cells release two principal isoforms of tryptase: alpha-tryptase and beta-tryptase (6). Beta-tryptase is considered the predominant factor in anaphylaxis, whereas alpha-tryptase exerts distinct effects, particularly influencing the recruitment of inflammatory cells, including a reduction in neutrophil recruitment.
In the context of anaphylaxis, mast cell degranulation is initiated by the cross-linking of IgE molecules attached to the cell's IgE receptors by specific antigens (4, 6). This cross-linking leads to the release of stored tryptase. The precise mechanisms governing the role of tryptase in anaphylaxis remain elusive, although it demonstrates a notable preference for cleaving substrates at the C-terminal side of arginine and lysine amino acids, a characteristic it shares with the pancreatic enzyme trypsin, from which it derives its name (6).

The metabolic pathways of tryptase are not yet comprehensively understood; however, it is well-established that renal clearance is not the primary mode of elimination, with the liver being responsible for its catabolism (1, 6).

Functions of tryptase in the human body

The primary role of tryptase is to cleave and activate specific proteins and peptides, serving as a crucial participant in a variety of physiological processes. These processes encompass the immune response, because tryptase plays a pivotal role in immune defense mechanisms, particularly in allergic reactions and inflammation and its function involves the degradation of allergens, activation of complement proteins, and facilitation of immune cell recruitment to the inflammatory site (1, 17), and the regulation of cell function, because tryptase possesses the capability to influence the behavior of diverse cell types, including epithelial cells, fibroblasts, and smooth muscle cells and it exerts an impact on processes such as cell proliferation and apoptosis (4, 6).

Tryptase and allergic reactions

In the context of allergic reactions, especially in cases of anaphylaxis, tryptase assumes a central role. Following exposure to an allergen, mast cells undergo degranulation, liberating tryptase in conjunction with other mediators, notably histamine (6, 17, 19).
This liberation initiates the characteristic manifestations of an allergic response, including vasodilation, heightened vascular permeability, and contraction of smooth muscle. Consequently, heightened levels of tryptase serve as a distinguishing feature of mast cell activation during allergic reactions (1, 12).

Regulation of tryptase release

The release of tryptase from mast cells is meticulously regulated and occurs under specific circumstances:

1. **Allergen exposure**: the most prevalent trigger for tryptase release is exposure to

particular allergens, leading to IgE-mediated mast cell degranulation (17, 19).
2. **Physical and emotional stress**: physical trauma or emotional duress can also induce non-IgE-mediated mast cell activation and subsequent tryptase release (1, 4, 6, 12).
3. **Pharmacological agents**: specific medications and toxins can either directly or indirectly stimulate mast cell degranulation, resulting in elevated tryptase levels. Examples of such substances encompass opioids, muscle relaxants, and particular antibiotics such as vancomycin (20, 21). Radiocontrast media, employed in medical imaging procedures, can also prompt mast cell activation. Moreover, certain toxins, such as those found in venomous bites or stings, can evoke a similar response.

Mechanisms of tryptase release in allergic reactions

The release of tryptase during allergic reactions is primarily mediated via an IgE-dependent mechanism. This process encompasses several pivotal stages.
The first phase is the sensitization, the initial exposure to an allergen that initiates the production of specific IgE antibodies, which bind to high-affinity receptors (FcεRI) situated on the surface of mast cells (1, 4, 6, 8, 19).
The second phase includes subsequent exposure and cross-linking; upon subsequent exposure to the same allergen, the allergen molecules form cross-links with the IgE antibodies bound to the mast cells, thereby initiating a signaling cascade (4, 6).
Subsequentially occurs the mast cell degranulation where this signaling cascade culminates in the degranulation of mast cells, resulting in the release of tryptase, as well as other mediators such as histamine and leukotrienes (1, 4, 6, 8, 19-21).
All this leads to an augmentation of the allergic response where tryptase, in conjunction with other mediators, intensifies the allergic response by augmenting vascular permeability, attracting immune cells, and inducing smooth muscle contraction (4, 6).

Tryptase's role in anaphylaxis

Anaphylaxis is the most severe manifestation of an allergic response, characterized by a systemic release of mediators from mast cells and basophils. Tryptase plays a fundamental role in this process, contributing to the broad manifestations observed in anaphylaxis, including cardiovascular effects, since tryptase could induce vasodilation and increased vascular permeability, resulting in hypotension and shock, both crucial characteristics of anaphylaxis. (1, 6, 8, 17). Respiratory effects, given its role in bronchoconstriction and high mucus production, which leads to breathing difficulties (1, 4, 8, 17) and skin symptoms, since it is implicated in the development of the skin manifestations characteristic of anaphylaxis, such as urticaria and angioedema (1, 4, 8, 12, 18).
Histological analysis in cases of elevated tryptase levels, especially anaphylaxis, typically reveals mast cell degranulation, evidenced by dispersed granules within tissues and spleen (22, 23). Accompanying this are increased eosinophils and other inflammatory cells, indicating an allergic response. Additional histological signs may include edema and vascular congestion, supporting an allergic or anaphylactic etiology (24-30).

TRYPTASE IN CLINICAL DIAGNOSIS

In a clinical context, serum tryptase concentrations are assessed to facilitate the diagnosis of allergic responses, notably anaphylaxis.
The precise timing of sample acquisition is of paramount importance, as tryptase concentrations usually reach their zenith 1 to 2 hours following exposure to an allergenic substance and subsequently regress to their baseline levels within a 24-hour period (6, 8).
The measurement of serial MCT is considered the gold standard in aiding with the distinction between anaphylaxis and its clinical mimics (11). There has been a high degree of variation reported for the performance characteristics of MCT assays for anaphylaxis due to multiple factors, including variance in the definition

of anaphylaxis, diversity in the approach to MCT interpretation, heterogeneity in the clinical context of anaphylaxis, different causative agents, and the time of blood sampling (6, 8). Furthermore, other new biomarkers other than tryptase have been investigated for potential use in anaphylaxis. These include platelet activating factor, chymase, carboxypeptidase A3, dipeptidyl peptidase, basogranulin, and CCL-2. Apart from anaphylaxis, other clinical conditions could have increased tryptase levels (8).
Other conditions are chronic renal failure, hematological disorders such as myelodysplastic syndromes, acute and chronic myeloid leukemia, chronic eosinophilic leukemia, acute cardiac deaths, and others (6, 31). It has also been estimated that approximately 20% of the general population has an elevated baseline tryptase due to hereditary alpha-tryptasemia, which is an autosomal dominant condition that results from an increased number of germline copies of the alpha-tryptase gene (8).
Table I shows post-mortem tryptase levels for various clinical conditions. This factor contributes significantly to the inability of tryptase levels to serve as a reliable diagnostic indicator of post-mortem anaphylactic conditions. For example, in the case of acute cardiovascular deaths (ACD), tryptase levels can vary significantly depending on whether they occur with or without acute coronary syndrome (ACS), as indicated in the study by Xiao *et al.* (31). These examples show us how there are confounding factors in the diagnosis of death from anaphylaxis and how tryptase, despite being a credible diagnostic criterion today, must be associated with other factors before considering death for allergic reasons.

Challenges in interpretation

What is crucial for the understanding of post-mortem analysis is that raised levels of tryptase may be caused by a wide range of non-anaphylactic factors (8).
The increase in the concentrations of tryptase as a result of prolonged agonal states may be quite significant (2). During these periods, the human body experiences substantial stress and a lack of oxygen, leading to a cascade of biochemical reactions, including the activation of mast cells. This is most evidently occurring in cases of cardiac arrest or respiratory failure, where a resultant extended lapse of oxygen plays a critical role.

Table I. The table outlines various clinical conditions alongside corresponding tryptase levels, the timing of findings, and their sensitivity and specificity, where applicable.

Clinical Condition	Tryptase Levels	Timing of finding *	Sensitivity	Specificity	References
Anaphylaxis	12.3-309.0 ng/mL	Post-mortem	90%	92.1%	Xiao *et al.*, Tse *et al.*
Acute cardiovascular death (ACD)	3.2-18.9 (non-ACS); 8.0-188 (with ACS)	Post-mortem	NA	NA	Xiao *et al.*
Acute aortic dissection	9.0-36.0 ng/mL	Post-mortem	NA	NA	Xiao *et al.*
Pneumonia	3.8-8.0 ng/mL	Post-mortem	NA	NA	Xiao *et al.*
Asphyxia	10.9 (1.6-57.2) Femoral	Post-mortem	NA	NA	McLean-Tooke *et al.*
Trauma	13.7 (2.7-131.2) Femoral	Post-mortem	NA	NA	McLean-Tooke *et al.*
Sepsis	9.6 (2.1-18.0) Femoral	Post-mortem	NA	NA	McLean-Tooke *et al.*
Intracranial hemorrhage (ICH)	14.9 (9.8-107.0) Femoral	Post-mortem	NA	NA	McLean-Tooke *et al.*

* The timeframe for tryptase measurement after death is not detailed beyond the general post-mortem status in the articles analyzed.

The reason for the increase in tryptase levels in the context of sepsis is the systemic inflammatory response of the body (6). Sepsis often evolves into a condition called systemic inflammatory response syndrome (SIRS); in this case the activation of the immune system is not localized but extensive, resulting in the release of various mediators, including tryptase. The increase in this indicator signals the body's response to a serious infection: it is not limited only to anaphylactic reactions.

As for the surge in tryptase levels prompted by trauma itself, the reason is the correlation between this factor with the actual physical damage to tissues and cells (1). There is no doubt that trauma, especially of a severe or blunt nature, causes immediate mast cell degranulation, which causes the synthesis of tryptase and its release into the bloodstream. The reaction is undoubtedly part of the body's immediate response to the injury and is aimed at speeding up the recovery process.

Challenges in interpreting tryptase levels in anaphylaxis

While elevated tryptase levels can serve as an indicative marker of mast cell activation, their interpretation within the context of anaphylaxis, especially in post-mortem scenarios, poses considerable challenges owing to the following factors:

1. **Variability in peak levels**: the presence of individual variability in the highest recorded tryptase levels further complicates the final formulation of a specific diagnostic threshold (5, 6, 9). This result can also be found in the study by Tse *et al*. (5), who identified an optimal cut-off of 53.8 ng/mL for post-mortem total tryptase in femoral blood to diagnose anaphylaxis, with a sensitivity of 89% and specificity of 93% (9).
2. **Influence of basal tryptase**: individuals with elevated levels of basal tryptase, often associated with conditions such as mastocytosis, may exhibit distinct peak tryptase levels during anaphylactic episodes (17, 32). Conditions that increase basal tryptase, such as acute myelocytic leukemia, various myelodysplastic syndromes, and end-stage renal disease, may affect the interpretation of peak levels.
3. **Timing of sample collection**: It is clear that the transient increase in tryptase compromises the independent assessment of tryptase levels unless it is collected within the specified period post-anaphylaxis (5, 6, 8, 12, 16). The study by Garland notes that tryptase begins to increase within 15-30 minutes of anaphylaxis, peaks in 2 hours, and then decreases to the normal range within 24 hours. Furthermore, the study reiterates that the time from when ante-mortem tryptase samples were obtained or the duration of survival post tryptase peak could affect results. If only post-mortem samples are available, then negative results may be biased (6).

Biochemical properties of tryptase and relevance in post-mortem analysis

Tryptase exists in various isoforms, with beta-tryptase being particularly pertinent in the context of anaphylaxis. The enzyme's resilience after death plays a pivotal role in its applicability for forensic investigations. Unlike numerous other proteins, tryptase retains a degree of durability following demise, enabling its analysis post-mortem. However, this durability is subject to variations influenced by factors such as the post-mortem interval, environmental conditions, and the manner of demise (6, 17).

For example, Xiao *et al*. found that tryptase levels were elevated in groups described as anaphylactic deaths, acute cardiovascular deaths, and acute dissecting aneurysm ruptures compared to control groups, and suggested a cutoff of 43 ng/mL owing to the high degree of sensitivity and specificity in discriminating anaphylactic deaths (31).

Garland *et al*. found that the reference range for post-mortem tryptase in groups that are non-anaphylactic deaths is <23 μg/L, and they further understood that age, post-mortem interval, resuscitation, and the cause of death did not significantly influence the tryptase levels presented that the design technique is used (6).

The fact that different studies use different cutoff levels for making their recommendations makes it clear why it is essential to continue researching and debating the topic. The range from a cutoff of 43 ng/mL to a high of over 100 ng/mL shows that the current cutoff levels are flawed and, when coupled with abnormal values, show how necessary it is to evaluate all the evidence when making this determination (5, 7, 9, 16).

The National Institute for Health and Clinical Excellence (NICE) has identified post-mortem serum tryptase testing as a very important tool in those situations where anaphylaxis emerges as a potential cause of death. Although NICE does not establish a specific cutoff value, it points out the necessity of considering elevated tryptase levels along with the clinical history and autopsy data to sustain the final diagnosis of anaphylactic reaction (33).

The Royal College of Pathologists (RCPath) in the United Kingdom also refers to post-mortem tryptase analysis in its guidelines on autopsy practice and claims that it should be requested in cases of sudden, unexplained death and anaphylaxis is a probable cause. RCPath also acknowledges the fact that elevated tryptase levels do confirm an anaphylactic reaction yet should be solely one warrant of the correct conclusions and be analyzed with the general understanding of the case, including possible confounders as a post-mortem interval (PMI) or an intensive resuscitation (34).

The European Academy of Allergy and Clinical Immunology (EAACI) has published a comprehensive guideline on the diagnosis and management of anaphylaxis which, although primarily focused on clinical settings, indirectly support the forensic application of the presented method because they emphasize the biological rationale of tryptase, its mechanism, and patterns of release during the anaphylactic reaction. Moreover, the Academy also highlights that this agent is a highly specific marker of mast-cell degranulation, which aids it in the identification of an anaphylactic phenomenon if used correctly (35).

Challenges in measuring post-mortem tryptase levels

One of the foremost challenges encountered when interpreting post-mortem tryptase levels lies in the inherent variability of measurement methodologies. Diverse assays and techniques may produce disparate outcomes, and the absence of a standardized post-mortem tryptase level adds an additional layer of complexity to the interpretation process (6, 12).

Furthermore, the post-mortem interval, which denotes the time elapsed between the individual's demise and the collection of samples, exerts a profound influence on tryptase levels. The processes of degradation initiated after death can induce alterations in tryptase concentrations, potentially leading to erroneous conclusions (8, 17). Additionally, the physiological condition of the deceased at the time of demise, such as factors like stress or hypoxia, can also exert an impact on mast cell activation, thereby affecting the release of tryptase (1) (**Table II**).

Table II. Various considerations affecting the measurement and interpretation of tryptase levels in both post-mortem and clinical contexts.

Issue	Post-mortem	Clinical	References
Reference values	Variability across studies and lack of universally accepted cut-off values complicate establishing a universal level for post-mortem diagnosis of anaphylaxis	The reference values established clinically cannot be used to determine if the tryptase levels are consistent with sampling time and hence may not be easily used for post-mortem analysis	Xiao *et al.*, 2017; Tse *et al.*, 2018

Influence of post-mortem interval (PMI)	The elapsed time after death before sample collection can significantly affect tryptase levels, adding a layer of complexity to their interpretation	The PMI, which refers to the time elapsed after death before sample collection, does not have a clinical context as tryptase is collected while living	Xiao *et al.*, 2017; Tse *et al.*, 2018; NICE clinical guideline
Effect of resuscitation efforts	Resuscitation efforts, such as cardiopulmonary resuscitation (CPR), may artificially elevate tryptase levels due to induced trauma to mast cells	Tryptase levels may be influenced by resuscitation efforts, such as CPR, due to trauma inflicted onto mast cells, thereby increasing the release; however, this does not have any clinical value as the main area of focus is immediate medical history	Xiao *et al.*, 2017; NICE clinical guideline
Sampling site and technique	The choice of sampling site and method can influence tryptase levels, with a preference for femoral blood to reduce variations	The technique and data collection site in clinical contexts are peripherally sited compared to the preference in post-mortem collection, which is femoral to minimize differences.	Tse *et al.*, 2018
Confounding conditions	Pre-existing conditions such as cardiovascular diseases, trauma, and substance use can complicate the interpretation of tryptase levels in a post-mortem context	Confounding or preexisting conditions in the clinical context, such as systemic or chronic cases also have an effect on tryptase, while post-mortem conditions show the likelihood of concomitant diseases such as cardiovascular disease (CVD)	Tse *et al.*, 2018; EAACI anaphylaxis guideline
Variability in levels	There is considerable variability in measured post-mortem tryptase levels, necessitating standardization in measurement methodologies	Tryptase levels in a clinical setting follow a more predictable pattern post-anaphylactic event	Tse *et al.*, 2018
Interpretation in the context of sudden death	Tryptase analysis can be particularly useful in cases of sudden death where the cause is not immediately apparent	Analysis in case of sudden death is mainly to ascertain that the cause is due to the anaphylaxis condition, but in clinical contexts, it helps confirm the suspicion	Xiao *et al.*, 2017; Tse *et al.*, 2018
Influence of post-mortem factors	Factors such as decomposition and environmental variations can affect tryptase levels, adding further interpretative challenges	Factors influencing PM the decomposition phase and environmental factors are not applicable in clinical cases	Xiao *et al.*, 2017

THE ROLE OF TRYPTASE IN FORENSIC PATHOLOGY

In the field of forensic pathology, the examination of tryptase levels is utilized to offer insights into potential factors contributing to the cause of death. Historically, heightened levels of tryptase in post-mortem specimens have conventionally been regarded as indicative of anaphylactic reactions (6, 12). Nevertheless, owing to the intricate and variable nature of post-mortem alterations, a careful approach must be adopted when interpreting this data (8, 17).

It is imperative to possess a comprehensive understanding of the intricate biochemistry of tryptase and the various factors that can impact its levels post-mortem in order to arrive at precise conclusions regarding the cause of death (1).

Relationship between anaphylaxis and post-mortem tryptase levels

Anaphylaxis, owing to its inherent characteristics, instigates a swift discharge of tryptase from mast cells. In individuals who are still alive, a noteworthy surge in serum tryptase concentrations subsequent to exposure to an allergen serves as a pivotal diagnostic indicator for anaphylaxis (3, 32).

Nevertheless, in the post-mortem scenario, this association assumes a more intricate nature. Elevated tryptase concentrations are indeed frequently detected in instances where anaphylaxis is established as the underlying cause of demise. However, it is imperative to grasp that these heightened levels are not solely confined to fatalities resulting from anaphylaxis (10).

In instances of fatalities attributed to anaphylaxis, post-mortem tryptase concentrations generally exhibit an elevated profile when compared to individuals who succumbed to alternate causes (3, 11, 12). Nevertheless, it is essential to note that the range of these concentrations can be considerably wide, and the establishment of a universally accepted 'threshold' value that unequivocally signifies anaphylaxis remains elusive (6, 36).

The research contributions by Xiao *et al*. (31), and Tse *et al*. (9) present valuable findings on the forensic applicability of post-mortem tryptase measurements.

Firstly, on the marked elevation of tryptase in anaphylactic deaths compared to other causes of high levels of the enzyme, Xiao *et al*. proposed a cut-off value of 43 ng/mL for distinguishing anaphylactic from acute cardiovascular deaths (31).

Secondly, Tse identified the optimal cut-off value to be 53.8 mg/L for diagnosing anaphylaxis in post-mortem while reporting sensitivity and specificity of 90% (9).

However, the widespread of factors such as the post-mortem interval, hemolysis, trauma, and swabbing affected by various substituent such as body conditions compounds the attainment of a single threshold value that holds across all cases (6, 7, 9). For example, the post-mortem interval and conditions of storage can affect the degradation or preservation of tryptases, and hence the ability to measure its levels.

Tryptase levels in non-anaphylactic deaths

Certainly, it is of utmost importance to note that elevated tryptase levels have also been detected in cases unrelated to anaphylaxis. Factors such as asphyxiation, cardiac arrest, and even traumatic injuries can result in elevated tryptase levels (3, 10, 37, 38). The underlying causes for this phenomenon are multifaceted, encompassing stress-induced mast cell degranulation, destabilization of mast cells due to hypoxic conditions, and post-mortem autolytic processes (2, 21, 37).

These discoveries underscore the necessity for prudence when ascribing heightened post-mortem tryptase levels exclusively to anaphylactic reactions (4, 6).

The literature stresses that several factors than anaphylaxis may elevate post-mortem tryptase levels. For example, the level of post-mortem tryptase following drug overdose, especially heroin or other opioids, may be raised. One of the reasons it could do so is the capability of

these substances to catalyze non-specific mast cell degranulation, which can discharge mast cell granule items including tryptase. Thus, the increase in the level of tryptase may signal about the prevalence of an anaphylactoid mechanism of death (6, 7).
Secondly, sudden infant death syndrome (SIDS) has been observed to be another cause of post-mortem tryptase levels. Nonetheless, not all circumstances of SIDS appear to have an association, and even when the IgE-mediated allergic or anaphylactic reaction is accompanied by an elevated tryptase level, it may be inferred that agonal asphyxia, or some other process was among factors also increasing the tryptase level. It remains unclear what causes SIDS to produce post-mortem elevation of tryptase, thus, it may signal the complexity of reading post-mortem tryptase responses (6).
In patients with trauma, the post-mortem level of tryptase is often increased. The trauma site does not have to be centralized to have a significant influence on the tryptase level. Thus, either direct mast cell injury or lysis causing the release of tryptase, or the huge physical stimulus induced by trauma that produced a release from pulmonary and gastrointestinal mast cells are suggested to be the likely mechanisms. Therefore, trauma, even if the sampling is peripheral, is a substantiated cause of increased post-mortem tryptase level (6, 7, 31).
Furthermore, post-mortem tryptase levels during cardiovascular death increase, especially in acute coronary syndrome and acute dissecting aneurysm. Accordingly, the median tryptase level is highest in acute dissecting aneurysm, then followed acute coronary syndrome and total acute cardiac death, with wide intervals within each group, which offers a small if any practicable use. (6).

Implications for forensic interpretation

In conclusion, the available evidence suggests that, despite the high post-mortem tryptase levels having some meaning as a strategically useful marker (11), it is crucial not to base the diagnosis of anaphylactic deaths solely on this parameter (6).
To ensure as comprehensive and accurate forensic assessment as possible, additional contributing factors, such as the victim's existing medical conditions and previous history, immediate circumstances of their death, and various post-mortem examination results, must be considered as well (6, 39).

Implications for post-mortem analysis

The timing of death in relation to the onset of anaphylaxis, the time interval between death and sample collection, and the phenomenon of postmortem tryptase redistribution markedly influence postmortem tryptase (P-MTP) levels and should be taken into consideration when evaluating P-MTP levels for post-mortem diagnosis of anaphylaxis. Tryptase, although always present at a certain background level, reaches its peak between 30 minutes and 2 hours. After release, tryptase levels are deactivated or removed and return to normal within 12 to 72 hours (5, 9).
This dynamic of early release, deactivation, and removal of tryptase generates early release, but longer-lived tryptase levels highlight the importance of time-of-death (DTOD) offset determination. PMI influences current sampling. The slight increase in P-MTP with PMI has been recorded in previous research, with one review finding an increase from 6 mg/L at death to 8.8 mg/L after two days post-mortem (6, 16). However, this increase is not strong enough to exceed the proposed threshold for post-mortem diagnosis of anaphylaxis and demonstrates that PMI has minor effects on P-MTP but can be minimized by early sampling.
The phenomenon of post-mortem preservation of various molecules in immune cells could hypothetically be intensified by collection from or near mast cell-rich organs such as the heart or lungs (6, 7).
All these factors demonstrate the need for careful consideration of P-MTP levels in forensic practice. Release and elimination kinetics,

the impact of PMI and PPP (post-mortem retention phenomenon) all require special attention to adequately evaluate the role of anaphylaxis in sudden death.
Therefore, although tryptase is a useful indicator for identifying anaphylactic reactions, its use in determining the cause of death must be carefully considered. This involves examining these issues in the broader field of forensic pathology.
The influence of sampling sites and method on post-mortem tryptase measurement significantly impacts the accuracy of forensic pathology assessments. Variations in sampling techniques and locations can affect tryptase levels, complicating the diagnosis of anaphylactic deaths.
Tryptase levels may increase slightly post-mortem, but timely sampling mitigates this effect. Femoral blood is preferred due to its lower susceptibility to contamination and post-mortem changes compared to cardiac blood (40, 41). Standardization of sampling procedures is essential to minimize variations caused by different techniques and ensure reliable results.
The femoral vein clamp and aspiration technique involves occluding the femoral vein immediately after death to prevent blood redistribution. The blood is then drawn up using a sterile syringe. This method preserves the integrity of ante-mortem tryptase levels (16, 42). Consistent sampling allows for the development of robust cut-off levels for postmortem tryptase, addressing variability issues noted in previous studies (*e.g.*, 43 ng/mL by Xiao *et al.*, and 53.8 ng/mL by Tse *et al.*). By standardizing the collection process, the technique reduces false positives and negatives, improving overall diagnostic accuracy.

DISCUSSION

Initial research has established tryptase as a key marker for anaphylaxis in post-mortem examinations, laying the foundation for its widespread acceptance in forensic pathology. This marker, introduced through pioneering work in the late 1990s, was initially celebrated for its potential to differentiate between anaphylactic deaths and other causes. However, as the field progressed, further studies began to uncover certain limitations in the use of post-mortem tryptase levels.
These investigations have highlighted the need for a nuanced interpretation of tryptase levels. It has become evident that while tryptase is a valuable indicator, its levels can be affected by various factors, including acute cardiovascular events and changes that occur after death. This understanding calls for a critical reassessment of the diagnostic reliability of tryptase in forensic pathology, especially in suspected cases of anaphylaxis.
An essential aspect of interpreting post-mortem tryptase levels accurately involves understanding the methodologies used for its measurement. The significance of the post-mortem interval and the method of sample collection has been emphasized. Variations in sampling techniques can significantly impact the measured levels of tryptase, suggesting that the selection of a collection method can greatly influence the results. Additionally, establishing reference values for MCT in post-mortem serum has helped address potential confounding factors in the measurement process.
The challenge of distinguishing deaths caused by anaphylaxis from those resulting from other causes, based solely on tryptase levels, has become increasingly apparent. Research has shown that elevated tryptase levels can be present in both anaphylactic and non-anaphylactic deaths, indicating that high levels of tryptase are not solely indicative of anaphylaxis. This revelation underscores the importance of careful interpretation, particularly in the analysis of suspected anaphylactic deaths.
Comparing tryptase with other biomarkers used in post-mortem examinations is crucial for understanding its relative effectiveness. This comparison is instrumental for forensic pathologists, enabling them to make informed decisions about the most reliable biomarkers for determining causes of death.

Investigating alternative biomarkers to tryptase in post-mortem analysis is essential for understanding their effectiveness in diagnosing anaphylactic deaths. Biomarkers such as IgE, histamine, and their derivatives have been examined for their potential in identifying anaphylactic reactions. Unfortunately, plasma histamine measurement is not feasible due to the short plasma half-life and the difficulties in handling the sample. In this regard, tryptase generally demonstrated better diagnostic performance than histamine. Research has highlighted the role of IgE as a potential supplementary indicator alongside tryptase, and serum total IgE has been used to confirm anaphylaxis when tryptase is elevated.
The effectiveness of tryptase compared to other biomarkers has been a continuous area of research. Studies have methodically evaluated the diagnostic use of serum tryptase in anaphylactic deaths, providing a comparative perspective with other markers used in forensic pathology. These comparative studies are crucial in assessing the reliability and accuracy of various biomarkers in distinguishing anaphylactic deaths from other causes.

CONCLUSIONS

A key future direction in this field is identifying and addressing research gaps. This includes exploring factors that affect post-mortem tryptase levels and the possibility of false positive results. There is a recognized need for further research on the stability and variability of post-mortem tryptase levels.
Future research could focus on large-scale studies comparing multiple biomarkers, including tryptase, in different post-mortem scenarios. Additionally, experimental designs aiming to understand the kinetics of tryptase release during various post-mortem intervals would be valuable. This could provide a clearer understanding of the biomarker's behavior and improve its diagnostic accuracy.
While tryptase remains a valuable biomarker, its interpretation in post-mortem cases is fraught with challenges due to various confounding factors and the lack of a standardized threshold level.
Despite the limited supporting data, forensic pathologists and investigators are encouraged to consider tryptase as part of a broader investigative framework, including additional clinical, pathological, and circumstantial evidence.
The future of post-mortem analysis of anaphylactic deaths depends on the development of more sophisticated and multidimensional diagnostic strategies. This includes further research into the mechanisms affecting tryptase levels and the exploration of additional biomarkers that can provide a more detailed and accurate insight into anaphylactic fatalities.

ETHICS

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The Authors declare that they have no conflict of interests.

Availability of data and material

Data and material are available on request from the Authors.

Authors' contributions

All Authors contributed to the study conception and design. The first draft of the manuscript was written by GC and all Authors commented on previous versions of the manuscript. All Authors read and approved the final manuscript.

Ethical approval

N/A.

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