



Review Article

Fatal anaphylactic shock: A review of postmortem biomarkers and diagnostics

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ABSTRACT

Diagnosing anaphylactic shock postmortem is challenging since differential diagnoses exist and the forensic pathologist often faces subtle findings and lacks relevant information which prevents reaching an opinion of certainty. This review provides an overview of the literature covering research and existing recommendations on the postmortem diagnosis of anaphylactic shock. In order to harmonize the approach and provide guidance for diagnosing deaths from anaphylactic shock in the six forensic centers in Sweden, a guidance protocol aligned with the notion of a holistic view in the approach was devised. Areas in need of further studies include both immunohistological and biochemical investigations to stratify quantitative approaches based on condition and anaphylactic trigger and to lay the ground for and possibly establish alternative matrices.

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1. Introduction

The postmortem diagnosis of anaphylactic shock is difficult due to the complex pattern of pathogenic factors and pathophysiological processes that characterizes it. Anaphylactic shock is a severe systemic hypersensitivity reaction that is rapid in onset and can be life-threatening or fatal [1]. Death is caused by the effects of inflammatory mediators on several organs resulting in mucosal edema in the upper airways, bronchospasm and shock due to massive fluid shifts [2]. Anaphylactic reactions usually occur as a result of acute allergic reactions following exposure to a specific allergen in food, animal venom or medications [2]. The allergen triggers an immunoglobulin E (IgE) mediated degranulation of mast cells and basophils and release of inflammatory mediators such as histamine, tryptase, chymases, leukotrienes and prostaglandins. The mediators, mainly histamine, are thought to be responsible for the pathophysiological process in anaphylactic shock including vasodilation, increased vascular permeability, bronchoconstriction, arrhythmias and, hypotension [1,3]. Some mediators also induce chemotactic migration of eosinophils releasing secretory granules e.g. major basic protein (MBP) [4,5].

Other mechanisms of anaphylactic shock involve IgE-independent induction of mast cell degranulation where it has been indicated that possible mechanisms include complement or IgG mediated activation or a direct mast cell membrane activation [6].

These mechanisms are referred to as IgE-independent anaphylaxis [1] or non-IgE mediated anaphylaxis [2] when anaphylaxis is mediated by a non-immunological mechanism [7]. Although anaphylactic shock usually presents as an acute episode, mast cells can release mediators hours after the initial reaction causing a biphasic or late phase anaphylactic reaction [1,8].

Fatal anaphylactic shock is rare in Sweden with approximately five cases per year according to the database of the Swedish National Board of Forensic Medicine [9], corresponding to 0.05 cases per 100,000 inhabitants per year. This can be compared to 0.01–0.11 cases per 100,000 inhabitants per year internationally [2]. However, an increase in the reported anaphylaxis related deaths may be seen as a consequence of the more detailed classification and coding of these deaths in the world health organization (WHO) international classification of diseases (ICD) version ICD-11 when it goes live as indicated by Tanno et al. [10]. Clinically, anaphylaxis is diagnosed and graded depending on the most severe symptom from any of the involved organ systems. Death from anaphylaxis may occur suddenly outside the hospital setting and may be triggered by external factors, wherefore it may be erroneously considered and classified as an accident [11,12] or possibly suicide or homicide based on prevailing circumstances. The postmortem diagnosis of anaphylactic shock is based on a combination of relevant information gathered about the event, known medical history, blood serum analyses, macroscopic and histological findings [12–14]. The autopsy should be performed urgently to increase the probability of detecting relevant findings before they dissipate, as this facilitates the best conditions for a correctly diagnosing anaphylactic shock. This review aims to discuss the scientific literature relating to postmortem diagnosis of deaths from anaphylactic shock and presents a guidance protocol for the diagnostic approach.

2. Methods

2.1. Review of the literature

A review of the available literature was performed and Fig. 1 displays the selection process. The selection is based on whether 1) a

full-text article is electronically available in English, 2) information on findings for postmortem diagnosis of anaphylactic shock is stated or 3) reviews not older than five years. Several of the forensic medical textbooks contain no specific information pertaining to the diagnostic approach of anaphylactic shock [15–22] while a few of the books dedicate a section to possible findings at autopsy [11–14].

2.2. Generation of a guidance protocol

Diagnosing anaphylactic shock postmortem is challenging since the forensic pathologist often faces subtle findings and lacks relevant information. In conjunction with existing differential diagnoses this precludes reaching an opinion of certainty when evaluating the cause of death. The diagnosis is therefore often based on exclusion and circumstantial evidence. However, methods of varying scientific evidence are used to support the diagnosis though these may differ between different centers due to a lack of consensus. In order to harmonize the approach and provide guidance when diagnosing deaths from anaphylactic shock in the six forensic centers in Sweden, a guidance protocol was developed after extensive review of the literature.

3. Results

3.1. Anaphylactic shock described in case reports

Similarly to many other forensic research areas the scientific literature describing postmortem diagnosis of anaphylactic shock consists of many case reports and some well-controlled trials. A plethora of reported allergens is found in case reports in the literature with drugs [23–32], food [33–35] and insect-stings [3,36,37] being the most common. Other allergens like hydatid cysts [38], ecstasy [39], dust mites [40] and hair dye [41] have also been reported. In most of the described cases, edema in the airways, lungs and/or brain is reported. Several of the case reports demonstrate increased number of mast cells and degranulated mast cells in the lungs or other organs [24,26,33,34,37,38,41,42] but normal levels of mast cells have been reported as well [40]. Infiltration of eosinophils and mast cells in the spleen have likewise been reported [43,44], though these may possibly also be detected in bodies with mild decomposition [44].

Increased serum-tryptase levels have been observed and reported in many cases [3,23,27–30,33,34,37,40] however, information of when and from which site the blood is collected is often missing. Nonetheless increased serum-tryptase levels have been used for many years to support the diagnosis of anaphylactic shock. Inter-individual as well as allergen specific differences have however hampered the interpretation of the analysis, since a reliable reference interval for postmortem tryptase is missing.

3.2. Macroscopic findings

Classical findings described in textbooks in conjunction with anaphylactic shock include edema of the airway and lungs, lung congestion, diffuse or focal lung emphysema alternated with collapse as well as a mucus plug in the trachea. The swelling of the airways can be generally spread or limited to the soft palate, pharynx, epiglottis, larynx or trachea [2]. Brain edema has been reported in several cases secondary to a lack of oxygen [45,46]. Reaction and swelling can also be observed at the site for a drug injection or insect-sting [13]. However, in many cases of anaphylactic

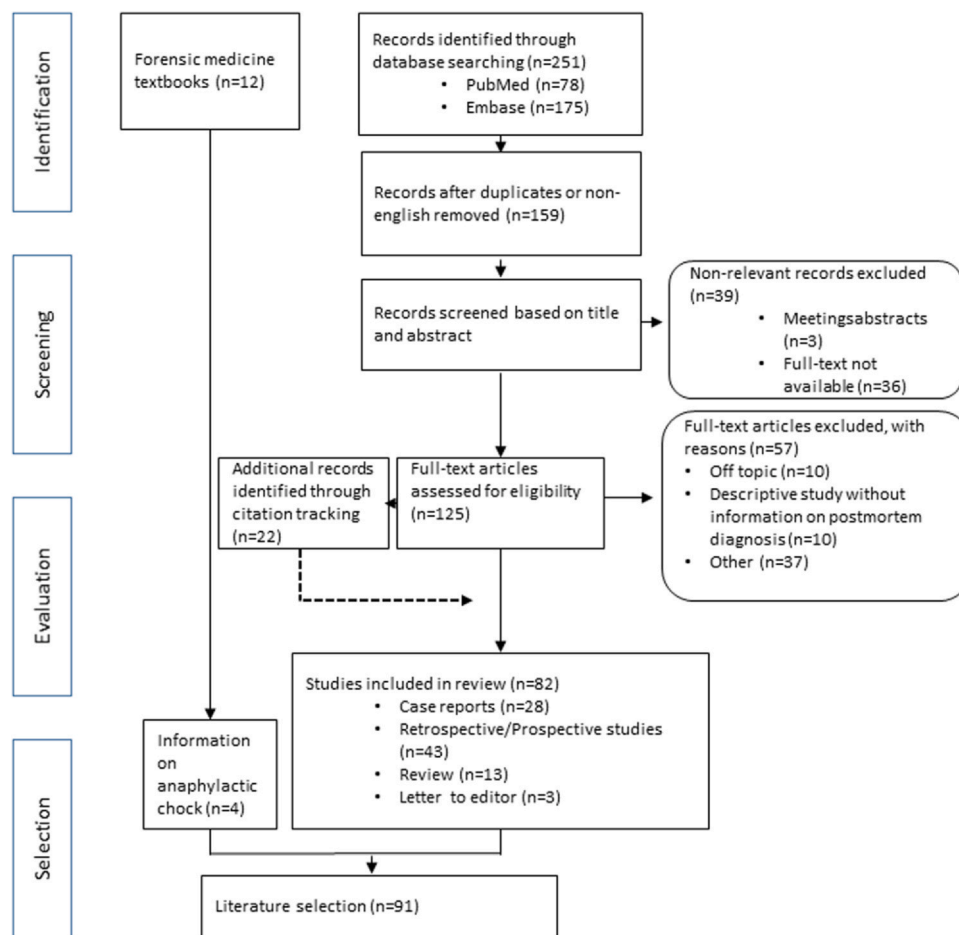


Fig. 1. Flowchart shows the process of literature search and selection.

shock, no macroscopic findings are observed at autopsy due to the rapid process or possibly due to postmortem de-swelling [2,45]. Also, the macroscopic findings, for example edema in the upper airway and bronchi, are not pathognomonic for anaphylactic shock and may occur in other conditions [45].

3.3. Biomarkers for postmortem analysis of anaphylactic shock

The ideal biomarker for anaphylactic shock from a forensic perspective is stable, cost effective, and able to withstand processing and handling postmortem. Several biochemical mediators are released from mast cells and other immune-cells which potentially could be detected and analyzed in body fluids. Out of the tested biomarkers, serum-tryptase has been proven to be an acceptable biomarker from a forensic perspective and has been used to support the diagnosis for more than 25 yr. Tryptase exists in two forms, α -tryptase which is secreted continuously from mast cells and β -tryptase which is stored in granules of mast cells and is secreted upon mast cell activation [47]. Total tryptase (α and β) is widely available as an established assay while the single forms are not and hence the total tryptase assay is used in routine analysis. However, the interpretation of the results can be challenging since there are indications that the levels are affected by several variables more or less linked to the degree of decay as well as sampling site and technique [48,49]. In addition, sampling technique, postmortem interval (PMI) as well as reference values have varied and are sometimes not specified in the studies that have been published on the use of serum-tryptase, making a comparison difficult. Postmortem use of tryptase has recently been discussed in an excellent review by

Garland et al. [50]. In addition to tryptase, analysis of chymase [51], histamine, diamine oxidase [52] and carboxypeptidase A [53] have been investigated as possible biomarkers in support of a post-mortem diagnosis of anaphylactic shock, see Table 1.

Increased IgE levels together with identification of specific IgE can be used for diagnosis of anaphylactic shock according to books in forensic medicine [13,14]. An increased specific IgE level indicates whether a person has been exposed to the specific allergen and the immune cells have reacted, which could support a diagnosis of anaphylactic shock. Conversely, a lack of increased specific IgE does not rule out anaphylactic shock and hence a negative result is inconclusive [50]. A retrospective study presented in 1983 by Hoffman et al. reported nine cases of deadly insect-stings in North Carolina which were analyzed for IgE and IgG levels. They showed that elevated IgE values are supportive of an anaphylactic process [54]. This study has been followed by further studies where increased general and specific IgE values are reported and increased levels support the diagnosis anaphylactic shock in cases where the process was IgE mediated.

In 2016, Tran et al. presented a study with the aim to characterize the distribution of total and specific IgE in postmortem serum, pericardial fluid and cerebrospinal fluid [55]. The authors found that total and specific IgE could be analyzed in serum and pericardial fluid while cerebrospinal fluid was unsuitable. They also point out that elevated IgE values are not conclusive as evidence in the diagnosis of death due to IgE mediated anaphylactic shock but rather that a sensitization of a specific allergen took place before death. This statement is also supported in a letter to the editor in 2017 by Koniari et al. who emphasized the importance of histologic analysis

Table 1

Studies investigating biomarkers for postmortem analysis of anaphylactic shock.

Study	Body fluid	Biomarker	Study population	Biomarker assay
Nishio et al. (2005) [51]	Cardiac blood	Chymase	40 (ctrl) 8 (ana)	ELISA Kit (Otsuka Pharmaceutical Co. (Tokushima, Japan)
Mayer et al. (2011) [52]	Femoral vein blood	Histamine Diamine oxidase	55 (ctrl) 3 (ana)	RIA with a lower sensitivity cut-off of 0.1 ng/ml (Immunotech, Marsailles, France). RIA using radio-labeled putrescine as a substrate (Sciotech, Vienna, Austria)
Guo et al. (2015) [53]	Cardiac blood Pericardial fluid	Carboxy-peptidase A	20 (ctrl) 15 (ana)	ELISA kit (Huamei Bio, Wuha, China)

Ctrl = Control, ana = Anaphylactic shock, ELISA = Enzyme-linked immunosorbent assay, RIA = Radioimmuno assay.

of several tissues to detect the presence of activated mast cells and eosinophils [56].

3.3.1. Sampling

Several studies have indicated that the sampling site as well as type of bodily fluid used can affect the detected tryptase levels [50]. In addition, the sampling technique has been shown to affect biochemical analysis in general and specifically also tryptase [57–59]. A standardized sampling technique with clamping of and aspiration from the femoral vein is therefore recommended in the literature [48,50,60] as it prevents backflow of central blood. This technique is also in alignment with research on other toxicological sampling [58,61].

Studies analyzing tryptase levels in bodily fluids other than blood are limited. In 2014 Comment et al. presented a retrospective study with the aim of investigating the possibility of analyzing beta-tryptase in urine, vitreous fluid and pericardial fluid in cases where access to serum is limited [62]. The authors concluded that pericardial fluid could be used as an alternative medium but that tryptase levels are generally lower compared to femoral blood, at least in the early postmortem period. The study also indicated that tryptase levels are not increased in urine or vitreous fluid, which would render these bodily fluids unsuitable.

Other studies have shown that analysis of heart blood generates higher tryptase levels compared to femoral blood, most likely due to diffusion of tryptase from cell lysis in the heart and other mast cell-rich tissues like lung [48,63].

3.3.2. Postmortem interval

The effect of the PMI on tryptase levels is inconclusive as studies have reported conflicting results. In 2004 Horn et al. presented a study where 57 non-anaphylactic deaths were analyzed to investigate the effect of PMI on serum tryptase and IgE levels and found that a longer PMI generated both higher tryptase and IgE levels [49]. As is the case in many studies in forensic medicine only one measured value per case was included and thus changes over time could not be followed. However, in 2018 Woydt et al. presented a prospective study where serial blood sampling at four predefined time points were analyzed for tryptase from 20 non-anaphylactic deaths [64]. The authors reported a minimal increase in some cases and a minimal decrease in other cases up to two days after death [64]. Decrease in tryptase levels with increased PMI have also been observed and reported after serial sampling in individual cases of anaphylactic shock despite tryptase levels remaining above a clinical reference value [65,66]. The reason for an increase in tryptase levels can be explained by postmortem cell-lysis and thereby a mast cell-release of tryptase which is supported by the demonstrated relationship between tryptase levels and hemolysis index [59,67]. A decrease in tryptase levels can, on the other hand, be explained by a potential degradation of tryptase [65,68]. The variations and differences observed could thus be explained by these simultaneously ongoing postmortem processes defined by case and time specific conditions. The authors of a recently published study investigated the amount of mast cells and degranulation as well as IgE levels in

the throat, lung, and gastrointestinal tract using tryptase and IgE staining of tissues, and correlated these markers with PMI in cases of fatal anaphylactic shock [69]. 20 cases of anaphylactic shock and 52 non-allergic deaths as control cases with varying PMI were included in the study. The conclusion of the study was that the anaphylactic group showed a significant increase in mast cell degranulation accompanied by an increase in IgE levels compared to the control group and that the correlation with PMI was tissue dependent. No correlation with PMI with regard to neither IgE nor mast cells was shown in laryngeal tissue while a significant correlation was observed between PMI and mast cell numbers and degranulation in lung tissue [69].

3.3.3. Reference values

Most of the clinical reference values for biochemical analyses are not applicable after death since postmortem processes cause biochemical changes [50,70]. Several attempts have been done to establish a reference or cut-off value for postmortem serum tryptase but some aspects still need to be investigated for comprehensive conclusions. Varying sampling techniques and PMI have given various reference values and it is also known that different allergens have different effects on serum tryptase levels, [33,71–74], possibly due to the difference in administration route. Parenteral administration of an anaphylactic trigger, i.e. drugs, imaging contrast agents or insect stings, generate higher tryptase levels than allergens in food [74,75]. The reference values that have been suggested are summarized together with sampling site, study population, PMI and criteria for diagnosis in Table 2. The performed studies suggest similar levels for a postmortem reference value for tryptase, especially for femoral vein blood (43.0–53.8 µg/L), see Table 2.

However, as with other situations within forensic medicine, biochemical analysis needs to be used together with all other relevant information to ascertain cause of death even when a reliable reference value is applied.

With regard to the other possible biomarkers (chymase [51], histamine and diamine oxidase [52]), there are no published studies establishing reference values for use in a forensic medical setting. Reference levels for tryptase and IgE included in the guidance protocol as a support for the diagnosis are summarized in Table 3.

3.3.4. Other conditions that can affect tryptase levels

Other conditions that can affect tryptase levels are heroin-related deaths [76], cases of sudden infant death syndrome (SIDS) [77–79], hyperthermia [80], amniotic fluid embolism [81] and heart conditions [71,82,83].

In 1994 Holgate et al. presented a study where the hypothesis that anaphylactic shock could be an underlying cause for SIDS was investigated [79]. The study included analysis of tryptase in heart blood collected in 106 infants (aged between 1 week and 2 yr) divided into groups of non-explained and explained deaths. The authors demonstrated increased tryptase levels in the group of non-explained cases compared to the group with explained cases and concluded that the results provided evidence that mast cell degranulation as a result of anaphylactic shock may possibly occur in

Table 2
Studies investigating reference values for postmortem tryptase.

Study	Sampling site	Established Reference value	Study population size	PMI (days)	Criteria for diagnosis
Edston et al. (2007) [63]	Femoral vein Heart ventricles	44.3 µg/L 93.2 µg/L	55 (ctrl) 5 (ana)	3–5	Primary criteria: Recent contact with identified allergen Secondary criteria: High level of tryptase and identification of specific IgE in serum Based on case history
Mayer et al. (2011) [52]	Femoral vein	45 µg/L	55 (ctrl) 3 (ana)	0–5	Not specified
McLean-Tooke et al. (2014) [67]	Aorta	110 µg/L	189 (ctrl) 10 (ana)	Not specified	Based on case history and edema in airways or lungs at autopsy
Xiao et al. (2017) [83]	Femoral vein	43 µg/L	54 (ctrl) 20 (ana)	0.5–3	Based on case history and exclusion of other pathology at autopsy
Tse et al. (2018) [96]	Femoral vein Femoral artery	53.8 µg/L	45 (ctrl) 9 (ana)	1–8 Median PMI ana: 6 Median PMI ctrl: 3.8	Not specified.
Sun et al. (2018) [99]	Not specified	30.4 µg/L	296 Metaanalysis of 7 studies	0–15	Not applicable, only controls
Garland et al. (2019) [100]	Femoral vein	23 µg/L	120 (ctrl)	0–6	

Ctrl = control, ana = anaphylactic shock, PMI = postmortem interval.

some SIDS cases [79]. In 1999, Edston et al. offered an alternative explanation to the anaphylactic shock hypothesis, based on a study in which tryptase and IgE were analyzed in 44 infants (up to 1.5 yr of age) diagnosed with SIDS [78]. This study showed that 40% of the infants had increased tryptase levels ($> 10 \mu\text{g/L}$) and 33% had increased IgE values. The authors concluded that the results did not support the hypothesis of anaphylactic shock being an underlying cause, instead they suggested that asphyxia as a consequence of a prone position causes mast cell degranulation and thereby increased tryptase levels [78].

In 2001, Buckley et al. presented a study where tryptase levels from 40 infants who died of SIDS were analyzed and compared with 32 infants with other causes of death [77]. The authors demonstrated an increased level of β -tryptase but not α -tryptase in the SIDS group and speculated that anaphylactic shock could be an explanation. However, another study from 2004 by Nishio et al. where tryptase levels were analyzed in 21 infants who died of SIDS and 14 infants with other causes of death concluded that they did not detect any statistical difference in the serum tryptase levels between the two groups [84]. The last study used a different method to measure total levels of tryptase, fluoroenzyme-immunoassay (FEIA) method instead of the radio immunoassay (RIA) kits that had been used in the earlier studies [77,84], which may account for the conflicting results.

Increased levels of serum tryptase have also been observed in cases where death was caused by coronary heart disease (43,56,58,63). The suggested mechanism for the increase in tryptase levels is degranulation of mast cells which exist in high quantity near atherosclerotic plaques [49,52,71]. The aim of a retrospective study in 2014 by Palmiere et al. was to investigate serum tryptase levels in 90 cases with coronary heart disease as a cause of death, compared with six cases of fatal anaphylactic shock [71]. Femoral vein blood was analyzed from all cases. The median tryptase level in the cardiac deaths was below the clinical reference value (7–8 µg/L), but in nine cases out of 90 they were increased (between 21 and 65 µg/L). For the six anaphylactic shock cases all values were far above the clinical reference value (146–979 µg/L). Importantly, these results indicate that increased levels may be observed in cardiac deaths though this is not generally seen [71]. A rare disorder that has been described as an acute coronary syndrome caused by strong immune reaction is referred to as Kounis syndrome [85,86]. Mast cell activation and tryptase have been suggested to be involved in the inflammatory cascade which leads to plaque rupture and formation of thrombosis and coronary infarction described in the syndrome [87,88]. Therefore, increased serum tryptase level can be expected in cases of Kounis syndrome [50].

Increased postmortem serum tryptase levels have also been reported in heroin-associated deaths [67,76,89,90]. A study from 2001 by Fineschi et al. compared tryptase levels in 48 cases of heroin-related deaths with two control groups, one consisting of 44 cases of head injury as cause of death, and one consisting of 32 cases of anaphylactic shock as the cause of death [76]. Increased tryptase levels were detected in the heroin-related death group (12–178 µg/L) as well as in the anaphylactic shock group (91–207 µg/L) whilst being below the clinical reference point in the head injury group (4–8 µg/L). Tryptase release from mast cells induced by heroin and possibly other opioids is most likely not an IgE-mediated process [76,90,91] and alternative non-allergic mechanisms like protein kinase signaling as suggested by Sheen et al. [92] may be responsible for the activation.

3.3.5. Microscopic findings

The symptoms of anaphylactic shock are mainly a consequence of a combination of activities by mediators released after mast cell activation, which causes vessel relaxation, increased permeability in capillaries and contractions in smooth muscles. Therefore, histology

Table 3
Postmortem reference levels in guidance protocol of biomarkers.

Biomarker	Body fluid	Reference level indicating anaphylaxis
Tryptase total	Femoral vein blood	>43–53 µg/L
Tryptase total	Pericardial fluid	Not determined, expected to be lower than serum
IgE specific, dependent on suspected allergen	Femoral vein blood	>0.35 kE/L (mix) >0.10 kE/L (single)

examination with a general staining like hematoxylin-eosin (HTX-E) can visualize edema in the larynx, narrowing of the bronchial lumen as well as lung emphysema in instances where anaphylactic shock is suspected. Mast cell degranulation can be detected by immunohistochemistry-staining with anti-tryptase where degranulation is seen as staining in the extracellular space [62,71,93]. However, the microscopic findings need to be interpreted in combination with all other findings in order to determine whether the finding is something the person has died with or from.

In a study from 2013 by Edston it was shown that staining and quantification of mast cells with anti-tryptase and eosinophils with anti-eosinophil major basic protein (MBP) in spleen tissue in combination with analyzed tryptase levels in serum increases the degree of certainty of a diagnosis of anaphylactic shock [93]. The aim of a prospective study in 2015 by Guo et al. was to examine the level of mast cell tryptase and mast cell secreted protease, carboxypeptidase A, in cases of fatal drug-related anaphylactic shock using immunofluorescent analysis of tissue samples from the stomach, jejunum, lungs, heart and larynx [53]. The study included 15 cases diagnosed as anaphylactic shock based on known clinical parameters and compared these to 20 control cases. The study showed a significant difference between the groups regarding tryptase levels in all studied tissue types, with the highest levels seen in the stomach and small intestine. No statistical difference in the expression of carboxypeptidase A was noted between the groups [53]. This study supports the use of a quantifiable immunofluorescent approach and interestingly indicates that detection of tryptase levels in organs may prove meaningful in cases where it is difficult to collect fluids due to advanced decomposition.

The goal of a study from 2015 by Reggiani-Bonetti et al. was to examine whether the accumulation of eosinophils and mast cells in the spleen differs between deaths caused by anaphylactic shock and other causes of death [94]. Two study groups were defined prospectively where 15 cases of anaphylactic shock caused by intramuscular injection of antibiotics, contrast medium or wasp stings were included in the experimental group and the control group constituted of 40 cases matched on age, race and gender. The authors demonstrated a significantly higher presence of eosinophils, mast cells and degranulated mast cells in the spleen in the experimental group compared to the control group. Interestingly, the authors observed no significant difference based on the triggering allergen in the anaphylactic shock cases which may suggest that the pattern of mast cell and eosinophil accumulation in the spleen is independent of the specific allergen according to the authors, however the number of allergen specific cases in the study was low. [94].

Also, the recent study by Wang et al. using a histological and immunohistological approach confirms that an increase of mast cell degranulation together with elevated tissue IgE level is a promising biomarker for a postmortem diagnosis of anaphylactic shock [69].

4. Discussion

Many of the implemented methods for postmortem diagnoses are based on experience in the field rather than evidence based research. With regards to the postmortem diagnosis of anaphylactic shock, the scientific literature provides a varying degree of evidence

for expected macroscopic and microscopic findings as well as biochemical analysis. There are obvious differences in prerequisites, methods and formulations of questions between clinical and forensic research since forensic research spans several disciplines including medicine, the natural sciences and the law [95]. A crucial difference between these is that research in the field of forensic medicine many times constitutes observations and data collected only at one point in time while clinical research allows for repeated patient contact with the possibility to study changes over time. Several of the conflicting results, including the effect of PMI on tryptase levels and the definition of a postmortem tryptase reference value may exist due to inter-individual variations, differences in sampling techniques but also suboptimal study designs. However, similar levels for tryptase concentrations were identified in the performed studies, see Table 2, which gives a benchmark value as a guidance for femoral vein blood [52,63,83,96]. Fatal anaphylactic shock is a rare event and sparse studies exist where a larger number of anaphylactic deaths are included. Studies stratified to investigate reference values regarding different allergens as well as studies with a well stratified control group regarding non-anaphylactic conditions which may affect both α - and β -tryptase levels are still missing. The difference noted in observed tryptase levels reported in older studies compared to reports in newer publications may partly be explained by differences in the form of tryptase analyzed or the used bioassays. In the attempts to identify other supportive biomarkers, serum chymase was demonstrated to correlate to tryptase levels and to be fairly specific in a few cases with anaphylactic shock compared to control cases [51]. However, detailed evidence on stability and reference values are needed as well as larger controlled studies to confirm and ascertain the extent to which chymase adds additional information to the diagnosis. Even though strongly elevated histamine levels may give a clue to fatal anaphylaxis [52], the use of histamine as a biomarker is not relevant due to a short half-life [97] and thus a limited time-window for sampling. While moderately decreased carboxypeptidase A levels were demonstrated in a limited number of anaphylactic shock cases [53] diamine oxidase has been shown to have an unexpectedly wide range of variation and is therefore unsuitable as a biomarker [52]. An elevated IgE value is not conclusive evidence of an anaphylactic event but it can be supportive in the diagnosis if there is enough information of a suspected allergen causing the shock. An IgE screening process where many known allergens are included has been suggested [49] but is not likely to be informative due to large intra-individual variation in serum-IgE which can vary over time making a reference value difficult to establish [52]. In addition, IgE produced as a response to an allergen may be consumed in the anaphylactic process and circulating levels may for that reason be low.

The guidance protocol developed in order to harmonize the approach between the six forensic medical centers in Sweden is aligned with the notion of a holistic approach to reach a postmortem diagnosis of anaphylactic shock with as high degree of certainty as possible. A very recent study by Esposito et al. concluded that the accuracy needed for forensic purposes was reached using a very similar approach [98].

In agreement with the literature, it is recommended the autopsy and sampling is performed as soon as possible in cases of suspected anaphylactic shock, as this increases the probability of detecting

findings thus providing the best prerequisite for correctly diagnosing the condition [12–14]. The literature review did not give rise to additional points to the existing guidance protocol for external examination based on the classical findings described in textbooks, e.g. edema in upper airway and lungs, lung congestion, diffuse or focal lung emphysema with varying degrees of collapse as well as a mucus plug in trachea. It has been noted though that these findings are unspecific and often not present. A major advantage that forensic medicine has compared to clinical medicine is the access to tissue samples from all organs and structures where histology and immunohistology is useful in the diagnosis. Many case reports and studies illustrate increased infiltration of mast cells and eosinophils in edematous tissues, lungs, and spleen after an anaphylactic shock event [24,26,33,34,37,41,42,93,94]. In addition to routine histology, staining of lung and spleen tissue with HTX-E and anti-tryptase is recommended together with anti-MBP staining of the spleen. This gives a general histological view and identifies infiltrations and degranulation of mast cells as well as eosinophils as shown in previous studies [93,94]. Blood to determine serum levels of tryptase and allergen specific IgE when there is a known allergen, should be sampled from the femoral vein with centrifugation as soon as possible to isolate serum. The scientific evidence for the use of tryptase measured in pericardial fluid is limited with undetermined reference levels, but may nonetheless be of use in when access to serum is limited or problematic [55,62]. Future well-designed studies are needed to increase the knowledge of alternative bodily fluids for tryptase analysis. In addition, there is limited knowledge of tryptase levels in serum depending on the characteristics of the allergen and further controlled studies with access to enough cases are needed. Furthermore, existing histological and immunohistological studies addressing tissue-infiltration of mast cells and eosinophils are small and based on few cases with a limited possibility for quantitative conclusions and hence larger studies are needed where infiltration and mast cell activity are quantified divided into groups based on the allergen. Finally, future studies should not be limited to the determination of tryptase as a reliable marker for postmortem use but ideally extended to the investigation of additional biomarkers which potentially could aid in assessing fatal anaphylactic shock with greater certainty.

5. Conclusion

Deaths due to anaphylactic shock are uncommon and it is a diagnosis based on findings which may not always be present and as such poses challenges for the forensic pathologist in reaching certainty with regard to the cause of death. A guidance protocol was devised based on the scientific literature, which aligns with a holistic view that all findings need to be considered in the approach to reach a definitive cause of death. An area in need of further immunohistological studies includes quantification of infiltration as well as activation of mast cells and eosinophils based on the type of allergen and the route of administration. In addition, the focus of future studies regarding tryptase analysis should be on identification of alternative bodily fluids and studies to correlate differing tryptase levels as well as tryptase forms with the type of anaphylactic trigger.

Ethical approval

This article does not contain any studies with living human participants or animals that were performed by any of the authors.

CRediT authorship contribution statement

N. Heldring: Conceptualization, Investigation, Writing – original draft. **L. Kahn:** Writing – review & editing. **B. Zilg:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors report no declarations of competing interest.

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Appendix A. Supporting information

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Supplementary material

Material and methods

Search strings

In MEDLINE the following strings were used:

1. (((("anaphylactic"[All Fields] OR "anaphylactically"[All Fields]) AND ("associate"[All Fields] OR "associated"[All Fields] OR "associates"[All Fields] OR "associating"[All Fields] OR "association"[MeSH Terms] OR "association"[All Fields] OR "associations"[All Fields]) AND ("death"[MeSH Terms] OR "death"[All Fields] OR "deaths"[All Fields])) OR "anaphylactic event"[All Fields] OR "anaphylactic shock"[All Fields] OR "anaphylactic reaction"[All Fields]) AND ("death"[MeSH Terms] OR "death"[All Fields] OR "deaths"[All Fields]) AND ("autopsied"[All Fields] OR "autopsy"[MeSH Terms] OR "autopsy"[All Fields] OR "autopsies"[All Fields])
2. "anaphylaxis"[MeSH Terms] OR "anaphylaxis"[All Fields]) AND ("autopsied"[All Fields] OR "autopsy"[MeSH Terms] OR "autopsy"[All Fields] OR "autopsies"[All Fields])
3. "anaphylaxis"[MeSH Terms] OR "anaphylaxis"[All Fields]) AND "post mortem"[All Fields]

In EMBASE the following string were used:

1. ('anaphylactic associated death' OR 'anaphylactic event' OR 'anaphylactic shock'/exp OR 'anaphylactic shock' OR 'anaphylactic reaction'/exp OR 'anaphylactic reaction') AND ('death'/exp OR death) AND ('autopsy'/exp OR autopsy)