



Post mortem tryptase cut-off level for anaphylactic death

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ABSTRACT

Serum mast cell tryptase is used to support the diagnosis of anaphylaxis. The recommended clinical cut-off for total tryptase ($<11.4 \mu\text{g/L}$) appears unsuitable in the post mortem setting due to largely unknown processes which result in significantly elevated levels in these samples. Consequently there is no widely accepted tryptase cut-off level for diagnosing an anaphylactic death.

This 5-year retrospective study compared total tryptase levels in post mortem femoral blood in anaphylactic deaths and control. Univariate and multivariate analysis was used to assess the relative contribution of other factors (age, gender, post mortem interval, and presence of resuscitation) on post mortem tryptase levels.

Nine anaphylactic deaths and 45 controls were identified. Receiver-operating characteristic (ROC) curve analysis identified an optimal cut-off of $53.8 \mu\text{g/L}$, with sensitivity of 89%, and specificity of 93%, for total post mortem tryptase in femoral blood to diagnosis anaphylaxis. No other factors showed any statistical significant contribution to post mortem tryptase elevation.

Femoral total post mortem tryptase level of $53.8 \mu\text{g/L}$ and above is a useful ancillary test in diagnosing an anaphylactic death.

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1. Introduction

Anaphylaxis is an immediate and severe hypersensitivity reaction which can cause death within minutes through cardiorespiratory collapse [1,2]. In Australia, both the numbers of presentations and fatality rates from anaphylaxis had increased in recent years [3]. An accurate diagnosis of anaphylaxis at post mortem requires the interpretation of medical history, scene investigation, complete autopsy and ancillary tests [4]. Mast cell tryptase is a commonly used ancillary test to support a diagnosis of anaphylaxis in both clinical and post mortem settings [1,5].

Tryptase exists in two forms being alpha (α) tryptase, secreted continuously from mast cells and beta (β) tryptase, stored in granules of mast cells and secreted upon mast cell activation [6].

Only total tryptase (α and β) is widely available as a commercial assay in Australia. During anaphylaxis, tryptase release may not be detected in the first 15–30 min following mast cell degranulation, but peaks between 30 min and 2-h. It then becomes deactivated or cleared, returning to baseline in 12–72 h [7,8].

At post mortem, the literature recommends sampling femoral blood for tryptase as early as possible after death [1,4,5]. Establishing a post mortem tryptase cut-off is challenging for multiple reasons. The clinically accepted 95% upper percentile value for tryptase of $11.4 \mu\text{g/L}$ is not applicable at post mortem setting as other process and conditions can alter tryptase level [1,5,9–16]. Furthermore, the incidence of anaphylactic death is relatively low which limits the data for establishing a cut-off level [3].

This study was carried out to establish a post mortem tryptase cut-off level in deaths from anaphylaxis using femoral blood samples. It also examines the relative contribution of different predictors on post mortem tryptase levels.

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2. Material and methods

This 5-year retrospective study (2012–2016 inclusive) included all autopsy cases from a single centre with a definitive diagnosis of anaphylaxis. A diagnosis of anaphylactic death was determined on the basis of a clear allergic history, circumstantial evidence of exposure to a trigger, death within hours after the exposure, and had no other significant pathology at autopsy apart from anaphylaxis related changes.

All deaths in which the final autopsy diagnosis was not anaphylaxis, but for whom a tryptase level was analysed were identified as controls. All traumatic, suspicious and homicidal deaths and bodies that were decomposed or incinerated were excluded.

Police and post mortem reports were reviewed, and the demographics (age and gender), days between death and autopsy (post mortem interval, PMI), the presence of cardiorespiratory resuscitation (CPR), and post mortem tryptase levels were recorded.

Total tryptase levels were measured from femoral blood samples collected with a wide bore needle (18-gauge) and syringe by gently aspirating from either or both femoral vessels, or collected from a femoral vein cut down during evisceration. The blood samples were placed into plain tubes and stored at 4 °C. The specimens were analysed within 7 days by the local pathology laboratory (NSW Health Pathology) using a commercially available immunofluorescent enzyme assay (ImmunoCAP™, Thermo Fisher Scientific, Sweden).

2.1. Statistical methods

Demographic information of participant groups was summarised with the use of simple descriptive statistics. Postmortem tryptase levels for anaphylactic deaths and controls were compared with Wilcoxon Rank-Sum Test, while levels comparing non-anaphylactic causes of death in controls used Kruskal–Wallis testing. A 2-tailed *p* value <0.05 was considered statistically significant. Univariate logistic regression models determined predictive values of analysed variables (tryptase, age, gender, CPR and PMI) for anaphylactic death, with receiver operating curve analysis used to define optimal diagnostic cut-offs. Variables with *p* values <0.1 were retained in multivariate models for anaphylactic death prediction. All analyses were performed using R statistics (version 3.4.1; 2017 The R Foundation for Statistical Computing).

Details of this study were submitted to the Hunter New England Human Research Ethics committee and it was deemed that formal approval was not required (approval au201709-15).

3. Results

A total of nine anaphylactic deaths and 45 controls were identified. In the anaphylactic death cases which the allergen was documented, two were from insect bite (tick and ant), one from erroneous antibiotic administration, one food related and one from unknown environmental exposure. The remaining cases had either no clear documentation of allergen involved or further immunology studies did not reveal a definite trigger. The exact time between allergen exposures, adverse response and death were not clearly documented in most anaphylactic death with missing time points in most cases. Only two cases had clear documentation of timing of allergen exposure, adverse response and death. Both cases (ant bite and antibiotic administration) had a reported adverse reaction within minutes and death within an hour. The remaining cases, after careful reviewing from the police report, can only establish the time between trigger exposure and death were within 6-h period.

Table 1

Summary of results between anaphylactic deaths and control.

Variable	Level	Deaths		<i>p</i>
		Anaphylactic death (n = 9)	Control (n = 45)	
Gender	Female	2 (22%)	21 (47%)	0.18
	Male	7 (78%)	24 (53%)	
Age	Mean (SD)	40 (18)	40.3 (17.2)	0.13
	Median (min, max)	46 (25, 68)	41 (1, 77)	
Time between death and autopsy (PMI, days)	Mean (SD)	6 (1.9)	3.8 (1.7)	0.09
	Median (min, max)	6 (2, 8)	4 (1, 7)	
CPR	Presence	7 (78%)	27 (60%)	0.31
	Absence	2 (22%)	18 (40%)	
Post mortem total tryptase, µg/L	Mean (SD)	121.6 (77.8)	18.1 (16.1)	<0.01
	Median (min, max)	90 (34.5, 300.0)	11.5 (2.2, 67.8)	

Total tryptase levels were significantly higher in anaphylactic death (*p* < 0.001) compared with control samples (Table 1). There was no demonstrable statistically difference (*p* = 0.414) in post mortem tryptase levels in the control group resulting from the cause of death (Table 2).

Logistic regression demonstrated that total tryptase was a significant univariate predictor of anaphylaxis as a cause of death (*p* = 0.004).

ROC curve analysis revealed an area under the curve (AUC) of 0.98 (Fig. 1). The optimal cut-off level was determined to be 53.8 µg/L resulting in a specificity of 93.3% and a sensitivity of 88.9%. Post mortem tryptase for corresponding levels of sensitivity and specificity is shown (Table 3).

Linear regression models assessing associations of total tryptase demonstrated that anaphylaxis as a cause of death was significant in both univariate (*p* < 0.001) and multivariate analyses (*p* < 0.001). Age, gender and the presence of CPR were non-significant. The relationship between the post mortem interval and tryptase level appears complex. It showed a positive prediction coefficient almost reaching statistical significance in initial univariate analysis (*p* = 0.05), but fails to demonstrate any significance in subsequent multivariate analysis (Table 4).

4. Discussion

Our data supports the findings of several previous studies in which serum total tryptase levels are higher and more variable in post mortem specimens [1,4,5,17].

Table 2

Post mortem tryptase in non-anaphylactic deaths.

Cause of death	n	Mean post mortem total tryptase (µg/L)	Min	Max	Kruskal–Wallis
Ischaemic heart disease	3	24.9	6.9	60.3	<i>p</i> = 0.41
Cardiac (non-ischaemic)	6	19.6	5.5	43.8	
Asthma	5	16	3.6	31.8	
Natural (non-cardiac, non-asthma)	11	16.5	2.2	67.8	
Drug and alcohol related death	16	19.2	2.5	51.6	
Others (incl. unascertained)	4	13.1	8.4	19.2	
Overall	45	18.1	2.2	67.8	

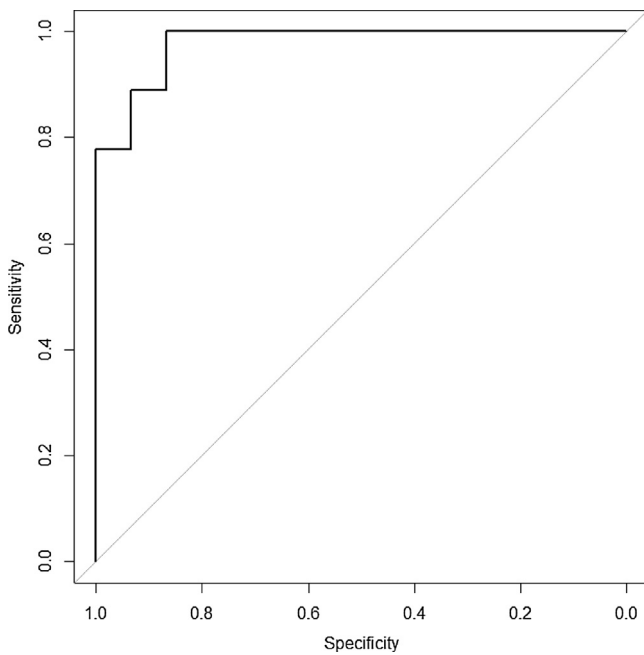


Fig. 1. Receiver operating curve (ROC) for total tryptase predicting anaphylaxis as a cause of death.

Table 3

Sensitivity and specificity of total post mortem femoral tryptase for anaphylactic deaths according to tryptase level.

Total tryptase level ($\mu\text{g/L}$)	Sensitivity	Specificity
>11.4	100%	47%
>20	100%	69%
>30	100%	82%
>40	89%	87%
>50	89%	91%
>60	78%	96%
>70	78%	100%
>90	44%	100%

An ideal laboratory reference range should be derived from a large number of 'healthy/non affected' individuals and adequate numbers (generally a minimum of 20) of well characterised disease controls. Although the number of anaphylaxis fatalities is rising in some jurisdictions, the rate remains low. A recent study of fatalities in Australia over a 15-year period identified 324 deaths attributed to anaphylaxis [3], while this may be an overestimate as forensic service will only make this diagnosis for several patients in a given year. In addition, serum tryptase levels are not a routine part of post mortem testing and are generally only requested when anaphylaxis is suspected.

Nevertheless several recent publications have suggested a modified total tryptase level ranges similar to the $54 \mu\text{g/L}$ found in our study. In 2007, Edston et al. noted that there were other

conditions that increase post mortem tryptase and provided a modified reference value of $44.5 \mu\text{g/L}$ in femoral samples using five anaphylaxis deaths [13]. In 2011 Mayer et al., studied three anaphylactic deaths and suggested a cut-off level of $45 \mu\text{g/L}$ [17]. In 2014, McLean-Tooke et al. established a cut-off level of $110 \mu\text{g/L}$ in aortic samples in 10 anaphylactic deaths however it's subsequently been recommended that femoral samples are preferable [18]. The most recent and largest study in 2017 from Xiao et al., which had 20 anaphylactic deaths, compared post mortem tryptase between anaphylactic deaths and cardiac deaths using femoral samples and was able to establish a cut-off level of $43 \mu\text{g/L}$ [9].

Multiple factors were likely to contribute to the variability observed including sample site (aortic samples higher than subclavian or femoral), cause of death and the post-mortem interval [9,18]. It has also been suggested that the use of CPR around the time of death might cause mast cell degranulation and elevated tryptase levels due to visceral trauma from chest compression and defibrillation [13]. In our study there was no statistical association. Sampling blood away from central areas such as aortic or subclavian blood may reduce the effects of mechanical trauma on visceral organs from CPR. A recent study also noted sampling technique may also influence post mortem tryptase levels [19]. Whether there are other confounding factors such as sampling time, interferences in sample (haemolysis), length of agonal period, specific type of trauma, rapidity of the dying process and cause of death needs further studies.

The effect of PMI on post mortem tryptase is not well understood. In the clinical setting, the change in serum tryptase is a dynamic process and the time between sampling and an anaphylactic reaction is crucial for interpretation [6,8,11]. The decline of tryptase after anaphylaxis in the clinical setting is also observed in the post mortem period as recent case reports have shown a steep decline in serial post mortem tryptase levels in anaphylactic deaths [10,11,20]. This highlights that PMI is also a dynamic process which may not be fully appreciated in either anaphylactic or non-anaphylactic deaths. The effects of PMI, however, may be underestimated in this study. Some of the cases had a PMI of up to eight days, due to the delays in Coroner's directions and body transportation. During that time the bodies were appropriately refrigerated which may reduce the effect of PMI. Our results show a PMI of up to eight days (with appropriate storage and refrigeration) had no significant association with elevated post mortem tryptase. More data and adequate documentation on the nature of PMI is required to further understand the different elements of PMI contributing to post mortem tryptase.

This study had several limitations, some of which are unavoidable due its retrospective nature and the relatively rare cause of death.

The number of anaphylactic death in our study is low ($n=9$), due the rarity of this condition. Despite having low number of cases, the cut-off level in the presented study ($54 \mu\text{g/L}$), was comparable with previous similar studies having a cut-off at around $45 \mu\text{g/L}$ [9,13,17]. There is confirmatory bias in both groups

Table 4

Associations of total post mortem tryptase levels.

Variable	Univariate linear regression coefficient (95% CI)	P Value	Multivariate linear regression coefficient (95% CI)	P Value
Anaphylactic death	103.50 (77.37–129.62)	<0.001	99.40 (71.72–127.08)	<0.001
Age	0.74 (–0.98 to 1.55)	0.08	0.26 (–0.34 to 0.86)	0.40
Male	18.93 (–9.90 to 47.76)	0.19		
PMI	7.61 (–0.13 to 15.35)	0.05	1.24 (–4.60 to 7.09)	0.67
CPR	–19.77 (–49.27 to 9.73)	0.18		

PMI = time between death and autopsy performed in days, CPR = the presence of cardiorespiratory resuscitation, OR = odds ratio, CI = confidence interval.

which cannot be entirely eliminated unless a more systematic approach in requesting tryptase together with robust study design.

The exact allergic trigger was not documented in all cases and all the anaphylactic deaths were grouped together for analysis. Different allergen triggers can result in different levels of tryptase in the clinical setting [1]. For example, anaphylaxis from contrast medium can result in very high tryptase level, followed by insect bite and food [1]. The exact timing between trigger, adverse reaction and death were not fully established in all anaphylactic deaths which is another limitation in this study.

Tryptase levels are reported to vary in non-anaphylactic death with elevated levels in cardiovascular deaths, drug deaths, sudden infant deaths and post traumatic deaths [1,5,9,12,15,21,22]. In the presented study, the mean total tryptase level in the control group appears to have higher levels compared to clinical reference level with no statistical difference between non-anaphylactic causes of death. Our study was not designed to investigate the differences in post mortem tryptase in non-anaphylactic deaths and had only one case less than 16 years old. Drug deaths, particularly opiates, are previously documented to be associated with an elevated post mortem tryptase level [21]. In the drug and alcohol related deaths included in the presented study ($n = 16$), all the deaths were mixed drug and/or alcohol toxicity. The drugs indicated in the deaths were from prescribed opioids (oxycodone, morphine, and fentanyl), psychiatric medications (benzodiazepine, antidepressants and antipsychotics) and illicit drugs (mainly novel psychoactive substances). There were no cases of heroin death or death in which only single opiates or opioids were indicated in the death. The total post mortem tryptase level in the drug and alcohol related death in this study may be lower than strict heroin and opiate deaths. Furthermore, the numbers of different types of non-anaphylactic death were small and may render a low power for statistical analysis to demonstrate any significance in tryptase between non-anaphylactic deaths. Further investigation in post mortem tryptase in non-anaphylactic deaths may clarify this.

Finally, it has been proposed that the β -isoform of tryptase would be a more useful measurement however to our knowledge it is not commercially available [5]. The β -isoform is only secreted after anaphylactic stimuli which increases in proportion to total tryptase, and is thought to be more specific in diagnosing anaphylaxis

Diagnosing anaphylaxis at post mortem and interpreting post mortem tryptase can be challenging. Further systematic studies are required to clarify the use of post mortem tryptase. The authors recommend clear documentation on the exact sequence and nature of events (i.e. time and type of anaphylactic trigger, subsequent clinical response, medical treatment, time of death, storage condition and time of blood sampling), strict use of femoral blood samples placed in appropriate sampling tubes, and sub-categorizing anaphylactic deaths into different allergen triggers and non-anaphylactic deaths into different causes of death for analysis in future studies.

5. Conclusion

This 5-year retrospective study compares post mortem total tryptase in anaphylactic deaths with controls and shows an elevated post mortem total tryptase is a good predictor for anaphylaxis. A total post mortem tryptase level of $53.8 \mu\text{g/L}$ provides a sensitivity of 89% and specificity of 93%. Age, gender, post mortem interval, and presence of CPR had relatively insignificant contributions on post mortem tryptase elevation compared to anaphylaxis. There are several limitations in this study with the most significant one being the small sample size in both the anaphylactic death and control such that further segregation or partitioning was not possible. Post mortem tryptase

should be considered in light of the circumstance of death, medical history and autopsy findings, and should never be interpreted alone.

Compliance with ethical standards

This study was not funded.

The authors declare that they have no conflict of interest.

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

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