

Postmortem Trypsin Level in 120 Consecutive Nonanaphylactic Deaths

Establishing a Reference Range as $<23 \mu\text{g/L}$

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Abstract: Postmortem trypsin is a useful biochemical test to aid the diagnosis of anaphylaxis. Multiple perimortem and postmortem factors have been documented to cause an elevation in postmortem trypsin level. One factor that was recently recognized to have an impact on postmortem trypsin level is correct sampling technique. A recent study recommended aspirating blood samples from a clamped femoral/external iliac vein to be used for reliable postmortem trypsin analysis. This study sampled 120 consecutive nonanaphylactic deaths in which all the peripheral bloods were sampled as recommended. Postmortem interval, resuscitation, different nonanaphylactic causes of death, sex, and age did not show any statistical significant relation to postmortem trypsin level in Student *t* test, Pearson correlation, and univariate and multivariate analyses. The mean (SD) postmortem trypsin level was 8.4 (5.2) $\mu\text{g/L}$ (minimum, 1.0 $\mu\text{g/L}$; maximum, 36.1 $\mu\text{g/L}$; median, 7.3 $\mu\text{g/L}$). Using nonparametric methods, the postmortem trypsin reference range in nonanaphylactic death was established as $<23 \mu\text{g/L}$ (97.5th percentile).

Key Words: anaphylaxis, postmortem, trypsin, sampling, venous, arterial, aspirate, cutdown

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Measuring mast cell trypsin in serum is a biochemical test used to aid in the diagnosis of anaphylaxis in both clinical and postmortem settings.^{1–5} The clinically accepted upper limit for trypsin (11.4 $\mu\text{g/L}$)⁶ is not applicable in the postmortem setting, and the proposed postmortem cutoff of around 45 $\mu\text{g/L}$ is used as a

threshold for postmortem biochemical diagnosis of anaphylaxis.^{2,7–10} The reason is that many nonanaphylactic causes of death and peri/postmortem conditions can increase postmortem trypsin levels.^{1,4,5,8,10–15} Trauma, ischemic heart disease, aortic dissection, drug-related death, inflammatory conditions, sudden infant death syndromes, increased postmortem interval (PMI), presence of resuscitation, and different sampling sites have been reported to cause a change in postmortem trypsin levels.^{1,3–5,8,10–18} To diminish the postmortem processes effecting postmortem trypsin levels, the current literature recommends the acquisition of femoral blood, a peripheral blood sample, for biochemical analysis but does not specify the exact sampling method.^{4,5,19} A recent study showed that the method of sampling femoral blood can effect postmortem trypsin levels,²⁰ but this was not accounted for in previous studies.^{2,7–10} A subsequent study on the impact of sampling technique on postmortem trypsin suggested that direct visualizing and clamping the femoral/external iliac vein followed by aspirating the vein is the most reliable and consistent method.²¹ This given study was subsequently carried out to establish the postmortem trypsin reference range using 120 consecutive nonanaphylactic deaths in which femoral blood was sampled by aspirating the clamped femoral vein only.

MATERIALS AND METHODS

This study was performed at the Department of Forensic Pathology, LabPLUS, Auckland City Hospital, and approved by the Chief Coroner of New Zealand at the Coroner's Office. All collections of postmortem samples in this study are authorized by the coroner. All biochemical investigations were performed as part of the examination. One hundred twenty consecutive femoral samples collected for postmortem trypsin analysis were used in this study. All postmortem examinations were performed by forensic pathologists, and all blood samples were collected by either the attending pathologists or qualified technical staffs. The venous femoral blood sampled for postmortem trypsin analysis was collected during evisceration by the recommended sampling method.²¹ A clamp was placed on the external iliac vein, and femoral blood was collected by aspirating the femoral/external iliac vein, distal to the clamp, with a sterile single-use 18-gauge needle and a 10- or 30-mL syringe as close to the inguinal ligament as possible with expression of the femoral vein if required.

The age, sex, cause of death, PMI, and presence of resuscitation were recorded as potential confounding aspects. The PMI was determined as the time between the deceased being declared dead and commencement of postmortem examination. The causes of deaths were stratified into 2 groups being (a) causes of death that are documented to cause an elevation in postmortem trypsin and (b) those

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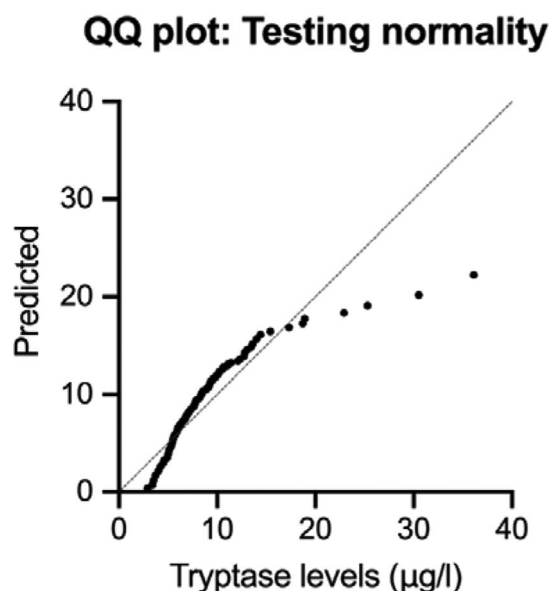


FIGURE 1. Distribution of all postmortem tryptase levels measured in this study with a QQ plot to determine normality.

that are not. Causes of death that are documented to cause an elevation in postmortem tryptase include asphyxia, ischemic heart disease, artery dissection, death from inflammatory conditions (eg, infection), trauma (chest involved), and opioid-related death.

Postmortem Tryptase Analysis

Samples collected were immediately transferred to serum separator collection tubes. The collected blood tubes were cooled and sent to an onsite local accredited laboratory (Department of Immunopathology, LabPLUS, Auckland City Hospital) for tryptase analysis. The separated serum was analyzed within 1 day using a commercially available sandwich immunoassay (ImmunoCAP) on a Phadia 250 analyzer (Thermo Fisher Scientific, Fyrislund, Sweden). Only total tryptase (sum of α and β) is available as a commercial laboratory assay in New Zealand.

Exclusion Criteria

1. All suspicious and homicide deaths, due to medicolegal implications
2. Any cases in which there was insufficient femoral blood samples, such as cases with advanced decomposition, incinerated bodies, and traumatic deaths with exsanguination
3. Any cases in which the samples were clotted or showed signs of hemolysis and were therefore not suitable for analysis

Statistical Analysis and Reference Range Determination

Statistical analysis was performed using R statistics (open source; version 3.4.1; 2017 The R Foundation for Statistical Computing) and PRISM 8 software (GraphPad, La Jolla, California), and a P value of <0.05 was considered significant.

Continuous variables were described using means and SD. Medians, minima (min), and maxima (max) are also presented. Categorical variables are described using counts.

To examine confounding factors for postmortem tryptase level, Pearson correlation coefficients were analyzed for continuous variables (age, PMI), and t test between dichotomous variables (sex, presence of resuscitation, and causes of death) after using a QQ plot for testing on normality showing bulk of the data following a linear line,

with occasional outliers (Fig. 1). Univariate and subsequent multivariate linear regression analyses were used to determine the association of postmortem tryptase level with age, sex, PMI, and cause of death.

A previous study assumed a γ distribution for postmortem tryptase; thus, a nonparametric method in establishing a reference range was used.¹⁰ The reference interval, confidence interval (CI), and sample size for this reference interval study were determined using the nonparametric method as described in the CLSI EP28-A3c guideline.²² The minimal sample size required to establish a reference range is 20, with 120 being the standard. Nonparametric method was used to increase the robustness of our results. The reference interval is established by determining the 2.5th percentile and 97.5th percentile of the sampled population and corresponding 90% CI on both end spectra.²²

RESULTS

The original data for this study are included in the Supplementary Data, <http://links.lww.com/FMP/A32>. In the 120 cases sampled (male-to-female ratio, 79:41), the mean (SD) age was 54.4 (17.2) years (min, 18 years; max, 94 years; median, 56 years), the mean (SD) PMI was 34.3 (21.7) hours (min, 7 hours; max, 144 hours; median, 27.5 hours), and 71 cases had documented resuscitation. Sixty-eight cases were classified to have causes of death that are documented to cause an elevation in postmortem tryptase (37 cases of coronary artery atherosclerosis, 20 cases of asphyxia death, 2 cases of pneumonia, 1 case of acute pyelonephritis, 3 cases of aortic dissection, 3 cases of trauma, 1 case of bone cement syndrome, and a case of coronary artery dissection), and the remaining 52 were classified as death not causing postmortem tryptase elevation.

Pearson correlation coefficients between total postmortem tryptase with age and PMI were 0.004 ($P = 0.96$) and 0.108 ($P = 0.24$), respectively (Fig. 2). The mean postmortem tryptase level in male sex was 8.75 $\mu\text{g/L}$, that in female sex was 7.6 $\mu\text{g/L}$ ($P = 0.29$). The mean postmortem tryptase level in death with documented resuscitation was 8.6 $\mu\text{g/L}$, and that without was 8.1 $\mu\text{g/L}$ ($P = 0.61$). The mean postmortem tryptase level in causes of deaths that were documented to cause an elevation in postmortem tryptase was 7.7 $\mu\text{g/L}$, and that was not was 9.3 $\mu\text{g/L}$ ($P = 0.13$). See Figure 3 for graphical illustration.

The coefficients for sex, age, PMI, presence of resuscitation, and cause of death with postmortem tryptase as the outcome in the univariate analysis were 0.97 ($P = 0.34$), 0.00 ($P = 0.96$), 0.02

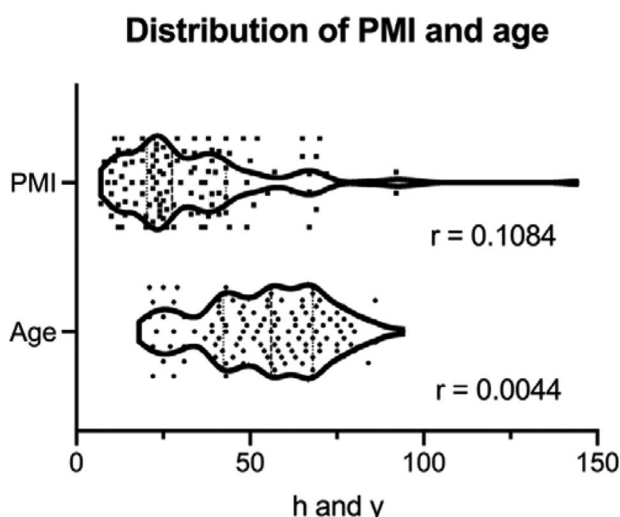


FIGURE 2. Missing correlation between postmortem tryptase levels and age of the deceased as well as their PMI until sampling.

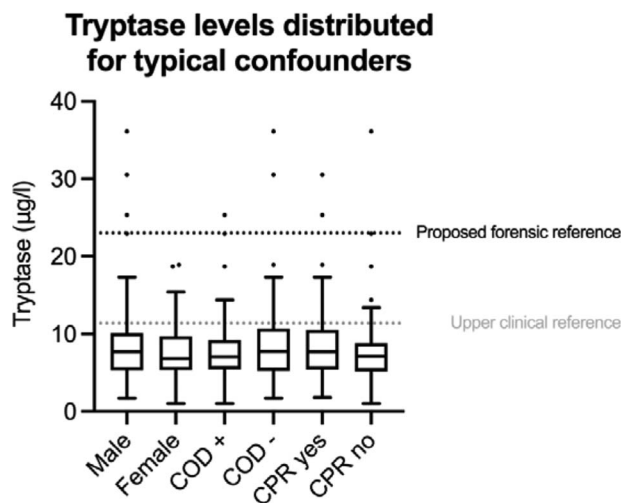


FIGURE 3. No relevant differences in postmortem tryptase levels for sexes, causes of death (COD) known to potentially elevate tryptase (COD+), and presence of cardiopulmonary resuscitation (CPR).

($P = 0.24$), 0.51 ($P = 0.61$), and -1.56 ($P = 0.11$), respectively. The coefficient for sex, age, PMI, presence of resuscitation, and cause of death in the subsequent multivariate calculation were 1.22 ($P = 0.24$), 0.01 ($P = 0.75$), 0.03 ($P = 0.22$), 0.43 ($P = 0.66$), and -1.68 ($P = 0.09$), respectively.

The mean (SD) postmortem tryptase was 8.4 (5.2) $\mu\text{g/L}$ (min, 1.0 $\mu\text{g/L}$; max, 36.1 $\mu\text{g/L}$; median, 7.25 $\mu\text{g/L}$). The 2.5th and 97.5th percentile for postmortem tryptase were 2.3 $\mu\text{g/L}$ (90% CI, 1–2.9 $\mu\text{g/L}$) and 23.0 $\mu\text{g/L}$ (90% CI, 17–36 $\mu\text{g/L}$), respectively. Because there is no known forensic value of a low postmortem tryptase in nonanaphylactic deaths, the reference value derived in this study was $<23.0 \mu\text{g/L}$.

DISCUSSION

This study established a reference range of $<23 \mu\text{g/L}$ in postmortem tryptase in nonanaphylactic deaths. Age and PMI did not show any significant correlation with total postmortem tryptase levels. There were no significant differences in total postmortem tryptase level between sex, presence of resuscitation, and causes of death. The regression coefficients for age, sex, PMI, presence of resuscitation, and causes of death on tryptase levels in the univariate and subsequent multivariate analyses were overall low and did not reach statistical significance.

This study is unique in many ways in comparison with other postmortem tryptase studies. It systematically established a reference range rather than a “critical” threshold, addressed a range of confounding factors (ie, PMI, resuscitation, different causes of death that were reported to increase postmortem tryptase), and most importantly used only one recommended blood sampling technique.

The use of peripheral cadaveric blood samples for postmortem tryptase is recommended in the literature.^{4,5,19} The rationale to do so is to decrease the effects of perimortem and postmortem artifacts. Conditions such as resuscitation, chest trauma, increased PMI, aortic dissection, inflammation, and coronary artery atherosclerosis were postulated to increase tryptase by cell lysis in visceral mast cell-rich organs.^{4,5,19} In 1998, Edston and van Hage-Hamsten^{14,23} first documented a difference in total postmortem tryptase in different sampling sites noting that heart blood appears to be elevated compared with femoral blood and raised the possibility of postmortem diffusion from mast cell-rich organs such as the lung or stomach due to cell lysis. They were the first to

recommend that femoral blood should be sampled for postmortem tryptase.²³ A follow-up study by Edston et al¹⁰ subsequently confirmed that heart blood definitely had elevated postmortem tryptase compared with femoral blood, rendering it hard to interpret. Thus, analyzing peripheral blood samples (in particular femoral vessel) is now recommended.^{4,5,19} However, the femoral sampling method in the context of postmortem tryptase analysis is not well described and seems variable between studies. Standard forensic textbook describes a range of femoral/peripheral blood sampling methods in which none is preferred.²⁴ Recent studies determining postmortem tryptase cutoffs^{3,7,8} had specified the site (femoral, aortic, subclavian) and type of vessel used for blood sampling (artery or vein), but the description on the actual method used for sampling is lacking. Difference in sampling technique on analysis is slowly being recognized and now demonstrated in toxicological blood samples²⁵ and recently in tryptase analysis.²⁰ A recently study recommended clamping and aspirating femoral/external iliac vein as the recommended sampling method for postmortem tryptase analysis, which is the same as for toxicological analysis.^{21,25} The reason for this is to prevent arterial contamination and backflow of central caval blood to the femoral/peripheral blood samples.²¹

Confounding Factors

Use of postmortem tryptase in the postmortem setting is riddled by confounding factors. In 1995, Randall et al¹³ noted that postmortem tryptase can be spuriously elevated (up to 106 $\mu\text{g/L}$) in 49 nonanaphylactic deaths, and occasional case reports of sudden infant and heroin deaths were reported to cause increased postmortem tryptase.^{26,27} This was followed by a series of studies by Edston and van Hage-Hamsten,^{14,23} who initially noted that the postmortem tryptase elevates in traumatic deaths. Edston et al¹⁰ further noted that asphyxia death elevated postmortem tryptase level, but resuscitation did not. That is in good comparison with the results presented here. Horn et al¹ noticed that increased PMI is associated with increased postmortem tryptase in the femoral vein. He further noted that chest trauma (inferring resuscitation) and coronary artery atherosclerosis were also associated with increased postmortem tryptase. Recently, Mayer et al,² McLean-Tooke et al,³ Xiao et al,⁸ and Tse et al⁷ showed increased total postmortem tryptase levels in hemolyzed samples, cardiac deaths, deaths from aortic dissection, inflammatory/infective causes of death, and traumatic deaths. However, Tse et al⁷ was unable to demonstrate that PMI and resuscitation were associated with a clinically significant increase in postmortem tryptase, which is again reproduced in this given study. This further agrees with a recent study by Woydt et al,¹⁷ who showed that serial sampling for postmortem tryptase showed a clinically insignificant elevation in nonanaphylactic death only. Age and sex have never been shown to have any association with an increase in postmortem tryptase.

This study did not show any statistically significant association or difference with age, sex, PMI, presence of resuscitation, and causes of death with respect to tryptase, and is contrary to some studies in the literature. It is possible that during the PMI, the process of decomposition causes cell lysis releasing tryptase and subsequently diffuses into the blood. However, its effect is likely negligible provided that the sample is not contaminated by central blood and no hemolysis had occurred as long as the recommended sampling method is used. Thus, sampling blood for postmortem tryptase analysis should still be done as early as possible to avoid the effects of postmortem hemolysis and cytolysis. Resuscitation and chest trauma cause tryptase increase, secondary to trauma to mast cell-rich visceral organs, and are more pronounced when defibrillation is used.^{4,5,19} Although our study did not stratify for the use of defibrillation in detail, postmortem tryptase was not elevated in individuals who have received resuscitation

compared with those who have not. A possible reason is that trauma to the visceral mast cell-rich organs causes a release in tryptase but is initially circulated in the arterial phase or back flowed from caval blood during postmortem, and by clamping and sampling from femoral vein, this artifact can be minimized.

In terms of cause of death, our study did not have any heroin deaths or any infant deaths. However, in the deaths from coronary artery atherosclerosis, death from artery dissection, inflammatory/infective causes, and asphyxia, none showed a relevant increased postmortem tryptase. It is possible that these conditions do result in increased tryptase according to former publications, but either are negligible or stay in the arterial circulation or central venous blood and not reaching the peripheral venous system. Thus, it is possible that the reported spurious increase in postmortem tryptase in nonanaphylactic death can be due to the aforementioned factors but can be significantly muted by adequate sampling technique. During the time of this study, we encountered one antibiotic-related anaphylactic death in which the deceased had a circulatory collapse shortly after the administration. Postmortem tryptase (collected with the recommend method) was $>100 \mu\text{g/L}$, supporting the autopsy diagnosis of anaphylaxis. This case gives evidence that the recommended sampling technique does not “mute” the effects of anaphylaxis in general but only affects potential confounding factors.

Reference Range

This study established a reference range for postmortem total tryptase level in nonanaphylactic death with $<23 \mu\text{g/L}$. In our cases, not a single death had a postmortem tryptase level above the recommended threshold in the literature of approximately $45 \mu\text{g/L}$, and this is contrary to previous studies in which the sampling method of femoral blood was not specified.^{1–3,7,8,23} This reference range is more reliable and less controversial than the previous studies. Nonetheless, this reference range must be used within the context of the wider pathological examination and findings and should not be used by itself to exclude or confirm anaphylaxis.

Previous studies focused on establishing a threshold for postmortem tryptase documented a range from <10 to $>1000 \mu\text{g/L}$ in nonanaphylactic deaths.^{1–3,7,8,23} The clinical threshold for postmortem tryptase was set at $11.4 \mu\text{g/L}$ by Yunginger et al⁶ in 1991. However, previous postmortem studies showed that many nonanaphylactic causes of death and peri/postmortem processes can increase postmortem tryptase, rendering the clinical threshold not translatable to postmortem setting.^{10,13,14,26} Since the 1990s, numerous studies attempted to establish a threshold for anaphylactic death, but the sampling methods between the studies were not uniform. Some studies would simply report that “femoral vessel blood” was sampled, some would indicate femoral vein or artery was sampled but does not specify the exact method, and some would use a mixed method in sampling femoral blood. For example, Edston and van Hage-Hamsten²³ reported that in asphyxia and sudden cardiac deaths, the postmortem tryptase in femoral vein was up to $57 \mu\text{g/L}$, but did not specify the sampling method. Horn et al¹ reported serum tryptase of almost $200 \mu\text{g/L}$ in transcutaneous sampling of femoral blood but did not record whether the blood was arterial or venous. Mayer et al² reported that serum tryptase in femoral vein of a traumatic death was $143 \mu\text{g/L}$, but did not specify the sampling method. Xiao et al⁸ reported a postmortem tryptase of up to $188 \mu\text{g/L}$ in death from acute coronary syndrome in femoral vein, but again did not specify the sampling method. Mclean-Tooke et al³ reported a postmortem tryptase of up to $1453 \mu\text{g/L}$ in femoral arteries in cardiac deaths, but again did not specify the sampling method. Tse et al⁷ reported a postmortem tryptase level of $67 \mu\text{g/L}$ in the femoral vein in nonanaphylactic death with a mixed method of sampling.

Furthermore, a recent meta-analysis performed did not include the method of sampling in their study.²⁸ A difference in femoral blood sampling on postmortem tryptase was first reported in 2018 by Tse et al,²⁰ who showed a difference between blood samples collected via transcutaneous sampling and internal collection. Garland et al²¹ subsequently compared different sampling methods and noted spurious tryptase elevation in aspirated arterial and venous cutdown samples. The reason was probably due to contamination from arterial blood and backflow from caval blood. It was observed that clamping of the femoral vein seemed to give a steadier postmortem tryptase level and recommended that method for further use and as rationale for this given study.

This study subsequently used the recommended sampling method for postmortem tryptase analysis and established a reliable reference range with no spurious elevation. With our observation, it is very probable that previous studies had overestimated the postmortem tryptase threshold, which limited its use in postmortem setting. Thus, when a strict sampling method is used, which prevents arterial contamination and backflow of caval blood, it is probable that the threshold for postmortem tryptase would be lower than previously documented, with corresponding improvement in sensitivity and specificity of the laboratory test. Further studies in reestablishing the cutoff for postmortem tryptase to diagnose anaphylaxis is strongly indicated.

Limitations

Study Methodology and Sample Cases

The design and data collection of this study have a few limitations. First, it is important to stress that this study was aimed to establish a reference range for nonanaphylaxis death (and also analyzing potential confounding factors) and not a critical threshold to diagnose anaphylaxis. To establish a threshold, anaphylactic and/or anaphylactoid deaths should be analyzed (with blood sampled using the recommended method). Thus, although can be used as a guide, the reference range established in this study cannot be definitively used to diagnose anaphylaxis.

In this study, the exact type (eg, presence of defibrillation) and timing of resuscitation were not analyzed, and the effects of PMI are not fully explored. The PMI was taken as the time between when the body was found and postmortem examination. Strictly speaking, it is not the exact PMI (ie, time between death and postmortem examination). Also, the effect of body storage, such as refrigeration time and temperature, was not explored. This study, because of technical and ethical constraints, is unable to do serial sampling for postmortem tryptase analysis. With an overall low postmortem tryptase level, no difference in postmortem tryptase level in groups with and without resuscitation, no significant association with PMI and postmortem tryptase levels, and insignificant coefficients in the univariate and multivariate analyses, resuscitation and PMI would at most cause only insignificant clinical difference in the interpretation of postmortem tryptase.

This study did not investigate whether body handling has impacts on postmortem tryptase. Although there is no difference being face up or down, body positions on an incline may possibly affect total postmortem tryptase levels, especially when the working hypothesis is that backflow of venous caval blood may cause an increase in postmortem tryptase. The authors recommend that clamping and limiting the degree of inclination of the upper torso may prevent backflow of caval blood.

Use of Total Tryptase

The use of β -isoform for the diagnosis of anaphylaxis is recommended in the literature⁴; however, it is not commercially

available in Australia and New Zealand. The β -isoform is only secreted after an anaphylactic stimulus, which increases in proportion to total tryptase, and is thought to be more specific in diagnosing anaphylaxis than total tryptase.¹ Our study used total tryptase (α - and β -isoforms) and not its β -isoform, which may limit the application of our results.

CONCLUSION AND RECOMMENDATION

This study established the postmortem tryptase reference range using 120 consecutive nonanaphylactic deaths being $<23 \mu\text{g/L}$ in which the recommended femoral blood sampling method was used only (direct visualization followed by clamping and aspirating the femoral/external iliac vein). Age, sex, PMI, presence of resuscitation, and causes of death are not associated with any significant differences and correlation with postmortem tryptase level. The reference range is below, with no cases over, the former reported postmortem threshold ($\sim 45 \mu\text{g/L}$), which is contrary to all previous studies.

It is possible that previously reported elevated postmortem tryptase level in nonanaphylactic deaths is at least partially related to sampling technique. Given our results, it is likely that the accepted threshold for postmortem tryptase can be lower than previously documented with corresponding improvement in sensitivity and specificity when the strict sampling method is used. This reference range is part of the wider context of pathological examination and findings and does not independently exclude or confirm anaphylaxis.

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