Suicide or not? Issues in the demonstration of anaphylaxis, a review of the literature

Veronika Králíková[1,2], Adriana Gavronová[1,2], Lukáš Hamerlik[1,2], Václav Svrchokryl[1,2], Marek Vitovják[1,2], Hajin Kim[3], Veronika Šidová[4,5], Jana Bednaříková[4,5], Martin Dobiáš[1,2]

[1] Ústav soudního lékařství a medicínského práva Fakultní nemocnice Olomouc
[2] Ústav soudního lékařství a medicínského práva Lékařské fakulty Univerzity Palackého v Olomouci
[3] Lab of Forensic Medicine, Department of Anatomy and Cell Biology, Sungkyunkwan University School of Medicine, Republic of Korea
[4] Ústav imunologie Lékařské fakulty Univerzity Palackého v Olomouci
[5] Ústav imunologie Fakultní nemocnice Olomouc

SUMMARY
The authors review the literature on the determination of post-mortem serum tryptase values and present the case of a young man who was hit by a train. However, his family believes he has no motivation to commit suicide. Collision with a train is one of the most common methods of suicide, especially among young men under 40 years of age. (1). The forensic autopsy showed that the man died due to the collision with the train, with traumatic hemorrhagic shock stated as a cause of death. Following toxicological, biochemical, and immunological tests created a supposition that the incident was not a result of suicidal action but a consequence of a possible allergic or anaphylactic reaction of the organism combined with a state of mild alcohol intoxication.

Keywords: tryptase – post mortem tryptase – suicide – anaphylactic reaction

Sebevražda nebo nehoda? Problematika anafylaxe s analýzou odborné literatury

SOUHRN
Autoři poskytují přehled literatury zabývající se problematikou stanovení post-mortem hodnot sérové tryptázy a prezentují případ mladého muže, který byl sražen vlakem, ačkoliv podle jeho rodiny neměl k sebevraždě žádnou motivaci. Srážka s vlakem je jedním z nejčastějších způsobů sebevražedného jednání, zejména u mladých mužů, přičemž skok pod auto nebo vlak představuje až 10 % všech dokonaných sebevražd u mužů mladších 40 let (1). Nařízená soudní pitva prokázala, že muž zemřel následkem srážky s vlakem úrazově-krvácivým šokem. Provedená toxikologická, biochemická a imunologická vyšetření posílila předpoklad, že ke smrti došlo nikoliv v důsledku sebevražedného jednání, ale v důsledku nehody při možné alergické či anafylaktické reakci organismu v kombinaci se stavem mírné alkoholové opilosti.

Klíčová slova: tryptáza – post-mortem tryptáza – sebevražda – anafylaktická reakce

Soud Lek 2023; 68(3): 30–32

Tryptase is a serine protease mainly synthesized and released by mast cells and can be used as a marker for mast cell number (α-tryptase) and the grade of mast cell activation (β-tryptase). (2) The routine examination includes both forms as so-called total tryptase. Serum mast cell tryptase is a reliable indicator in the postmortem diagnosis of anaphylaxis. (3,4) Tryptase shows relatively good stability postmortem in serum and could be measured up to four days after death. (5). The study by Horn et al. demonstrated a median increase of 6-8.8 µg/L up to two days after death. (6) Due to the publication by the Mayer group, levels of system tryptase in anaphylaxis can reach a value of up to 880 µg/L. (7)

✉ ***Correspondence address:***
MUDr. Martin Dobiáš, Ph.D.:
Ústav soudního lékařství medicínského práva FN a LF UP v Olomouci
Hněvotínská 3, 779 00 Olomouc
tel.: 585 632 603
e-mail: martin.dobias@fnol.cz

Received: April 25, 2023.
Accepted: July 13, 2023.

CASE REPORT

On 6 October 2019 at 4:40 a.m., a train set consisting of a locomotive with four wagons collided with a 25-year-old man on track. The man was seated on the left rail, his head bent towards the ground and his feet facing the track. Immediately after spotting the person on the track, the train driver used the warning horn and applied the speed brake, despite which the train was stopped up to 200 m from the point of collision with the person. The ambulance doctor called to the scene and stated that there were devastating injuries to the whole body incompatible with life. The police's Crime Scene Investigation Report states, „...The body is severely deformed in the right and left hip and thigh areas. There are large lacerations, deformation of the legs at the knees, missing pieces of tissue..."

On October 9, 2019, in the Official Record, the deceased's mother told the investigating officer that she ruled out the possibility of her son committing suicide. She states that knowing her son, he would not have committed suicide and had no reason to do so. She further said that her son was medically stable but was allergic to bees and wasp stings and carried pills with him in case he was stung by a wasp or bee.

The deceased's medical records show he sought medical treatment for a wasp sting in 2015. He observed facial swelling, skin

tension, body exanthema, and breathing difficulties 30 minutes after the sting to the toe of his right foot. Internal medicine department examination concluded a generalized allergic reaction to the wasp sting. A medical report prepared by an immunologist, whom the deceased had seen regularly, stated, among other things, that at the first sign of a wasp or bee sting, he should lie down on the floor, take two tablets of Prednisone 20 mg, try to remove the sting and at the first sign of a systemic reaction take Emerade in the upper outer quadrant of the thigh and call the ambulance.

Autopsy findings

The external and internal examination revealed almost complete separation of the right lower limb in the thigh area as the most severe injury, followed by open fractures of the right tibia, practically complete separation of the left lower limb in the midthigh area, and rib fractures in several anatomical lines, fracture of the right scapula, contusion of the lungs, liver, spleen, and contusion of the right adrenal gland with the presence of 200 ml of liquid blood in the left and 150 ml of liquid blood in the right pleural cavity and 400 ml of blood in the abdominal cavity, fractures of the cranial bones, contusion of the cerebral pituitary gland.

All the relevant injuries on the deceased's body showed signs of a vital reaction and occurred during his lifetime.

The immediate cause of the man's death was a traumatic hemorrhagic shock, which had developed in particular due to almost complete traumatic amputations of the lower limbs in the thighs, rib fractures, and injuries to the organs of the thoracic and abdominal cavities.

Furthermore, toxicological examination revealed 1.31 ‰ (g/kg) of ethanol and 0.01 ‰ (g/kg) of n-propanol in the blood of the deceased and 2.37 ‰ (g/kg) of ethanol and 0.01 ‰ (g/kg) of n-propanol in the urine. No addictive substances of amphetamines, benzodiazepines, cannabinoids, cocaine with metabolites, or opiates were detected in the urine. No analgesic, hypnotic, psychopharmaceutical drugs or other drugs of toxicological significance or their metabolites were detected in urine, gastric contents, intestinal contents, liver, and kidney tissue. Only nicotine with metabolite (cotinine) and caffeine with metabolites (theophylline and theobromine) were detected in the blood.

The above results show that the deceased was negatively affected by the ethanol, and in the period immediately before death, he was in a state of mild alcoholic intoxication, which is usually characterized by a slight decrease in judgment and attention, increased self-confidence, and multilingualism.

Examination of tryptase at the Institute of Immunology revealed a serum with a resulting concentration of 160 ug/l by fluorescence immunoassay (FEIA).

A sample was collected from the femoral vein 54 hours after death.

Mechanism of death

The external and internal examination revealed that the injuries were mainly caused by a massive blunt or blunt-edged external mechanical force applied to the deceased's body from several directions. It is typical for railway accidents, where the victim's body is run over and eventually thrown off by a passing railway vehicle. All the injuries found on the deceased's body can be satisfactorily explained by the collision with the railway train, the almost complete amputation of the lower limbs being caused by them being run over by the railway carriage wheels.

Objectively demonstrated, significantly elevated tryptase values indicate a probability of an ongoing allergic or anaphylactic reaction of the organism, which the deceased may have been adversely affected by at the time of the accident in question.

DISCUSSION

Tryptase is a relatively stable (8) biochemical marker that should be tested when an allergic or anaphylactic reaction is suspected, even in deceased persons up to 4 days after death (5). Combining results for tryptase with a more specific assay for allergen sensitivity, such as specific serum IgE may prove more conclusive. (6) Total serum Ige with tryptase serum levels may support a postmortem diagnosis of anaphylaxis. (9-11) However, the interpretation of IgE postmortem levels faces several problems. The value of total IgE may be elevated in many atopic individuals during the season, along with the amount of allergen or duration of allergen exposure. (6) Postmortem level of mast cell chymase was also discussed in the study performed by Nisho et al. (12)

Under physiological conditions, the serum tryptase level is low, averaging 3.4 µg/l. Basal levels of tryptase in the range of 10-20 µg/l at rest are associated with an increased risk of severe allergic reactions and reflect an increased mast cell burden. Tryptase elevation is caused by massive systemic mast cell activation. After the mast cell activation, tryptase levels rise sharply. The maximum concentration is reached within 15-120 minutes after the onset of the reaction, and during the following 3 - 6 hours, its value slowly decreases. A return to baseline concentrations usually occurs within 12 - 24 hours after the reaction. Tryptase levels are directly proportional to the severity of anaphylaxis. (8)

Interpretation of the results of antemortem and postmortem determinations differs substantially. (13) Tryptase shows relatively good stability postmortem in serum. However, the association with postmortem elevated tryptase values has been discussed in the literature leading to questioning the appropriateness of this parameter. (14,15)

Postmortem elevated values can be seen mainly in anaphylactic reactions but also to some extent in myocardial infarction, SIDS, trauma, especially fractures, after heroin administration. (14) The 1998 Edston group study reported median tryptase values in a control group of deceased patients of 6 µg/l ranging from 2 to 42 µg/l. Due to anaphylactic/anaphylactoid reaction, the deceased group showed a higher median of 23 µg/l ranging from 11 - 37 µg/l. A value in the normal reference range of 6 µg/l was measured in an isolated case of food allergy. (14) Other studies have reported much higher tryptase values for anaphylactic reactions, detectable only after serum dilution. A 2011 publication by the Mayer group describes extremely high values in anaphylaxis, up to 880 µg/L. (7)

The Edston group study observed tryptase levels in trauma-related deaths with a mean of 227±146 µg/l. Those samples were collected from the site of tissue injury (14). An explanation of the mechanism is mast cell degranulation as an early response to trauma. It can be caused by mast cell lysis due to local mechanical trauma or trauma-induced hemolysis, and bleeding may be accompanied by mast cell lysis. (16)

In cases of death due to trauma, values may be elevated to various levels compared to physiological values in a significant proportion of the population. In a 2020 article, Garland et al. reported that multi-trauma and short survival time after an accident might increase tryptase levels. In particular, devastating injury to the lungs and gastrointestinal tract leads to an elevation of this parameter. (17)

A modest increase in the parameter over time has been demonstrated in the literature in a control group of decedents without anaphylactic reaction. (17,18) The study by Horn et al. demonstrated a median increase of 6-8.8 µg/L up to two days after death. The increase in the parameter with prolonged

post-mortem interval is due to lysis and degranulation of tryptase-containing mast cells. The increase in the parameter is obvious but not essential for data interpretation. (6)

Studies between 1990 and 2000 have also demonstrated the importance of the sample collection site. (18) A survey by Edson et al. in 1998 and 2007 demonstrates differences in tryptase levels between samples taken from the heart and femoral vein, with the heart showing higher tryptase levels. For reproducibility reasons, sampling from the femoral vein is usually recommended. (18)

Postmortem examination of tryptase is essential for diagnosing anaphylactic and anaphylactoid death, even in trauma cases. However, the values must be assessed individually and in the context of the circumstances. (19)

Every year, almost 1 400 people commit suicide in the Czech Republic, of whom more than 80 % are men. (1) At our workplace in Olomouc, we encounter various types of suicides. Despite the work of the police, it is sometimes difficult to distinguish whether it is an accidental death or a completed suicide. Several markers can be used to exclude accidental death due to health problems, for example, to determine whether an anaphylactic or anaphylactoid reaction has occurred in the deceased. A suitable parameter for investigation is tryptase, a stable mediator released from mast cells after their activation by the allergen. This marker can bring essential information to the case under investigation.

In our case in the context of the deceased's previous medical report and the mother's testimony, it was essential to prove or disprove an allergic reaction that, combined with alcohol, could have affected the deceased's behavior before the train impact.

This was to be done by the indicated immunological testing of serum tryptase. Although in the case we presented, it was impossible to detect the bee or wasp sting location due to body damage, tryptase examination could explain why a young man with no motive for suicidal action was sitting on the train track and did not respond to the train horn.

CONCLUSION

The diagnosis of anaphylaxis may seem difficult in the case report described above, mainly because there is no evidence of a sting. Therefore, considering all the facts reported, an interdisciplinary approach is necessary for a correct interpretation. Given the high tryptase value in the deceased's serum, it is probable that an anaphylactic or anaphylactoid reaction occurred, which may have affected the dead's behavior and judgment. It is important to note, that the examination of the sample occurred 54 hours after death, which made it virtually impossible to interpret other markers that might have better specified the cause of the elevated tryptase. Interpretation of post-mortem tryptase levels faces a dearth of literature, and further studies are needed to quantify the destruction of body structures and the amount of tryptase measured.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

Poznámka redakce

Jeden z recenzentů vyslovil kritické připomínky k některým závěrům článku, s publikováním v časopise však souhlasil.

REFERENCES

1. **Czech statistical office**, 2022 [online]. ČSÚ. [cit. 18.10.2022]. Dostupné z: https://www.czso.cz/csu/czso/sebevrazdy_zaj.
2. **Schwartz LB, Sakai K, Bradford TR, Ren S, Zweiman B, Worobec AS, Metcalfe DD.** The alpha form of human tryptase is the predominant type present in blood at baseline in normal subjects and is elevated in those with systemic mastocytosis. *J Clin Invest* 1995; 96(6): 2702-2710.
3. **Schwartz LB, Metcalfe DD, Miller JS, et al.** Tryptase levels as an indicator of mast-cell activation in systemic anaphylaxis and mastocytosis. *N Engl J Med* 1897; 316: 1622-1626.
4. **Schwartz LB, Younginger JW, Miller J, Bokhari R, Dull D.** Time course of appearance and disappearance of human mast cell tryptase in the circulation after anaphylaxis. *J Clin Invest* 1989; 83: 1551-1555.
5. **Wilkins B.** Guidelines on Autopsy Practice: Autopsy for Suspected Acute Anaphylaxis (Includes Anaphylactic Shock and Anaphylactic Asthma) [(accessed on 20 January 2021)]; 2018 Available online: https://www.rcpath.org/uploads/assets/47841b6b-891f-450a-b968889ff3e0a7d1/G170-DRAFT-Guidelines-on-autopsy-practice-autopsy-for-suspected-acute-anaphalaxis-For-Consultation.pdf.
6. **Horn KD, Halsey JF, Zumwalt RE.** Utilization of serum tryptase and immunoglobuline assay in the postmortem diagnosis of anaphylaxis. *The American journal of forensic medicine and pathology* 2004; 25(1): 37-43.
7. **Mayer DE, Krauskopf A, Hemmer W, Moritz K, Jarisch R, Reiter C.** Usefulness of post mortem determination of serum tryptase, histamine and diamine oxidase in the diagnosis of fatal anaphylaxis. *Forensic science international* 2011; 212(1-3): 96-101.
8. **Payne, V, Kam PCA.** Mast cell tryptase: a review of its physiology and clinical significance. *Anaesthesia* 2004; 59: 695-703.
9. **Schwartz HJ, Squillace DL, Sher TH, et al.** Studies in stinging insect hypersensitivity: postmortem demonstration of antivenom IgE antibody in possible sting-related sudden death. *Am J Clin Pathol* 1986; 85: 607-610.
10. **Hoffman DR, Wood CL, Hudson P.** Demonstration of IgE and IgG antibodies against venoms in the blood of victims of fatal sting anaphylaxis. *J Allergy Clin Immunol* 1983; 71: 193-196.
11. **Younginger JW, Nelson DR, Squillace DL, et al.** Laboratory investigation of deaths due to anaphylaxis. *J Forensic Sci* 1991; 36: 857-865.
12. **Nishio H, Takai S, Miyazaki M et al.** Usefulness of serum mast cell–specific chymase levels for postmortem diagnosis of anaphylaxis. *Int J Legal Med* 2005; 119: 331–334.
13. **Palmiere C, Mangin P.** Postmortem chemistry update Part II. *International Journal of Legal Medicine* 2012; 126(2): 199-2015.
14. **Edston E, van Hage-Hamsten M.** beta-Tryptase measurements post-mortem in anaphylactic deaths and in controls. *Forensic science international* 1998; 93(2-3): 135-142.
15. **Woydt L, Bernhard M, Kirsten H. et al.** Intra-individual alterations of serum markers routinely used in forensic pathology depending on increasing post-mortem interval. *Sci Rep* 2018; 8: 12811.
16. **Edston E, van Hage-Hamsten M.** Mast cell tryptase and hemolysis after trauma. *Forensic Sci Int* 2003; 131: 8–13.
17. **Garland J, Ondruschka B, Da Broi U, Palmiere C, Tse R.** Post mortem tryptase: A review of literature on its use, sampling and interpretation in the investigation of fatal anaphylaxis. *Forensic science international* 2020; 314: 110415.
18. **Edston, E, Eriksson O, van Hage M.** Mast cell tryptase in postmortem serum-reference values and confounders. *Int J Legal Med* 2007; 121(4): 275–80.
19. **Da Broi U, Moreschi C.** Post-mortem diagnosis of anaphylaxis: A difficult task in forensic medicine. *Forensic Science International* 2011; 204: 1-5.