

Decline in Postmortem Serum Tryptase Levels in Anaphylactic Deaths

To the Editor:

We read the article by Sravan et al¹ published in the *American Journal of Forensic Medicine and Pathology* with considerable interest.¹

In this article, the authors described a case of fatal anaphylaxis in which 2 separate tryptase determinations were performed in blood samples taken from the same sampling site (femoral vein) but 24 hours apart. The 2 measurements revealed a substantial decrease in tryptase from 130 µg/L at day 2 postmortem to 84.8 µg/L at day 3.

The authors hypothesized that this decline might be the consequence of molecule degradation and therefore recommended that early blood sample be obtained as soon as possible after death in situations of suspected anaphylaxis because a significant downward trend in blood tryptase levels may be expected with increasing postmortem interval in anaphylactic deaths (in contrast to the increasing trend observed by other authors in nonanaphylactic cases).

We found the conclusions made by Sravan et al¹ to be of utmost interest and deserving of some comments.

Firstly, human mast cell (βII)-tryptase stored in the secretory granules of mast cells is a tetrameric, neutral serine protease with a molecular weight of 140 kDa, made up of 4 identical subunits of 30 to 36 kDa. The precursor βII-preprotryptase is converted to the βII-protryptase by removing the 18-amino acid signal peptide. The further activation of βII-protryptase involves 2 proteolytic steps: the first is an autocatalytic intermolecular cleavage that occurs optimally at acidic pH and in the presence of heparin or dextran sulfate. The resulting product is a monomer (β-protryptase), which is approximately 50 times less active than the final tetramer.

The second step involves the removal of the remaining precursor dipeptide by the enzyme dipeptidyl peptidase I, which functions at acidic pH values, to form the mature monomer. In the presence of heparin, the monomers come together on a heparin backbone to form an active tetramer. These events probably take place in the Golgi and post-Golgi secretory granules in mast cells. The mature, heparin-stabilized, packaged β-tryptase is then stored until

mast cell activation causes granular content release.²⁻⁴ When tryptase is released, it is secreted as a large-sized active proteoglycan complex (200–250 kDa) that limits diffusion away from mast cell activation sites.⁵

It has been observed that active tryptase tetramer is stabilized by heparin proteoglycan and other polymers with high anionic charge densities. Indeed, tryptase binds to heparin or other proteoglycans through its cationic groove. In the absence of heparin, the tryptase tetramer rapidly loses activity in a process that is accompanied by tetramer dissociation into inactive monomers. The exact mechanism for the inactivation/regulation of tryptase activity is unknown. The tetrameric structure of β-tryptase with the active site of each of the 4 monomers oriented toward the inner face of a central pore makes it resistant to inactivation by endogenous protease inhibitors of serine proteases. The most probable regulation mechanism is postulated as spontaneous β-tryptase inactivation associated with destabilization/conformational molecule changes and slow β-tryptase dissociation from heparin proteoglycan. This dissociation would be promoted by the neutral pH to which β-tryptase is exposed after its release from mast cells and the consequent, weaker affinity for heparin proteoglycan, mediated by basic proteins such as antithrombin III.^{2,5,6}

β-Tryptase determination as a support of the clinical diagnosis of anaphylaxis has been emphasized as highly valuable if correctly performed and completely useless if blood collection timing is inappropriate. Two elements must be considered for informative serum β-tryptase determination as an anaphylactic marker: the comparison of each patient's peak serum β-tryptase level with his/her own baseline β-tryptase level and the knowledge of serum β-tryptase kinetics. The first point is crucial, because a "normal" β-tryptase level (ie, the clinical reference value of 11.4 µg/L) in an anaphylactic patient may conceal an important rise when compared with the baseline value of that precise patient. Hence, when assessing serum β-tryptase for clinical diagnosis of anaphylaxis, sequential β-tryptase determination should be mandatory, with at least 2 blood samples (peak and baseline).⁷

The second paramount point is serum β-tryptase kinetics. Because β-tryptase is released from activated mast cells as an active proteoglycan complex whose large size limits diffusion away from mast cell activation sites, elevation of serum β-tryptase concentration may not be detectable during

the first 15 or 30 minutes after anaphylaxis onset. Once in the blood, β-tryptase's half-life is approximately 2 hours. Hence, the optimal delay for peak β-tryptase collection is best estimated between 30 minutes and 2 hours after anaphylaxis onset. This time interval is considered long enough to allow degranulated β-tryptase to reach peripheral blood and short enough to avoid missing an elevated serum β-tryptase due to serum clearance. Considering a serum β-tryptase half-life of 2 hours and a peak serum level that may reach 100 µg/L or more, a significant decrease in β-tryptase levels is usually seen 24 hours after the anaphylactic episode, although serum β-tryptase may not reach the "individual" baseline values until 48 to 72 hours after symptom onset in cases of very high peak values or prolonged mast cell activation. This leads to the conclusion that accurate baseline β-tryptase assessment in the clinical setting should ideally be performed more than 48 to 72 hours after the end of mast cell activation induced symptoms.^{5,7}

In our own experience, we observed a case of fatal anaphylaxis after contrast material administration, in which 7 serial β-tryptase determinations were performed during patient hospitalization at different sampling times. The patient died 2 days after contrast material administration, and a forensic autopsy was performed the same day of the death. The results of the laboratory investigations performed at hospital are summarized in Table 1 and show a substantial, progressive decrease in β-tryptase concentrations between the first (taken 2 hours after contrast material administration) and last determination (taken 28 hours apart from the same sampling site).

Furthermore, systematic β-tryptase determination performed in our facility in femoral blood and postmortem serum samples

TABLE 1. Summarized β-Tryptase Concentrations Measured in Seven Serial Samples During Patient Hospitalization

June 16th, 10 pm:	70.7 µg/L
June 17th, 4 am:	19.8 µg/L
June 17th, 11 am:	12.6 µg/L
June 17th, 3 pm:	10.5 µg/L
June 17th, 6 p.m.	9.88 µg/L
June 17th, 10 p.m.	9.33 µg/L
June 18th, 4 a.m.	8.91 µg/L

The first and the last measurements were taken 28 hours apart from the same sampling site.

obtained from corpses with different post-mortem intervals and causes of death unrelated to anaphylaxis failed to reveal β -tryptase increases with increasing post-mortem intervals.

We would like to congratulate the authors for the published case report and emphasize their finding of a significant β -tryptase decrease in 2 blood samples taken 24 hours apart from the same sampling site in a case of fatal anaphylaxis. On the other hand, this significant downward trend in blood β -tryptase levels with increasing postmortem intervals observed by the authors seems to be quite similar to that expected in the clinical setting in situations of anaphylaxis, in which substantial decreases in β -tryptase concentrations are usually seen 24 hours post anaphylaxis onset.

In our opinion, the decrease in blood β -tryptase levels observed by Sravan et al¹ in 2 postmortem blood samples taken

24 hours apart from the same sampling site is not necessarily the consequence of β -tryptase degradation that might occur after the onset of decompositional changes but could instead reflect the spontaneous, slow β -tryptase molecule inactivation that has been postulated in the clinical setting and might potentially continue in the early postmortem period.

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