β-Tryptase measurements post-mortem in anaphylactic deaths and in controls

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Abstract

The reliability of measuring mast cell tryptase in post-mortem blood to diagnose anaphylactic deaths has been questioned because moderate elevation of tryptase can be seen also in control cases. Very high tryptase concentrations have been recorded even in a few control cases with known cause of death such as myocardial infarction or trauma. Aiming to compare findings we measured tryptase in 193 cases: 176 with known cause of death, 10 unexplained deaths and seven anaphylactic or anaphylactoid deaths (AADs). Using binary logistic regression we calculated the sensitivity and specificity of the tryptase test at different cut-off values, and found 10 μg/l to be optimal, the sensitivity being 86% and the specificity 88%. Traumatic deaths (n=23), sudden infant death syndrome (n=40) or deaths after heroin-injection (n=22) showed elevated tryptase values in 35%, 35% and 32%, respectively, and in 40% of the unexplained deaths (n=10), which was higher than expected (12%). Heart blood tryptase level was elevated in 22% of the controls and femoral blood tryptase in 10%. No correlations were seen with age or post-mortem delay. It is concluded that tryptase measurements are useful in confirming death from AAD, and that blood should be sampled from the femoral vessels. In unexplained deaths tryptase measurement is a useful indicator, but the diagnosis is not to be based on the test alone. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Anaphylactic or anaphylactoid reactions appear suddenly and death may ensue within minutes. Autopsy findings are few and unspecific [1]. The post-mortem diagnosis of death by anaphylactic or anaphylactoid shock has therefore previously relied mainly on the circumstances of death.

Analyses of total-immunoglobulin E (IgE) and specific-IgE by the radio allergo sorbent technique (RAST) are possible in post-mortem serum but can merely verify the atopic disposition and the degree of sensitisation of the patient. Histamine, the mediator of the acute anaphylactic or anaphylactoid reactions, degrades too rapidly in post-mortem blood to be a reliable marker of anaphylactic or anaphylactoid death (AAD).

Mast cell tryptase, a serine protease stored in the mast cell granules and released together with histamine, is relatively stable post-mortem and can be found in blood from a few minutes up to several hours after mast cell degranulation [2]. Analyses of tryptase in post-mortem samples of suspected anaphylactic deaths have consistently shown elevated values [3,4]. Increased tryptase levels have also been demonstrated in a few cases of death from non-allergic causes, however [5].

In the present study we tested the reliability of β-tryptase analysis post-mortem as a marker of AAD by comparing the results in a group of 58 controls with those in seven cases of AAD. We also used data from 186 forensic autopsy cases with various causes of death for comparisons of influence of sampling site and post-mortem delay.

2. Material and methods

Between 1992 and 1996, blood samples from 193 autopsy cases were analyzed for tryptase.

During four periods in 1996, femoral blood was sampled from consecutive autopsies; 15 cases in February, 14 in May, 14 in August and 15 in October 1996 in a series of 58 patients. Seven cases of certain AAD were found on forensic autopsy during the study period. The criteria for AAD were elevated total IgE, a positive RAST and/or a temporal relationship between death and a preceding challenging incident, e.g., the injection or ingestion of a therapeutic agent, ingestion of certain foods or a bee sting.

Other cases were selected by diagnosis, i.e., suicidal hanging (n=21), traumatic deaths (n=23), deaths after heroin injection (n=22), coronary thrombosis and acute myocardial infarction (n=54), sudden infant death syndrome (SIDS) (n=40), sudden unexplained deaths (n=10), deaths by drowning (n=7) and intoxications (n=9). Since femoral blood was scarce in many of the SIDS cases, blood was sampled from the heart and peripheral veins. In 36 control cases blood was sampled from the femoral vein and artery and the right ventricle of the heart, and fluid was collected from the pericardial sac. Twelve of these cases were acute coronary deaths.

The mean time between death and autopsy was 3.8±1.0 (S.D.) days. The majority of the bodies had been stored at 4°C within 24 h after death.

Blood or pericardial fluid was sampled in test tubes without additives. It was centrifuged and the supernatant was frozen at −20°C until analysis.
β-Tryptase was determined in all cases with a commercial kit (Pharmacia Tryptase RIACT, Pharmacia Diagnostics, Uppsala, Sweden) a radioimmunoassay with a lower detection limit of 2 μg/l.

Statistical analysis was done with binary logistic regression, comparing anaphylactic deaths with 58 control cases. For comparing the different diagnosis groups the Mann–Whitney U-test for nonparametric data was used. Correlations between tryptase on the one hand and age and post-mortem delay, on the other, were calculated using linear regression [6], as were effects of sampling site.

3. Results

3.1. Controls versus anaphylactic/anaphylactoid deaths

The mean tryptase value in the control group ($n=58$) was $7.9\pm22.6$ (S.D.) μg/l and $21.0\pm11.7$ μg/l in the AAD group ($n=7$) (Table 1). The difference between groups was not significant ($p>0.05$). The sensitivity and specificity of the method was calculated at different cut-off values: at a tryptase concentration of 5 μg/l sensitivity was 100% whereas specificity was 71%. At 10 μg/l sensitivity was 86% and specificity 88%, which means that a value above 10 μg/l would be falsely attributed to AAD in about one case of 10 in a blind sample, and in samples from apparent AAD, one out of 10 would show a tryptase value below 10 μg/l. At 20 μg/l the sensitivity decreased to 71% while the specificity increased to 93% (Fig. 1).

3.2. Tryptase values in femoral blood in non-AAD by diagnoses other than AAD

Using the 10 μg/l cut-off value, increased tryptase levels were found in 7% of 54 cardiac deaths, in 5% of 21 suicides by hanging, in 10% of nine intoxication deaths and in none of seven deaths by drowning. These findings correspond to the expected ratios. Larger than expected proportions with elevated tryptase concentrations were seen in other diagnostic groups: 35% in 23 traumatic deaths, 35% of 40 deaths from SIDS, 32% of 22 heroin-deaths and in 40% in 10 cases of sudden unexplained deaths in apparently healthy individuals (Fig. 2). The mean values in the four latter groups differed

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Delay</th>
<th>Tryptase</th>
<th>IgE</th>
<th>RAST</th>
<th>Circumstances</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>Male</td>
<td>Shock</td>
<td>4d</td>
<td>16</td>
<td>Not done</td>
<td>Not done</td>
<td>Infusion of Macrodex</td>
</tr>
<tr>
<td>2</td>
<td>82</td>
<td>Female</td>
<td>Shock</td>
<td>7d</td>
<td>33</td>
<td>60</td>
<td>Not done</td>
<td>Injection of Carbocain</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>Male</td>
<td>Sudden death</td>
<td>4d</td>
<td>11</td>
<td>15</td>
<td>Neg (carrot, nuts)</td>
<td>Ingestion of NSAD</td>
</tr>
<tr>
<td>4</td>
<td>44</td>
<td>Male</td>
<td>Sudden death</td>
<td>4d</td>
<td>28</td>
<td>300</td>
<td>Pos (Pc-V, Pc-G)</td>
<td>Ingestion of Pc-V</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>Female</td>
<td>Sudden death*</td>
<td>3d</td>
<td>37</td>
<td>8</td>
<td>Neg (cacao,milk)</td>
<td>Ingestion of cocopops</td>
</tr>
<tr>
<td>6</td>
<td>17</td>
<td>Male</td>
<td>Shock</td>
<td>3d</td>
<td>6</td>
<td>3100</td>
<td>Pos (nuts,paprika)</td>
<td>Ingestion of kebab</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>Female</td>
<td>Shock</td>
<td>3d</td>
<td>17</td>
<td>Not done</td>
<td>Pos (bee venom)</td>
<td>Bee sting</td>
</tr>
</tbody>
</table>

*aHad urticaria pigmentosa.
significantly from that of either suicidal hanging ($p<0.05$) and the group of cardiac deaths ($p<0.05$). Six of eight cases with elevated tryptase concentrations in the group with traumatic deaths, including the overall top value, 170 μg/l, had multiple fractures and organ damage. One died of extensive bleeding from a severed radial artery under water and brain laceration due to a gun-shot wound in another. There were, however, multiple traumata in six of the 15 individuals with tryptase below 10 μg/l, and the other nine with low tryptase concentrations exhibited isolated chest or cranial injuries. The only elevated tryptase value in the intoxication group was seen in a case of cyanide poisoning.

3.3. Analysis of 10 sudden unexpected deaths

Four of 10 sudden deaths had elevated tryptase concentrations, including two cases totally without pathologic findings. All 10 were free from clinically significant cardiovascular disease. Three of six sudden deaths with low tryptase values had pathological findings or a medical history (such as a defective aortic valve and asthma) that was probably not of any significance, whereas no such clues were found in the three other cases (Table 2).
Fig. 2. Box-plot showing β-tryptase values in deaths by different causes (x-axis). Minimum and maximum values (bottom and upper symbols); bottom, median and top of the lines of the boxes mark the 25th, 50th and 75th percentiles, respectively; the vertical lines from the top of the boxes marks the 90th percentile. Individual values above the 90th percentile are marked by symbols. *Significantly different from myocardial infarct, ○=significantly different from hanging and myocardial infarct ($p<0.05$).

Table 2
A presentation of the ten cases of unexplained deaths

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Delay</th>
<th>Tryptase</th>
<th>Circumstances</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>Male</td>
<td>No pathology</td>
<td>5d</td>
<td>2</td>
<td>Found dead, drug abuse</td>
</tr>
<tr>
<td>2</td>
<td>53</td>
<td>Female</td>
<td>No pathology</td>
<td>1d</td>
<td>2</td>
<td>Died in her sleep</td>
</tr>
<tr>
<td>3</td>
<td>46</td>
<td>Male</td>
<td>Asthma?</td>
<td>3d</td>
<td>2</td>
<td>Found dead outside</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>Female</td>
<td>No pathology</td>
<td>3d</td>
<td>5</td>
<td>Found dead on the floor</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>Male</td>
<td>Laryngeal edema</td>
<td>4d</td>
<td>6</td>
<td>Found dead on the floor</td>
</tr>
<tr>
<td>6</td>
<td>29</td>
<td>Male</td>
<td>Defect heart valve?</td>
<td>3d</td>
<td>7</td>
<td>Died outside during activity</td>
</tr>
<tr>
<td>7</td>
<td>46</td>
<td>Female</td>
<td>Epilepsy?</td>
<td>6d</td>
<td>10</td>
<td>Found dead, alcohol abuse</td>
</tr>
<tr>
<td>8</td>
<td>48</td>
<td>Male</td>
<td>No pathology</td>
<td>7d</td>
<td>13</td>
<td>Died in his sleep</td>
</tr>
<tr>
<td>9</td>
<td>31</td>
<td>Male</td>
<td>No pathology*</td>
<td>7d</td>
<td>38</td>
<td>Found dead on the floor</td>
</tr>
<tr>
<td>10</td>
<td>58</td>
<td>Male</td>
<td>Thin circumflex artery</td>
<td>4d</td>
<td>42</td>
<td>Died outside during activity</td>
</tr>
</tbody>
</table>

*Had diabetes mellitus.
3.4. Tryptase and sampling site

Twenty-two percent of the 58 control samples had elevated tryptase concentrations in heart blood, 11% in femoral blood and 3% in pericardial fluid. The differences between heart blood, femoral blood and pericardial fluid were not significant (p > 0.05). In non-cardiac deaths the correlation coefficient (r) was 0.08 for femoral blood vs. heart blood but 0.66 for heart blood vs. pericardial fluid.

3.5. Post-mortem interval and age vs. tryptase values

These variables were not intercorrelated (r values 0.11 and 0.06, respectively).

4. Discussion

We have shown that a cut-off value of 10 μg/l of β-tryptase gives a sensitivity of 86% and a specificity of 88%, while either the specificity or the sensitivity decreases at a lower or higher cut-off value. Although 10 μg/l appeared to be the optimal value in our study our AAD-group was rather small (n = 7) though, and the cut-off value might be calculated more exactly in a larger group. Ten μg/l was also suggested as the cut-off point in a previous study by Yunginger et al. [4].

A complex pattern of variations in tryptase values was revealed when control samples were analyzed separately in different diagnostic categories. In traumatic deaths, for instance, 8/23 had elevated tryptase concentrations, whereas only 3/23 were expected. Many elevated values were also found in SIDS and deaths by heroin injection, which has been reported previously [7–9]. The highest tryptase value in the whole material, 170 μg/l, was found in a case of multiple fractures and organ injuries after a car accident. IgE was not elevated and RAST for bee/wasp venom was negative. The elevated values of β-tryptase in this case and in other traumatic deaths remain to be explained. In a clinical study, Ennis et al. [10] showed that elevation of histamine was common in polytraumatized patients and a poor prognostic factor. One hypothesis is that agonal physiological stimuli and/or multiple crush injuries may cause mast cell degranulation or disruption, with leakage of histamine and tryptase into the circulation.

In sudden unexplained deaths, mainly in young and middle-aged adults, tryptase was elevated in 4/10. Since the expected value was 1/10, it is probable that AAD was responsible for the other three of these four unexplained deaths. Schwartz et al. [11] showed, in a retrospective study, that at least 13% of sudden unexpected deaths could be attributed to anaphylaxis.

In the anaphylaxis group, tryptase values ranged between 6 and 37 μg/l, with the lowest value in a case of food allergy. This is consistent with the findings of Yunginger et al. [4], who reported tryptase values below 10 μg/l in cases of anaphylaxis due to food allergy. This implies that tryptase might not be significantly elevated even in clear-cut cases of witnessed death by anaphylaxis, and with a well-defined allergenic agent.

The sampling site seems to be of importance. Tryptase was elevated in 22% of control
samples from right heart blood, but only in 11% (expected value) from femoral blood samples. Nor was there any correlation between the blood samples from the right heart and from the femoral vessels. A weak positive correlation between heart blood and pericardial fluid indicated that post-mortem diffusion might occur between these compartments. The reason for the difference between heart blood and femoral, might be attributed to post-mortem diffusion from ruptured mast cells in mast cell rich organs such as the lung or stomach. If so, however, this seems to occur haphazardly. Our conclusion is that blood for tryptase analysis should be sampled from the femoral vessels.

Neither the post-mortem delay before autopsy nor the age of the subject seems to influence the tryptase measurements, which agrees with our previous results [9].

Why β-tryptase is sometimes elevated above 10 μg/l in cases with no circumstantial evidence of AAD remains unclear. Some of these cases may reflect an undiagnosed anaphylactic or anaphylactoid reaction. Anaphylactoid reactions after exercise [12,13] and of unknown cause, (idiopathic anaphylaxis) [14] have been reported.

We believe that tryptase measurements are valuable not only for confirmation of suspected AAD but also in sudden unexpected or unwitnessed deaths without significant pathological findings. In cases with a tryptase value about 10 μg/l or higher, however, additional information is needed on the subject’s history, the circumstances of death and IgE and/or RAST should be analyzed.

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References


