

Accumulation of eosinophils, mast cells, and basophils in the spleen in anaphylactic deaths

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Abstract Death in anaphylactic shock cannot be diagnosed by autopsy alone. Morphological diagnosis of anaphylactic death by counting mast cells in the lung and airways have failed to give consistent results. Previously it has been observed that eosinophils seem to accumulate in the spleen in anaphylaxis. The purpose of this study was to investigate if it is possible to safely diagnose anaphylactic deaths by counting eosinophils, mast cells, and basophils in the spleen. In 43 forensic autopsy cases specific antibodies to mast cells, eosinophil-, and basophil granulocytes were used on sections from lung and splenic tissue. The cells were counted in 20×40 fields in a Leica photo-microscope. Presumed deaths in anaphylaxis were compared with sudden deaths after intravenous injection of opiates, and sudden cardiac deaths (control group). The main result was that significant ($p < 0.05$) increases of both eosinophil granulocytes (mean 26.6 ± 17.8 /SD/) and mast cells (3.2 ± 2.0 /SD/) versus controls (eosinophils mean 7.0 ± 10.5 and mast cells mean 0.9 ± 1.1) were seen in splenic tissue in anaphylactic deaths. Comparing cases with high and low concentrations of mast cell tryptase in serum showed a similar increase in eosinophils and mast cells in the spleen in cases with elevated tryptase, but not in the lung. The numbers of pulmonary mast cells and eosinophils were not different in anaphylactic deaths compared with controls. It is concluded that by quantifying eosinophil granulocytes and mast cells in the spleen in combination with tryptase measurements in serum it is possible to diagnose anaphylaxis with a high degree of certainty.

Keywords Anaphylaxis · Postmortem · Spleen · Eosinophils · Mast cells · Basophils

Introduction

Deaths in anaphylaxis occur suddenly, and in many cases are they un-witnessed. The diagnosis of anaphylactic shock was formerly a diagnosis of exclusion, and sometimes based on circumstantial evidence such as a history of allergy, external signs such as urticarial efflorescences or marks after wasp stings.

The role of mast cells in allergy and anaphylaxis has been known for a long time. The main mediator of the allergic reaction, histamine, is however difficult to analyze post mortem due to its short half-life. With the work of Schwartz and others [1] assays of the neutral protease tryptase, contained in and released from mast cell granules, have come into use. As tryptase is relatively stable in postmortem blood it was possible to prove that mast cells had been activated pre mortem, indicating that the cause of death was anaphylaxis [2, 3]. However, it was also found that a few false positive cases occurred, in deaths proven to be unrelated to allergy [4, 5]. Because of this uncertainty it is not safe to diagnose anaphylaxis from an elevated tryptase value alone, especially when the circumstances of death and a medical history of allergy are unknown.

Therefore, in adjunct to tryptase analysis it would be useful to find histological markers of anaphylaxis. In a study by Pumphrey and Roberts [6] the most common finding in anaphylactic deaths was edema and eosinophilia in the upper airways, but the histology of suspected or confirmed anaphylaxis was highly variable and non-specific. In a work by Heard et al. [7] using the toluidin blue stain for mast cells they reported an increased amount of those cells in the lung in

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cases of anaphylaxis. In a study by Delage and Irely [8] they demonstrated increased numbers of eosinophils in the red pulp of the spleen. In a more recent study by Trani et al. [9] that phenomenon was corroborated. The present study uses immunohistochemistry to identify mast cells, eosinophil-, and basophil granulocytes in the spleen and in the lung in deaths from anaphylaxis, cardiac complications, and deaths associated with abuse of opiates and also correlated the morphological findings with serum tryptase concentrations.

Methods

The lung and spleen were studied in 43 forensic autopsy cases. Eight cases were anaphylactic deaths, verified by circumstantial evidence and high concentrations of mast cell tryptase in serum (45–520 $\mu\text{g/L}$); 20 cases were deaths from cardiovascular disease and were used as controls; 15 cases were deaths ascribed to intravenous injection of opiates (heroin, morphine, and methadone). In 41 cases serum tryptase was measured and this population was divided into high tryptase $\geq 45 \mu\text{g/L}$ ($n = 19$) and low tryptase $< 45 \mu\text{g/L}$ ($n = 22$).

Mast cell tryptase was measured in serum from blood collected from the femoral vein 3–5 days post mortem. Analysis of serum tryptase was done at the laboratory of Clinical Immunology at the Karolinska Hospital in Stockholm (Tryptase RIACT, Pharmacia Upjohn AB, Uppsala, Sweden).

Tissue samples from both lungs and the spleen were processed routinely at our histological laboratory and stained with hematoxylin-erythrosine-saffron [10] and Pagoda red [9] stains. Parallell sections were treated with antibodies to tryptase (Dako), chymase (Nordic BioSite, Stockholm, Sweden) for identification of mucosal and connective tissue mast cells, major basic protein (MBP, Nordic BioSite, Stockholm, Sweden) for identification of eosinophils, and proMBP1 (Nordic BioSite, Stockholm, Sweden) for basophils [11].

The cells were counted in 20 random $\times 40$ fields with a Leica photo microscope DFC295.

The data were analyzed using the Prism 5 for Mac (GraphPad Software, Inc). Column statistics, mean-values and standard deviations ($\pm\text{SD}$), and the Mann–Whitney U -test for non-Gaussian distributed data were used. Correlations were expressed as r values and significant positive correlations were expressed in p values. Significant differences between groups were defined as $p < 0.05$.

Results

The mast cells in the lung were unevenly distributed and mainly located in the bronchial walls and in the

perivascular spaces. The tryptase positive mast cells were more numerous than chymase positive ones. Very few MBP positive cells (eosinophils) and proMBP positive cells (basophils) were seen in the lung sections. Comparing the number of mast cells in lung sections in anaphylactic deaths with controls yielded no significant differences ($p > 0.05$), whereas cases with high serum tryptase had significantly more mast cells than low serum tryptase cases ($p < 0.05$; Fig. 1). In opiate associated deaths there was no significant difference compared with controls ($p > 0.05$).

In sections from the spleen the number of mast cells (Fig. 2) and eosinophils (Fig. 3) were highly variable from case to case. Those cells were mainly located to the red pulp. In a few cases a small number of basophils (Fig. 4) were found. In splenic tissue tryptase positive mast cells were more numerous than chymase positive ones. The number of eosinophils were significantly higher in anaphylactic deaths (mean $26.6 \pm 17.8/\text{SD}$; range 10–64 per $\times 40$ field), and in

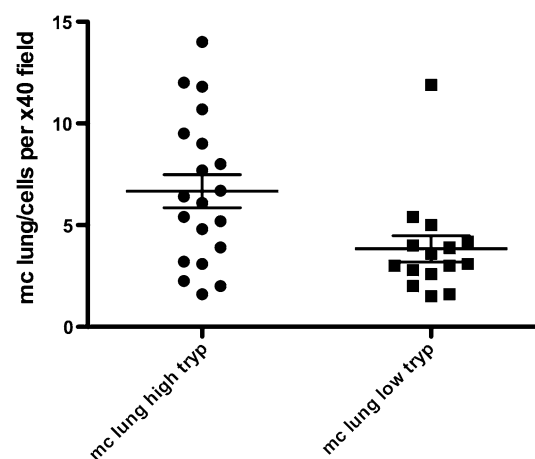


Fig. 1 Mast cells in the lung: high versus low serum tryptase ($p < 0.05$)

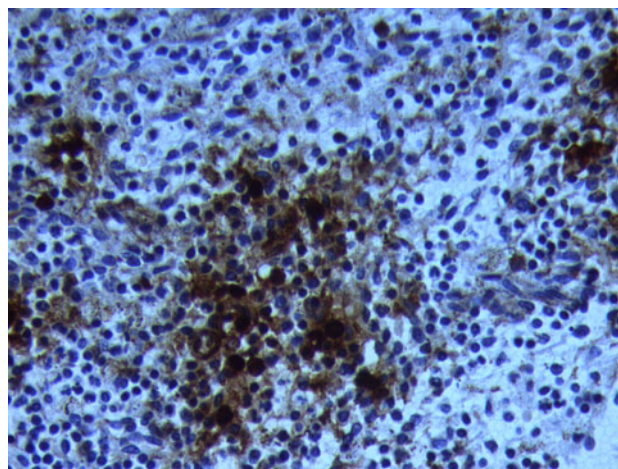


Fig. 2 Major Basic Protein (MBP)-positive cells (eosinophil granulocytes) in the splenic red pulp (brown dots)

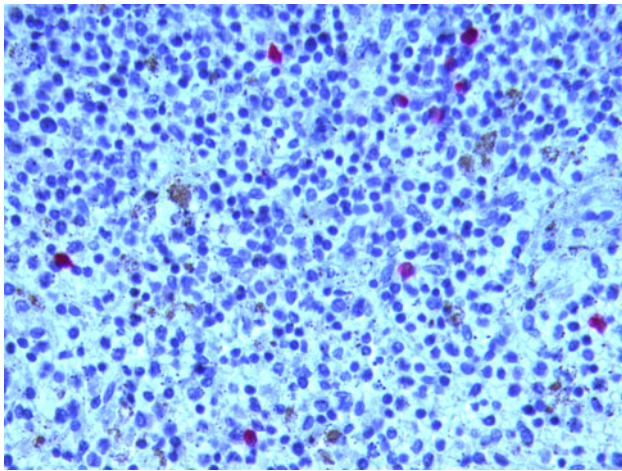


Fig. 3 Tryptase-positive cells (mast cells) in the splenic red pulp (red dots)

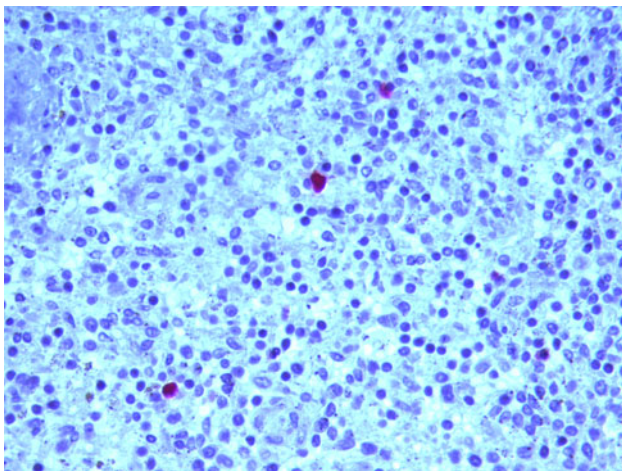


Fig. 4 pro-MBP-positive cells (basophil granulocytes) in the splenic red pulp (red dots)

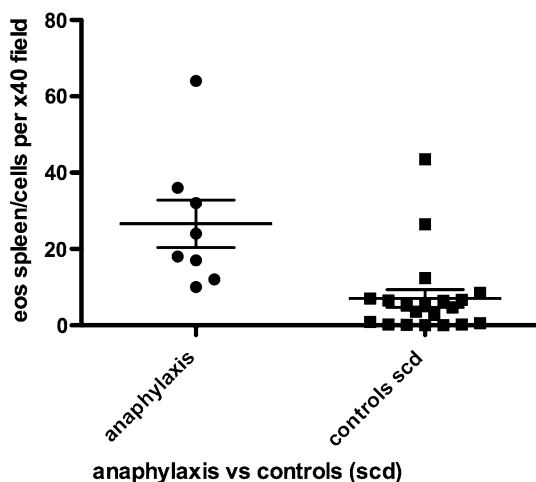


Fig. 5 Eosinophils in the spleen: anaphylaxis versus scd-controls ($p < 0.05$)

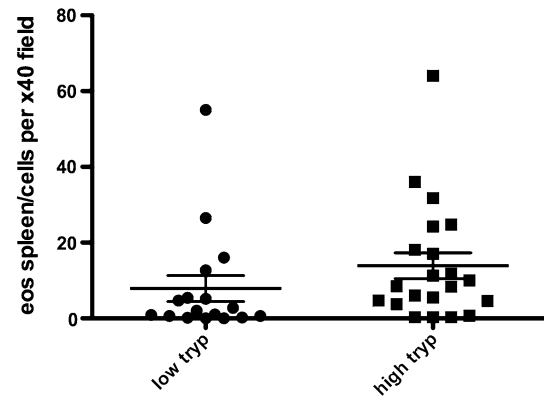


Fig. 6 Eosinophils in the spleen: high versus low serum tryptase ($p < 0.05$)

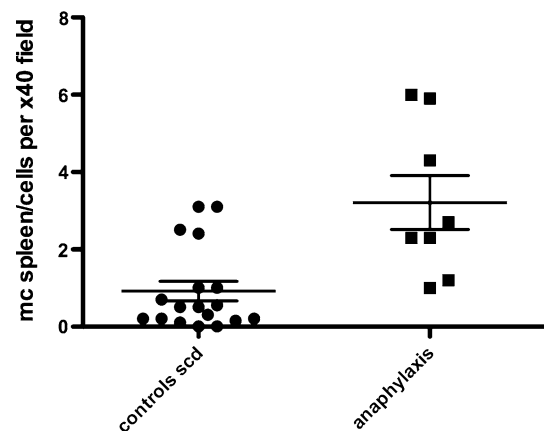


Fig. 7 Mast cells in the spleen: anaphylaxis versus scd-controls ($p < 0.05$)

cases with elevated tryptase (mean $13.9 \pm 15.5/\text{SD}$; range 0.2–64 per $\times 40$ fields) compared with controls (mean 7.0 ± 10.5 ; range 0–43.5) (Figs. 5, 6). In opiate associated deaths the number of splenic eosinophils was not significantly different compared with controls (mean 8.8 ± 13.2 ; range 0.3–55 per $\times 40$ fields) ($p < 0.05$). The numbers of tryptase positive mast cells in splenic tissue were significantly elevated in cases of anaphylaxis (mean $3.2 \pm 2.0/\text{SD}$; range 1–6 per $\times 40$ fields) versus controls (mean $0.9 \pm 1.1/\text{SD}$; range 0–3.1), and in cases with elevated serum tryptase (mean $1.8 \pm 1.6/\text{SD}$; range 0–6 per $\times 40$ fields) compared with the low serum tryptase group (mean 0.7 ± 0.8 ; range 0–3.1 per $\times 40$ fields) ($p < 0.05$) (Figs. 7, 8), but the number of splenic mast cells in opiate associated deaths did not differ from the controls ($p > 0.05$). The number of basophils in the spleen was not significantly different between the study groups.

In Table 1 the correlations between the number of eosinophils, basophils, and two types of mast cells expressed as r values are presented. Eosinophils in the spleen were positively correlated to both mast cells and basophils in splenic tissue but not in lung tissue. The two

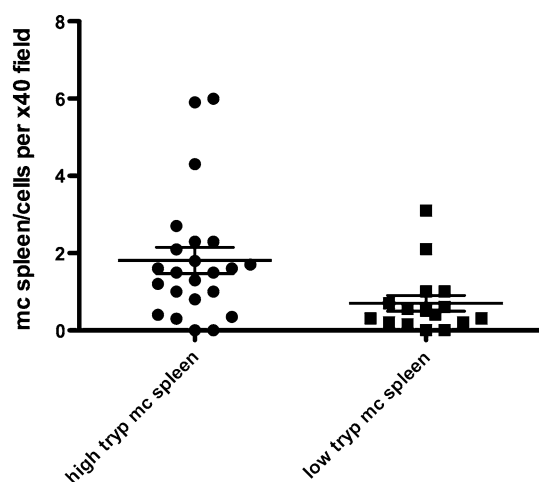


Fig. 8 Mast cells in the spleen: high versus low serum tryptase ($p < 0.05$)

Table 1 Correlations between eosinophils in spleen versus mast cells and basophils in spleen and lung

Comparisons ($N = 43$)	r value	p value ($ns = >0.05$)
Eos spleen versus mc-t spleen	0.34	<0.05
Eos spleen versus baso spleen	0.30	<0.05
Eos spleen versus mc-c spleen	− 0.11	ns
Eos spleen versus eos lung	0.57	<0.05
Eos spleen versus baso lung	0.06	ns
Eos spleen versus mc-t lung	0.14	ns
Eos spleen versus mc-c lung	−0.15	ns
Mc-t lung versus mc-c lung	0.66	<0.05
Mc-t spleen versus mc-t lung	0.24	ns

MC-t tryptase positive mastcell, MC-c chymase positive mastcell, Eos eosinophil granulocyte, Baso basophil granulocyte

types of mast cells were positively correlated in the lung, but not in splenic tissue.

Discussion

The results confirm previous observations that eosinophils and mast cells appear to accumulate in the spleen in cases of anaphylactic death [8, 9, 12]. Voigt used cytometric methods to count eosinophils and compared anaphylactic deaths with controls [12]. Delage and Irej [8] used only common histochemical stains and included no controls. Trani et al. [9] used a special stain, Pagoda red, but that stain does not discriminate between eosinophils and mast cells. Furthermore Pagoda red is not as sensitive as immunohistochemical staining with monoclonal antibodies. Although small in numbers the occurrence of basophils in the spleen in anaphylactic deaths was not unexpected as basophils are the third cell directly involved in IgE-mediated anaphylaxis [13]. No significant

increases in the numbers of mast cells or eosinophils were observed in the lung in anaphylactic deaths. However, individuals with a high serum level of tryptase had more mast cells in the lungs than the scd-controls. The negative result regarding anaphylactic deaths might have been due to the rather small number of cases. In a few previous studies mast cells have been counted in the lung and correlated to levels of serum tryptase, but in contrast to the present study no correlation was found [14, 15]. One cause of this might be the difficulty to correctly count mast cells in pulmonary parenchyma due to its irregular architecture, compared with the splenic red pulp. Another may be that many mast cells might have degranulated and thus become invisible. The fact that the amount of resting mast cells in the lung apparently shows great individual variation is a factor that renders statistical calculation unfeasible on small samples. Regarding eosinophils and basophils, those cell types were largely absent in the pulmonary tissue in the majority of cases.

Is the accumulation of eosinophils, basophils, and mast cells in the spleen caused by recruitment of those cells due to chemotactic factors released locally or does it reflect a general increase of those cells in the circulating blood and tissues due to an allergic disposition? The correlation study showed a significant positive correlation between eosinophils in lung and spleen, whereas no correlation was seen regarding mast cells. From these results one cannot answer this question with certainty, but they imply that apart from blood eosinophilia there might also be an active chemotactic process leading to the observed accumulation of eosinophils and mast cells in the splenic red pulp.

In the present study a group of opiate associated sudden deaths with elevated tryptase values ($>45 \mu\text{g/L}$) were included, because evidence for mast cell activation have been indicated in previous studies of drug related sudden deaths [15, 16]. However, this group did not differ from the controls regarding splenic eosinophilia and mastocytosis. This is indirect evidence that the sequence of events leading to mast cell activation and tryptase release is different from anaphylactic, IgE-mediated reactions. As yet the mechanisms and receptors involved in opiate-induced mast cell degranulation are not entirely clear [17].

The important conclusion of this study is that quantification of eosinophils, basophils, and mast cells in splenic tissue can be of help in the postmortem diagnosis of death in anaphylaxis. There were some false positives in the control group (2 cases or 5 % exceeded 10 eosinophils per $\times 40$ field). They were primarily classified as scd and had normal serum tryptase in one case (in the other case tryptase was not measured).

A finding of eosinophilia (>10 per $\times 40$) and mastocytosis (>2 per $\times 40$), and an occasional basophil (optional) in the spleen in combination with elevated serum tryptase ($>45 \mu\text{g/L}$ serum) is nearly conclusive that death was

caused by anaphylaxis. Therefore it is recommended that splenic tissue should be sampled for immuno-histological examination in all cases of un-witnessed sudden death.

Key points

1. Accumulation of eosinophils, mast cells (and basophils) in the splenic red pulp is seen in cases of anaphylactic death, and can be used in the post mortem diagnosis of this condition.
2. A high concentration of mast cell tryptase in serum and increased numbers of eosinophils and mast cells in the spleen make the diagnosis of anaphylactic shock almost conclusive.
3. The number of mast cells in lung tissue is highly variable and these cells are unevenly distributed, therefore they cannot be used in the diagnosis of anaphylaxis.
4. In deaths by intravenous injection of opiates there are no significant increases of eosinophils and mast cells in the spleen, which indicates that the mechanism behind mast cell activation is different from IgE mediated allergy.

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