

## Histopathological markers for the diagnosis of anaphylactic death

S Luongo MD, C Frontalini MT, M Pesaresi MD, M Valsecchi MD and A Tagliabracci MD

Sezione Dipartimentale di Medicina Legale, Università Politecnica delle Marche, via Conca, 60126, Ancona, Italy  
Correspondence: A Tagliabracci. Email: a.tagliabracci@univpm.it

### Abstract

The postmortem diagnosis of anaphylactic death may be frustrating when victims are not hospital patients, even more so when they are recovered dead. The frequent lack of specific morphological findings in such cases means that diagnosis by the forensic pathologist must rely solely on exclusion criteria or circumstantial evidence. However, a diagnostic approach based on case history, analysis of circumstances, available clinical and necropsy findings, as well as toxicology, histopathology and biohumoral data, often allows demonstration of the cause of death. Some useful reflections on microscopic morphological data have come from two recent cases, where thorough data collection provided a reasonably certain diagnosis of anaphylactic death and systemic inflammatory response syndrome-related cardiac arrest, respectively. In both cases tissue histopathology proved crucial, since histochemical (Giemsa) and immunohistochemical analysis (CD117 and tryptase) documented a large number of mast cells in tissues, particularly the laryngeal wall, and a discrepancy between cells positive for Giemsa and tryptase and those positive for CD117. Staining for CD117 was also detected in cells with dendrite morphology and in a subpopulation of small lymphocytes with incised nuclei. The morphological findings of these cases are discussed, especially those obtained with immunohistochemistry, and the need for the latter data to be interpreted by experienced medical staff in the framework of a thorough analysis of all the data collected is highlighted.

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### Introduction

The postmortem diagnosis of anaphylactic death may be frustrating for the forensic pathologist, due to the non-specific morphological findings and to the fact that it is often largely based on circumstantial data and the exclusion of other causes.<sup>1,2</sup>

However, a diagnostic approach based on case history, analysis of circumstances, the available clinical and necropsy findings, as well as toxicology, histopathology and biohumoral data often allows demonstration of the cause of death.

Two recent postmortem examinations that were performed at our institution for suspected anaphylactic death are described with a view to contributing to the definition of a set of diagnostic criteria.

### Case reports

The records of two postmortem examinations requested by the legal authorities for suspected anaphylactic death were reviewed.

A thorough history, information regarding the circumstances of death, and emergency room medical records were collected. A complete postmortem examination was carried out according to the protocol of our institution. Fresh tissue specimens for histology were placed in 10% buffered formalin. Samples of biological fluids (blood,

urine, vitreous humour) were collected and frozen at  $-20^{\circ}\text{C}$  ( $\pm 2^{\circ}\text{C}$ ) until use. Blood was smeared on a cloth fragment, air-dried and stored at ambient temperature in a Petri dish for future reference.

Tissue sections were stained for haematoxylin–eosin (H–E). Giemsa staining, commonly used for the morphological study of haematopoietic cells, was applied for its metachromatic properties. Immunohistochemical analyses were conducted with anti-tryptase and anti-CD 117 antibodies (Cell Marque® products and kit) according to the manufacturer's recommendations.

Blood was analysed for ethanol and volatile organic compounds using headspace gas chromatography. Urine was screened for illicit drugs using the enzyme multiplied-immunoassay technique.

Blood samples were subjected to quantitative determination of beta-tryptase. Total and specific IgE were determined in the first case. Both determinations were performed using 'Immuno CAP® 250'.

### Case 1

A 30-year-old Caucasian man with congenital polyneuropathy, osteoarthropathy and chronic left foot osteomyelitis reported epigastric pain and generalized pruritus before losing consciousness a few minutes after an intramuscular injection of a beta-lactam antibiotic.

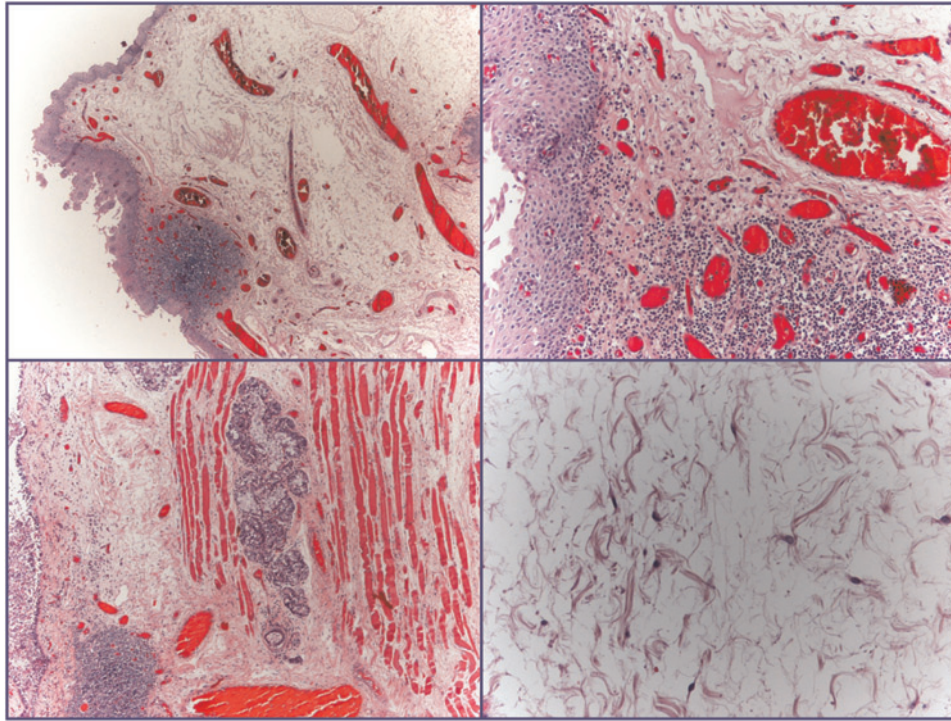


**Figure 1** Severe oedema of the glottis (EE)

An ambulance arrived 20 minutes later to find absent vital parameters. Cardiopulmonary resuscitation (CPR) was applied unsuccessfully for 60 minutes.

The postmortem examination was performed 44 hours after death.

Gross examination disclosed slight steatotic hepatomegaly, oedema of glottis, aryepiglottic fold and vocal cords, abundant mucus on the tracheal and bronchial mucosae, focal lung haemorrhage and significant congestion of all organs.

Sections of the glottis (Figure 1) disclosed marked oedema without significant inflammation, non-activated mucosa associated lymphoid tissue and marked blood vessel dilation and congestion. The liver exhibited alcoholic steatosis, marked hydropic degeneration of hepatocytes, platelet clotting in small parenchymal vessels and significant congestion. The lungs demonstrated significant congestion, focal haemorrhagic oedema in the alveoli and marked platelet clotting in small vessels (Figure 2). The other organs examined showed a general dilation of the microcirculation and intense congestion. There was no leukocyte accumulation in small vessels. Mast cell numbers were considerably increased in all tissues, particularly in the laryngeal mucosa, where they were demonstrated both in the submucosal layer, around small vessels, and in deeper layers (Figure 3). Mast cells, which often displayed metachromatic granules scattered in the pericellular region (Figure 4), were also numerous at the level of gland structures, muscle and small nerve bundles, where they were detected in nerve sheaths and fibres. There was a similar number of Giemsa-positive mast cells and tryptase-positive cells. CD117-positive cells were significantly more numerous (Figure 5), particularly in lymphoid tissue, where cells

with dendritic morphology and small lymphocytes with incised nuclei were also positive (Figure 6).

The toxicology report was negative.

Beta-tryptase 240.5 µg/L (n.r. < 11.5 µg/L), total IgE 248 UI/mL (n.r. < 130 UI/mL) and specific IgE (to penicillo-lyl V) 0.410 U/mL (Class n. 1–0.35–0.70 U/mL) were determined in the blood samples.

## Case 2

A 47-year-old man with no previous history for any pathological condition bought a homeopathic preparation containing propolis (a substance collected by honey bees from various botanical sources), which he self-administered to

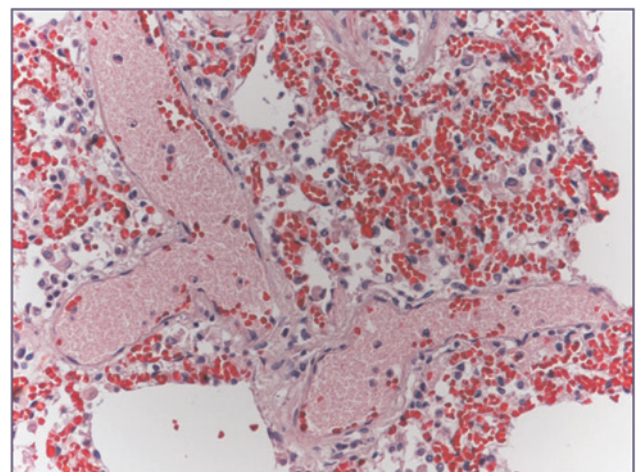


**Figure 2** Marked platelet clotting in pulmonary small vessels

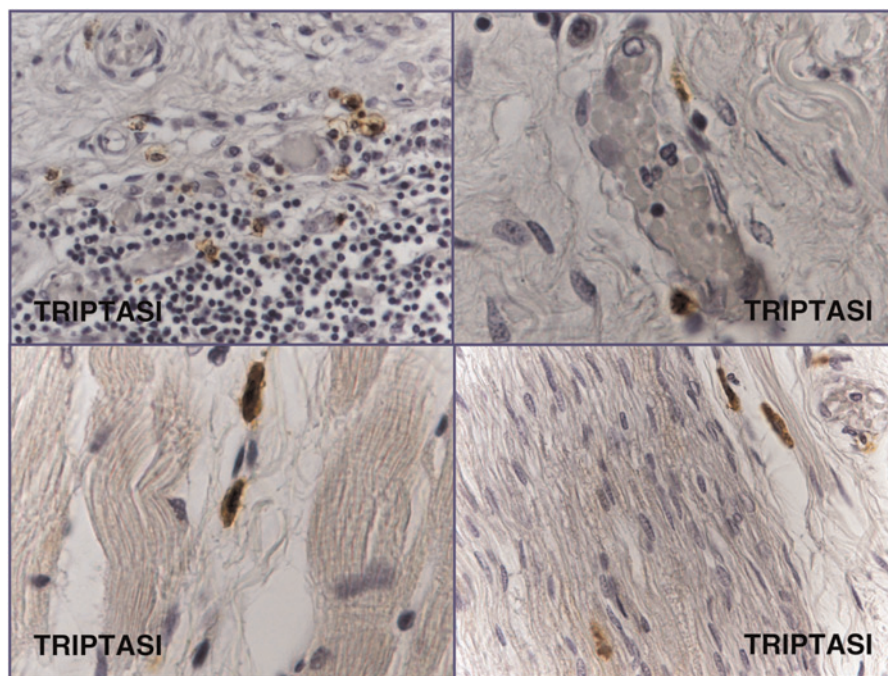


**Figure 3** Mast cells in laryngeal wall

treat a sore throat that had been painful for several days. His symptoms worsened after a fish dinner, and he presented to the emergency department. Physical examination did not show evidence of hyperaemia and/or oedema of the upper airways and all vital parameters were in the normal range. Beta-tryptase was  $2.5 \mu\text{g/L}$ . The man was kept under observation. After about 40 minutes he experienced increasingly severe dyspnoea followed by a sudden cardiac arrest that did not respond to prolonged CPR.

The post-mortem examination, requested by the magistrate, was performed 57 hours from death.

Gross examination showed marked glottis oedema, significant multivisceral congestion, pulmonary oedema and sub-pleural petechiae.

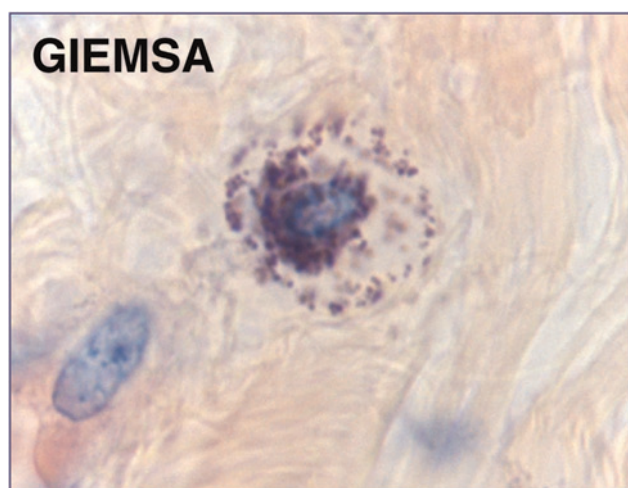


**Figure 4** Mast cell with granules scattered in the pericellular region

Severe, extensive glottic inflammation was demonstrated on histology (Figure 7). Chronic features of inflammation were predominant at the level of the mucosa, with clearly activated lymphatic follicles and intense plasmacytosis; acute inflammatory aspects were prevalent deeper in the mucosa, with abscesses that extended into the muscle layers. Slight, focal hydropic degeneration of hepatocytes and marked intravascular granulocyte accumulation were seen in the small vessels of the other organs. A marked mast cell increment was detected in all tissues, particularly the laryngeal mucosa, where they were abundant around the peripheral vessels of abscesses (Figure 8). There was a similar number of mast cells staining for Giemsa and of tryptase-positive cells. CD117-positive cells were significantly increased, especially at the level of the inflamed mucosa (Figure 9), where cells with a dendritic morphology and small lymphocytes with incised nuclei were also CD117-positive (Figure 10).

The toxicology data were negative.

Blood beta-tryptase was  $2.7 \mu\text{g/L}$  (n.r.  $< 11.5 \mu\text{g/L}$ ). Total and specific IgE were not measured.

## Discussion

Anaphylaxis is an acute, multiorgan systemic reaction with potentially fatal consequences, triggered by an abnormal, massive release of vasoactive chemical mediators from tissue mast cells and basophilic granulocytes,<sup>3,4</sup> which are responsible for the subsequent symptoms.

There is no universally accepted definition of anaphylaxis. The adjective 'anaphylactic' has traditionally been used to refer to IgE-dependent reactions, and 'anaphylactoid' to the, clinically indistinguishable, IgE-independent events (e.g. those mediated by IgG and immunocomplexes). In 2003 the World Allergy Organization recommended their

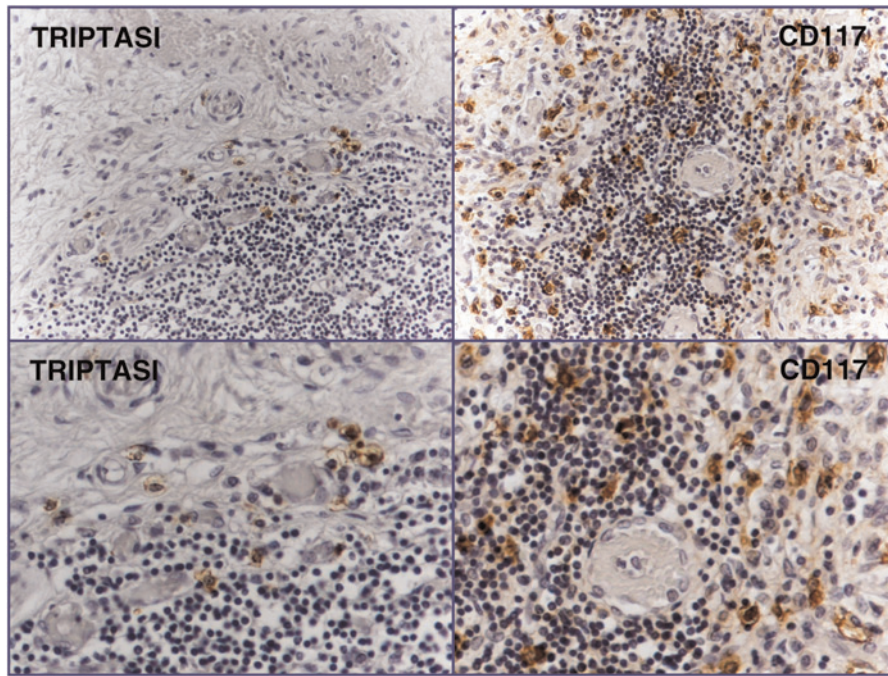


**Figure 5** Comparison between tryptase and CD117-positive cells in case 1

substitution with 'immunological' and 'non-immunological' anaphylaxis, respectively.<sup>5</sup>

The underlying pathophysiological mechanism, e.g. massive release of vasoactive mediators responsible for the subsequent symptoms, is similar in both processes.

Mast cells and basophilic granulocytes contain granules endowed with surface IgE receptors, which are an additional source of vasoactive mediators. Mast cells are mainly localized in connective tissue, lung and GI mucosa, dermis and the microcirculation. Basophilic granulocytes are found in small amounts in the circulation and can migrate to tissues.

Stimulation of mast cells and basophilic granulocytes by antigens induces degranulation, whereby the inflammation mediators contained in their dense cytoplasmic granules are secreted into the extracellular substance, releasing acid mucopolysaccharides (such as heparin), serine proteases,

chemotactic mediators for neutrophils and eosinophils, and histamine. Stimulation of mast cells and basophilic granulocytes also induces release of products of arachidonic acid metabolism, including LTC<sub>4</sub>, LTD<sub>4</sub> and LTE<sub>4</sub>, platelet-activating factor and cytokines such as tumour necrosis factor-alpha and interleukin-4.<sup>6</sup>

It should be noted that besides allergens, degranulation is also induced by exogenous factors such as cold, trauma, cationic proteins derived from platelets and from the lysosomal granules of neutrophils.<sup>6</sup>

Anaphylaxis is characterized clinically by respiratory insufficiency, pruritus, urticaria, mucosal oedema, gastrointestinal manifestations (nausea, vomiting, pain and diarrhoea) and cardiocirculatory collapse. In principle, any allergen can trigger immunological and non-immunological anaphylaxis; however, most cases are related to protein substances such as antisera, hormones, pollen derivatives, hymenoptera venom, food, drugs (particularly antibiotics) and radiological contrast media. The interval from allergen exposure to symptom onset commonly ranges from seconds to minutes.<sup>7</sup>

Data on the incidence and prevalence of anaphylaxis are scanty, also due to the fact that they often come from studies with different designs. These factors have contributed to a broad frequency range, which is 0.01–2% according to different reports.<sup>8,9</sup> Neugut *et al.*<sup>10</sup> estimated that 1–15% of the US population is at risk of immunological and non-immunological anaphylaxis, based on rates of 0.0004% for food-related anaphylaxis, 0.7–10% for penicillin, 0.22–1% for radiological contrast agents and 0.5–5% for insect bites.

Fatalities are not numerous but they are by no means rare, ranging from 0.65% to 2% of individuals with anaphylaxis.<sup>11,12</sup> In a study conducted in Olmsted County, Minnesota (USA), mortality was 0.65%.<sup>13</sup>

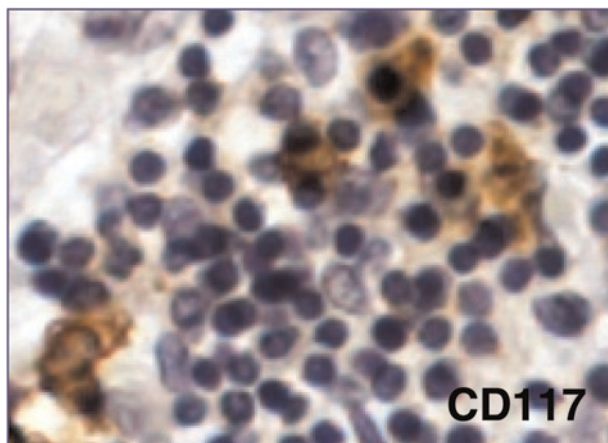


**Figure 6** CD117-positive cells with dendritic morphology and small lymphocytes with incised nuclei in case 1

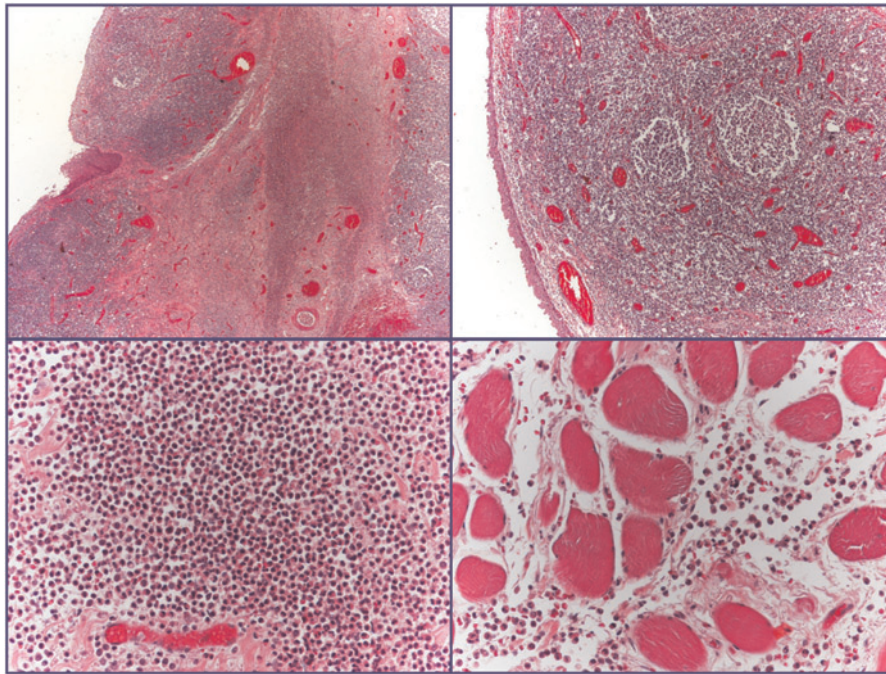


**Figure 7** Severe, extensive glottic inflammation with abscesses that extended into the muscle layers

In both our cases the circumstances of death were considered consistent with anaphylaxis.

In the first, the pathology report described glottic oedema secondary to intense mast cell reaction throughout the wall layers and an increase in beta-tryptase, total IgE and specific IgE (to penicillolyl V). An interesting finding was significant platelet accumulation in the lumen of small vessels of lungs and liver, which has been reported to be characteristic of anaphylaxis in animal studies.<sup>14</sup> In

case 1 all findings agreed with the man's history of antibacterial drug treatments, also considering his osteomyelitis. Symptom onset after injection of a synthetic beta-lactam antibiotic was rapid: gastric manifestations and generalized pruritus, which are frequent early events of anaphylactic reactions, were soon followed by loss of consciousness and cessation of cardiac and respiratory activity, which could not be reversed by the prompt arrival of the ambulance staff.

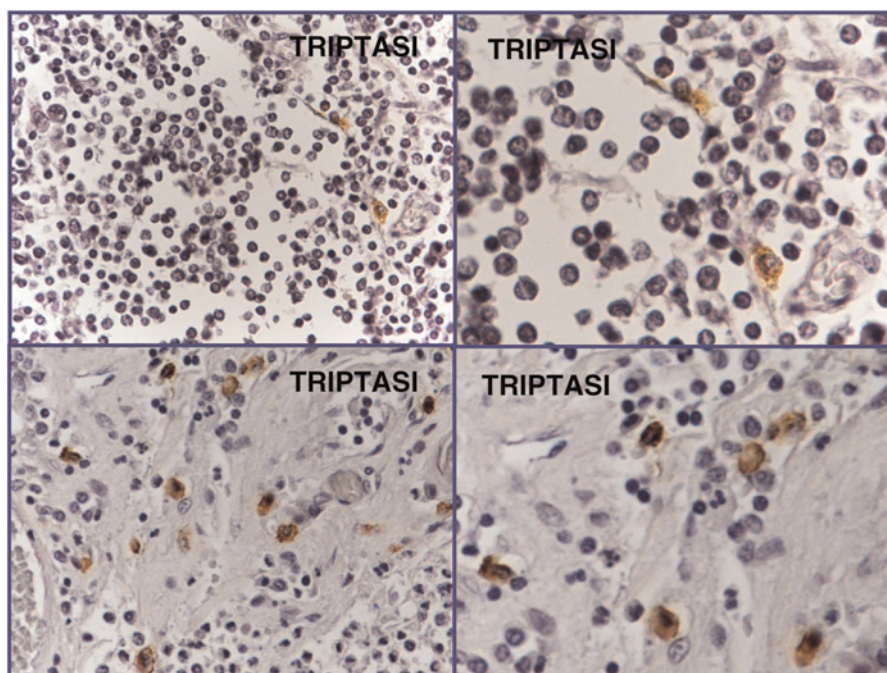


**Figure 8** Mast cells around the peripheral vessels of abscesses

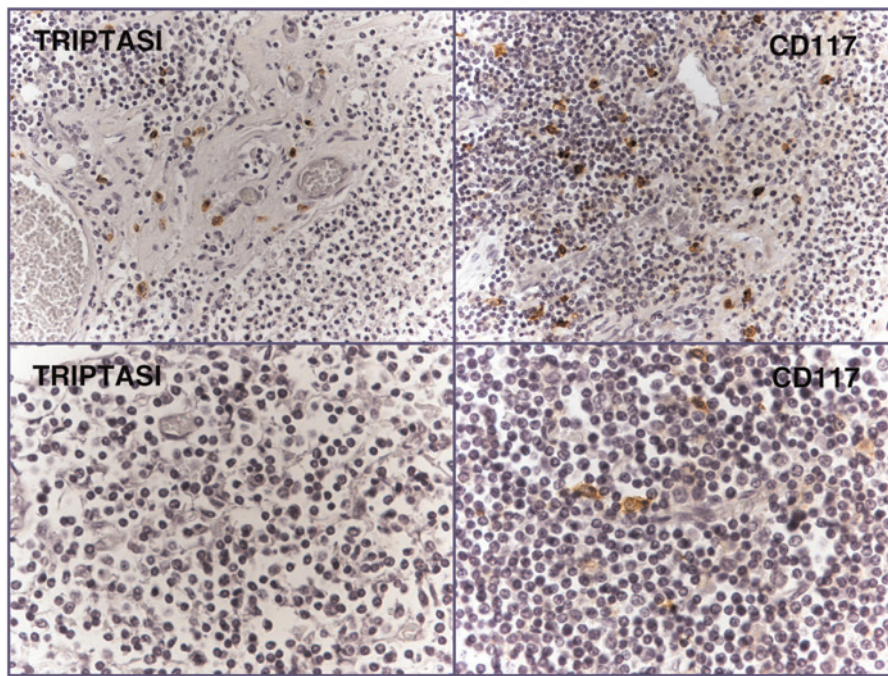


**Figure 9** Comparison between tryptase and CD117-positive cells in case 2

In the second case the postmortem findings were extensive swelling of the glottis due to severe chronic infection and abscesses, in the presence of not significantly increased beta-tryptase levels. The pathology report agreed with the clinical data both with regard to the persistent upper airways pain, which is not typical of anaphylactic reactions, and its worsening, which was the reason for the hospital admission. The single discordant element was the absence of any upper airways alteration during the short hospital stay, which did not appear to be consistent with the severe and extensive inflammation seen on autopsy.

The body of data collected led the two fatalities to be attributed with reasonable certainty to anaphylactic reaction and systemic inflammatory response syndrome, respectively.

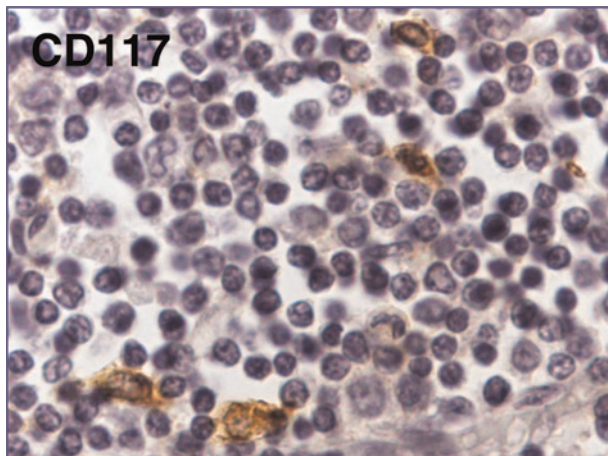


**Figure 10** CD117-positive cells with dendritic morphology and small lymphocytes with incised nuclei in case 2

In both cases microscopic examination demonstrated an increased number of mast cells in the glottis. This finding is non-specific, and can be documented in necrotic inflammatory processes not related to immunological reactions as well as in conditions secondary to immunomediated disturbances, such as anaphylaxis and mastocytosis.

Mast cell detection was based both on histochemical and immunohistochemical (tryptase and CD117) approaches. Comparison of their results showed that in tissues from both victims CD117-positive cells in each field greatly exceeded the number of Giemsa-positive mast cells and tryptase-positive cells, which were present in similar proportions.

Besides cells with a clear mast morphology, cells with the appearance of reticular macrophages and small lymphocytes with an incised nucleus were positive for CD117, and were especially numerous where lymphoid tissue was particularly abundant.

CD117 is a tyrosine kinase receptor found in several cell types, such as interstitial cells of Cajal, germ cells, bone marrow stem cells, melanocytes, breast epithelial cells, mast cells and subpopulations of lymphocytic dendritic cells.<sup>15,16</sup> It is also expressed in several tumour cells (follicular and papillary thyroid carcinoma, adenocarcinoma of endometrium, lung, ovary, pancreas and breast, malignant melanoma, endodermal sinus tumour, small-cell carcinoma, B-cell lymphoma), and is very useful in distinguishing gastrointestinal stromal tumours from Kaposi's sarcoma and smooth muscle tumours.

Despite the value of CD117 in studying mast cells, its findings, like the findings of all ancillary immunohistochemical techniques, must be interpreted in terms of cell morphology, of the location and morphological context where the positive cells are detected, and of the data from

the other analytical approaches. In addition, it is essential to be aware of which cell populations can express the reaction being studied, avoiding uncritical adherence to product datasheets.

More precise identification of positive cells can be obtained by a multiple immunohistochemical approach using several antibodies and detection systems with different chromogens in the same section.

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