



Diagnosis of anaphylactic death in forensics: Review and future perspectives



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ABSTRACT

The diagnosis of anaphylaxis in a pre- or post-mortal phase involves the formulation of problems not yet solved by the international scientific literature, due to the complexity of pathogenic factors and pathophysiological processes that characterizes it. For forensic autopsies, further problems of differential diagnosis arise and often leave the forensic pathologist unable to express an opinion of certainty, as a result of lack of case history, circumstantial and autopsical-histopathological data. Nevertheless, in routine cases the postmortem diagnosis of anaphylactic death continues to be based on exclusion and circumstantial evidence.

The author, after an extensive review of the literature relating to deaths from anaphylaxis of forensic pathological interest, and a discussion of the microscopical and biochemical findings, proposes a diagnostic protocol for forensic purposes and evaluates the diagnostic perspectives enabled by the newly available analytic techniques and markers.

Maybe, the application of omics methodologies could help in the future for anaphylaxis diagnosis.

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1. Introduction

Anaphylaxis is a type I hypersensitivity reaction which clinical symptoms can be mild to severe. It can cause death within few minutes due to cardiovascular collapse (anaphylactic shock), with symptoms like paleness, loss of consciousness, shallow breathing and frequent insensitivity to external stimuli, imperceptible pulse and severe hypotension, as well as angioedema, airway obstruction due to laryngeal oedema. In other occasions, it is presented with

more subdued symptoms like nausea, vomiting, giant hives, asthma and wheezing.

It follows contacts with the allergen through the respiratory tract (inhalation) and digestive tract (ingestion), through skin contact or blood. To the latter belongs the iatrogenic anaphylaxis, already reported by several authors in literature [1–9]. Anaphylactic deaths related to hepatic hydatid cyst have recently been published [10–12]. Very uncommon cases concern anaphylactic death secondary to an accidental sting by crown-of-thorn starfish [13], and a bee sting during paragliding activity [14]. Known allergies can be used for suicidal purposes [15].

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The diagnosis of anaphylaxis in a pre- or post-mortal phase involves the formulation of problems not yet solved by the international scientific literature, due to the complexity of pathogenic factors and pathophysiological processes that characterizes it. For forensic autopsies, further problems of differential diagnosis arise and often leave the forensic pathologist unable to express an opinion of certainty, as a result of lack of case history, circumstantial and autoptical-histopathological data [16]. Nevertheless, in routine cases the postmortem diagnosis of anaphylactic death continues to be based on exclusion and circumstantial evidence, and the knowledge of the patient's history and circumstances of death remains of major importance for the forensic pathologist when investigating suspected anaphylactic deaths [17].

Experimental research lines so far covered with histological and chromatographic-electrophoretic analytical techniques, coupled with multiple detection systems, on tissues and body fluids, have underlined the importance of the functional and pathophysiological role of mast cells, eosinophils, IgE, tryptase, in particular β -tryptase, chymase, eosinophilic cationic protein, histamine, etc... Their specific, individual or synergistic, role has not been, however, useful for formulating a diagnosis of certainty and/or high likelihood of anaphylaxis, which is relevant in the causation of the pre-mortal syndrome and the death [3,18–20].

2. Microscopical findings

The symptoms of anaphylaxis are mainly due to the combined action of vasodilatation, increased permeability of capillaries and smooth muscle contraction caused by the activation of effector cells: mast cells and blood basophils, which are activated through IgE-mediated or IgE-independent pathways (C5a complement, IL-3 cytokines and nerve growth factor) [21].

Consequently, histopathological examination will concern laryngeal oedema, bronchial constriction and acute pulmonary emphysema (Fig. 1a), tracheobronchial secretions, which combined with the exfoliation of the bronchial epithelium, determine

the formation of mucus plugs (Fig. 1c), congestion and intra-alveolar haemorrhages, congestion and oedema of major organs, etc. . .

Nevertheless, these findings are not exhaustive and specific for the diagnosis of fatal anaphylaxis, and a differential diagnosis with asthma can be necessary (Fig. 1b and 1d).

So far, studies have mainly focussed on mast cells, the most important effector cells of anaphylaxis. The evaluation of basophils – other key cells implicated in anaphylaxis-bounces technical problems, and their low blood levels resulted in their role being consistently underestimated [22].

As known, mast cells are found in connective tissue, especially skin and perivascular, and in the bronchial, respiratory and intestinal mucosa. Commonly, immunohistochemistry (IHC) is used for detecting the presence of mast cells in tissues by anti-tryptase and anti-chymase antibodies. Sandwich IHC techniques allow to identify both simultaneously. While IHC with anti-tryptase antibodies is largely used, chymase has been poorly studied in the post-mortem tissues probably because of technical problems related to the paraffin-embedded tissue processing [8,23,24].

Nevertheless, it is important to bear in mind that the diagnosis of an anaphylaxis case only based on the anti-tryptase+ mast cells number is not reliable. Mast cells live in many tissues and play a role in tissue remodelling, angiogenesis, fibrosis and tumor growth, aside from anaphylaxis and allergic inflammation. Recently, the number of mast cells has been proved increased in lungs perivascular areas following asphyxia [25]. Their number is individual-dependent. For this reason, only the tryptase degranulation detection, through IHC with anti-tryptase antibody, if very abundant, can be related to anaphylactic processes (Fig. 2) [26].

It could be useful to compare the number of mast cells and their degranulation rate by performing IHC with specific markers for mast cells, in addition to anti-tryptase antibody. anti-stem cell factor (SCF) antibody is specific for the activated form of the most important mast cell growth factor and differentiation marker (stem cell factor, or KIT-ligand). Using IHC with anti-SCF antibody allows

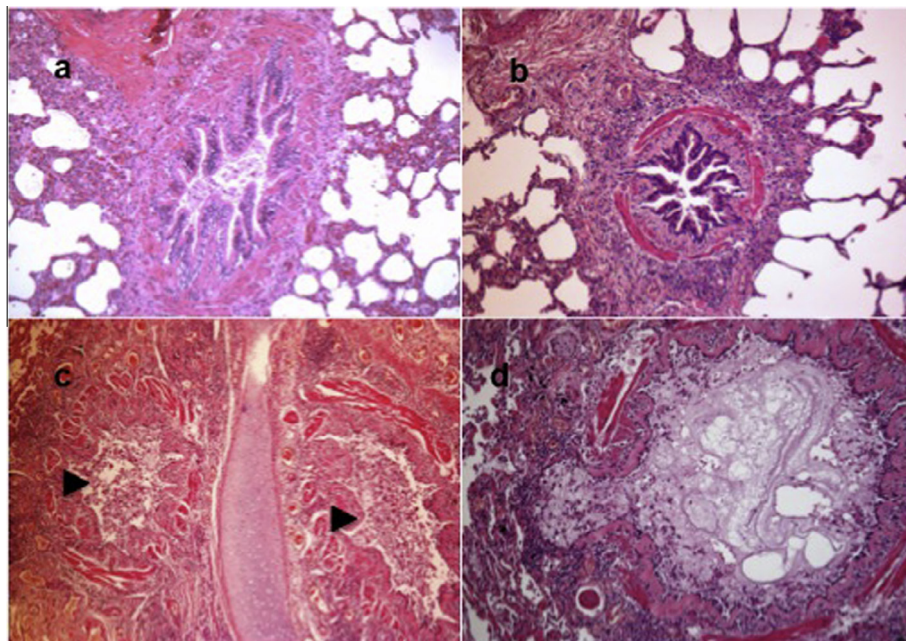


Fig. 1. Bronchial constriction and acute emphysema in a case of anaphylaxis (1a) and in asthma (1b). Note the hypertrophy of the smooth muscle layer and the intense contraction of the ciliated cuboidal epithelium in asthma (1b) compared to a looser one in anaphylaxis. Tracheobronchial secretions and exfoliation of the bronchial epithelium (arrowheads) results in a plug of mucus in a case of anaphylaxis (1c). The inspissated mucus, in which no cells are found inside the plug, is typical of the chronicity of asthma (1d) (H.E., 10x).

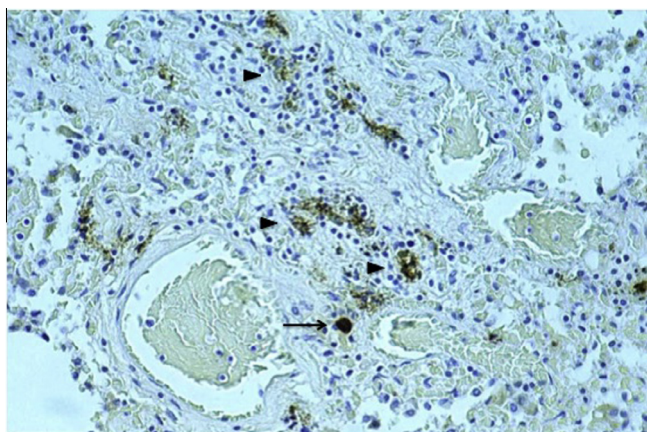


Fig. 2. Lung mastcell's tryptase degranulation (arrowheads) in a case of anaphylaxis. The arrow indicates a non-degranulated mastcell (IHC with anti-tryptase antibody, 10x).

identifying mature forms of mast cells in tissues. Experimental data suggest that the local release of SCF following an allergic stimulus may activate mast cells for IgE-mediated release of inflammatory mediators, especially LTB₄, that would drive the recruitment of eosinophils [27]. Anti-CD117 antibody is specific for the activated form of cKIT, gene that codifies the transmembrane receptor (KIT) of SCF. IHC with anti-CD117 antibody identifies the mast cells present in the tissue.

Systematic studies on the number of mast cells resident in healthy tissues, compared to those present in anaphylactic death cases, along with the rate of degranulation in both tissues, are required to give IHC with anti-tryptase antibody value of anaphylaxis evidence.

An attempt has been recently performed using the abovementioned approach: a study in two forensic cases, using anti-CD117 and anti-tryptase antibodies combined with GEMSA histochemical staining, documented a large number of mast cells in tissues, particularly in the laryngeal wall. The discrepancy between cells positive for GEMSA and tryptase and those positive for CD117 allowed making diagnosis of anaphylactic death and of systemic inflammatory response syndrome-related cardiac arrest [28].

Nevertheless, Edston [29] recently found out that the number of pulmonary mast cells and eosinophils was similar in anaphylactic deaths compared to controls. This result might confirm that only degranulation gives evidence of anaphylaxis. Furthermore, Edston showed that, in cases of anaphylactic death and in controls, where high serum concentrations of mast cell tryptase (MCT) occurred, an increase in eosinophils and mast cells was detected in the spleen, but not in the lung by comparing the accumulation of eosinophils, mast cells and basophils in such organs. Therefore, he assumed that by quantifying eosinophils 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.

The role of the spleen in anaphylaxis was also studied by Trani et al. [30] who found a splenic eosinophilia as the main feature for the differential diagnosis in 4 cases of antibiotic-induced anaphylaxis. Authors recommend the use of the pagoda red stain to show the concomitant massive presence of mast cells and degranulated mast cells, the latter mainly located in splenic sinuses, proving tryptase to be released in the blood flow.

3. Biochemical findings

Analytical techniques in biological fluids can overcome the limitations of IHC by evaluating the concentrations of blood and/or

urinary mediators secreted by mast cells or basophils, which are in turn affected by the timing of secretion and the timing of disappearance from blood circulation of the mediator, and the concentrations achieved in each case. Recently, bone marrow was investigated as an alternative matrix in postmortem toxicology because of its good resistance to autolysis and contaminations. Few autolytic changes were found and notable cell changes were also detected in anaphylactic reactions [31].

α -tryptase is constitutively secreted by mast cells in its proenzyme form, α -protryptase, and its blood concentration indicates the number of circulating mast cells. β -tryptase is secreted only after anaphylactic stimuli and shows a slow release because it is stored and linked to the proteoglycane-protease complex. It is therefore identifiable in the blood after 30', with a peak at 1–2 h. β -tryptase basal serum level is 1 ng/ml, whereas total tryptase level (α -protryptase and β -protryptase) is 1–15 ng/ml. Mast cell activation in living people is given by blood concentrations of β -tryptase > 13.5 ng/ml (b.v. 0 ng/ml) and proportional increase in total tryptase. β -tryptase values are not influenced by high levels of total tryptase, as in mastocytosis, since it needs an activating stimulus to the mast cells for the release of the mediator to be induced [18,32–34].

The ideal sample for the study of blood tryptase in living people consists of a blood draw performed about 1–2 h after the allergic reaction, when it achieves its maximum expression. Nevertheless, several studies attest its utility even when performed 3–6 days after death, but a substantial decline, probably due to degradation of the degranulated MCT, is seen with the increase of post mortem interval (PMI). Therefore it is suggested to collect a blood sample as soon as possible in cases of suspected anaphylactic death [35].

Moderately elevated MCT levels are common in postmortem sera, and the collection site is important for results evaluation. A significant correlation exists between blood tryptase and the degree of sample haemolysis.

When evaluating post-mortem blood tryptase, it should also be taken into account that β -tryptase blood concentration is very high in anaphylaxis due to contrast medium [36], and less in food anaphylaxis [37]. Intermediate values are obtained in cases of insect bites [22], but also in cases not clearly related to allergen contact, such as heroin-related deaths [38–40], or several cases of SIDS [19,20,41], or post-traumatic deaths and in cases of heart diseases [36,42]. This implies obvious difficulties of differential diagnosis, which greatly diminish the probative value of blood tryptase [43].

Despite all the precautions that must be taken in evaluating the tryptase in post mortem blood, these are its strenghts: tryptase is stable, the sample does not require freezer storage and can be collected into most common blood collection tubes. High concentrations of hemoglobin, due to haemolysation of post mortem samples, do not seem to influence tryptase analysis in itself, but could indicate the rate of cell lysis that may in itself increase a tryptase release [44]. Postmortem samples should be taken from femoral blood vessels in order to avoid the increase of tryptase released by mast cells during a prolonged cardiac massage or defibrillation. Other sites of sampling show higher level of tryptase and a cut-off of 110 μ g in aortic blood samples seems to be acceptable as diagnosis of anaphylaxis-related death because, although few cases could be missed, a sensitivity of 80% and specificity of 92.1% has been published [43]. However, it is important to report the blood collection site, the possible reasons for the higher tryptase concentration, e.g. sample degradation, release secondary to the resuscitation process (particularly cardiac defibrillation), or a nonimmune mechanism. Of course, the interpretation of these results must be considered together with all other information available [44].

Histamine is another important mediator secreted by mast cells and can cause vasodilatation, bronchial and laryngeal spasm or

hives within 5 min. It is secreted by many cell types, therefore, is not very specific of mast cells. High levels of histamine are found in basophils, and its increase in serum may also be a sign of basophils activation, or post-mortem release from basophil [18,33]. It can be studied by microfluorimetry, HPLC, spectrophotometry, spectrofluorimetry and with fluorimetric, radioenzymatic and immunologic assays, however, it is of little use in anaphylaxis diagnosis because of its 2 min half-life and the blood concentration peak occurs at 5 min, afterwards it returns to baseline values within 5–30 min and its metabolite 1-methyl-4-imidazole Acetic Acid does not have enough time to appear in the urine.

The eosinophilic cationic protein shows lack of specificity because it is secreted by eosinophils and therefore presents high blood levels even in asthma deaths and heroin-related deaths, while the IgE, which, although stable in biological fluids in post-mortem and in putrefied corpses, are not however specific to anaphylaxis [24]. Of course, when evaluating specific IgE with high tryptase blood levels, and degranulation of mast cells in tissues, anaphylaxis-related death can be diagnosed with a good accuracy.

Refinements of analytical techniques allowed the identification and the study of the biological fluids concentrations of other mediators synthesized by effector cells – mast cells and basophils – after an allergen stimulus.

Mast cells, in fact, besides preformed mediators such as histamine, serotonin, proteoglycans (heparin, chondroitin sulfate), proteases (tryptase, chymase, carboxypeptidase), also release newly synthesized mediators like cytokines (particularly TNF- α , IL-4, IL-5, IL-6), leukotrienes (LTE4 is the most specific for mast cells), prostaglandins (PGs) (9 α , 11 β -PGF2 is specific of mast cells) and platelet-activating factor (PAF) [22,41,45]: these substances are newly produced in response to the antigen contact.

Cytokines can be studied with ELISA Multiplex, IHC, RT-PCR and In Situ Hybridization (ISH) and PAF can be studied with LC-MS. The remodelling of the plasmatic membrane leads to the production of prostaglandins and leukotrienes that can be studied with HPLC [22,41,45,46].

Proteomic approaches may create a platform not only for identification and characterization, but also for quantitation of allergy-related proteins [47]. The new mass spectrometry (Energie) [MS (E)] approach is suitable for quantitative analysis and allergens profiling in food matrices [47]. In MS(E) the energy of the gas-filled travelling-wave collision cell is dynamically switched between a low-energy and an elevated-energy status, producing composite mass spectra of all intact molecular ions, followed by chimeric mass spectra of all precursors.

Recently, thanks to comprehensive metabolomics, new insights in the monitoring of the metabolic status of anaphylaxis help in understanding its pathophysiological processes and give new approaches to diagnosis. Potential biomarkers of anaphylaxis are searched with gas-chromatography mass-spectrometry (GC-MS) in serum and the metabolic profiling of anaphylaxis is achieved. Hu et al. [48] measured the IgE level in the serum with ELISA kits and then detected the major metabolic changes in serum by GC-MS. By doing so, metabolomic features are identified as metabolites related to energy metabolism and signal transduction in anaphylaxis. In another study, the alarmin uric acid was identified as a critical signal for the induction of peanut allergy, using GC-MS technique [49].

Plasma proteins in food allergy as biomarkers for use in evaluating the allergenicity of novel foods were studied. Specific IgE and specific IgG studied by ELISA, were further investigated through differential protein expression assessed in plasma and in organ tissue by two-dimensional difference gel electrophoresis (2D-DIGE). Differentially expressed proteins were identified by liquid chromatography coupled with tandem mass spectrometry. This

methodology proves to be reliable, although further validation of the proteins is needed [50].

Gel-free and label-free methods, peak integration and spectral counting, using a linear trap mass spectrometer showed to be linear and reproducible with protein standards from 5 to 200 ng, and was applied towards the analysis of transgenic peanut lines targeting the reduction of a prominent allergen [51].

All these methods should be applied to forensic cases in order to evaluate their utility in anaphylaxis-related deaths. Studies in this direction are analyzing the new synthesized mediators of mast cells in plasma and urine in both living and dead patients.

Urinary LTE4, 9 α , 11 β -PGF2, eosinophil-derived neurotoxin (EDN) and leukotriene B4 glucuronide (LTBG) concentrations were determined by enzyme immunoassay [45]. A significant increase was seen in urinary LTE4 and 9 α , 11 β -PGF2 concentrations during anaphylaxis respect to asthmatic patients on acute exacerbation and healthy control subjects, while no significant differences were found in concentrations of EDN and LTBG. Urinary LTE4 correlated significantly with 9 α , 11 β -PGF2 concentrations in the anaphylactic patients, and LTE4 concentration was significantly elevated in anaphylactic shock following anaphylaxis compared with that in patients without anaphylactic shock [45]. These results confirm the specificity of LTE(4) and 9 α , 11 β -PGF2 as mediators specific for mast cells' activation.

Aspirin-intolerant asthma patients show significantly higher LTE(4) and lower PGE(2) concentrations in urine than the aspirin-tolerant asthma group. During the reaction, the urinary concentrations of LTE(4) and PGD(2) metabolites correlatively increased, but with differently marked patterns of the mediator release: the urinary PGD(2) metabolites and primary PGs were significantly decreased in the aspirin-tolerant asthma group. Urinary enantiomers-PGF(2) α concentrations were significantly increased in the anaphylaxis group but not in the aspirin-intolerant asthma group [52].

9 α , 11 β -PGF2 is actively generated during the early allergic response to Bee Venom provoked in allergic patients, as assessed through gas chromatography-mass spectrometry negative ion chemical ionization on fluids taken from skin chamber [53].

Table 1 proposes a timetable of cell mediators in blood after mast cell activation.

Basophils are potent leukocytes in allergy and asthma. They contain high concentrations of histamine, which result in massive bronchial spasm and vasodilatation within 5 min in the anaphylaxis basophil-mediated. Basophil-mediated anaphylaxis results in rapid death due to the synergy between histamine, immediately released, followed by the action of leukotrienes-stimulated COX and 5-LO.

In allergen IgE-mediated activation the maximum activation of the basophils can be expected within 10–15 min. Expressions of CD203c or activations by non-IgE mechanisms (e.g. C5a, fMLP) are faster and require only few minutes to reach their peak [54].

In a case report on anaphylactic intraoperative death due to Thymoglobulin carbohydrate excipient, recruitment of both mastocytes and basophils, activated and degranulated, were found. Authors underline the important role played by basophils in this type of anaphylaxis, and suggest the basophil activation test (BAT) for preventing it [9].

The flow cytometric BAT is useful for in vitro allergologic diagnosis. It analyzes the expression of basophil markers and provides important advantages in patients with anaphylaxis to beta-lactams, non-steroidal anti-inflammatory drugs, neuromuscular blocking agents and drugs where there is no technique available to measure specific IgE [55]. Blood samplings require many precautions, and more studies in post mortem samples of blood are needed.

Table 1

Timetable of mast cells mediators in blood after mast cell activation. Studies in post mortem blood samples are needed for confirmation.

- > tryptase: identifiable in the blood after 30', with a peak at 1–2 h (normal values in rapid deaths)
- > 9 α , 11 β -PFG2: mast cell prostaglandin metabolite (max C: 3 h)
- > LTE4: leukotriene specific mast cell (max C: 3–6 h)
- > IL-4: at 2 h, unidentifiable at 30 min
- > specific IgE (if the reaction is supported by IgE)
- > LTE4 and 9 α , v 11 β -PFG2 in serum and/or urine (index of certain mast cell activation, lower in asthma)
- > of LTE4 correlates the severity of bronchial spasm (1000 times stronger than histamine), the increased secretion of mucus and the increased vascular permeability

Table 2

Timetable of basophils mediators in blood after basophil activation. Studies in post mortem blood samples are needed for confirmation.

- basal values of serum tryptase levels
- LTC4: increased at 30 s and significantly increased after 5 min
- IL-4: increased at 2 h, undetectable at 30 min
- specific IgE (if the reaction is supported by IgE)
- increased leukotrienes and prostaglandins serum and urine metabolites (certain index of basophil activation)

Markers of new synthesis are leukotrienes (particularly LTC4), prostaglandins, cytokines, especially IL-4, and PAF [22,41,45].

Table 2 proposes a timetable of basophils mediators in blood after basophil activation.

PAF is an important mediator in the lethality of systemic anaphylaxis. The intravenous injection of PAF increases the myeloperoxidase activity of neutrophils in lungs, and provokes death in mice. The administration of PAF acetylhydrolase reduces mortality in a dose-dependent manner [56]. Thus, failure of PAF acetylhydrolase to inactivate PAF may contribute to the severity of anaphylaxis.

Studies in patients and in fatal cases demonstrate that mean serum PAF levels are significantly higher in patients with anaphylaxis induced by peanuts (805 ± 595 pg per ml) than in patients in control groups (127 ± 104 pg per ml) and are correlated with the severity of anaphylaxis. Low levels of PAF acetylhydrolase values correlate with the severity of anaphylaxis and with fatal cases [57].

Currently, the role of the PAF/NO system is recognized as crucial in some forms of anaphylaxis. In particular, studies indicate that PAF is able to activate endothelial nitric oxide synthase (eNOS) which in turn stimulates NO within only 10–15 min, subsequently leading to a massive vasodilatation and highly increased vascular permeability with death in 30 min [58]. High levels of mRNA for induced NO synthase (iNOS) are found only after few hours, and lead to the endothelial damage and interstitial lung oedema observed in cases with longer survival [59,60].

Certainly, the knowledge of these markers'behaviour will provide new and important information that will help in the diagnosis of anaphylaxis. To achieve this goal, it is essential to consider the information given by other branches of medicine, which focus on the study of those mechanisms that induce anaphylaxis and the role played by secreted mediators, also favoured by the progress of analytical and molecular biological techniques. These prevalently concern experimental studies, mainly on animals, which focus on leukotrienes, prostaglandins, PAF, IL-1 α and TNF- α . Forensic medicine, therefore, has the opportunity to contribute with human case studies.

4. Omics methodologies in anaphylaxis diagnosis

The development of the so called analytical morphology can pave the way for a meeting point in which different skills come together and each can contribute. The IHC supported by ISH, in situ PCR, antigen retrieval, image analysis, confocal microscopy, etc. . . will enable forensic histopathology to maintain its role as a major advance in spite of the contributions of genetic and analytic techniques.

Laser Scanning Confocal Microscopy, in particular, could play a central role in contributing to the study e.g. of the sodium channel, which changes, triggered by NO, cause the increase in permeability in anaphylaxis. There are already studies which document the pre-disposition to anaphylaxis derived from alterations in sodium channels [42]. It also allows the observation of T lymphocytes behaviour, that play a well known key role in anaphylaxis, or to localize in tissues proteins involved in anaphylaxis.

Besides, with the advent of nanotechnology, an exciting area of research is offered to the problems related to the diagnosis of anaphylaxis.

Forensic genetics can make an important contribution, thanks to the innovations provided by the development of Real-Time PCR System techniques, in the study of mRNA, that shows the degree of cell activation after an allergen stimulus, by investigating the production of proteins that play a role in anaphylaxis. A study using the LightCycler Real-Time PCR System points out an increase in mRNA for the mIL-9 in the intestine of rats with anaphylaxis by oral allergens, as well as an increase of mast cells and the mast cell associated gene mMCP1, in the jejunum. These data have also been confirmed histologically by the increase of CAE positive mast cells in intestinal mucosa [46].

Studies on anaphylaxis using in situ PCR and RT-PCR techniques, by amplification of RNA, exploit the opportunity to demonstrate molecules that are expressed at low levels (e.g. growth factors, receptors, and developmental signals) [61]. Such studies open a wide field of possibilities to photograph the anaphylactic grade arisen at the moment of death, gaining with this approach an important proof at Court. Microarray on material obtained from tissues or cells make the analysis of gene expression profiles possible and hence to identify the degree of allergic reaction. In fact, the detection of RNA, which may or may not, be just translated into protein, reflects the "intentions" of the cell at the time of the death, or in other words what protein the cell was about to synthesize and what quantities.

Various immunoassays for the detection of specific IgE in serum, such as ELISA, the Radio Allergo Sorbent Test, and high-capacity solid-phase tests, e.g., the CAP system, are currently used in the diagnosis of allergies. The development of high-density ordered arrays of molecules (microarrays) in anaphylaxis studies could allow simultaneous and multi-parametric analysis of serum reactivity against a variety of antigens, opening the field to a more complex interpretation of pathological phenomena and molecular mechanisms by giving information about DNA, RNA, SNP, oligonucleotides, proteins and peptides. Although there are still problems of data analysis, it will be certainly very useful in the diagnosis of anaphylaxis. IgE specific proteomic Immuno-CAP dosage, in which

protein microarrays in combination with fluorochrome-labelled secondary antibodies are then excited by a laser and quantified with a software, have been used to reveal the presence in serum of specific IgE [62,63].

The Affymetrix integrated GeneChip array consists of a high-density oligonucleotide array synthesized directly onto glass slides by photochemical methods. The synthetic DNA oligonucleotides sequences are able to identify qualitatively and quantitatively the RNA, which is visualized with confocal scanning microscopy [64].

Combining proteomic and genomic technologies, such as MALDI (matrix-assisted laser-desorption ionization) mass spectrometry and Reverse transcription and in vitro transcription labelling of the resulting cDNA, reveals simultaneously both the protein production already planned but not implemented, and that one already in place [65,66].

The MALDI-TOF (Mass Spectrometer Protein identification by peptide mass fingerprinting), the ESI-IT (Protein and Peptide Identification and Characterisation) and NanoHPLC-MS/MS (Nano Liquid Chromatography, Mass Spectrometry Coupling) are certainly techniques of great interest in proteomics. Finally, the possibility of identifying metabolites of anaphylaxis mediators with metabolomics techniques such as GC-MS (Gas Chromatography/Mass Spectrometry system), la GC-C-IRMS (Gas Chromatography/Combustion/Isotopic Ratio Mass Spectrometry system), la LC-QqQ (Liquid Chromatography/Triple Quadrupole mass spectrometry system), il Metabolic Profiler (LC/RMN/ToF), il LTQ Orbitrap et FT-ICR MS e la LC-QToF: Liquid Chromatography/Quadrupole Time of Flight mass spectrometry system will open new diagnostic perspectives, leading to the identification of the infinitely small that still eludes the traditional and modern techniques.

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