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# Dosage des IgE dans le domaine forensique

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## Determination of specific IgE in pericardial and cerebrospinal fluids in forensic casework



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### ABSTRACT

The aim of this study was to characterize total and specific IgE distribution in postmortem serum as well as pericardial and cerebrospinal fluid samples and evaluate the diagnostic usefulness of total and specific IgE determination in pericardial and cerebrospinal fluids in the forensic setting. Three groups were investigated (non-allergic deaths in non-atopic individuals, fatal allergic anaphylaxis deaths and non-allergic deaths in individuals without medical records). In the first group (non-allergic deaths in non-atopic individuals), total IgE concentrations in postmortem serum from femoral blood, pericardial and cerebrospinal fluids were lower than 40, 32 and 11 kU/l, respectively. No specific IgE were identified in any of the sampled fluids. In the second group (fatal allergic anaphylaxis deaths), total IgE concentrations in postmortem serum from femoral blood ranged from 139 kU/l to 818 kU/l, in pericardial fluid from 89 kU/l to 622 kU/l and in cerebrospinal fluid from 4 kU/l to 11 kU/l. A positive Phadiatop<sup>®</sup> test and specific IgE antibodies >0.35 kU/l were found exclusively in postmortem serum from femoral blood and pericardial fluid. In the third group (non-allergic deaths in individuals without medical records, possibly including atopic individuals), total IgE concentrations ranged from 42 kU/l to 516 kU/l in postmortem serum from femoral blood, from 34 kU/l to 417 kU/l in pericardial fluid and from 3 kU/l to 12 kU/l in cerebrospinal fluid. A positive Phadiatop<sup>®</sup> test and specific IgE antibodies >0.35 kU/l were found exclusively in postmortem serum from femoral blood and pericardial fluid. These results seem to suggest that total and specific IgE may be measured in postmortem serum from femoral blood and pericardial fluid to estimate total and specific IgE titers at the time of death. Conversely, cerebrospinal fluid total and specific IgE measurement in suspected IgE mediated fatal anaphylaxis cases is of no value for diagnostic purposes.

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### 1. Introduction

Postmortem identification of anaphylaxis as the cause of death remains a challenge for forensic pathologists due to the absence of supportive morphological findings and specific biochemical markers [1–4].

In the clinical setting, the diagnosis of anaphylaxis is based on consistent symptoms following exposure to potential triggering agents and may be confirmed by increased levels of histamine and mast cell tryptase in plasma or serum. In the realm of forensic pathology, elevated mast cell-derived tryptase levels in postmortem serum have been considered by numerous research teams to

reflect antemortem mast cell activation. Indeed, increased mast cell tryptase values in postmortem serum have been reported in several papers regarding suspected fatal hypersensitivity reactions [1–3].

Nevertheless, other authors have claimed that increased mast cell tryptase measured in samples collected at autopsy (i.e. postmortem serum) may be an artifact caused by postmortem release of the molecule from tissues due to decomposition changes. Furthermore, increased value of mast cell tryptase in postmortem serum can occasionally be found in individuals who had died from various causes other than allergic or non-allergic anaphylaxis [4–9].

Combining results from mast cell tryptase determination in postmortem serum with a more specific assay for allergen sensitivity (such as allergen-specific postmortem serum IgE assays or total postmortem serum IgE assay when the allergen is not known) has been proposed to support the hypothesis of IgE-mediated fatal anaphylaxis [2,10,11].

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The IgE is the antibody associated with allergy and type I hypersensitivity. Although IgE is the least abundant immunoglobulin isotype (normal serum IgG levels are almost 270 times that of serum IgE levels), it can trigger powerful immunological response. Measurement of specific IgE in plasma or serum may be useful for identifying the allergen involved in the allergic reaction. Nevertheless, very few studies in the forensic setting have investigated total and specific IgE antibody behavior in postmortem serum and other biological samples collected during autopsy [12,13].

According to some observations, total and specific IgE antibodies appear to be relatively stable in peripheral blood/serum after death. However, determination of total and specific IgE in postmortem serum can only provide information pertaining to the atopic disposition and degree of sensitization to a particular allergen in individual cases, thereby not proving death preceded by IgE-mediated anaphylaxis. In addition, some authors have reported that average levels of postmortem serum total IgE would tend to increase proportionally with the postmortem interval [2,4,10,11].

Postmortem serum from femoral, cardiac or aortic blood may be partially or completely unavailable for collection and IgE determination at autopsy. Hence, there is a need to find alternative biological fluids to sample and analyze should postmortem serum prove unavailable during postmortem investigations.

In the study herein described, levels of total and specific IgE were measured in postmortem serum from femoral blood as well as pericardial and cerebrospinal fluid samples collected at autopsy in a series of deaths that underwent medicolegal investigations.

The aim of our analyses was to characterize total and specific IgE distribution in tested biological samples, thereby evaluating the usefulness of IgE determination in pericardial and cerebrospinal fluid samples for diagnostic purposes in the forensic setting.

## 2. Materials and methods

### 2.1. Study design and study populations

The present study was performed in 2015. A total of 54 autopsy cases (44 males and 10 females) with a mean age of 47 years (range 18–78 years) were both prospectively and retrospectively selected. Three study groups were formed.

The first group consisted of 16 cases (13 males, 3 females) of non-allergic death in non-atopic individuals. According to the information obtained from general practitioners, local health services, and relatives, all these individuals were non-atopic. Investigated atopic diseases and allergies included atopic dermatitis, allergic rhinitis, rhinosinusitis, bronchial asthma, atopic and allergic conjunctivitis and keratoconjunctivitis, eczema, urticaria, drug allergies, food allergies and multiple allergies.

None of these cases had been admitted to hospital prior to death, and all individuals died without cardiopulmonary resuscitation. Causes of death included hanging and abdominal stab wounds.

Case inclusion criteria consisted of postmortem interval (not exceeding 48 h) and availability of postmortem serum from femoral blood as well as pericardial and cerebrospinal fluids during autopsy. Postmortem interval was defined as the interval between death and peripheral blood sampling.

The second group consisted of 8 cases (6 males, 2 females) of fatal allergic anaphylaxis death. The selected cases encompassed fatal anaphylaxis following insect stings and fatal anaphylaxis following drug administration. According to the information obtained from general practitioners, local health services, and relatives, 5 out of 8 cases suffered from drug allergy, 2 cases from wasp/honey bee venom allergy, 2 cases from bronchial asthma and 1 case from multiple allergies.

Case inclusion criteria consisted of postmortem interval (not exceeding 48 h) and availability of postmortem serum from femoral blood as well as pericardial and cerebrospinal fluids during autopsy. Postmortem interval was defined as the interval between death and peripheral blood sampling.

Death from fatal anaphylaxis was defined as macroscopic evidence of laryngeal edema and/or acute pulmonary emphysema and/or mucous bronchial secretion, histological and/or immunohistochemical evidence of eosinophils and degranulated mast cells in the spleen, consistent laboratory findings, and exclusion of alternative causes of death based on all postmortem investigation findings.

The third group included 30 autopsy cases (25 males, 5 females) of non-allergic deaths in individuals without available medical records. None of these cases had been admitted to hospital prior to death, and all individuals died without cardiopulmonary resuscitation.

Case inclusion criteria consisted of postmortem interval (not exceeding 48 h) and availability of postmortem serum from femoral blood as well as pericardial and cerebrospinal fluids during autopsy. Postmortem interval was defined as the interval between death and peripheral blood sampling.

Causes of death included hanging and drug intoxication. Since increased tryptase levels have been occasionally observed in cases of death related to heroin-injection following intravenous administration of heroin, opiate intoxication cases were also excluded.

All cases selected for the study originated from forensic practice and underwent medicolegal autopsies as requested by local inquiring authorities (the public prosecutor). Laboratory analyses, including total and specific IgE measurement, were performed as part of the medicolegal investigations. Medical records and clinical histories of the deceased as well as police reports were consistently reviewed before conclusions were made.

### 2.2. Postmortem investigations and sample collection

Complete, conventional medicolegal autopsies, histology, toxicology and biochemical investigations were performed in all cases. Medicolegal autopsies were jointly performed by two forensic pathologists (at least one board-certified) as in accordance with both local standards and international guidelines for medicolegal cases. Conventional histology included hematoxylin-eosin (HE) stains of brain, heart, lung, liver and kidney samples. Splenic eosinophils and degranulated mast cells were specifically sought out with Pagoda Red (PR) stain. HE and PR stains were performed after tissue fixation in formaldehyde. Immunohistochemistry using anti-tryptase antibodies was also performed on spleen tissue samples.

Biochemical investigations systematically included measurement of mast cell tryptase in postmortem serum from femoral blood and total IgE determination in postmortem serum from femoral blood, pericardial fluid and cerebrospinal fluid.

The presence of specific IgE to inhalant allergens, seafood, peanuts, tree nuts, and seeds as well as wasp and honey-bee stings was systematically investigated in postmortem serum from femoral blood, pericardial and cerebrospinal fluids.

Peripheral blood from the femoral veins was systematically collected for toxicology and postmortem biochemistry as soon as possible upon arrival of the bodies at the morgue and prior to autopsy. Femoral blood samples were collected by aspiration with sterile needles and syringes from the femoral vein(s). Blood samples were drawn after clamping the vein(s) at the proximal end and lifting the lower limb(s) for several minutes. Samples were stored in tubes containing sodium fluoride and preservative free gel serum separator tubes. These latter were centrifuged immediately post

collection at  $3000 \times g$  for 15 min. After centrifugation, the separated supernatant (postmortem serum) was collected and stored in preservative-free tubes. Postmortem serum samples were transferred to the laboratories immediately post collection. When analyses were delayed, samples were stored at  $-20^\circ\text{C}$ . No specimens were excluded due to insufficient sample volume.

Undiluted cerebrospinal fluid samples were collected by aspiration using a sterile needle and syringe by suboccipital puncture as soon as possible upon arrival of the bodies at the morgue and prior to autopsy. All samples were immediately centrifuged at  $3000 \times g$  for 15 min. After centrifugation, the separated supernatant was collected and stored in preservative-free tubes. Cerebrospinal fluid samples were transferred to the laboratories immediately post collection. When analyses were delayed, samples were stored at  $-20^\circ\text{C}$ . No specimens were excluded due to insufficient sample volume.

Undiluted pericardial fluid samples were collected by aspiration using a sterile needle and syringe immediately post pericardium incision during postmortem examination. All samples were immediately centrifuged at  $3000 \times g$  for 15 min. After centrifugation, the separated supernatant was collected and stored in preservative-free tubes. Pericardial fluid samples were transferred to the laboratories immediately post collection. When analyses were delayed, samples were stored at  $-20^\circ\text{C}$ . No specimens were excluded due to insufficient sample volume.

### 2.3. Laboratory assays and statistical analyses

Mast cell tryptase and total IgE were measured with a commercial fluoroenzyme immunoassay method (Pharmacia & Upjohn, Fisher Scientific, Thermo Fisher Scientific, USA, Pittsburgh, PA).

Phadiatop<sup>®</sup> test was systematically used to detect the presence of IgE specific to inhalant allergens. Results were expressed as positive or negative. Specific IgE antibodies to both wasp (*Vespa*) and honey-bee (*Apis*) venoms were determined by using ImmunoCAP<sup>®</sup> Specific IgE. Results were expressed in kU/l. Specific IgE antibodies to seafood were measured by using UniCAP<sup>®</sup> Specific IgE kit. Results were expressed in kU/l. Specific IgE antibodies to peanuts, tree nuts, and seeds were measured by using ImmunoCAP<sup>®</sup> Specific IgE. Results were expressed in kU/l.

Total and specific IgE levels in postmortem serum from femoral blood, pericardial fluid and cerebrospinal fluid in the studied groups were compared using non-parametric tests.

All statistical analyses were performed using GraphPad Prism 4.0 (GraphPad Software, La Jolla, CA, USA). Statistical significance was defined as a *P* value of less than 0.05. Receiver-operator characteristic (ROC) curves, sensitivity and specificity values were calculated to assess the diagnostic performance of the selected parameters.

For assessing the efficacy of postmortem serum from femoral blood mast cell tryptase, the cutoff value proposed by Edston et al. [14] was also used for comparison.

### 2.4. Ethics

All relevant ethical issues were identified and discussed with the local Ethical Committee. All cases included in this study underwent medicolegal autopsies as requested by the inquiring authorities. Postmortem serum, pericardial fluid and cerebrospinal fluid samples are routinely collected during autopsy for toxicological and/or biochemical purposes in our facility. Moreover, postmortem biochemical analyses are routinely performed as part of medicolegal investigations. All biological samples were anonymized prior to analysis and analyzed in the same laboratory. Hence, no ethical approval was necessary to perform biochemical analyses in the collected cases.

### 3. Results

The main results obtained in all studied groups are summarized in Table 1.

In the first group (16 non-allergic deaths in individuals who were non-atopic according to medical records and available information), mast cell tryptase concentrations in postmortem serum from femoral blood were lower than 16 ng/ml. Total IgE concentrations in postmortem serum from femoral blood, pericardial and cerebrospinal fluids were lower than 40, 32 and 11 kU/l, respectively. There was neither evidence of increased eosinophils or degranulated mast cells in the spleen nor any positive Phadiatop<sup>®</sup> test cases in any of the tested samples. Specific IgE concentrations were lower than 0.35 kU/l in all the tested samples.

**Table 1**  
Summarizes the main results obtained in all studied groups.

Postmortem investigations	Studied groups		
	Non-allergic deaths in non atopic individuals (N=16)	Allergic deaths (fatal allergic anaphylaxis) (N=8)	Non-allergic deaths (medical information unavailable) (N=30)
<i>Autopsy/histology/immunohistochemistry</i>			
Laryngeal edema	0 cases	6 cases	0 cases
Eosinophils/mast cells in the spleen	0 cases	7 cases	0 cases
<i>Biochemical investigations results (postmortem serum)</i>			
Mast cell tryptase	4–16 ng/ml	21–200 ng/ml	4–56 ng/ml
Total IgE	6–40 kU/l	139–818 kU/l	42–516 kU/l
Positive Phadiatop <sup>®</sup> test	0 cases	2 cases	4 cases
Specific IgE antibodies (>0.35 kU/l)	0 cases	4 cases	5 cases
<i>Biochemical investigations results (pericardial fluid)</i>			
Total IgE	3–32 kU/l	89–622 kU/l	34–417 kU/l
Positive Phadiatop <sup>®</sup> test	0 cases	2 cases	4 cases
Specific IgE antibodies (>0.35 kU/l)	0 cases	4 cases	5 cases
<i>Biochemical investigations results (cerebrospinal fluid)</i>			
Total IgE	4–11 kU/l	4–11 kU/l	3–12 kU/l
Positive Phadiatop <sup>®</sup> test	0 cases	0 cases	0 cases
Specific IgE antibodies (>0.35 kU/l)	0 cases	0 cases	0 cases

No statistically significant differences between postmortem serum and pericardial fluid total IgE values were noticed. Differences between total IgE levels in postmortem serum from femoral blood and cerebrospinal fluid as well as between total IgE levels in pericardial and cerebrospinal fluids were statistically significant ( $P < 0.05$  for both comparisons).

In the fatal anaphylaxis group (8 cases), mast cell tryptase concentrations in postmortem serum from femoral blood ranged from 21 ng/ml and 200 ng/ml. Laryngeal edema was noticed in 6 out of 8 cases. Significantly increased eosinophils and degranulated mast cells in the spleen were observed in 7 out of 8 cases.

Total IgE concentrations in postmortem serum from femoral blood ranged from 139 kU/l to 818 kU/l, in pericardial fluid from 89 kU/l to 622 kU/l and in cerebrospinal fluid from 4 kU/l to 11 kU/l.

The highest pericardial fluid total IgE concentration (622 kU/l) was observed in the case with the most elevated postmortem serum total IgE value (818 kU/l).

No statistically significant differences between postmortem serum and pericardial fluid total IgE values were noticed. Differences between total IgE levels in postmortem serum from femoral blood and cerebrospinal fluid as well as between total IgE levels in pericardial and cerebrospinal fluids were statistically significant ( $P < 0.05$  for both comparisons).

A positive Phadiatop® test in both postmortem serum from femoral blood and pericardial fluid was observed in 2 cases but was systematically negative in cerebrospinal fluid.

Specific IgE antibodies ( $>0.35$  kU/l) were found in both postmortem serum from femoral blood and pericardial fluid in 4 cases. Specific IgE antibodies were systematically lower than 0.35 kU/l in cerebrospinal fluid in all cases of this group.

No case was noticed that had specific IgE antibody level higher than 0.35 kU/l in either postmortem serum or pericardial fluid exclusively.

In the third group (30 cases of non-allergic deaths in individual with no available medical information, possibly including atopic individuals), mast cell tryptase concentrations in postmortem serum from femoral blood ranged from 4 ng/ml and 56 ng/ml. No increased eosinophils or degranulated mast cells were noticed in the spleen.

Total IgE concentrations ranged from 42 kU/l to 516 kU/l in postmortem serum from femoral blood, from 34 kU/l to 417 kU/l in pericardial fluid and from 3 kU/l to 12 kU/l in cerebrospinal fluid.

The highest pericardial fluid total IgE concentration (417 kU/l) was observed in the case with the most elevated postmortem serum total IgE value (516 kU/l).

No statistically significant differences between postmortem serum and pericardial fluid total IgE values were noticed. Differences between total IgE levels in postmortem serum from femoral blood and cerebrospinal fluid as well as between total IgE levels in pericardial and cerebrospinal fluids were statistically significant ( $P < 0.05$  for both comparisons).

A positive Phadiatop® test in both postmortem serum from femoral blood and pericardial fluid was observed in 4 cases but was systematically negative in cerebrospinal fluid.

Specific IgE antibody levels were higher than 0.35 kU/l in both postmortem serum from femoral blood and pericardial fluid in 5 cases. No case was noticed that had specific IgE antibody level higher than 0.35 kU/l in either postmortem serum or pericardial fluid exclusively. Specific IgE concentrations in cerebrospinal fluid were systematically lower than 0.35 kU/l.

Using the cutoff value proposed by Edston et al. [14] in the three groups included in our study, one false negative case could be identified in the fatal allergic anaphylactic group and 2 false positive cases in the third group (non-allergic deaths in individuals

without medical records), with a sensitivity of 87.50% and a specificity of 93.33%.

If a cutoff value of 48  $\mu$ g/l is considered, we obtained the best sensitivity and specificity, with 1 false negative case in the fatal allergic anaphylactic group and 1 false positive case in the third group, with a sensitivity of 87.50% and a specificity of 96.67%.

As far as total IgE concentrations are concerned, using the cutoff value of 50 kU/l (corresponding to the upper limit reference value for total IgE in the blood in the living in the laboratory where the analyses were performed), total IgE concentrations in all tested fluids were lower than 50 kU/l in all the cases of the first group whereas all those included in the second group had total IgE concentrations in both postmortem serum and pericardial fluid higher than 50 kU/l. Total IgE concentrations in cerebrospinal fluid were systematically lower than 50 kU/l in all cases in the second group.

Concerning the third group (non-allergic deaths in individuals without available medical records, possibly including atopic individuals), total IgE concentrations in both postmortem serum and pericardial fluid were higher than 50 kU/l in 9 out of 30 cases. All these individuals had total IgE concentrations higher than 50 kU/l in both postmortem serum and pericardial fluid. No case was noticed that had total IgE concentrations higher than 50 kU/l in postmortem serum or pericardial fluid alone. In all the cases of the third group, total IgE concentrations in cerebrospinal fluid were systematically lower than 50 kU/l.

If a value of 60 kU/l (in postmortem serum from femoral blood) and 52 kU/l (in pericardial fluid) is considered, we obtained the best sensitivity and specificity, with no false negative case in the fatal allergic anaphylactic group (considering both postmortem samples) and no false negative cases in the third group (considering both postmortem samples in 9 out of 30 cases).

As regards specific IgE concentrations, levels were lower than 0.35 kU/l in all tested fluids of all the cases included in the first group using the cutoff value of 0.35 kU/l (corresponding to the upper limit reference value for specific IgE in the blood in the living in the laboratory where the analyses were performed).

4 out of 8 cases included in this second group and 5 out of 30 cases included in the third had specific IgE concentrations in both postmortem serum and pericardial fluid higher than 0.35 kU/l. In all cases included in the second and third group, specific IgE concentrations in the cerebrospinal fluid were systematically lower than 0.35 kU/l.

#### 4. Discussion

Anaphylaxis is an acute, severe, life-threatening multisystem syndrome caused by the sudden release of mast cell and basophil-derived mediators into the systemic circulation. It most often results from immunologic reactions to food, insect venom and saliva as well as medications, but any agent capable of promoting a sudden, systemic mast cell or basophil degranulation can produce it. Anaphylaxis can be initiated by specific immunologic mechanisms, which may be IgE-dependent or IgE-independent, or by non-immunologic mechanisms [14–17].

IgE antibodies are key mediators for allergic diseases and allergic anaphylaxis. The classic form of IgE-mediated anaphylaxis involves prior sensitization to an allergen, which causes specific IgE formation, and renewed contact with the allergen, which causes symptoms by means of an IgE-mediated immunologic mechanism [15,18,19].

In the living, increased plasma total IgE concentrations are usually found in atopic individuals, with the highest levels generally being measured in atopic dermatitis, followed by atopic asthma, perennial allergic rhinitis, and seasonal allergic rhinitis. Nevertheless, higher total IgE values can be frequently found in

other diseases, including parasitic infections, non-parasitic infections, hematologic malignancies, skin diseases, primary immunodeficiency states, and numerous other conditions including nephrotic syndrome, cystic fibrosis and Kawasaki's disease. Elevated total IgE levels have also been measured following hematopoietic stem cell transplantation, in individuals suffering from chronic ethanol misuse, and smokers, especially males, where the physiologic, age-dependent IgE blood level decrease was not noticed [20–24].

In the clinical setting, the diagnosis of anaphylaxis is based on consistent symptoms following exposure to potential triggering agents and may be further confirmed by increased levels of histamine and mast cell tryptase in plasma or serum [8,19,25–28].

In the realm of forensic pathology, the identification of fatal anaphylaxis is extremely challenging for several reasons. Factors consistent with the hypothesis of fatal IgE-mediated anaphylaxis may include an immediately preceding challenge with an allergen known to cause reactions, clinical features consistent with or suggesting anaphylaxis, a previous history of reactions to similar or cross-reactive allergens, increased levels of mast cell-derived tryptase in postmortem serum, increased postmortem serum levels of specific IgE antibodies to the allergen suspected of causing the reaction as well as the histologically and immunohistochemically documented accumulation of activated mast cell and eosinophils in the red pulp of the spleen [4,8,25,29–33].

Total and specific IgE antibodies have been demonstrated to be relatively stable in postmortem serum samples and their measurements have proven useful for diagnostic purposes in the forensic setting, though increased postmortem serum levels of total IgE can only provide information pertaining to atopic disposition in individual cases and do not prove that death was preceded by IgE-mediated anaphylaxis [4,5,8,11,25].

The human pericardial cavity contains 20–60 ml of fluid under normal conditions, with a balance between production and removal determining this volume. Though the composition of physiologic human pericardial fluid is difficult to define, it is considered to be a plasma ultrafiltrate derived from epicardial capillaries. A further small amount derives from the interstitial fluid within the underlying myocardium. According to the available literature, the concentration of small molecules (such as urea nitrogen, creatinine, uric acid, electrolytes and glucose) in the pericardial fluid corresponds to that of plasma ultrafiltrate. In contrast, protein concentration is higher than expected in a plasma ultrafiltrate. Immunoglobulin presence and isotype in the human pericardial fluid was occasionally investigated in the clinical setting. It has been demonstrated that pericardial fluid may contain IgM, IgG and immune complex in specific diseases. Moreover, pericardial fluid IgE and IgE-containing immune complexes were found in patients with rheumatoid pericarditis and chronic Chagas disease [34–37].

Cerebrospinal fluid is secreted by the choroid plexus situated in the lateral, third and fourth ventricles of the brain. The secretion process starts with a passive plasma filtration due to a pressure gradient from choroid capillaries. The cerebrospinal fluid is then actively secreted into the ventricular spaces through the choroid epithelial cells. In normal adults, the total cerebrospinal fluid volume is completely replaced three or four times each day and is estimated to be 100–150 ml [38].

Several cerebrospinal fluid constituents are maintained at concentrations that are different from those in plasma, thus indicating that this fluid is not simply a protein-free plasma ultrafiltrate. Protein composition and total protein concentration are different from plasma. The main fraction of proteins in the normal, human cerebrospinal fluid originates from the plasma. Protein transfer from the plasma follows the laws of diffusion depending on the size of the molecule. Albumin constitutes

35–80% of total proteins. There is normally a small concentration of immunoglobulins in the cerebrospinal fluid, which are mainly represented by small size immunoglobulins. Cerebrospinal fluid immunoglobulins derive both from plasma or may be synthesized intrathecally. This latter fraction is rather constant and depends on underlying pathologies and their localization within the brain. IgE in the cerebrospinal fluid have been observed in the course of cerebral infections by *Toxoplasma gondii*, *Cysticercus cellulosae* and *Schistosoma mansoni* [39–49].

Within the limits of the number of subjects involved in the study, the results of the study presented herein suggest that:

- total IgE measurements in the forensic setting using a cutoff value of 60 kU/l in postmortem serum from femoral blood and 52 kU/l in pericardial fluid may allow atopic individuals to be identified. It must be highlighted, however, that cutoff values and diagnostic efficacy depend on study population and institutional provisions,
- specific IgE measurements in the forensic setting using a cutoff value of 0.35 kU/l in both postmortem serum and pericardial fluid allow atopic individuals to be identified. Again, it must be highlighted that cutoff values and diagnostic efficacy depend on study population and institutional provisions,
- pericardial fluid can be considered an alternative to postmortem serum for total and specific IgE measurement should postmortem serum prove unavailable or insufficient and the hypothesis of IgE-mediated fatal anaphylaxis must be investigated by biochemical analyses,
- total and specific IgE measurements in the forensic setting using a cutoff value of 50 kU/l and 0.35 kU/l respectively in cerebrospinal fluid do not allow atopic individuals to be identified and are of no diagnostic value in cases of suspected IgE-mediated anaphylaxis.

This is the first study, to our knowledge, to have investigated total and specific IgE in pericardial and cerebrospinal fluids in a series of cases of fatal anaphylaxis that had undergone forensic investigations. We were unable to find similar studies in the forensic setting with which to compare our results.

Our present study has some limitations. The most important is the relatively small number of studied cases, which may limit the accuracy of our research. However, precise selection criteria were applied during the recruitment process in all study groups to minimize heterogeneity in the study populations. Prospective investigations including a greater number of subjects are therefore needed to confirm our findings.

## 5. Conclusions

Though further studies are required to confirm these preliminary observations, our results seem to suggest that total and specific IgE may be measured in both postmortem serum from femoral blood and pericardial fluid for estimating total and specific IgE titers at the time of death.

It is worth highlighting, however, that increased total and specific IgE levels in postmortem serum (and pericardial fluid) do not prove that death was preceded by IgE-mediated anaphylaxis but can only suggest antemortem atopic disposition and the degree of specific allergen sensitization in individual cases. The hypothesis of IgE-mediated fatal anaphylaxis as the cause of death can therefore be formulated exclusively based on consistent circumstantial evidence (when available), in-depth forensic investigations (including measurement of mast cell-derived tryptase, total IgE and specific IgE in postmortem serum samples as well as histological and immunohistochemical documentation of activated mast cell and eosinophil accumulation of in the red pulp of the

spleen) and exclusion of alternative causes of death based on all postmortem investigation findings.

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# Postmortem serum levels of total IgE

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**Abstract** The first aim of this study was to assess whether non-allergic deaths in non-atopic individuals with increasing postmortem intervals are characterized by progressively greater concentrations of total IgE in postmortem serum from femoral blood. Our second goal was to determine whether traumatic deaths with different survival times, septic deaths, and deaths in individuals suffering from diseases with significant systemic inflammation are systematically characterized by increased concentrations of total IgE in postmortem serum from femoral blood. Four study groups were prospectively and retrospectively formed (non-allergic deaths in non-atopic individuals with increasing postmortem intervals, traumatic deaths in non-atopic individuals with different survival times, deaths possibly related to sepsis in non-atopic individuals, and deaths occurring in non-atopic individuals with disseminated malignancies at autopsy). Unenhanced computed tomography, autopsy, histology, and biochemistry were performed in all cases. First results indicate that increasing postmortem intervals are not associated with progressively increasing postmortem serum IgE levels. Moreover, the obtained results do not reveal that severe trauma, bacterial sepsis, and disseminated malignancies are systematically associated with increased postmortem serum IgE levels, irrespective of survival time duration. Though the usefulness of increased total IgE concentrations in postmortem samples to assess any underlying atopic disposition or death preceded by acute IgE-mediated allergic reaction remains questionable, measurements of total IgE are possible in postmortem serum samples.

**Keywords** IgE · Death · Autopsy · Postmortem biochemistry

## Introduction

The identification of fatal anaphylaxis in the forensic setting remains challenging due to the absence of specific biochemical markers [1]. Elevated mast cell-derived tryptase levels in postmortem serum from femoral, subclavian, and aortic blood have been reported to reflect antemortem mast cell activation, compatible with an anaphylactic reaction [1, 2].

On the other hand, some authors have postulated that higher than clinical reference levels in postmortem samples cannot be considered as reliable as those in the clinical setting due to decompositional changes. In addition, increased concentrations of mast cell-derived tryptase were demonstrated in postmortem serum in individuals with causes of death unrelated to allergy or IgE-mediated allergic anaphylaxis [3–7].

Accordingly, combining results from postmortem serum assay for mast cell-derived tryptase with a more specific assay for allergen sensitivity, such as allergen-specific postmortem serum IgE assays (or total postmortem serum IgE assay when the candidate allergen is unknown), has been suggested to support a postmortem diagnosis of IgE-mediated allergic anaphylaxis [2, 8, 9].

It is well known that atopic diseases are characterized by IgE antibody overproduction. Hence, high titers of total IgE measured after death may provide information on antemortem atopic disposition, regardless of possible IgE-mediated allergic reactions prior to death. It is worth noting that, in the clinical setting, increased levels of total IgE have been observed in infections with certain parasites. Moreover, high concentrations of total IgE have been reported in patients with sepsis after traumatic injury and various other diseases characterized by immune deficiencies or significant inflammatory

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components (i.e., neoplasms and some cutaneous disorders). In the realm of forensic pathology, it has been demonstrated that both total and specific IgE can be measured in postmortem serum from femoral blood. However, according to the results of some authors, average levels of postmortem serum total IgE would tend to increase proportionally with postmortem interval [8, 10–13].

Since the usefulness of postmortem serum total IgE for the diagnosis of IgE-mediated fatal anaphylaxis is quite limited, few investigations have been dedicated to this topic in forensic literature. As a result, little is known about total IgE concentrations that can be found in postmortem serum correlated to postmortem interval or cause of death.

In the study herein described, levels of total IgE were measured in femoral blood postmortem serum samples collected at autopsy in a series of deaths that underwent medicolegal investigations. Our first aim was to determine whether non-allergic deaths in non-atopic individuals with increasing postmortem intervals are characterized by progressively greater concentrations of postmortem serum total IgE. Our second aim was to assess whether traumatic deaths with different survival times, septic deaths, and deaths in individuals suffering from diseases with significant systemic inflammation (i.e., disseminated malignancies) are systematically characterized by increased postmortem serum total IgE levels.

## Materials and methods

### Study design and study populations

The present study was performed during 2015. A total of 110 autopsy cases (95 males and 15 females) with a mean age of 48 years (range 19–79 years) were both prospectively and retrospectively selected and included the following:

- 60 cases of non-allergic deaths in non-atopic individuals with increasing postmortem intervals (20 cases with a postmortem interval up to 24 h, 20 cases with a postmortem interval ranging between 24 and 48 h, and 20 cases with a postmortem interval greater than 48 h). Postmortem interval was defined as the interval between death and peripheral blood sampling at autopsy. None of these cases had been admitted to hospital prior to death, and all individuals died without cardiopulmonary resuscitation. Causes of death included hanging, thoracic gunshot wounds, and drug intoxication. Cases of heroin, methadone, and other opioid intoxication were excluded
- 30 cases of traumatic deaths in non-atopic individuals (10 cases with a survival time at hospital up to 4 h, 10 cases with a survival time ranging between 4 and 8 h, and 10 cases with a survival time greater than 8 h). All these cases were admitted to hospital and included road traffic

victims (crash-involved cyclists or pedestrians) and high falls. None received transfusion with blood products. Patients with the shortest survival intervals were admitted in a state of hemodynamic instability due to massive, active bleeding and died rapidly after arrival to the intensive care units. In those with the longest survival times, multiple traumas led to progressive, multiple organ failure. In all these cases, the cause of death was determined to be multiple traumas. None of these cases developed a septic condition. In 15 out of 30 cases (5 cases for each of the tested subgroups), antemortem serum samples were obtained and analyzed for total IgE concentrations. These samples were dispatched refrigerated to the laboratory in charge of analysis

- 10 cases of deaths possibly related to sepsis in non-atopic individuals. None of these cases had been admitted to the intensive care units of local hospitals. The cause of death was attributed in all cases to multiple organ failure, possibly related to sepsis based on the results of postmortem investigations. Underlying bacterial infections and sepsis were postulated as the cause of multiple organ failure. Alternative causes of death were excluded based on autopsy and other investigation findings. Respiratory tract infections were the infectious foci most often identified by means of autopsy, histology, and bacteriology. Determination of procalcitonin and C-reactive protein in postmortem serum from femoral blood revealed increased levels of both parameters
- 10 cases of natural deaths occurring in non-atopic individuals with disseminated malignancies at autopsy (8 cases) and non-Hodgkin lymphoma (2 cases)

All cases selected for the study originated from forensic practice and underwent medicolegal autopsies as requested by local inquiring authorities (the public prosecutor). Intervals between deaths and autopsies ranged from 12 to 56 h. Laboratory analyses, including total IgE measurement, were performed as part of the medicolegal investigations. Availability of postmortem serum was the most important criterion for case inclusion. Medical records and clinical histories of the deceased as well as police reports were consistently reviewed before conclusions were made. According to the information obtained from general practitioners, local health services, and relatives, all individuals were non-atopic. Investigated atopic diseases and allergies included atopic dermatitis, allergic rhinitis, rhinosinuitis, asthma, atopic and allergic conjunctivitis and keratoconjunctivitis, eczema, urticaria, drug allergies, food allergies, and multiple allergies.

### Postmortem investigations and sample collection

Unenhanced computed tomography (CT) scans were performed before any manipulation of the corpses in all cases

included in this study. Complete, conventional medicolegal autopsies, histology, toxicology, and biochemical investigations were performed in all cases. Bacteriology was performed in all cases with suspected sepsis. Specimens for bacteriology were collected from at least two different sampling sites. Medicolegal autopsies were jointly performed by two forensic pathologists (at least one board-certified) as in accordance with both local standards and international guidelines for medicolegal cases. Conventional histology included hematoxylin-eosin (HE) stains of brain, heart, lung, liver, and kidney samples.

Peripheral blood from the femoral veins was systematically collected for toxicology and postmortem biochemistry as soon as possible upon arrival of the bodies at the morgue and prior to autopsy. Femoral blood samples were collected by aspiration with sterile needles and syringes from the femoral vein(s). Blood samples were drawn after clamping the vein(s) at the proximal end and lifting the lower limb(s) for several minutes. Samples were stored in tubes containing sodium fluoride and preservative-free gel serum separator tubes. These latter were centrifuged immediately post collection at 3000g for 15 min. After centrifugation, the separated supernatant (postmortem serum) was collected and stored in preservative-free tubes. No specimens were excluded due to insufficient sample volume. Postmortem serum samples were transferred to the laboratories immediately post collection. When analyses were delayed, samples were stored at  $-20^{\circ}\text{C}$ .

### Laboratory assays

Biochemical investigations systematically included measurement of total IgE in postmortem serum from femoral blood. Total IgE were measured with a commercial fluoroenzyme immunoassay method (Pharmacia & Upjohn, Fisher Scientific, Thermo Fisher Scientific, USA, Pittsburgh, PA). For the purpose of this study, an increased level of total IgE in postmortem serum from femoral blood was defined as that exceeding the highest upper limit of the clinical reference value indicated by the clinical laboratory where the analysis was performed (5–50 kU/l).

### Results

Data were analyzed by using the STATA statistical software package (STATA Corporation, College Station, TX, USA). Postmortem serum total IgE levels among and within the selected groups and subgroups were compared using non-parametric tests. Statistical significance was set at  $p < 0.05$ .

Table 1 summarizes the main results obtained in all studied groups and subgroups.

In the first studied population (non-allergic deaths in non-atopic individuals with increasing postmortem intervals), the

obtained results do not indicate that increasing postmortem intervals are associated with progressively increasing post-mortem serum IgE levels due to molecule release after death from autolyzed cells or tissues. Postmortem serum total IgE levels do not display significant differences relating to post-mortem interval length and/or cause of death.

In the second studied group (traumatic deaths in non-atopic individuals with increasing survival time at the hospital), the obtained results do not indicate that severe trauma is systematically associated with increased blood IgE levels. Postmortem serum total IgE levels do not show significant differences relating to survival time duration. In addition, no statistically significant differences are noticed when comparing the results obtained from total IgE determination in antemortem and postmortem serum samples. It is worth noting that antemortem serum samples were obtained exclusively in 15 out of 30 cases (5 cases for each of the tested subgroups). Antemortem serum was unavailable in the only case having a survival time greater than 8 h with postmortem total IgE concentration higher than clinical reference values (total IgE concentration 65 kU/l).

Lastly, the results of our analysis indicate that cases of bacterial sepsis and disseminated malignancies are not systematically associated with increased blood total IgE levels, at least within postmortem intervals up to 56 h.

Globally considered, these results suggest that total IgE are relatively stable in peripheral blood after death irrespective of postmortem interval length and do not increase depending on trauma relevance, survival time, or cause of death. This can be deducted pertaining to the survival times, causes of death, and diseases considered in this study.

### Discussion

Anaphylaxis is an acute, potentially life-threatening multisystem syndrome caused by the sudden release of mast cell- and basophil-derived mediators into systemic circulation. It most often results from immunologic reactions to food, medications, and insect stings, but any agent capable of promoting a sudden, systemic mast cell or basophil degranulation can produce it. Anaphylaxis can be initiated by specific immunologic mechanisms (allergic anaphylaxis) or by non-immunologic mechanisms (non-allergic anaphylaxis). Allergic (immunologic) anaphylaxis may be IgE-dependent or IgE-independent (IgG-mediated or immune complex-mediated) [14–17].

The pathogenesis of anaphylaxis is arguably quite obscure, though a murine model has demonstrated two distinct mechanisms that most likely apply to humans as well. The first IgE-dependent reaction is both interleukin (IL)-4 and IL-4 receptor-dependent and is initiated by activation of the high-affinity receptor Fc $\epsilon$ RI present on mast cell and basophil membranes.

**Table 1** Summary of the main results obtained in all studied groups and subgroups

Study populations	IgE levels higher than the clinical reference values (5–50 kU/l)	
	PM sample	AM sample
Non-allergic deaths in non-atopic individuals PMI < 24 h (n = 20)	3/20 (1 hanging, 1 intoxication, 1 gunshot wound) IgE concentrations	
Non-allergic deaths in non-atopic individuals PMI > 24 h < 48 h (n = 20)	3/20 (1 hanging, 2 intoxications) IgE concentrations 285, 76, and 187 kU/l, respectively	
Non-allergic deaths in non-atopic individuals PMI > 48 h (n = 20)	2/20 (1 intoxication, 1 gunshot wound) IgE concentrations 175 and 126 kU/l, respectively	
Traumatic deaths in non-atopic individuals ST < 4 h (n = 10)	0/10	0/5
Traumatic deaths in non-atopic individuals ST > 4 h < 8 h (n = 10)	0/10	0/5
Traumatic deaths in non-atopic individuals ST > 8 h (n = 10)	1/10 IgE concentration 97 kU/l	0/5
Sepsis (n = 10)	2/10 IgE concentrations 189 and 222 kU/l	
Malignancies (n = 10)	2/10 IgE concentrations 216 and 88 kU/l	

PM sample postmortem sample, AM sample antemortem sample, PMI postmortem interval, ST survival time

FcεRI aggregation caused by polyvalent antigen-induced IgE cross-linking incites phosphorylation cascades within the cells, promoting significant morphological and transcriptional modifications as well as rapid release of preformed mediators along with de novo synthesis of lipid mediators. These act on target tissues to trigger the early phase of the allergic response, which occurs within minutes of allergen exposure. The released inflammatory mediators and cytokines are responsible, among others, for increased vascular permeability (leading to edema of airway walls), smooth muscle contraction, and mucus secretion by epithelial cells (leading to the further airflow/airway narrowing) that clinically characterize anaphylaxis. Hours later, FcεRI-activated cells produce proinflammatory and immunomodulating cytokines and chemokines that promote recruitment of other cells including eosinophils, monocytes, and T cells. These events can elicit “late-phase” allergy symptoms in which a second wave of allergic symptoms is experienced 8–12 h after the acute reaction. The second mechanism of anaphylaxis is IgE-independent and involves antigen ligation of IgG receptor FcγRs bound on mast cell membranes. As for IgE receptor FcεRI, the activation of IgG receptors causes a complex cascade of signals culminating in mast cell degranulation [14, 18–20].

IgE antibodies are key mediators for allergic diseases. Their production is tightly regulated at molecular and cellular levels, resulting in a serum concentration that is several orders of magnitude lower than that of either IgG or IgA. In the

absence of disease, the concentration of IgE in serum is the lowest of the five human immunoglobulin isotypes and is age dependent: cord blood IgE levels are low (<2 kU/l) and they gradually increase throughout childhood, with a peak at 10–15 years of age. Levels decrease from the second to the eighth decade of life. Approximately 50 % of total body IgE is intravascular, with a half-life of free IgE of about 2 days. Once IgE has bound to mast cells, the half-life is extended to about 2 weeks due to the high affinity of this interaction [21, 22].

IgE shares a similar molecular structure with other immunoglobulin isotypes with two pairs of identical, heavy and light chains. IgE antibodies exert their biological effects via several receptors. The two main ones are commonly referred to as the “high-affinity receptors,” FcεRI, which is expressed on mast cells and basophils as a αβγ<sub>2</sub> tetramer, and the low-affinity receptor (FcεRII; CD23), which is present on B cells. A trimeric αγ<sub>2</sub> form of FcεRI exists on the surface of dendritic cells, Langerhans cells, and eosinophils in humans [20, 21].

Total IgE levels are influenced by genetic makeup, race, immune status, environmental factors (e.g., season of the year, pollen exposure), and the presence of diseases. Elevated IgE concentrations are usually observed in individuals with atopic conditions, with the highest levels generally being seen in atopic dermatitis, followed by atopic asthma, perennial allergic rhinitis, and seasonal allergic rhinitis. For seasonal allergens, peak IgE levels occur 4–6 weeks after pollen season peak. Increased IgE concentrations are also seen in other

situations, including parasitic infections (e.g., strongyloidiasis, ascariasis, schistosomiasis); non-parasitic infections (e.g., EBV, CMV, HIV, *Mycobacterium tuberculosis*); hematologic malignancies (e.g., Hodgkin's lymphoma, IgE myeloma); skin diseases (systemic lupus erythematosus, generalized pustular psoriasis, bullous pemphigoid); primary immunodeficiency states; and numerous other conditions including nephrotic syndrome, cystic fibrosis, and Kawasaki's disease. Elevated IgE levels have also been observed following hematopoietic stem cell transplantation, in individuals suffering from chronic ethanol misuse, and smokers, especially males, where the physiologic, age-dependent IgE blood level decrease was not observed [21, 23–27].

T-helper 2 (Th2) cell responses have been shown to play a critical role in both protection against helminth infections and atopic diseases. Th2 cells stimulate high titers of antibody production. In particular, IL-4, IL-10, and IL-13 activate B cell proliferation, antibody production, and class-switching. Indeed, class-switching from IgG to IgE cannot occur without the presence of IL-4 or IL-13, making the production of IgE a perfect bioassay for the presence of Th2 cells in vivo. Unlike inflammation stimulated by type 1 cytokines, type 2-mediated inflammation is characterized by eosinophilic and basophilic tissue infiltration, as well as extensive mast cell degranulation [28–31].

Concentration modifications pertaining to total blood proteins, albumin/globulin ratio, IgE, and other immunoglobulins after death have rarely been the subject of in-depth examinations in forensic literature. It is generally agreed that postmortem electrophoresis patterns tend to reproduce antemortem patterns, save when significant hemolysis is present. Gamma globulin has been found to be the most stable blood protein fraction, with a good correlation between antemortem and postmortem levels [32–34].

Brazinsky and Kellenberger [33] compared IgG, IgA, and IgM concentrations in antemortem and postmortem paired heart blood sera from 32 autopsies and observed that there was no distinct trend to increase or decrease in any of the measured immunoglobulins, except in the highest ranges, which showed a slight trend to increase. According to these results, the authors concluded that specific immunoglobulin deficiency or monoclonal gammopathy might be reliably diagnosed using postmortem blood samples. Analogously, McCormick [34] failed to observe appreciable loss of immunoglobulins A, G, and M when comparing antemortem samples and postmortem serum samples obtained from right-ventricle heart blood in a series of hospital autopsy cases, thus concluding that, within the limits of occasional changes in individual cases, postmortem antibody titers tend to reflect the antemortem state [32–34].

The presence of IgE antibodies against the appropriate venom in the sera of individuals who died from sting-induced anaphylaxis was demonstrated for the first time

by Hoffman et al. [35] and subsequently confirmed by other research teams, along with the presence of specific drug- and food-reactive IgE antibodies [10, 36–43]. Moreover, Hoffman et al. [35] reported that IgE antibodies appeared to be able to survive in unseparated blood samples stored in tubes with the preservative sodium fluoride at refrigerator temperatures for up to 6 months before being assayed. On the other hand, these authors recommended promptly drawing freezing serum samples should laboratory analysis be delayed. Yunginger et al. [2] tested IgE antibody stability in blood obtained from three living persons who were allergic to different allergens under a variety of storage conditions and temperatures. These authors found that IgE antibodies were moderately stable during storage in tubes containing a variety of anticoagulants at room temperatures for up to 11 weeks. According to this study, IgE antibody titers tended to decrease to a greater degree in fluoride/heparin tubes. Nevertheless, this decline did not produce a false-negative result in any of the tested cases.

The results of the study presented herein confirm that total IgE are relatively stable in peripheral blood after death, at least with postmortem intervals up to 56 h.

On the other hand, our findings do not confirm the conclusions formulated by Horn et al. [8], who observed average levels of postmortem serum total IgE proportionally increased depending on postmortem interval length. Lastly, our results failed to indicate that elevated concentrations of total IgE in peripheral blood may be systematically found in individuals with severe trauma injury (irrespective to survival time) or bacterial sepsis.

The limitations of our study must be acknowledged. The most important is the relatively small number of studied cases, which may limit the accuracy of our research. However, precise selection criteria were applied during the recruitment process in all study groups and subgroups in order to minimize heterogeneity in the study populations. Prospective investigations including a greater number of subjects are therefore needed to confirm or invalidate our findings. The second limitation concerns the reliability of the information that all individuals included in the study were non-atopic. Even though the decedent's medical records were obtained from general practitioners, local health services, and family members in most of the studied cases, in some cases, clinical data were relatively scarce.

To conclude, measurements of total IgE are possible in postmortem serum samples, though a positive result will not prove that death was preceded by acute IgE-mediated allergic reactions. Moreover, the usefulness of increased total IgE concentrations in postmortem serum from femoral blood to assess any underlying atopic disposition remains questionable. Given the apparent variation of this parameter within a population with a high prevalence of atopic diseases as well as the

observed elevations of total IgE concentrations in non-atopic individuals suffering from a large variety of infectious and inflammatory conditions, the interpretation of positive analysis results may be confounded [6, 8, 10, 40–43].

**Compliance with ethical standards** All relevant ethical issues were identified and discussed with the local Ethical Committee. All biological samples were anonymized prior to analysis and analyzed in the same laboratory. No ethical approval was necessary to perform biochemical analyses in the collected cases.

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