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REVIEW ARTICLE

Postmortem chemistry update part II

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Abstract As a continuation of “Postmortem Chemistry Update Part I,” Part II deals with molecules linked to liver and cardiac functions, alcohol intake and alcohol misuse, myocardial ischemia, inflammation, sepsis, anaphylaxis, and hormonal disturbances. A very important array of new material concerning these situations had appeared in the forensic literature over the last two decades. Some molecules, such as procalcitonin and C-reactive protein, are currently researched in cases of suspected sepsis and inflammation, whereas many other analytes are not integrated into routine casework. As in part I, a literature review concerning a large panel of molecules of forensic interest is presented, as well as the results of our own observations, where possible.

Keywords Postmortem chemistry · Liver function · Cardiac function · Sepsis · Inflammation · Anaphylaxis

Introduction

As a continuation of “Postmortem Chemistry Update Part I,” Part II deals with a large panel of molecules implicated in many forensic situations. As in part one, our attention has been placed on molecules which could provide information in determining the cause of death and not those concerning the time of death. Some of the molecules investigated have already been explored by Coe, though many others are more recent in forensic practice. Numerous publications have hence appeared over the last two decades. A literature review concerning the postmortem evaluation of liver and cardiac functions, markers of alcohol misuse and alcohol intake, myocardial ischemia, sepsis, inflammation, infection and anaphylaxis as well as some hormones is herein presented, along with results of our experiences, where possible.

Liver

Liver function

The metabolic functions of the liver include the processing of amino acids, carbohydrates, lipids, and vitamins; serum protein synthesis; detoxification; and excretion into bile of endogenous waste products and xenobiotics [1]. Measurements of total cholesterol and triglycerides in postmortem serum and other fluids have been performed in the past and have been associated with the presence of coronary heart disease and sudden cardiac death [2]. Coe [3] reported that total serum cholesterol stays in the normal range after death and postmortem levels correlate closely with antemortem values, in both high and low concentrations. Särkioja et al. [4] investigated the stability of total cholesterol, triglycerides, and apolipoproteins B and A-I in postmortem serum from peripheral blood and found that unpredictable fluctuations occurred in the postmortem values of lipids and lipoproteins already in the first 24 h after death, rendering the estimation of antemortem lipid levels from postmortem samples difficult. Uemura et al. [5] investigated 11 clinically available biochemical markers (including total cholesterol and triglycerides) in postmortem serum from three sampling sites (left cardiac blood, right cardiac blood, and femoral vein blood) in 164 consecutive autopsy cases.

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The mean triglyceride levels were comparable among the sampling sites though higher than in living subjects and easily affected by ingestion or starvation, suggesting that this parameter was not useful as a postmortem marker. Total cholesterol levels showed a tendency to decrease time dependently and showed differences according to sampling sites. Among them, the highest values were observed in postmortem serum from left cardiac blood, which was therefore recommended as the sample of choice. Total cholesterol levels were reliable as postmortem markers when carefully interpreted in an appropriate limit range. Bilirubin levels slightly increase after death though, in individuals with jaundice, the postmortem increase is relatively small and does not interfere with determining the degree of antemortem jaundice [2, 3]. Uemura et al. [5] investigated total bilirubin in postmortem serum from three sampling sites (left cardiac blood, right cardiac blood, and femoral vein blood) in 164 consecutive autopsy cases and found that this parameter tended to show a time-dependent increase, although not significant, and could provide reliable data useful in a clinical context. Again, postmortem serum from femoral vein blood was proposed as the sample of choice. Measurements of total proteins in postmortem serum provide valid data, exploitable in the clinical context [2, 3]. Uemura et al. [5] investigated total proteins in postmortem serum from three sampling sites (left cardiac blood, right cardiac blood, and femoral vein blood) in 164 consecutive autopsy cases and found that postmortem protein levels could provide reliable data. Postmortem serum from femoral vein blood was proposed as the sample of choice.

Postmortem serum transaminase levels increased rapidly and unpredictably [2, 3]. γ-Glutamyl transpeptidase (γ-GT) in postmortem serum was designated as stable by Piette and De Schrijver [6] and unpredictable by Sadler et al. [7]. Uemura et al. [5] investigated γ-GT levels in 164 autopsy cases at various sampling sites and found that the concentrations tended to be too dissimilar from those in living subjects to use this parameter as a reliable postmortem marker.

Markers of alcohol misuse and alcohol intake

Carbohydrate-deficient transferrin

Serum transferrin (Tf) is an iron-transporting glycoprotein synthesized mainly by hepatocytes. It is composed of 679 amino acids with two potential glycosylation sites that usually bind two biantennary and/or triantennary carbohydrate chains of variable compositions, containing four different carbohydrates (*N*-acetylglucosamine, mannose, galactose, and sialic acid terminals). Sialic acid is the only charged moiety in these chains, and, when present, gives a negative charge to the Tf molecule. Tf occurs in at least seven isoforms: hexasialo, pentasialo, tetrasialo, trisialo, disialo, monosialo, and asialo transferrin. In healthy organisms, the tetrasialo isoform accounts for about 80% of the total circulating Tf. Elevated alcohol intake decreases the hepatic glycosylation of Tf, and consequently increases the amounts of Tf isoforms deficient in terminal oligosaccharides (mainly asialo and disialo Tf), referred to as carbohydrate-deficient transferrin (CDT). Alcohol intake of >50–80 g/day for 1–2 weeks is believed to lead to an increase in serum CDT, which has a half-life of about 15 days [8–11]. A review of the literature concerning the determination of CDT in postmortem samples has been recently reported by Rainio et al. [10] who examined the studies performed on this topic by several authors on different biological substrates between 1996 and 2008.

Simonnet et al. [12] investigated the influence of several factors (haemolysis, sampling site, storage, and repeated cycles of freezing and thawing) on CDT concentration in serum samples obtained from living individuals and autopsy cases (postmortem serum samples). Their results showed that the site of sampling had no influence on the CDT concentrations and that the molecule remained stable even under stringent experimental storage conditions, whereas haemolysis and repeated freezing and thawing were responsible for decreased CDT levels.

Malcolm et al. [13] evaluated the stability of CDT in 25 forensic autopsy cases and did not find any correlation between postmortem serum CDT levels (postmortem serum from heart blood or other main sources as the aorta) and the time elapsed from death to the sample collection, suggesting that CDT levels were stable for at least 36 h after death.

Osuna et al. [14] and Berkowicz et al. [15, 16] investigated the usefulness of the postmortem determination of CDT in vitreous. Osuna et al. [14] performed a study on vitreous samples collected from 66 autopsy cases (38 alcohol abusers and 28 control cases). Berkowicz et al. performed a study on vitreous samples collected from 28 autopsy cases (21 alcohol abusers and seven control cases). Both studies showed that the determination of vitreous CDT could be useful in detecting heavy alcohol consumption. The results of the study of Berkowicz et al. [15, 16] also suggested the possibility that time-dependent changes in vitreous Tf could interfere with vitreous CDT values.

Rainio et al. [17] compared the concentrations of CDT in postmortem serum from various sampling sites (left and right femoral vein and inferior vena cava) and concluded that CDT was not subject to substantial postmortem redistribution. Results of this study also revealed that CDT concentrations were mostly unchanged after several days of cold body storage, implying that the postmortem time did not influence analytical results to any significant degree.

Ethyl glucuronide and ethyl sulphate

Ethyl glucuronide (EtG) is a non-volatile, water-soluble, stable-upon-storage, direct metabolite of ethanol which can be detected in body fluids and tissues (as well as in postmortem samples) for an extended time period after the complete elimination of ethanol from the body. EtG has been detected up to 8 h in blood and up to 80 h in urine after ethanol intake. Moreover, the determination of EtG in hair offers a comparatively long period of detection up to several months. EtG is considered a marker of ethanol intake rather than misuse. Several studies have been performed in the last decade on numerous postmortem substrates, including hair, blood, liver, skeletal muscle, adipose tissue, urine, bile, and rib bone marrow [10, 18–41].

Yegles et al. [25] determined the concentrations of EtG and fatty acid ethyl esters (another product of non-oxidative ethanol metabolism) in the hair specimens of several categories of living subjects, including ten alcoholics in withdrawal treatment and 11 samples from autopsies. They compared both markers with self-reported data pertaining to alcohol consumption. Their results showed different distributions in the hair for both markers, leading to the conclusion that a time-resolved drinking history of subjects could not be ascertained by the corresponding segmental concentrations of EtG and fatty acid ethyl esters.

Schloegl et al. [27] examined the stability of EtG in several substrates including urine from volunteers and postmortem specimens. The study was carried out on urine samples of nine volunteers who had consumed different amount of alcohol during the previous evening, stored up to 5 weeks at room temperature and at 4°C. Postmortem blood, liver, and skeletal muscle samples from seven autopsies, with positive and negative blood alcohol concentrations, stored after sampling at room temperature for up to 4 weeks were also included in the study. Ethanol was added to a part of the blood and liver samples of corpses with negative blood alcohol concentrations. In urine samples, EtG was a stable marker when stored at 4°C for 5 weeks, whereas in samples stored at higher temperatures larger variations of EtG concentrations were detected during 5 weeks of storage. In EtG-positive tissue material, a slow decrease in EtG concentration over 4 weeks of storage at room temperature was observed. Lastly, all EtG-negative tissue samples remained negative for up to 4 weeks of storage at room temperature, suggesting that the presence of EtG in body liquid or tissues prove alcohol consumption prior to death. In a subsequent study performed on several substrates (rib bone marrow, liver and skeletal muscle samples, fat tissue, urine, blood, and bile) from 12 autopsies, they observed that rib bone marrow could be helpful in detecting EtG concentrations in cases where other material was not available [28].

Høiseth et al. [31, 32] studied the pertinence of EtG measurements in postmortem blood samples. They found that EtG had a high specificity for alcohol ingestion, indicating that no EtG was produced endogenously, even when ethanol was formed postmortem. The authors also emphasized that the absence of EtG in postmortem blood samples, especially in heavily putrefied cases, must be interpreted with caution due to EtG degradation. This would further suggest the importance of analysing additional media to determine whether or not ethanol was ingested. However, Helander et al. [41] reported postcollection synthesis of EtG in urine in the presence of ethanol and *Escherichia coli*.

Ethyl sulphate (EtS), a direct ethanol metabolite formed by sulphate conjugation, has been recently indicated as a biomarker for recent alcohol consumption, with excretion characteristics similar to those of EtG. Moreover, since EtS and EtG are formed via different pathways, either may be used to identify recent ethanol intake [38, 42–46].

Baranowski et al. [43] performed a study with in vitro experiments, in order to ascertain EtG and EtS degradation from bacterial colonies isolated from autopsy material (13 cases). Their results showed that EtG was completely degraded by bacteria with β-glucuronidase activity in a range of 3–4 days, whereas EtS was not affected by degradation within 11 days of incubation. However, Halter et al. [44] showed the possibility of EtS bacterial degradation in extreme conditions, leading to the conclusion that EtS degradation should be taken into account when alcohol intake some hours prior to death needs to be excluded, especially in putrefied corpses.

Thierauf et al. [46] collected samples of urine, femoral vein blood, and vitreous from 26 deceased cases with assumed ethanol consumption prior to death, in order to determine EtG, EtS, and ethanol concentrations and to compare the vitreous levels with blood and urine levels. No evidence of a concentration or distribution pattern of ethanol, EtG, and EtS was observed and a constant relationship among the three analytes could not be established. However, this study showed that vitreous could be used as a substrate for the detection and determination of ethanol, EtG, and EtS.

Cardiac function

ANP, BNP, and N-terminal propeptides

Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are small peptides consisting of 28 and 32 amino acid residues, synthesized and secreted from the atrial and ventricular myocardium, respectively. Atrial and ventricular cardiomyocytes produce proANP and proBNP, which are cleaved into the biologically active ANP and BNP and their amino-terminal counterparts (NT-proANP and NT-proBNP). Increased cardiac pressure or volume as well as neurohumoral factors can trigger the synthesis and release of peptides derived from proANP and proBNP. Circulating concentrations of ANP and NT-proANP primarily reflect increased preload, whereas BNP and NT-proBNP concentrations primarily reflect increased heart afterload [47–59].

Zhu et al. [59] investigated ANP and BNP levels in the pericardial fluid of 263 medico-legal autopsy cases and found that both markers showed negative correlations with cardiac troponin T levels in pericardial fluid, suggesting that their production by cardiac tissue was reduced depending on the severity of myocardial damage. A significant elevation in pericardial ANP levels, in contrast to mildly elevated BNP levels, was observed in drowning cases. According to the authors, this result depended on multiple factors, including cardiac dysfunction due to electrolyte disturbance, acute atrial overload involving increased wall tension and pulmonary hypertension caused by water aspiration, or stretched atrial chambers due to increased circulatory blood volume, especially in freshwater drowning. The postmortem pericardial BNP levels and BNP/ANP ratio were markedly elevated in chronic congestive heart disease cases, in which BNP levels correlated with the grade of ventricular dilatation and heart and lung weights, suggesting a relationship with gradually developing cardiac hypertrophy, terminal pulmonary congestion, and oedema as a consequence of fatal cardiac dysfunction.

Michaud et al. [60] investigated the postmortem stability and levels of NT-proBNP in 96 forensic cases classified into four groups according to autopsy and histological findings (acute coronary ischemia, 18 cases; acute and chronic coronary ischemia, 13 cases; chronic coronary ischemia, 25 cases; and control group, 40 cases). NT-proBNP levels in pericardial fluid, femoral blood, and postmortem serum from femoral blood were similar, suggesting that any of these fluids could be used for postmortem testing, whereas NT-proBNP vitreous levels were of no value. The highest NT-proBNP levels were observed in individuals who had suffered from acute coronary thromboembolism in association with chronic cardiac ischemia.

Biochemical markers of myocardial ischemia

Biochemical markers of myocardial ischemia (cardiac troponin I, cardiac troponin T, myosin, myoglobin, creatine kinase, and creatine kinase MB) have been investigated by several authors in postmortem serum from different sampling sites and pericardial fluid, with sometimes controversial results [61–67].

Osuna et al. [61] evaluated the diagnostic efficacy of cardiac troponin I (cTnI) determination to diagnose acute myocardial ischemia in pericardial fluid and postmortem serum from femoral blood. This was carried out on 89 medico-legal autopsy cases, 25 of which were myocardial infarction, classified into four groups according to autopsy and histological findings. Their results suggested a high diagnostic specificity for cTnI in pericardial fluid.

Ellingsen and Hetland [62] performed a study on 102 autopsy cases in order to assess the importance of measuring cardiac troponin T (cTnT) in postmortem serum from femoral blood and found that increased cTnT levels in an otherwise negative autopsy could support a diagnosis of sudden cardiac death.

Zhu et al. [64–66] analysed the cTnT, cTnI, and CK-MB levels in pericardial fluid and postmortem serum from different sampling sites in three series of medico-legal autopsies. In an initial study performed on 405 medico-legal autopsy cases, the authors observed that cTnT measurements might be useful in investigating the severity of myocardial damage due to various causes of death. In general, cTnT levels in postmortem serum from heart blood and pericardial fluid were higher than in postmortem serum from peripheral blood, with an increase depending on postmortem time, suggesting that the severity of myocardial damage at the time of death contributed to the postmortem leakage of cTnT from the myocardium. In a subsequent study performed on 171 medico-legal autopsy cases (96 sudden cardiac deaths and 75 control cases), the authors observed that increased cTnT levels in postmortem serum from heart blood and pericardial fluid in sudden cardiac deaths were correlated to the severity of ischemic myocardial damage. In a third study performed on 234 medico-legal autopsy cases, the authors observed that increased cTnI levels in postmortem serum from heart blood and pericardial fluid were linked to the morphological severity of myocardial damage. On the contrary, CK-MB levels seemed to be independent of the morphological severity of myocardial damage.

Sepsis, inflammation, and infection

Procalcitonin

Serum procalcitonin (PCT), a protein of 116 amino acids with a molecular weight of 13 kDa, is normally produced in the C cells of the thyroid gland as the precursor to calcitonin. A specific protease cleaves serum PCT to calcitonin, catacalcin, and a N-terminal residue. Serum PCT levels are therefore undetectable (<0.1 ng/ml) in healthy humans. During severe bacterial infections with systemic manifestations, however, serum PCT levels may increase to over 100 ng/ml. In these situations, serum PCT is probably produced by extra-thyroid tissues. In fact, patients who have previously undergone total thyroidectomy still produce high levels of serum PCT during severe infection. The exact origin of serum PCT during sepsis is uncertain and the pathophysiological role of PCT during sepsis is not clear. Serum PCT levels increase during severe generalised bacterial, parasitic, or fungal infections with systemic manifestation. In severe viral infections, or inflammatory reactions of non-infectious origin, serum PCT levels do not increase or only show a moderate increase. Compared with the relatively short half-lives of cytokines such as tumour necrosis factor (TNF)-α and interleukin-6 (IL-6), the half-life of serum PCT in the systemic circulation is rather long, 25–30 h. Bearing in mind these properties, PCT has been proposed as an indicator of severe generalised infections or sepsis. Infections with no systemic manifestation cause a limited, if any, increase in serum PCT levels. Systemic inflammatory syndrome of non-infectious etiologies also leads to increases in serum PCT levels though increased serum PCT levels may be present without any evidence of severe infection in patients subsequent to major trauma or surgery as well as post-cardiopulmonary bypass. However, the median values under these conditions are usually lower than those found during severe sepsis and septic shock [68–74].

Tsokos et al. [75] investigated the use of PCT as a postmortem marker of sepsis in 61 autopsy cases (eight sepsis cases and 53 control cases). Postmortem serum was obtained from femoral blood and postmortem PCT concentrations were compared with PCT concentrations measured in antemortem serum samples. All sepsis cases showed increased PCT levels, whereas PCT levels were undetectable in control cases. According to the authors, measurements of PCT levels in sepsis-related fatalities seemed to be reasonable up to at least approximately 140 h after death. The authors also emphasized that, compared with other biochemical markers (TNF-α, IL-6, and C-reactive protein), PCT was very stable even at room temperature and had a long half-life (25 to 30 h). Moreover, repeated freezing and thawing of the blood samples did not significantly influence its concentration.

In a study performed by Ramsthaler et al. [76], postmortem serum PCT levels were determined in 70 forensic and 78 clinical-pathological autopsy cases, in order to evaluate whether a semi-quantitative test (rapid diagnostic test) was a reliable indicator of PCT levels. Postmortem serum was obtained from femoral vein blood. The results showed that the introduction of a rapid diagnostic test could be useful in achieving a rapid distinction between sepsis and non-sepsis related causes of death, especially in conjunction with the medical case history and further autopsy results.

Acute-phase proteins and cytokines

The proinflammatory cytokine IL-6 is made up of a series of phosphoglycoproteins with a molecular weight ranging from 21 to 45 kDa. The serum reference limit of IL-6 is less than 10 pg/ml in healthy individuals. It is a mediator of the acute-phase response to inflammatory tissue injury. C-reactive protein (CRP), the classical acute-phase protein, is a non-glycosylated protein consisting of identical, 21 kDa non-covalently bound subunits. It is predominantly produced and secreted by hepatocytes in response to the release of inflammatory cytokines. The reference limit of serum CRP concentrations in healthy individuals is less than 10 mg/l [77].

IL-6 and CRP levels in peripheral blood or postmortem serum from femoral blood have been investigated by several authors. The results have showed that both markers, particularly CRP, can be used in forensic routine similar to clinical practice [78–82].

Astrup and Thomsen [80] investigated CRP levels in 50 forensic autopsy cases in different samples, including liver. They found that liver CRP levels correlate well with postmortem serum levels, suggesting the possibility of using liver samples as an alternative to postmortem serum for CRP determination.

Maeda et al. [81] analysed urea nitrogen, creatinine, and CRP postmortem serum levels in 429 medico-legal autopsy cases and found that the combined use of these three markers could be useful for postmortem investigations of death due to hyperthermia, especially in the absence of pathological and toxicological evidence.

Reichelt et al. [83] investigated interleukin-1β (IL-1β), soluble interleukin-2 receptor (sIL-2R) and lipopolisaccharide binding protein (LBP) in postmortem serum from femoral blood in two series of autopsy cases (eight septic cases and 16 control cases). IL-1β is predominantly produced by monocytes/macrophages as an inactive 31 kDa precursor protein (pIL-1β) and is processed to its active 17.5 kDa form by the specific interleukin-1β converting enzyme. The reference limit of IL-1β serum concentrations in living individuals is less than 5 pg/ml. sIL-2R is a marker of lymphocyte activation, with a molecular weight between 40 and 45 kDa and is produced by T cells and granulocytes. The reference value of sIL-2R in healthy individuals is below 1,000 U/ml. The acute-phase protein LBP is a 60-kDa serum glycosylated protein, forming high-affinity complexes with bacterial endotoxins (lipopolysaccharide), functioning as an opsonin, that is produced and secreted by hepatocytes. The reference limit of LBP in healthy individuals is less than 10 μg/ml [83–85]. The results of this study showed increased sIL-2R and LBP postmortem serum levels over the clinical reference limits in sepsis-related fatalities, suggesting that these markers could be an appropriate diagnostic tool, in combination with other biochemical markers, in the postmortem diagnosis of sepsis in forensic autopsy practice.

Schrag et al. [86] investigated CRP, PCT, TNF-α, IL-6, and IL-8 levels in postmortem serum from femoral blood in two series of autopsy cases (eight septic cases and ten control cases), in order to evaluate their applicability as postmortem markers of sepsis. They also compared CRP and PCT values in postmortem serum, vitreous, and cerebrospinal fluid in a series of sepsis-related fatalities and control subjects. The results showed that PCT was more reliable than the other mentioned parameters in the postmortem diagnosis of sepsis, thus allowing a better differentiation between sepsis-related fatalities and non-sepsis-related fatalities. Moreover, PCT penetrated the blood–vitreous barrier, with detectable values in cases of sepsis. Detectable CRP values could also be found in vitreous and cerebrospinal fluid in cases of sepsis with increased CRP and PCT levels.

Multiple organ failure of various non-infectious causes (including trauma, burn, myocardial infarction, and pancreatitis) are well known to induce a rise in inflammatory cytokine levels. Mimasaka et al. [87–90] performed several studies on postmortem cytokine levels (including granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-γ, TNF-α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, and IL-13) in postmortem serum from cardiac blood and concluded that IL-6 and IL-8 could differentiate traumatic and non-traumatic deaths, supporting the diagnosis of traumatic death in the former.

Neopterin

Neopterin is a biochemical product of the guanosine triphosphate pathway produced primarily in monocyte/macrophage and related cells, upon stimulation of interferons, especially Th1-type cytokine IFN-γ. High neopterin concentrations in serum and urine have been shown to be a reliable indicator of the severity of viral, bacterial, protozoic, parasitic, or fungi-induced infections including the ensuing systemic inflammatory response syndrome. Correlations between neopterin levels and disease state have also been reported for rheumatoid arthritis, insulin dependent diabetes mellitus, systemic lupus erythematosus, multiple sclerosis, celiac disease and rheumatic fever. In various types of malignant diseases, increased neopterin concentrations can be predictive in tumour progression, metastasis development, and mortality. Although not produced by tumour cells themselves, increased neopterin concentrations most likely reflect the host defense reaction elicited by the aggressiveness of the tumour. Furthermore, augmenting concentrations of neopterin have been found to be a valid indicator of graft rejection and/or infectious complications following kidney, heart, liver, lung, pancreas, and bone marrow transplants [91, 92].

Forensic use of neopterin has been investigated by Ishikawa et al. [82] and Ambach et al. [93, 94] in postmortem serum from various sampling sites and urine. Autopsy cases with diagnoses indicating cellular immunological background showed increased urine and postmortem serum levels comparing to control cases. Postmortem serum neopterin levels over 500 nmol/l were observed in cases of delayed death due to trauma involving the systemic inflammatory response syndrome as well as fatal bacterial and viral infections.

Anaphylaxis

Tryptase and chymase

Mast cells are heavily granulated, wandering cells found in connective tissues and in abundance beneath epithelial surfaces. They circulate in blood as precursors and are recruited into peripheral tissues such as the dermis of the skin and lungs as well as the mucosa and submucosa of the intestine, where they differentiate and mature. Mast cell granules contain heparin, histamine, and many proteases such as tryptase and chymase. The extracellular release of mediators, known as degranulation, may be induced by immune mechanisms (IgE dependent and IgE independent) as well as other factors. IgE receptors are present on the mast cell membranes. When IgE-coated antigens bind to surface receptors, mast cell degranulation occurs. Anaphylaxis is defined as a condition caused by an IgE-mediated reaction, whereas anaphylactoid reactions are defined as those reactions that produce the same clinical picture as anaphylaxis but are not IgE mediated. Mast cell tryptase is a tetrameric neutral serine protease with a molecular weight of 134 kDa and made up of four non-covalently bound subunits, each one with an active site. There are two main types of mast cell tryptase, α-tryptase and β-tryptase. β-Tryptases are classified into βI-, βII- and βIII-tryptases and the α-tryptases into αI- and αII-tryptases. βII-Tryptase is stored in the secretory granules of mast cells. In contrast, α-tryptase is not stored and is secreted constitutively from mast cells as an active proenzyme. Hence, it is the major form of tryptase found in the blood of normal subjects and does not contribute to the increase in tryptase levels following acute mast cell degranulation [95–98].

Analyses of total immunoglobulin E and specific IgE are possible in postmortem serum though only atopic disposition and the individual's degree of sensitisation can be verified, hence not proving that death was preceded by acute anaphylactic reaction. Histamine degrades too rapidly after death to be a reliable postmortem marker. Tryptase is more stable, has a longer half-life and can be detected from a few minutes up to several hours after mast cell degranulation [99, 100]. Analyses of tryptase in postmortem serum samples of suspected anaphylaxis and anaphylactoid reactions have consistently shown elevated values [99–112]. However, increased tryptase levels have also been demonstrated in other situations, including sudden death infant syndrome, acute death after heroin injection, atherosclerotic cardiovascular disease, and traumatic death [100, 111, 113–122].

Some studies have indicated that tryptase may have a correlation with increased postmortem intervals, with concentrations varying on sampling site. Edston and van Hage-Hamsten [100] found that tryptase levels were generally higher in postmortem serum than in the serum from living patients, possibly due to postmortem passive diffusion. A weak positive correlation between heart blood and pericardial fluid also suggested the possibility of postmortem diffusion between these compartments. In a study performed in 2007 on 60 cases, including five anaphylactic deaths, Edston et al. [111] measured tryptase in postmortem serum from femoral and heart blood and proposed a value of 45 μg/l in postmortem serum from femoral vein blood as a reference limit in order to eliminate the possibility of false positives.

Da Broi and Moreschi [123] reviewed the literature concerning the postmortem diagnosis of anaphylaxis and emphasized that, because of the artificial increases of biochemical markers induced by the onset of postmortem cytolytic processes, postmortem serum from blood samples immediately obtained during resuscitation procedures as well as urine samples obtained immediately before or after death should be used to measure tryptase (in serum) and *N*-methylhistamine (in urine).

Mayer et al. [124] measured tryptase, histamine, and diamine oxidase in postmortem serum from femoral blood in 58 forensic cases including three anaphylactic deaths. They concluded that while moderately elevated tryptase levels were also common in control cases, values above 45 μg/l, as suggested by Edston et al., would support the diagnosis of fatal anaphylaxis. Strongly elevated histamine levels could further corroborate this hypothesis, whereas diamine oxidase did not prove helpful in confirming the diagnosis.

The forensic use of chymase, a mast cell-derived serine protease, was investigated by Nishio et al. [125], who tested the usefulness of this marker in the postmortem diagnosis of anaphylaxis. Postmortem serum from heart blood was analysed in 112 cases, including eight anaphylactic deaths. Chymase was detected in all anaphylactic cases but only in two control cases (myocardial infarction in both cases). The authors also examined the relationship between chymase and tryptase levels in all eight chymase-positive cases and found a significant positive correlation between the two markers, suggesting that measurement of serum mast cell-specific chymase levels could be an additional method for postmortem diagnosis of anaphylaxis.

In our medico-legal centre, we observed a case of fatal anaphylactoid reaction following the injection of 10 ml of a gadolinium-based contrast agent in a 63-year-old man. Some minutes after the injection, he developed a generalised itch, became flushed, started coughing, and showed signs of respiratory difficulties before losing consciousness. All attempts at reanimation proven unsuccessful and the patient died after 1 h. Postmortem serum was immediately obtained from femoral blood. Tryptase level was 181 μg/l. Vitreous tryptase was undetectable. Gadolinium concentrations in femoral blood and postmortem serum from femoral blood were similar and consistent with the administration of 10 ml of a gadolinium-based contrast agent.

Hormones

Adrenocorticotropic hormone

Ishikawa et al. [126] investigated adrenocorticotropic hormone (ACTH) levels in postmortem serum from cardiac blood and cerebrospinal fluid in 162 forensic autopsy cases. They also compared the results with immunohistochemical investigations in the anterior pituitary gland. Postmortem serum levels varied depending on the cause of death. Cerebrospinal fluid levels were usually higher than postmortem serum levels, but significantly lower in cases of hypothermia. A decrease in the ACTH-immunopositivity rate in the anterior pituitary gland was also revealed in these cases, dependent on the postmortem serum levels. In accordance with the results of a previous study [127], the authors postulated that in a cold environment, ACTH production could initially increase to generate heat to then be suppressed due to metabolic disorders involving abnormal lipid metabolism in advanced hypothermia. They concluded that low ACTH levels in cerebrospinal fluid could suggest an exhausted pituitary function due to prolonged exposure to cold.

Thyroid stimulating hormone, thyreoglobulin, and thyroid hormones

Several studies have been performed in order to detect increased thyreoglobulin (Tg), thyroxine (T4), and triiodothyronine (T3) levels in postmortem serum or blood in subject who died by hanging, ligature, or manual strangulation [128, 129]. Müller et al. [128] observed the highest Tg concentrations in the postmortem serum from heart blood in cases of incomplete suspension and manual strangulation. Similarly, Şenol et al. [129] found increased heart blood Tg and T3 levels and normal heart blood thyroid stimulating hormone (TSH), T4, calcitonin, parathormone, and amylase levels in hangings.

Edston et al. [130] investigated TSH, T3, and T4 levels in femoral blood and vitreous humor in 38 forensic autopsy cases and found that T3 and T4 did not cross the vitreous–blood barrier, whereas blood levels were fairly comparable to antemortem clinical reference values.

Dressler and Müller [131] studied Tg levels in cases of fatal traumatic brain injuries in serum from mixed blood from both cardiac ventricles. They also compared the results with immunohistochemical investigations in the anterior pituitary gland and thyroid. The results showed increased Tg serum levels, closely linked to a low TSH activity in the hypophysis and Tg reactions in the thyroid, suggesting that damages to the hypothalamus and hypophysis through trauma caused acute and excessive hormonal release (TSH and Tg) as well as disturbances in the feedback mechanism.

Ishikawa et al. [132] investigated TSH levels in postmortem serum from cardiac blood and cerebrospinal fluid in 120 forensic autopsy cases. They also compared the results with immunohistochemical investigations in the anterior pituitary gland. Postmortem serum levels varied depending on the cause of death but were significantly lower in hypothermia cases, which also showed lower TSH levels in cerebrospinal fluid and a low TSH-immunopositivity rate in the anterior pituitary gland. The authors postulated that in a cold environment, TSH production, as with ACTH production, could initially increase to generate heat only to be subsequently suppressed by metabolic disorders involving abnormal lipid metabolism in advanced hypothermia. They concluded that low TSH levels in cerebrospinal fluid could suggest exhausted pituitary function due to prolonged cold exposure.

Catecholamines

Postmortem serum catecholamine levels in relation to the cause of death have been investigated by Zhu et al. [133] and Wilke et al. [134]. Zhu et al. [133] analysed catecholamine levels in 542 autopsy cases in postmortem serum from different sampling sites and found that increases in catecholamine fractions (adrenaline, noradrenaline, and dopamine) depended on the cause of death as well as the magnitude of physical stress responses during the death process in individual cases. Wilke et al. [134] analysed catecholamine levels in femoral blood, heart blood, cerebrospinal fluid, urine, and vitreous in 98 autopsy cases divided into four groups (short agony, long agony, hanging asphyxiation, and cardiopulmonary resuscitation). Absolute values for adrenaline and noradrenaline in heart and femoral blood displayed no significant differences in relation to the cause of death and length of agony. In a similar manner, recorded catecholamine values in cerebrospinal fluid and urine did not allow any conclusions pertaining to the cause of death or the length of agony to be made. On the contrary, vitreous values warranted significant conclusions at least in cases that underwent cardiopulmonary resuscitation, and could be clearly distinguished from cases with short and long agony. Ishikawa et al. [135] investigated catecholamine levels in postmortem serum from cardiac blood and cerebrospinal fluid in 290 forensic autopsy cases. They also compared the results with immunohistochemical investigations in the hypothalamus, adenohypophysis, and adrenal medulla. Postmortem serum and cerebrospinal fluid levels were reduced in hypothermia cases. Moreover, a positive correlation between cerebrospinal fluid noradrenaline levels and noradrenaline immunopositivity in the adrenal medulla in hypothermia cases suggested a systemic, progressive deterioration of the sympathetic/adrenomedullary system due to fatal cold exposure.

Cortisol

No extensive investigations have been performed on cortisol levels in postmortem serum and urine, with the exception of the study carried out by Finlayson [136] on 35 autopsy cases (15 infants and children and 20 adults). Similar values in postmortem serum from femoral and right atrial blood, averaging 18 μg/dl (497 nmol/l, not unlike normal values obtained during life), were found in both study groups. Finlayson emphasized that a small decrease in cortisol levels was also found during the first 18 h after death. Some cases of adrenocortical insufficiency have been mentioned in literature where the postmortem diagnosis was usually obtained by combining immunohistochemical investigations in the adrenal cortex with the measurement of cortisol in peripheral blood or in postmortem serum from peripheral blood and the measurement of cortisol and 17-hydroxycorticosteroid in urine [137–140].

Chorionic gonadotropin

Fanton et al. [141] investigated human chorionic gonadotropin (HCG) in postmortem serum (from peripheral or cardiac blood), vitreous, and bile in 39 forensic autopsy cases (five pregnant women and 34 presumed non-pregnant women) and observed HCG-positive results in all substrates from pregnant women and no HCG-positive results in the control cases.

Erythropoietin

Erythropoietin (EPO) is a glycoprotein hormone made up of 165 amino acids with a molecular weight of 30–35 kDa that regulates erythropoiesis by binding to a receptor on the surface of erythroid progenitor cells. The primary sites for EPO synthesis is the peritubular fibroblasts of renal cortex cells in adult humans and hepatocytes in foetuses. The main stimulus for increased EPO production is tissue hypoxia. EPO levels increase as early as 2 h post-anaemia and/or systemic hypoxia initiation with a half-life of about 5–9 h. Most anaemic patients have an elevated plasma EPO concentration that depends on the degree and type of anaemia. However, plasma EPO in patients with anaemia from chronic renal failure is usually inappropriately low due to reduced EPO synthesis by the kidney [142, 143].

Quan et al. [143] investigated EPO levels in postmortem serum from various sample sites (left and right heart and external iliac vein) in 536 forensic autopsy cases. No difference was observed depending on the sampling site, and values were within the clinical reference range in most cases. A survival time-dependent increase was mainly seen in protracted deaths due to blunt injury and fire fatality, suggesting the systemic influence of anaemia/hypoxia following massive bleeding and/or tissue damage. Similar findings in subacute deaths from gastrointestinal bleeding and infectious diseases were related to the severity and duration of bleeding/anaemia or advanced hypoxia in the death process. In a subsequent study, the authors [144] investigated EPO levels in postmortem serum from different sampling sites in 185 injury death cases (sharp instrument injury and blunt injury) and compared them with CRP levels. Survival time-dependent increases in EPO levels within about 6 h were observed in cases of sharp instrument injury to the heart or a proximal major vessel as well as blunt injuries with massive haemorrhages. These findings suggested that bleeding velocity was the main factor in early EPO level elevation, possibly due to the rapid progression of renal ischemia and hypoxia. In contrast, serum CRP levels gradually increased about 12–24 h after a blunt injury, suggesting that postmortem serum EPO could be a marker for investigating survival time within 6 h of major injury involving acute, massive haemorrhage.

Miscellaneous

Chromogranin A

Chromogranin A (CgA) is a 49–68-kDa calcium-binding glycoprotein, originally isolated as a major soluble protein in adrenal medullary chromaffin granules. It is widely distributed in the secretory granules of endocrine and neuroendocrine cells and cosecreted with hormones such as catecholamine. Yoshida et al. [145] investigated CgA and catecholamine levels in postmortem serum from cardiac blood and cerebrospinal fluid in 298 forensic autopsy cases and compared the results with immunohistochemical investigations in the hypothalamus, adenohypophysis, and adrenal medulla. Postmortem serum CgA levels were generally higher than clinical reference values. Hypothermia cases tended to show lower CgA levels in postmortem serum and increased levels in cerebrospinal fluid. Low hypothalamus neuronal CgA immunopositivity with a positive correlation to CSF CgA levels was also found, suggesting a terminal state of hypothalamus dysfunction involving the depletion of CgA-containing secretory granules in prolonged death due to cold exposure.

S100B

The S-100 protein is a 10–12-kDa calcium-binding protein. Its subunit B (S100B) is highly specific for astrocytes, oligodendrocytes, and ependymocytes in the central nervous system. S-100B is clinically used as a serum marker of brain damage due to cerebral injury and hypoxia/ischemia in the evaluation of neurological prognosis. Li et al. performed several studies [146–149] to evaluate S100B levels in postmortem serum and cerebrospinal fluid, as well as the immunohistochemical distribution of S-100 protein in the cerebral cortex, with regard to the cause of death. In an initial study performed on 283 medico-legal autopsy cases, the authors measured S100B levels in postmortem serum from various sample sites and found increased S100B levels in postmortem serum from right heart blood and subclavian vein in cases of acute deaths from head injury and asphyxiation due to neck compression. In a subsequent study performed on 286 medico-legal autopsy cases, the authors observed an inverse relationship between S100 positivity in the astrocytes and serum S100B levels in cases of acute deaths, suggesting that astrocytes were more rapidly and severely injured than neurons during fatal brain damage. In a third study performed on 216 medico-legal autopsy cases, the authors measured S100B levels in postmortem serum from heart blood and cerebrospinal fluid and concluded that CSF S100B levels could be used in assessing brain damage severity due to injury and cerebral hypoxia/ischemia as a consequence of fatal trauma.

Serotonin

Quan et al. [150] investigated serotonin (5-HT) levels in cerebrospinal and pericardial fluids with regard to the cause of death in 351 medico-legal autopsy cases. The postmortem 5-HT concentration in cerebrospinal fluid was similar to that described in a previous study carried out by Musshoff et al. [151] and higher than the clinical reference value, whereas the postmortem 5-HT level in pericardial fluid overlapped with the clinical serum level. Particularly

Table 1 Summary of reports describing postmortem analysis of markers of liver and cardiac function, alcohol misuse, sepsis, inflammation, infection, anaphylaxis, hormones, chromogranin A, and S100B

Marker analysed	Number of cases	Samples analysed	Time of sampling after death	Analytical method	Concentration range proposed and other suggestions	Reference
Total cholesterol	164	Postmortem serum from left cardiac blood	0–72 h	Enzyme method	Clinical range (150–219 mg/dl)	[5]
Total bilirubin	164	Postmortem serum from femoral blood	0–72 h	Vanadinate oxidation method	Clinical range (0–2–1.0 mg/dl)	[5]
Total proteins	164	Postmortem serum from femoral blood	0–72 h	Biuret method	Clinical range (6.7–8.3 g/l)	[5]
CDT	64	Postmortem serum from femoral blood	6–96 h	IEF/laser densitometry MAEC/RIA (CDTect™)	18% for CDTq 50 U/l for CDTect™	[7]
	25	Postmortem serum from heart/aorta blood	2–37 h	RIA (CDTect™)	CDT levels >17 U/l in male and >25 in female	[13]
	10	Postmortem serum from femoral and heart blood	Not indicated	RIA (CDTect™)	Clinical range not indicated	[12]
	70	Postmortem serum from femoral blood and inferior vena cava	1–6 days	CZE, HPLC	Clinical range not indicated	[17]
	66	VH	4–72 h	RIA (CDTect™)	4.7–24.5 U/l (cases of previous diagnosis of alcohol misuse) 3.4–13.9 U/l (no previous diagnosis of alcohol misuse)	[14]
	28	VH	Not indicated	RIA (CDTect™)	5 U/l (detection limit of the commercially available test)	[15, 16]
FAEE and EtG	10 alcoholics in withdrawal treatment and 11 autopsy cases	Hair	Not indicated	GC-MS	Insignificant quantitative correlation between EtG and FAEE concentration in hair	[25]
EtG	7 and 12 (autopsy cases)	Urine, blood, liver, and skeletal muscle	Not indicated	LC-MS/MS	Stable marker in urine when stored at 4°C. No new EtG formation after addition of ethanol. Rib bone marrow useful for Etg determination	[27, 28]
EtG	146	Blood	Not indicated	LC-MS	No EtG observed in cases with no ethanol ingestion of and postmortem ethanol formation	[31]
EtG and EtS	36	Blood	Not indicated	LC-MS	EtG and EtS together reliable criterion for antemortem ethanol ingestion	[38]
EtG and EtS	26	Femoral vein blood, urine, and VH	Not indicated	LC-MS/MS	VH useful for EtG and EtS determination	[46]
ANP, BNP, NT-proANP, and NT-proBNP	263	ANP and BNP in PF	3–72 h	RIA	Clinical reference range	[59]
	96	NT-proBNP in postmortem serum from femoral blood, whole femoral blood, VH, and PF	24 h	Chemoluminescent immunoassay	Clinical reference range	[60]
cTnI, myosin, myoglobin, and CK-MB	89	Postmortem serum from femoral blood and PF	1–29 h	Enzyme immunoassay (cTnI), RIA (myosin), RIA (myoglobin), and immunoinhibition technique (CK-MB)	Clinical serum reference range	[61]
cTnT	102	Postmortem serum from femoral blood	3–75 h	Immunoassay	Reference values for autopsy samples higher than clinical reference	[62]
cTnT, cTnI, and CK-MB	405, 171, and 234	Postmortem serum from different sampling sides and PF	48 h	Electro-chemoluminescent immunoassay (cTnT) Available clinical laboratory methodologies (cTnI and CK-MB)	Clinical serum reference range	[64–66]

Table 1 (continued)

Marker analysed	Number of cases	Samples analysed	Time of sampling after death	Analytical method	Concentration range proposed and other suggestions	Reference
Procalcitonin	61 (8 sepsis cases)	Postmortem serum from femoral blood	0.3–139 h	Immunoluminometric assay	Clinical reference range. Detection limit 0.3 ng/ml	[75]
	148	Postmortem serum from femoral blood	96 h	Semi-quantitative test	Clinical reference range (cut-off point 2 ng/ml)	[76]
	18	Postmortem serum from femoral blood, VH, and CSF	Not indicated	Available clinical laboratory methodologies	Clinical reference range. Detection limit 0.25 ng/ml	[86]
CRP IL6		Peripheral blood or postmortem serum from femoral blood			Clinical reference ranges	[78–82]
CRP	50	Postmortem serum from femoral blood and liver	24–264 h	Immunoturbidimetric method	Clinical reference range (10 mg/l)	[80]
GM-CSF, IFN-γ, TNF-α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, and IL-13	90, 71, 156, and 43	Postmortem serum from cardiac blood	2 days	Available clinical laboratory methodologies (ELISA and cytometric bead array kit)	Clinical reference ranges	[87–90]
sIL-2R LBP	24	Postmortem serum from femoral blood	48 h	Chemoluminescent immunometric assays	Clinical reference ranges (1,000 U/ml for sIL-2R, 10 μg/ml for LBP)	[83]
Neopterin	129	Urine and postmortem serum from peripheral blood	9.5–28 h	HPLC	Generally higher than clinical serum and urine reference range	[93]
	32	Urine, postmortem serum from different sampling sites (peripheral)	3–69 h	HPLC	Generally higher than clinical serum and urine reference range	[94]
	474	Postmortem serum from different sampling sites (peripheral and cardiac)	2.8 h–3 days	HPLC	Postmortem serum level over 500 nmol/l in deaths involving SIRS and fatal bacterial and viral infections	[82]
Tryptase	60 (5 anaphylactic death)	Postmortem serum from femoral and heart blood	3–5 days	Fluoroenzymeimmunoassay method	Postmortem serum from femoral blood is proposed as sample of choice; 45 μg/l is proposed as reference	[111]
	58 (3 anaphylactic death)	Postmortem serum from femoral blood	4–110 h	Immunofluorescent enzyme assay	45 μg/l is proposed as reference	[124]
Chymase	112 (8 cases of anaphylaxis)	Postmortem serum from heart blood	17 h (for anaphylactic deaths)	Available clinical laboratory methodologies	Chymase detectable levels: 3 ng/ml. Anaphylactic deaths: 8–648 ng/ml	[125]
ACTH	162	Postmortem serum from cardiac blood and CSF	4.85.5 h	Immunoradiometric assay	Clinical serum reference range	[126, 127]
TSH, Tg, T3, and T4		Femoral and cardiac blood and postmortem serum from femoral blood			Clinical reference range	[128–131]
TSH	120	Postmortem serum from cardiac blood and CSF	24 h	Electro-chemoluminescent immunoassay	Clinical serum reference range	[132]
Catecholamines	542	Postmortem serum from different sampling sites	3.47 h	HPLC	Clinical reference range	[133]
	98	Femoral blood, heart blood, CSF, urine, and VH	Not indicated	HPLC	Clinical serum reference range	[134]
	290	Postmortem serum from cardiac blood and CSF	3 days	HPLC	Clinical serum reference range	[135]
Cortisol	35	Postmortem serum from femoral and right cardiac blood	9–29 h	Fluorescent method	Clinical reference range	[136]
HCG	39	Postmortem serum from peripheral or cardiac blood	5 days	EIA sandwich method with a final fluorescent detection	Clinical reference range	[141]
		Bile				
		VH				
EPO	536	Postmortem serum from different sampling sites	48 h	RIA	Clinical reference range (<36 mU/ml)	[143]

Table 1 (continued)

Marker analysed	Number of cases	Samples analysed	Time of sampling after death	Analytical method	Concentration range proposed and other suggestions	Reference
	185	Postmortem serum from different sampling sites	48 h	RIA	Clinical reference range	[144]
CgA, catecholamines	298	Postmortem serum from cardiac blood and CSF	3 days	ELISA HPLC	Postmortemserum levels higher than clinical serum levels	[145]
S-100B	283, 286, and 216	Postmortem serum from different sampling sites and CSF	48–72 h	ELISA	Postmortem serum levels higher than clinical serum levels and CSF levels higher than postmortem serum levels	[146–149]
Serotonin	351	CSF and PF	48 h	HPLC	Postmortem CSF levels higher than clinical reference values; postmortem PF level similar to serum level	[150]
Myoglobin	210 (urine) 72 (postmortem serum)	Urine and postmortem serum from cardiac blood	5 h to 22 days	RIA	No correlation between urine and serum levels	[154]

VH vitreous humor, *CSF* cerebrospinal fluid, *PF* pericardial fluid, *SIRS* systemic inflammatory response syndrome, *GC-MS* gas chromatography–mass spectrometry, *HPLC* high-performance liquid chromatography, *IEF* isoelectric focusing, *MAEC* micro-anion-exchange chromatography, *CDTq* CDT quotient, *CZE* capillary zone electrophoresis, *RIA* radioimmunoassay, *EIA* enzyme immunoassay, *ELISA* enzyme-linked immunosorbent assay, *LC-MS* liquid chromatography–mass spectrometry, *LC-MS/MS* liquid chromatography coupled to tandem mass spectrometry

higher serotonin values in both fluids were observed in cases of sedative-hypnotic drug intoxication as well as of hyperthermia cases, whereas hypothermia cases tended to show low 5-HT levels. According to the authors, the results of the study would indicate that a systemic neuronal dysfunction might be involved in these causes of death, as already suggested by previous investigations which indicated in the same situations similar changes in catecholamine levels observed in postmortem serum from cardiac blood and cerebrospinal fluid [133–135].

Myoglobin

Myoglobin levels in postmortem serum from femoral and cardiac blood with special references to electrical fatalities have been investigated by Püschel et al. [152] and Fieguth et al. [153]. Zhu et al. [154] evaluated urinary myoglobin levels in 210 forensic autopsy cases, partially in comparison with concentrations observed in postmortem serum from cardiac blood (72 cases). Myoglobin levels in urine did not correlate with serum levels. Postmortem changes including bladder wall muscle autolysis, tissue damage at time of death and prolonged survival time were identified as factors capable of inducing postmortem urinary myoglobin elevations. Increased myoglobin levels in urine were observed in heat stroke cases, fatal burns, and situations with massive skeletal muscle damages, muscle hyperactivity or convulsive disorders associated with hypoxia immediately before death.

Conclusions

In this article, a review of the literature concerning several biochemical markers which can be implicated in many current forensic situations (liver insufficiency, alcohol misuse and intake, myocardial ischemia, inflammation, infection, sepsis, anaphylaxis, hormonal diseases, anaemia or hypoxia, and brain damage) has been presented (Table 1). The contribution that these markers can provide in investigating the cause of death is not negligible. For instance, increased postmortem serum PCT levels, combined with cytokines and acute-phase proteins serum raises, histological and immunohistochemical findings consistent with infection and positive microbiology investigation results, can corroborate the hypothesis of death following sepsis. At the same time, the observation of increased PCT levels, without any other macroscopical and microscopical finding, can represent a source of confusion and be of no value in understanding the mechanisms leading to death. The same observation can be extended to other biochemical markers, e.g. tryptase and cardiac troponins. Of course, increased CDT levels can easily be interpreted as the biochemical sign of alcohol misuse and severe perturbations in total cholesterol, bilirubin, and total protein levels in postmortem serum obviously represent the result of liver disease. The presence of macroscopical and microscopical finding consistent with liver cirrhosis and the perturbation of the biochemical parameters linked to the liver function corroborate the hypothesis of liver insufficiency as cause of death. However, it could be pretentious to conclude that the cause of death is "liver insufficiency" only on the basis of increased postmortem serum levels of these markers and without any other radiological or morphological findings. Maximum prudence is also required in interpreting increased postmortem levels of cardiac markers to avoid yielding to the temptation of establishing direct links between postmortem cardiac troponin levels and sudden deaths. Once again, we strongly suggest an "intelligent" approach to postmortem chemistry, consisting of incorporating these analyses among the routine forensic and medico-legal investigations, with the same importance as radiology, histology, and toxicology, but above all in interpreting the results in a larger clinical and forensic context. The aim of postmortem chemistry must not be "limited" to determining the cause of death, but extended to understanding the pathophysiological mechanisms involved in death process.

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