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The Postmortem Diagnosis of Alcoholic Ketoacidosis

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Abstract — Aims: The aim of this article is to review the forensic literature covering the postmortem investigations that are associated with alcoholic ketoacidosis fatalities and report the results of our own analyses. **Methods:** Eight cases of suspected alcoholic ketoacidosis that had undergone medico-legal investigations in our facility from 2011 to 2013 were retrospectively selected. A series of laboratory parameters were measured in whole femoral blood, postmortem serum from femoral blood, urine and vitreous humor in order to obtain a more general overview on the biochemical and metabolic changes that occur during alcoholic ketoacidosis. Most of the tested parameters were chosen among those that had been described in clinical and forensic literature associated with alcoholic ketoacidosis and its complications. **Results:** Ketone bodies and carbohydrate-deficient transferrin levels were increased in all cases. Biochemical markers of generalized inflammation, volume depletion and undernourishment showed higher levels. Adaptive endocrine reactions involving insulin, glucagon, cortisol and triiodothyronine were also observed. **Conclusions:** Metabolic and biochemical disturbances characterizing alcoholic ketoacidosis can be reliably identified in the postmortem setting. The correlation of medical history, autopsy findings and biochemical results proves therefore decisive in identifying pre-existing disorders, excluding alternative causes of death and diagnosing alcoholic ketoacidosis as the cause of death.

INTRODUCTION

Alcoholic ketoacidosis: definition and clinical features

The entity of alcoholic ketoacidosis, sometimes called alcoholic acidosis in the literature, was first described by Dillon *et al.* in 1940. In this report, the authors described a series of nine patients who had episodes of severe ketoacidosis in the absence of diabetes mellitus. All of these patients also had evidence of prolonged, excessive alcohol consumption (Dillon *et al.*, 1940; Höjer, 1996; Tanaka *et al.*, 2004; McGuire *et al.*, 2006).

The suggestion by Dillon *et al.* that ketoacidosis might occur in non-diabetic, chronic alcohol abusers was not supported by further observations until 1970 when Jenkins *et al.* (1971) described a series of three non-diabetic individuals with a history of chronic, heavy alcohol misuse and recurrent episodes of ketoacidosis. Similar case studies were subsequently reported by Levy *et al.* (1973), Cooperman *et al.* (1974), Fulop and Hoberman (1975), Platia and Hsu (1979), Soffer and Hamburger (1982), Halperin *et al.* (1983), Fulop *et al.* (1986) and Wrenn *et al.* (1991), with remarkably consistent features: all patients described in these reports had a history of chronic alcohol abuse and recent, particularly excessive intake, abruptly terminated some days prior due to abdominal pain and repeated vomiting.

Alcoholic ketoacidosis only affects patients who have a history of chronic alcohol abuse (Höjer, 1996; Tanaka *et al.*, 2004). The clinical features are very similar to those of diabetic ketoacidosis (Smith *et al.*, 1999). Diabetics who abuse alcohol can also develop alcoholic ketoacidosis, though there is no documented connection between diabetic and alcoholic ketoacidosis. In exceptional cases, the two conditions can be coexistent (Höjer, 1996; Tanaka *et al.*, 2004). Some of the patients who develop alcoholic ketoacidosis appear to be prone to repeated episodes (Höjer, 1996). In typical cases, the onset of the syndrome is preceded by prolonged, massive alcohol intake followed by abrupt cessation of ethanol consumption a few days earlier due to nausea, severe vomiting and abdominal pain, often induced by gastritis and pancreatitis (Halperin *et al.*, 1983; Duffens and Marx, 1987; Wrenn *et al.*, 1991; Smith *et al.*, 1999; Tanaka *et al.*, 2004; McGuire *et al.*, 2006). This common presenting feature of vomiting is probably the result of ethanol-induced oesophagitis or gastritis and may inhibit all or most food and fluid intake, in some cases for several days (Halperin *et al.*, 1983; Duffens and Marx, 1987; Smith *et al.*, 1999).

On physical examination, beyond the typical signs of chronic alcohol abuse, these patients may present hyperventilation, tachycardia, hypotension and signs of dehydration due to the decreased fluid intake and severe vomiting described above. Though these subjects are in overall, poor conditions, the syndrome does not include any actual loss of consciousness. In contrast to patients with diabetic ketoacidosis, subjects with alcoholic ketoacidosis are usually alert and lucid despite the severity of the acidosis and marked ketonemia (Höjer, 1996; McGuire *et al.*, 2006; Rehman, 2012).

When altered mental status and loss of consciousness do occur, they are typically attributable to other underlying, complicating factors such as hypoglycemia or severe infection (McGuire *et al.*, 2006). Abdominal pain may be seen as part of the syndrome and may be caused by a myriad of entities, including acute alcoholic hepatitis, pancreatitis and alcoholic gastritis (Duffens and Marx, 1987; Höjer, 1996; Smith *et al.*, 1999; McGuire *et al.*, 2006; Rehman, 2012).

It can be difficult at presentation to distinguish between ethanol, methanol and ethylene glycol toxicity in an alcoholic patient with an increased anion gap, metabolic acidosis and a higher serum osmolal gap. Additional diagnostic possibilities, which may be concurrent abnormalities, include lactic acidosis and diabetic ketoacidosis (Höjer, 1996; Tanaka *et al.*, 2004; McGuire *et al.*, 2006). Main laboratory findings reflect the severity of the ketoacidosis as well as that of fluid and electrolyte disturbances secondary to vomiting and dehydration (Fulop, 1993). The mechanisms giving rise to alcoholic ketoacidosis are numerous. The syndrome arises through the various metabolic effects of alcohol in the fasted, volume-depleted, alcoholic who has abruptly stopped his alcohol intake. The ketoacids responsible for alcoholic ketoacidosis (beta-hydroxybutyrate and acetoacetate) are in part formed because of the metabolism of ethanol in the liver. Ethanol is oxidized to acetaldehyde in the

liver cytoplasm by alcohol dehydrogenase. In the liver mitochondria, acetaldehyde is oxidized to acetic acid by aldehyde dehydrogenase. These oxidative processes lead to an accumulation of reduced form of nicotinamide-adenine dinucleotide (NADH), and resultant increased NADH/nicotinamide-adenine dinucleotide (NAD) ratio. Acetic acid (acetate) is converted to acetyl coenzyme A, which can enter the citric acid cycle, form ketone bodies or be converted to fat. Acetoacetate and beta-hydroxybutyrate formation in alcoholic ketoacidosis has several causes. An increase in production follows from the oxidation of ethanol. It also occurs in response to starvation and the extracellular fluid volume depletion arising from vomiting, decreased fluid intake and inhibition of antidiuretic hormone secretion by alcohol. Moreover, dehydration and volume contraction impair the excretion of ketones by the kidneys, leading to further elevation in ketone levels. Numerous hormonal changes in alcoholic ketoacidosis mediate free fatty acid release through lipolysis, which provides substrate for subsequent ketone body formation (Duffens and Marx, 1987).

Variably severe metabolic acidosis with an increased anion gap is generally present. The main source of the large anion gap is the accumulation of acetoacetate and beta-hydroxybutyrate in blood that can be detected, along with acetone, in both blood and urine. Various acid-base disturbances have also been reported. In some series, most patients presented with metabolic acidosis with compensatory hyperventilation. Other patients may have combinations of metabolic acidosis, primary respiratory alkalosis due to abdominal pain and metabolic alkalosis secondary to vomiting (Fulop, 1993).

Decreased serum chloride and bicarbonate levels reflect the acid-base disturbances as well as the presence and severity of vomiting. Glucose concentrations in the blood and urine are usually normal, though both hypoglycemia and mild hyperglycemia may occur. Since abdominal pain and vomiting prevent the patient from drinking alcohol, serum ethanol is often low or undetectable on presentation (Fulop, 1979, 1989; Soffer and Hamburger, 1982; Palmer, 1983; Adams, 1990; Caspar *et al.*, 1993; Höjer, 1996; Lu *et al.*, 1997; Umpierrez *et al.*, 2000; Sibaï and Eggiman, 2005; McGuire *et al.*, 2006).

Liver tests may be pathological as a result of chronic alcoholism (Höjer, 1996). Serum lactate concentrations have been reported to be moderately increased in most cases and severely elevated only in patients who have concomitant, underlying complications responsible for increased lactate production or impaired clearance, such as pneumonia with hypoxia, sepsis or severe hepatic disorders. Some authors have reported patients with alcoholic ketoacidosis having higher blood lactate concentrations and a higher lactate to pyruvate ratio than those seen in patients with diabetic ketoacidosis (Fulop, 1979, 1989; Soffer and Hamburger, 1982; Palmer, 1983; Adams, 1990; Caspar *et al.*, 1993; Höjer, 1996; Lu *et al.*, 1997; Umpierrez *et al.*, 2000; Sibaï and Eggiman, 2005; McGuire *et al.*, 2006).

Many patients with alcoholic ketoacidosis have been found to have extremely elevated concentrations of plasma free fatty acids, with mean levels much higher than those observed in patients with diabetic ketoacidosis (Levy *et al.*, 1973; Cooperman *et al.*, 1974; McGuire *et al.*, 2006). Subjects with alcoholic ketoacidosis also have markedly raised cortisol, glucagon, catecholamine and growth hormone levels as well as relatively low or normal plasma insulin levels, as is common in starvation, whose presence is also supported by the finding of subnormal plasma triiodothyronine levels (Fulop *et al.*, 1986; McGuire *et al.*, 2006). Elevated serum amylase has been observed in about 50% of these patients. A higher serum osmolal gap has been described in some cases and attributed to the accumulation of glycerol, acetone and acetone metabolites such as acetol and 1,2-propanediol. Serum uric acid, sodium, potassium, calcium, magnesium and phosphate level disorders have also been described, though not systematically (Fulop, 1979, 1989; Soffer and Hamburger, 1982; Palmer, 1983; Adams, 1990; Caspar *et al.*, 1993; Höjer, 1996; Lu *et al.*, 1997; Umpierrez *et al.*, 2000; Sibaï and Eggiman, 2005; McGuire *et al.*, 2006).

When treated, alcoholic ketoacidosis may be rapidly and completely resolved with no apparent sequelae. However, numerous forensic studies have shown that untreated cases in individuals with severe alcoholism may be associated with sudden death (McGuire *et al.*, 2006).

Alcoholic ketoacidosis in the forensic setting: acetone and beta-hydroxybutyrate determination

Though sudden unexplained deaths in chronic alcohol abusers with hepatic steatosis, also reported as fatty liver-related sudden deaths, had been commonly reported by pathologists worldwide over the years, the pathogenesis remained unclear. Low or undetectable blood ethanol levels were frequently measured in these cases, raising the question of whether the fatal outcome was related to the metabolic consequences of ethanol withdrawal. The difficulties in establishing the pathogenesis of death with precision in such situations were mainly attributed to two factors. Firstly, most of these cases were not witnessed, contributing to the paucity of clinical information. Secondly, potentially lethal metabolic and biochemical disturbances existing at the time of death could not be reliably identified and diagnosed by postmortem investigations (Kuller *et al.*, 1974; Randall, 1980a,b; Copeland, 1985; Clark, 1988; Petersson, 1988; Hansen and Simonsen, 1991; Yuzuriha *et al.*, 1993, 1997).

The first report in the forensic field suggesting that ketoacidosis could be partially responsible for unexplained deaths in alcoholics dates back to 1993 and concerns a study performed by L.N. Denmark on 49 autopsy cases that included chronic alcohol abuse-related deaths. Some of the latter were found to have increased vitreous and urine beta-hydroxybutyrate concentrations as well as low or undetectable blood ethanol. Based on these results and similar features in the clinical field, Denmark postulated that ketoacidosis may play a role in the multi-factorial, metabolic catastrophe leading to death in situations of alcohol withdrawal (Denmark, 1993).

Succeeding Denmark, numerous researchers investigated blood ethanol levels as well as acetone and beta-hydroxybutyrate in blood and alternative biological fluids in suspected alcoholic ketoacidosis deaths. Different reference values have been proposed for acetone and beta-hydroxybutyrate in blood, vitreous, pericardial and cerebrospinal fluids to ascribe the cause of death to alcoholic ketoacidosis in the presence of other consistent data and exclusion of alternative causes of death. Vitreous and pericardial fluid have been shown to be reliable alternative to blood in case of blood unavailability and beta-hydroxybutyrate a more suitable indicator of alcoholic ketoacidosis than acetone. Blood beta-hydroxybutyrate reference values can be used for vitreous and pericardial fluid for diagnostic purposes. Some researchers

also emphasized that blood ethanol is not systematically low or absent in alcoholic ketoacidosis deaths and that low or absent acetone levels do not preclude the presence of pathologically significant beta-hydroxybutyrate concentrations (Thomsen *et al.*, 1993, 1995, 1997; Thomsen and Frohlich, 1995; Thomsen, 1996; Brinkmann *et al.*, 1998; Pounder *et al.*, 1998; Kadiš *et al.*, 1999; Iten and Meier, 2000; Kanetake *et al.*, 2005; Buszewicz *et al.*, 2007; Felby *et al.*, 2008; Teresiński *et al.*, 2009; Elliott *et al.*, 2010; Molina, 2010; Heninger, 2012; Hockenhull *et al.*, 2012; Palmiere *et al.*, 2012, 2013a) (Table 1).

Other biochemical and histological markers for alcoholic ketoacidosis

Isopropyl alcohol may be considered a marker of ketoacidosis and a product of acetone metabolism in clinical conditions presenting ketonemia. The compound can be detected in several situations of forensic interest, beyond direct exposure to isopropyl alcohol itself, which are characterized by increased acetone levels and an elevated NADH/NAD+ ratio, such as diabetic and alcoholic ketoacidosis as well as hypothermia fatalities and starvation (Palmiere *et al.*, 2012).

The results of isopropyl alcohol determination in cases of sudden death in chronic alcoholics were reported among others by Teresiński *et al.*, with measured concentrations in femoral blood ranging from 1 to 38 μmol/l (0.06–2.29 mg/l), and Palmiere *et al.*, with measured concentrations in femoral blood ranging from 18 to 116 μmol/l (1.1–7.0 mg/l) (Teresiński *et al.*, 2009; Palmiere *et al.*, 2012).

Significantly higher blood and vitreous isopropyl alcohol concentrations in chronic ethanol users were found by Molina, with measured blood values ranging from 0 to 71 mg/dl (median value 15 mg/dl) and vitreous values ranging from 0 to 81 mg/dl (median value 12 mg/dl), Petersen *et al.*, who identified an average concentration of isopropyl alcohol of 18.5 ± 22.1 mg/dl in 79 alcoholic ketoacidosis cases, and Dwyer and Tamama, who found a blood concentration of isopropyl alcohol of 6 mg/dl in a case of severe alcoholic ketoacidosis (Molina, 2010; Petersen *et al.*, 2012; Dwyer and Tamama, 2013).

C-reactive protein (CRP) levels in alcoholic ketoacidosis have been investigated by Lindroos-Jokinen *et al.*, who observed that CRP can successfully be measured in blood samples collected during autopsy up to 18 days after death and that ketoacidosis itself, either diabetic or alcoholic, is associated with increased CRP values without any other obvious, underlying causes, such as infection or trauma, which typically lead to higher CRP concentrations. Additionally, the authors highlighted that some degree of liver disease (mainly hepatic steatosis or incipient liver cirrhosis), commonly present in chronic alcoholics, does not seem to elevate CRP levels substantially (Lindroos-Jokinen *et al.*, 2012).

Recent reports by Parai *et al.* and Zhou *et al.* focused on histological findings consistent with the existence of ketoacidosis (either diabetic or alcoholic) at the time of death, namely the sub-nuclear vacuolation of the proximal tubules in the kidneys, mostly associated with diabetic ketoacidosis, and formalin pigment deposition in the vacuolated epithelial cells of the proximal tubules in the kidneys. According to the authors, the latter finding can prove extremely useful in cases of ketoacidosis and severe renal tubular epithelium degradation due to postmortem autolysis and decompositional changes (Parai *et al.*, 2012; Zhou *et al.*, 2013).

MATERIAL AND METHODS

Study design

With the hope of providing a more complete approach pertaining to the postmortem biochemical analyses that can be performed in cases of suspected alcoholic ketoacidosis, we selected eight cases that had undergone medico-legal investigations in our facility and for which the cause of death had been determined to be alcoholic ketoacidosis based on data obtained from the medical and social histories of the deceased as well as scene investigations and postmortem findings.

Alternative causes of death were excluded based on all postmortem investigation results. Circumstantial elements, autopsy and histology did not suggest exposure to cold or hypothermia as a contributing factor to death in any of these cases.

All cases were selected among the medico-legal autopsies performed in our center from 2011 to 2013. The main criterion for selection was the availability of femoral blood, postmortem serum from femoral blood, cardiac blood, vitreous humor, urine and cerebrospinal fluid during autopsy. The cases included eight males between 49 and 69 years of age, with a mean age of 58. According to the medical records, all individuals were non-diabetic. Additionally, glycated hemoglobin levels were measured and found to be normal in all cases.

All autopsies were performed within 24 h after body discovery. The interval between the supposed times of death and autopsies did not exceed 72 h.

A series of laboratory parameters were measured in order to obtain a more general overview on the biochemical and metabolic changes occurring during alcoholic ketoacidosis.

Most of these parameters were chosen among those that had been described in clinical and forensic literature associated with alcoholic ketoacidosis and its complications. These may include starvation, extracellular fluid volume depletion, impaired renal function, various hormonal changes and bacterial infections. In order to investigate chronic alcohol abuse, concomitant diabetes mellitus as well as cardiac ischemia and cardiac failure, other biochemical markers among those that are currently measured in the postmortem setting were also determined (Coe, 1991; Palmiere and Mangin, 2012a,b).

The tested parameters include:

- glucose, sodium, chloride and lactate in vitreous,
- glucose in urine,
- glycated hemoglobin in femoral whole blood,
- beta-hydroxybutyrate in femoral whole blood and vitreous,
- ethanol in blood,
- carbohydrate-deficient transferrin (CDT) in postmortem serum from femoral blood,
- cortisol in postmortem serum from femoral blood,
- free cortisol in urine,
- free fatty acids in postmortem serum from femoral blood,
- procalcitonin (PCT), CRP, lipopolysaccharide-binding protein (LBP), interleukin-6 (IL-6) and interleukin-10 (IL-10) in postmortem serum from femoral blood,
- pancreatic amylase (P-amylase) and gamma glutamyl transferase (gamma-GT) in postmortem serum from femoral blood,
- insulin, C-peptide and glucagone in postmortem serum from femoral blood,

Table 1. Beta-hydroxybutyrate and acetone determination results in vitreous, blood and urine as well as the proposed reference values for the diagnosis of alcoholic ketoacidosis

Author	Vitreous BHB	Blood BHB	Urine BHB	Acetone (vitreous-blood-urine)	Proposed reference value(s)
Denmark (1993)	1825–2585 µmol/l (19–26.9 mg/dl)		2565–47,353 µmol/l (26.7–493 mg/dl)		
Thomsen *et al.* (1993)					Blood ketone sum 531 µmol/l
Pounder *et al.* (1998)					Blood ketone sum 10,000 µmol/l Vitreous ketone sum 5000 µmol/l
Brinkmann *et al.* (1998)				Blood acetone 1655–6896 µmol/l (9.6–40 mg/dl)	Blood acetone 9 mg/dl (1548 µmol/l)
Kadiš *et al.* (1999)					Blood-urine-vitreous BHB 3000 µmol/l (31.2 mg/dl)
Iten and Meier (2000)		1260–47,200 µmol/l (13.1–491.4 mg/dl)			Blood BHB <500 µmol/l (5.2 mg/dl): normal 500–2500 µmol/l (5.2–26 mg/dl): increased >2500 µmol/l (26 mg/dl): pathologic
Kanetake *et al.* (2005)					Blood BHB 1000 µmol/l (10.4 mg/dl)
Teresiński *et al.* (2009)				Blood acetone 487–5150 µmol/l (2.8–29.9 mg/dl)	
Hockenhull *et al.* (2012)		3343–17,097 µmol/l (34.7–178 mg/dl)			
Palmiere *et al.* (2012)	1065–1130 µmol/l (11.1–11.8 mg/dl)	1650–2400 µmol/l (17.2–25 mg/dl)	1100–3180 µmol/l (11.5–33.1 mg/dl)	Blood acetone 431–1172 µmol/l (2.5–6.8 mg/dl) Urine acetone 724–3259 µmol/l (4.2–18.9 mg/dl) Vitreous acetone 328–1224 µmol/l (1.9–7.1 mg/dl)	
Molina (2010)				Blood acetone 0–195 mg/dl Vitreous acetone 0–231 mg/dl	
Elliott *et al.* (2010)					Blood BHB <480 µmol/l (5 mg/dl): normal 480–2400 µmol/l (5–25 mg/dl): increased >2400 µmol/l (25 mg/dl): pathologic
Palmiere *et al.* (2013a)					Blood-vitreous-pericardial fluid 2500 µmol/l (26 mg/dl) Cerebrospinal fluid 2000 µmol/l (20.8 mg/dl)
Heninger (2012)					Vitreous BHB 1200–2000 µmol/l (12.5–20.8 mg/dl): moderately elevated 2000–6000 µmol/l (20.8–62.6 mg/dl): significantly elevated >6000 µmol/l (62.6 mg/dl): life-threatening

BHB, beta-hydroxybutyrate

- urea nitrogen, creatinine and uric acid in postmortem serum from femoral blood,
- free triiodothyronine (fT3) in postmortem serum from femoral blood,
- troponin I (cTnI), *N*-terminal pro-brain natriuretic peptide (NT-proBNP) in postmortem serum from femoral blood,
- pre-albumin and albumin in postmortem serum from femoral blood.

All cases selected for this study underwent complete autopsies preceded by unenhanced CT-scans. Histology, toxicology, neuropathology, microbiology and biochemical investigations were performed in all cases. Specimens for microbiology were collected from at least two different sampling sites and always included cardiac blood and cerebrospinal fluid. Medical records and social histories of the deceased as well as police reports were reviewed consistently before conclusions were made.

Since all cases selected for this study originated from forensic practice with deaths occurring outside the hospital, data on antemortem biochemical results were not available.

Sample collection

Undiluted vitreous samples (between 1 and 3 ml) were obtained by aspiration using a sterile needle and syringe as soon as possible after arrival of the bodies at the morgue. Right and left vitreous samples were collected through a scleral puncture at the lateral canthus, aspirated from the center of each eye, pooled in the same syringe and mixed together. After collection, the vitreous samples were immediately centrifuged at 3000 g for 15 min. The separated supernatant was collected and stored in preservative-free tubes. No specimens were excluded due to insufficient sample volume. All samples were transferred to the laboratories immediately post collection. When analyzes were delayed, samples were stored at −20°C.

Urine samples were collected by bladder aspiration during the autopsy, stored in preservative-free tubes and frozen at −20°C until analysis.

Femoral blood samples were collected by aspiration with a sterile needle and a syringe from the femoral vein(s) during autopsy. Blood samples were drawn after clamping the vein(s) at the proximal end and lifting the lower limb(s) for several minutes. Femoral blood was stored in tubes containing sodium fluoride (for ethanol, acetone, acetoacetate, beta-hydroxybutyrate and isopropyl alcohol determination) and tubes containing ethylenediaminetetraacetic acid (for glycated hemoglobin determination). All samples were transferred to the laboratories immediately post collection. When analyses were delayed, samples were stored at −20°C. Blood samples were also collected in preservative-free tubes and centrifuged immediately after collection at 3000 g for 15 min. After centrifugation, the separated supernatant (postmortem serum) was collected and stored in preservative-free tubes.

The external side of the right atrium of the heart was sterilized by searing with a heated scalpel blade and cardiac blood was aspirated using a syringe. Once collected, cardiac blood was stored in blood-culture bottles (aerobic and anaerobic) and immediately incubated at 37°C.

Cerebrospinal fluid was collected by aspiration using a sterile needle and a syringe by suboccipital puncture as soon as possible after arrival of the bodies at the morgue or from the lateral ventricles and cisternal space during autopsy. Post collection, cerebrospinal fluid was stored in blood-culture bottles (aerobic and anaerobic) and immediately incubated at 37°C.

Analytical techniques

Glucose was analyzed in vitreous and urine stored in preservative-free tubes on the Roche Modular P clinical chemistry system (glucose hexokinase method). Lactate was determined in vitreous stored in preservative-free tubes on the Roche Modular P clinical chemistry system (lactate oxydase method).

Sodium and chloride were analyzed in vitreous stored in preservative-free tubes on the Roche Modular P clinical chemistry system. Concentrations were determined by an indirect potentiometry assay (ion selective-electrode using indirect potentiometry).

Ethanol, acetone and isopropyl alcohol were determined in whole femoral blood stored in tubes containing sodium fluoride by the use of headspace gas chromatography with flame ionization detection on an Agilent 1888 headspace and a 6850 GC (Palo Alto, CA, USA). The samples were incubated for 20 min at 80°C and then expanded to the GC column.

The CDT was analyzed in postmortem serum from femoral blood using capillary zone electrophoresis equipped with a UV detector set at 210 nm and using a commercial assay kit (CEofix™ CDT, Analis).

Glycated hemoglobin was determined in femoral whole femoral blood samples stored in tubes containing ethylenediaminetetraacetic acid by ion-exchange high-performance liquid chromatography (Bio-Rad D-10 Dual Program, Hercules, CA, USA).

Beta-hydroxybutyrate and acetoacetate concentrations were determined in femoral whole blood anticoagulated with sodium fluoride and vitreous stored in preservative-free tubes on a Cobas Mira Plus (Roche Diagnostics, Switzerland) by an enzymatic photometric method adapted in house from the technique described by Ruell and Gass (1991). Refrigerated or frozen samples thawed overnight at 4°C were deproteinized with perchloric acid and supernatant was used for analysis.

NT-proBNP and PCT were measured in postmortem serum from femoral blood with the commercially available immunoassays on the Roche Modular E170 system (Roche Diagnostics GmbH, Mannheim, Germany).

Creatinine (Jaffé method, rate-blanked and compensated), urea nitrogen (kinetic enzymatic UV assay for urea/urea nitrogen), uric acid (enzymatic uricase colorimetric AU Plus) and CRP (immunoturbidimetric Tina-quant CRP) were determined with the Roche standard methods on the Roche Modular P system (Roche Diagnostics GmbH, Mannheim, Germany).

Troponin I was analyzed in postmortem serum from femoral blood with the Access® AccuTnI™ assay on Access II (Beckman Coulter, Fullerton, CA, USA).

Free fatty acids were quantified in postmortem serum from femoral blood by the enzymatic colorimetric method 'NEFA-HR(2)' (Wako Diagnostics, USA) adapted on a Cobas MIRA Plus (Roche Diagnostics, Switzerland).

Albumin concentration was determined by the bromocresol green method. Prealbumin was measured by immunoturbidimetric method (Spectra East, Rockleigh, NJ, USA).

Postmortem serum cortisol was determined by fluorescent polarization immunoassay available on the Axsym analyzer (Abbott laboratories, Abbott Park, IL, USA).

Determination of insulin, C-peptide and glucagon in postmortem serum from femoral blood was performed by radioimmunoassay (RIA) method.

Pancreatic amylase activity in postmortem serum from femoral blood was determined by immunoprecipitation with reagents supplied by Roche (Roche Diagnostics, Switzerland). Catalytic concentration of gamma glutamyl transferase (GGT) in postmortem serum from femoral blood was determined by standard enzymatic procedures (Roche Diagnostics, Switzerland, International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference measurement procedure at 37°C).

Determination of free urinary cortisol was performed by RIA method.

Determination of free triiodothyronine (fT3) in postmortem serum from femoral blood was performed by chemiluminescent microparticle immunoassay (Abbott Architect analyser, Abbott reagents).

LBP was determined in postmortem serum from femoral blood by chemiluminescent immunometric assay Immulite®2000 (Siemens Medical, Germany).

IL-6 was measured in postmortem serum from femoral blood by the enzyme-linked immunosorbent assay (ELISA) technique using a commercially available kit (R&D System, Inc., Minneapolis, MN, USA).

IL-10 was measured in postmortem serum from femoral blood by the ELISA technique using a commercially available kit.

Ethical issues

All relevant ethical issues were identified and discussed with the local Ethical Committee. All cases collected for this study underwent medico-legal autopsies as requested by the public prosecutor. Biochemical analyses were performed as part of the medico-legal investigations. All biological samples were anonymized prior to analysis. No further ethical approval was necessary to perform biochemical investigations in the selected cases.

RESULTS

Detailed results for each analyzed marker and reference values, if applicable, are reported in Table 2. Blood ethanol was undetectable in all subjects, whereas postmortem serum CDT levels were increased in all individuals studied. All cases had blood beta-hydroxybutyrate concentrations over 2500 µmol/l and vitreous beta-hydroxybutyrate concentrations of at least 2400 µmol/l. Glycosuria was not detected in any of the cases included in this study and glycated hemoglobin concentrations were normal in all individuals examined. Vitreous glucose levels were lower than <1 mmol/l and suggested the absence of hyperglycemia at the time of death. Additionally, based on toxicology results, none of these individuals had taken any agent (other than ethanol) capable of causing hypoglycemia.

Vitreous lactate levels ranged from 26 to 32 mmol/l and were not considered diagnostic evidence of antemortem lactic acidosis, since values within this range are commonly found in vitreous humor after death. However, it should be emphasized that lactic acidosis can barely be diagnosed in the postmortem setting in the absence of consistent antemortem clinical data. Generalized, bacterial infections and sepsis, which are among causes of lactic acidosis and may themselves be responsible for death, were excluded in all cases based on autopsy and histology findings as well as normal PCT and LBP concentrations. On the other hand, some degrees of gastrointestinal bleeding, pancreatitis and liver disease, which can also be responsible for antemortem increased lactate production, are common findings in chronic alcoholics at postmortem examination and may in part contribute to increased lactate levels.

CRP, IL-6 and IL-10 levels were increased in all individuals studied. These results are not unexpected and appear to be unrelated to bacterial infections or sepsis, as confirmed by unremarkable macroscopy, microscopy and bacteriology as well as normal PCT and LBP concentrations. Moreover, similar findings have recently been described in a series of fatal cases of diabetic ketoacidosis in the absence of underlying bacterial infections (Palmiere *et al.*, 2013b).

Postmortem serum troponin I and NT-proBNP were at normal levels. Pre-albumin concentrations were decreased in three cases, not surprisingly, confirming that undernourishment can be considered one of the typical features of chronic alcohol abusers. Conversely, albumin concentrations were not diminished and even increased in one case, which might also be related to the effect of hemoconcentration secondary to dehydration.

Postmortem serum insulin and C-peptide were appropriately low in all subjects, as one would expect in individuals who are starved and normoglycemic, while glucagon concentrations were increased in four individuals. Postmortem serum cortisol and urine free cortisol were elevated in all individuals studied, along with decreased postmortem serum free triiodothyronine. In living individuals, plasma triiodothyronine decreases soon after the onset of fasting, but also during many serious illnesses. Low triiodothyronine levels are therefore by no means a specific indicator of starvation. The decreased plasma triiodothyronine in fasting and starvation (which may be an appropriate adaptive response) in humans seems to result from decreased hepatic thyroxine conversion, though decreased hepatic uptake of thyroxine may also be a factor (Fulop, 1979; Fulop *et al.*, 1986). Cortisol levels in subjects with fast-induced ketosis have been reported as unchanged or only mildly increased, in contrast to patients with either alcoholic or diabetic ketosis, whose cortisol levels tend to be high-normal or elevated. Cortisol elevations may promote lipolysis and ketogenesis, but this finding cannot be considered specific since hypercortisolemia is common in many acutely ill patients. In chronic alcoholics, increased blood cortisol levels may be due to anxiety, aggressiveness and agitation that can be observed during the alcohol withdrawal (Fulop, 1979). In the postmortem setting, elevated cortisol levels in both postmortem serum and urine have been formerly described in hypothermia fatalities (Bańka *et al.*, 2013; Palmiere *et al.*, 2013c).

Pancreatic amylase activity and gamma glutamyl transferase were elevated in all subjects. These results may suggest the existence of underlying pancreatic and liver diseases, such as alcoholic hepatitis of fatty liver, not completely unexpected in chronic alcoholics. However, as observed by Michiue *et al.*, increased pancreatic amylase and gamma glutamyl transferase in postmortem samples may also indicate leakages from respective tissues damaged by circulatory failure and hypoxia in the death process (Michiue *et al.*, 2013).

All subjects studied had increased postmortem serum free fatty acids. In the clinical setting, increased blood free fatty acid levels have been observed in patients with diabetic ketoacidosis and starvation ketosis, albeit possibly not as much as in patients

Table 2. The results for each analyzed marker and reference values in alcoholic ketoacidosis cases

Specimen and analyte	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Blood ethanol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Serum CDT 1.6%	2.2%	3.8%	3.6%	2.9%	3.0%	3.2%	3.3%	3.4%
Blood BHB 50–170 µmol/l (0.52–1.77 mg/dl)	2600 µmol/l (27.07 mg/dl)	3300 µmol/l (34.36 mg/dl)	4200 µmol/l (43.73 mg/dl)	2900 µmol/l (30.19 mg/dl)	5000 µmol/l (52.06 mg/dl)	5100 µmol/l (53.10 mg/dl)	3000 µmol/l (31.23 mg/dl)	4400 µmol/l (45.81 mg/dl)
Glycated hemoglobin 5–6.6% (31–49 mmol/mol)	5.8% (40 mmol/mol)	5.4% (36 mmol/mol)	6.0% (42 mmol/mol)	5.8% (40 mmol/mol)	5.2% (33 mmol/mol)	5.2% (33 mmol/mol)	5.6% (38 mmol/mol)	5.2% (33 mmol/mol)
Serum cortisol 170–630 nmol/l (µg/dl) (8:00) 40–260 nmol/l (µg/dl) (17:00)	1620 nmol/l (58.71 µg/dl)	1260 nmol/l (45.67 µg/dl)	2300 nmol/l (83.36 µg/dl)	1100 nmol/l (39.90 µg/dl)	960 nmol/l (34.80 µg/dl)	2600 nmol/l (94.24 µg/dl)	1440 nmol/l (52.19 µg/dl)	1820 nmol/l (66.00 µg/dl)
Serum free fatty acids 0.1–0.6 mmol/l	1.2 mmol/l	1.4 mmol/l	1.2 mmol/l	1.6 mmol/l	2.0 mmol/l	1.8 mmol/l	1.6 mmol/l	1.6 mmol/l
Serum PCT <0.06 µg/l	<0.06 µg/l	<0.06 µg/l	<0.06 µg/l	<0.06 µg/l	<0.06 µg/l	<0.06 µg/l	<0.06 µg/l	<0.06 µg/l
Serum CRP <10 mg/l	39 mg/l	45 mg/l	51 mg/l	26 mg/l	36 mg/l	81 mg/l	40 mg/l	48 mg/l
Serum LBP <10 µg/ml	6 µg/ml	8 µg/ml	5 µg/ml	6 µg/ml	4 µg/ml	7 µg/ml	6 µg/ml	8 µg/ml
Serum IL-6 <10 pg/ml	36 pg/ml	49 pg/ml	51 pg/ml	39 pg/ml	45 pg/ml	48 pg/ml	19 pg/ml	28 pg/ml
Serum IL-10 <10 pg/ml	28 pg/ml	54 pg/ml	24 pg/ml	44 pg/ml	49 pg/ml	55 pg/ml	18 pg/ml	37 pg/ml
Serum P-amylase 13–53 U/l	587 U/l	314 U/l	490 U/l	308 U/l	449 U/l	390 U/l	250 U/l	670 U/l
Serum gamma-GT 10–71 U/l	121 U/l	137 U/l	99 U/l	106 U/l	115 U/l	129 U/l	102 U/l	111 U/l
Serum insulin 5.0–18.0 µIU/ml (34.7–125.0 pmol/l)	5.6 µIU/ml (38.9 pmol/l)	6.2 µIU/ml (43.1 pmol/l)	10.2 µIU/ml (70.8 pmol/l)	6.6 µIU/ml (45.8 pmol/l)	8.4 µIU/ml (58.3 pmol/l)	9.1 µIU/ml (63.2 pmol/l)	8.8 µIU/ml (61.1 pmol/l)	6.4 µIU/ml (44.4 pmol/l)
Serum C-peptide 0.7–3.0 µg/l or ng/ml (0.23–1.0 nmol/l)	0.9 µg/l (0.30 nmol/l)	1.1 µg/l (0.37 nmol/l)	1.9 µg/l (0.63 nmol/l)	1.0 µg/l (0.33 nmol/l)	1.2 µg/l (0.40 nmol/l)	1.6 µg/l (0.53 nmol/l)	1.3 µg/l (0.43 nmol/l)	0.9 µg/l (0.30 nmol/l)
Serum glucagon 59–177 pg/ml or ng/l	182 pg/ml	107 pg/ml	201 pg/ml	156 pg/ml	199 pg/ml	170 pg/ml	255 pg/ml	114 pg/ml
Serum fT3 2.7–5.8 pmol/l (175–377 pg/dl)	2.4 pmol/l (156 pg/dl)	2.6 pmol/l (169 pg/dl)	1.8 pmol/l (117 pg/dl)	1.4 pmol/l (91 pg/dl)	1.2 pmol/l (78 pg/dl)	1.6 pmol/l (104 pg/dl)	2.0 pmol/l (130 pg/dl)	2.2 pmol/l (143 pg/dl)
Serum urea nitrogen 2.9–7.7 mmol/l (8.1–21.6 mg/dl)	8.2 mmol/l (23.0 mg/dl)	6.8 mmol/l (19.0 mg/dl)	12.1 mmol/l (33.9 mg/dl)	7.2 mmol/l (20.2 mg/dl)	6.4 mmol/l (17.9 mg/dl)	13.4 mmol/l (37.5 mg/dl)	6.6 mmol/l (18.5 mg/dl)	7.6 mmol/l (21.3 mg/dl)
Serum creatinine 62–106 µmol/l (0.70–1.20 mg/dl)	112 µmol/l (1.27 mg/dl)	99 µmol/l (1.12 mg/dl)	135 µmol/l (1.53 mg/dl)	88 µmol/l (1.00 mg/dl)	79 µmol/l (0.89 mg/dl)	141 µmol/l (1.60 mg/dl)	80 µmol/l (0.90 mg/dl)	104 µmol/l (1.18 mg/dl)
Serum uric acid 202–416 µmol/l (3.40–7.00 mg/dl)	528 µmol/l (8.88 mg/dl)	630 µmol/l (10.60 mg/dl)	668 µmol/l (11.23 mg/dl)	702 µmol/l (11.80 mg/dl)	590 µmol/l (9.92 mg/dl)	621 µmol/l (10.44 mg/dl)	780 µmol/l (13.11 mg/dl)	715 µmol/l (12.02 mg/dl)
Serum cTnI <0.03 µg/l or ng/ml	0.07 µg/l	0.09 µg/l	0.06 µg/l	0.06 µg/l	0.10 µg/l	0.12 µg/l	0.08 µg/l	0.08 µg/l

Continued

Table 2. *Continued*

Specimen and analyte	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Serum NT-proBNP <115 ng/l	125 ng/l	130 ng/l	148 ng/l	120 ng/l	98 ng/l	110 ng/l	90 ng/l	110 ng/l
Serum pre-albumin 18–45 mg/dl	15 mg/dl	31 mg/dl	16 mg/dl	38 mg/dl	15 mg/dl	30 mg/dl	39 mg/dl	26 mg/dl
Serum albumin 35–52 g/l	44 g/l	48 g/l	36 g/l	61 g/l	42 g/l	44 g/l	36 g/l	38 g/l
Urine free cortisol 100–400 nmol/l (3.62–14.50 µg/dl)	940 nmol/l (34.07 µg/dl)	720 nmol/l (26.10 µg/dl)	1140 nmol/l (41.32 µg/dl)	1000 nmol/l (36.25 µg/dl)	680 nmol/l (24.65 µg/dl)	2080 nmol/l (75.39 µg/dl)	880 nmol/l (31.90 µg/dl)	1260 nmol/l (45.67 µg/dl)
Urine glucose <0.8 mmol/l (<14 mg/dl)	<0.8 mmol/l (<14 mg/dl)	<0.8 mmol/l (<14 mg/dl)	<0.8 mmol/l (<14 mg/dl)	<0.8 mmol/l (<14 mg/dl)	<0.8 mmol/l (<14 mg/dl)	<0.8 mmol/l (<14 mg/dl)	<0.8 mmol/l (<14 mg/dl)	<0.8 mmol/l (<14 mg/dl)
Vitreous sodium 135–145 mmol/l or mEq/l	136 mmol/l	138 mmol/l	134 mmol/l	136 mmol/l	135 mmol/l	130 mmol/l	134 mmol/l	139 mmol/l
Vitreous chloride 98–110 mmol/l or mEq/l	90 mmol/l	88 mmol/l	86 mmol/l	84 mmol/l	92 mmol/l	78 mmol/l	80 mmol/l	92 mmol/l
Vitreous glucose	<1 mmol/l	<1 mmol/l	<1 mmol/l	<1 mmol/l	<1 mmol/l	<1 mmol/l	<1 mmol/l	<1 mmol/l
Vitreous lactate	28 mmol/l	30 mmol/l	32 mmol/l	28 mmol/l	26 mmol/l	32 mmol/l	24 mmol/l	26 mmol/l
Vitreous BHB 50–170 µmol/l (0.52–1.77 mg/dl)	2400 µmol/l (25.00 mg/dl)	3000 µmol/l (31.23 mg/dl)	3900 µmol/l (40.60 mg/dl)	2700 µmol/l (28.11 mg/dl)	4800 µmol/l (49.97 mg/dl)	4800 µmol/l (49.97 mg/dl)	2600 µmol/l (27.07 mg/dl)	4000 µmol/l (41.64 mg/dl)

Blood ethanol: n.d.: not detected. Limit of decision: 0.1 g/kg.
Serum: postmortem serum from femoral blood.
BHB, beta-hydroxybutyrate. Other abbreviations are reported in the text.

with alcoholic ketoacidosis (Fulop, 1979). In a study focusing on the biochemical markers of hypothermia fatalities, increased postmortem serum free fatty acid concentrations were observed not only in subjects who died from hypothermia but also in control individuals, possibly indicating the postmortem leakage of these molecules in the bloodstream following cellular autolysis and decompositional change onset, irrespective of the cause of death (Palmiere *et al.*, 2013c).

Most postmortem serum creatinine and urea nitrogen levels were normal in the subjects studied, although intravascular volume depletion and mild renal insufficiency may be seen in subjects with alcoholic ketoacidosis. These findings concur with those of former reports, which highlighted that blood urea nitrogen may be low, normal or elevated depending on the degree of starvation, intravascular volume depletion, malnutrition, presence of blood in the gastrointestinal tract and chronic liver disease (Duffens and Marx, 1987).

Vitreous sodium concentrations were normal or only slightly decreased, likely reflecting total body sodium depletion, whereas hypochloremia was systematically observed, possibly secondary to prolonged vomiting.

Lastly, all individuals studied were hyperuricemic, a condition that has also been found in patients with diabetic and starvation-induced ketosis (Fulop and Hoberman, 1975). Hyperuricemia is not surprising in cases of alcoholic ketoacidosis since decreased renal perfusion due to dehydration, increased tissue catabolism and competitive inhibition of renal excretion of uric acid by beta-hydroxybutyrate and acetoacetate (and lactate, when present) are all prevalent in such situation (Soffer and Hamburger, 1982; Caspar *et al.*, 1993).

DISCUSSION

To date, no extensive studies have been performed in the forensic field focusing on the metabolic and endocrine disorders that characterize the syndrome of alcoholic ketoacidosis and can be detected by postmortem biochemical investigations. Exceptions are the reports pertaining to acetone and beta-hydroxybutyrate determination and, more recently, CRP measurement. This lack of literature may seem surprising, especially considering that alcoholic ketoacidosis is commonly found in ethanol abusers in emergency departments worldwide. Furthermore, deaths related to chronic ethanol consumption account for a significant part of the forensic work (Denmark, 1993; Höjer, 1996).

It is known that several situations may be encountered in forensic pathology routine with negative autopsy and histology findings. Some of these otherwise unexplained cases concern sudden deaths in chronic alcoholics (Pounder *et al.*, 1998; Teresiński *et al.*, 2009). Although numerous observations have been made in the clinical setting describing the correlation among chronic alcohol abuse, repeated vomiting, alcohol withdrawal, prolonged fasting, poor fluid intake, endocrine and biochemical disorders, severe ketoacidosis and death, the medico-legal literature on alcoholic ketoacidosis is relatively scant and probably belies the true frequency of this condition. The paucity of publications on this topic may be in part attributable to the fact that biochemical analyses are not integrated in routine autopsy investigations in most medico-legal centers or are limited to the determination of specific compounds (acetone) exclusively in blood.

When the diagnosis of alcoholic ketoacidosis is suspected based on medical and social histories of the deceased, scene investigation findings as well as negative autopsy, pathologists should consider the following:

- Blood beta-hydroxybutyrate concentration seems to be a more suitable indicator of ketoacidosis than blood acetone and, therefore, should be the preferred biochemical marker in the postmortem setting.
- Beta-hydroxybutyrate concentrations over 25 mg/dl (2400 µmol/l) in the blood can be considered pathologically significant according to reviewed reports. The cause of death can be attributed to alcoholic ketoacidosis when medical records, social history and scene investigation findings are consistent with this hypothesis and postmortem investigation results allow alternative causes of death to be reasonably excluded.
- Low levels of acetone in blood do not preclude the presence of pathologically significant beta-hydroxybutyrate concentrations. Hence, acetone and beta-hydroxybutyrate should both be systematically measured in order to have a more complete metabolic overview of the case.
- Isopropyl alcohol should be systematically determined since some excess acetone undergoes endogenous reduction to isopropyl alcohol via the reversible action of alcohol dehydrogenase. Isopropyl alcohol can be detected in several situations of forensic interest, beyond direct exposure to isopropyl alcohol itself, which are characterized by increased acetone levels and an elevated NADH/NAD+ ratio, such as diabetic and alcoholic ketoacidosis as well as hypothermia fatalities and starvation. It must be emphasized, however, that low or undetectable levels of isopropyl alcohol in blood do not preclude the diagnosis of alcoholic ketoacidosis.
- Blood ethanol should be systematically determined. Ethanol can be undetectable or present at low concentrations in alcoholic ketoacidosis.
- Vitreous humor and pericardial fluid can be considered reliable alternatives to blood for acetone and beta-hydroxybutyrate (and isopropyl alcohol) determination should blood be unavailable during autopsy or reserved for toxicological purposes.
- Alcoholic ketoacidosis is generally characterized by normal glucose levels in both vitreous and urine, with the exception of diabetic individuals who abuse ethanol.
- Vitreous glucose and glycated hemoglobin should be systematically measured. Diabetic ketoacidosis should always be considered in the differential diagnosis since clinical presentations of diabetic and alcoholic ketoacidosis may be similar. Significant increases in vitreous and urine glucose concentrations may reflect the existence of uncontrolled diabetes mellitus and must be evaluated in the context of a more general metabolic dysfunction, along with glycated hemoglobin, beta-hydroxybutyrate, acetoacetate, acetone and isopropyl alcohol concentrations.
- Increased lactate levels in vitreous humor require careful interpretations. Vitreous lactate concentrations within the range of 26–30 mmol/l (230–270 mg/dl) may be a common finding after death. It has been shown that vitreous lactate levels rise with increasing postmortem time and that the postmortem vitreous lactate concentrations are due

not only to the antemortem vitreous lactate levels and the postmortem metabolism of glucose, but other sources can also be responsible for lactate formation in vitreous and subsequent increased vitreous lactate concentrations after death. Pancreatic and liver disease is frequently observed in chronic alcoholics at autopsy and can be responsible for increased lactate levels. Generalized, bacterial infections and sepsis can also contribute to increased lactate levels and be responsible for death themselves.

- Bacterial infections and sepsis as the sole or main contributing cause of death should be systematically sought out through microbiology and biochemical marker determination, including at least PCT.
- Elevated CRP, IL-6 and IL-10 concentrations may be observed in the absence of underlying bacterial infections.
- CDT should be systematically measured to support and corroborate the hypothesis of chronic alcohol abuse.
- Adaptive endocrine reactions in alcoholic ketoacidosis with prolonged fasting may include normal or low insulin and decreased free triiodothyronine as well as increased glucagon and cortisol. Hormonal responses that can be diagnosed in living individuals should also be sought out in appropriate postmortem samples.
- Sodium and chloride in vitreous as well as urea nitrogen, creatinine and uric acid in postmortem serum should be systematically measured in order to estimate the severity of volume depletion.
- Pre-albumin and albumin should be systematically determined to estimate the severity of undernourishment. However, relative increases in albumin concentrations may be related to hemoconcentration and dehydration.
- The determination of other biochemical markers such as free fatty acids, pancreatic amylase and gamma glutamyl transferase in postmortem serum is by no means diagnostic. Increases in pancreatic amylase and gamma glutamyl transferase may be the consequence of terminal heart failure and cannot be related to alcoholic ketoacidosis in itself.
- Increased concentrations of certain molecules might be an expression of preexisting diseases or follow the onset of decompositional changes. Isolated findings should therefore never be overestimated and always require careful interpretation in the context of all biochemical results, postmortem investigation findings and exclusion of other causes of death.
- Elevated blood beta-hydroxybutyrate, acetone and isopropyl alcohol, along with increased blood and urine cortisol as well as normal vitreous and urine glucose levels, may also be found in hypothermia fatalities. Considering that chronic alcoholics are not only at risk of alcoholic ketoacidosis but also fatal hypothermia, biochemical data must always be integrated and interpreted in the context of global findings.

To conclude, alcoholic ketoacidosis is a recognized acute complication in ethanol-dependent subjects and should always be considered as a possible cause of death when examining chronic alcohol abusers who suddenly died unwitnessed. In the clinical field, the syndrome is associated with a variety of laboratory abnormalities. Some of these can also be reliably investigated and diagnosed in the postmortem setting, provided that biochemical analyzes are integrated in routine autopsy investigations, particularly ethanol, ketones and CDT as well as markers of volume depletion and undernourishment. The correlation of medical history and scene investigation findings as well as autopsy, histology, microbiology, toxicology and biochemical results thus prove decisive in formulating appropriate hypothesis concerning the cause of death as well as identifying precipitating conditions and predisposing disorders. Exhaustive postmortem investigations can thereby allow the exclusion of alternative causes of death (namely diabetic ketoacidosis, hypothermia and ethanol intoxication) to be established and the conclusion of alcoholic ketoacidosis as the cause of death to be reasonably reached.

Conflict of interest statement. None declared.

REFERENCES

Adams SL. (1990) Alcoholic ketoacidosis. *Emerg Med Clin North Am* **8**:749–60.

Bańka K, Teresiński G, Buszewicz G. *et al*. (2013) Glucocorticosteroids as markers of death from hypothermia. *Forensic Sci Int* **229**:60–5.

Brinkmann B, Fechner G, Karger B. *et al*. (1998) Ketoacidosis and lactic acidosis—frequent causes of death in chronic alcoholics. *Int J Legal Med* **111**:115–9.

Buszewicz G, Teresiński G, Bańka K. *et al*. (2007) Diagnostic usefulness of the beta-hydroxybutyrate/acetone ratio in medico-legal diagnostics of sudden deaths. *Arch Med Sadowej Kryminol* **57**:289–93.

Caspar CB, Risti B, Iten PX. *et al*. (1993) Alcoholic ketoacidosis. *Schweiz Med Wochenschr* **123**:1929–34.

Clark JC. (1988) Sudden death in the chronic alcoholic. *Forensic Sci Int* **36**:105–11.

Coe JI. (1991) Postmortem chemistry update. Emphasis on forensic application. *Am J Forensic Med Pathol* **14**:91–117.

Cooperman MT, Davidoff F, Spark R. *et al*. (1974) Clinical studies of alcoholic ketoacidosis. *Diabetes* **23**:433–9.

Copeland AR. (1985) Sudden death in the alcoholic. *Forensic Sci Int* **29**:159–69.

Denmark LN. (1993) The investigation of beta-hydroxybutyrate as a marker for sudden death due to hypoglycemia in alcoholics. *Forensic Sci Int* **62**:225–32.

Dillon ES, Dyer WW, Smelo LS. (1940) Ketone acidosis of non-diabetic adults. *Med Clin N Am* **24**:1813–22.

Duffens K, Marx JA. (1987) Alcoholic ketoacidosis—a review. *J Emerg Med* **5**:399–406.

Dwyer JB, Tamama K. (2013) Ketoacidosis and trace amounts of isopropanol in a chronic alcoholic patient. *Clin Chim Acta* **415**:245–9.

Elliott S, Smith C, Cassidy D. (2010) The postmortem relationship between beta-hydroxybutyrate (BHB), acetone and ethanol in ketoacidosis. *Forensic Sci Int* **198**:53–7.

Felby S, Nielsen E, Thomsen JL. (2008) The postmortem distribution of ketone bodies between blood, vitreous humor, spinal fluid and urine. *Forensic Sci Med Pathol* **4**:100–7.

Fulop M. (1979) Serum potassium in lactic acidosis and ketoacidosis. *N Engl J Med* **300**:1087–9.

Fulop M. (1989) Alcoholism, ketoacidosis, and lactic acidosis. *Diabetes Metab Rev* **5**:365–78.

Fulop M. (1993) Alcoholic ketoacidosis. *Endocrinol Metab Clin North Am* **22**:209–19.

Fulop M, Hoberman HD. (1975) Alcoholic ketosis. *Diabetes* **24**:785–90.

Fulop M, Ben-Ezra J, Bock J. (1986) Alcoholic ketosis. *Alcohol Clin Exp Res* **10**:610–5.

Halperin ML, Hammeke M, Josse RG. *et al*. (1983) Metabolic acidosis in the alcoholic: a pathophysiologic approach. *Metabolism* **32**:308–15.

Hansen AU, Simonsen J. (1991) The manner and cause of death in a forensic series of chronic alcoholics. *Forensic Sci Int* **49**:171–8.

Heninger M. (2012) Postmortem vitreous beta-hydroxybutyrate: interpretation in a forensic setting. *J Forensic Sci* **57**:1234–40.
Hockenhull J, Dhillo W, Andrews R. *et al*. (2012) Investigation of markers to indicate and distinguish death due to alcoholic ketoacidosis, diabetic ketoacidosis and hyperosmolar hyperglycemic state using postmortem samples. *Forensic Sci Int* **214**:142–7.
Höjer J. (1996) Severe metabolic acidosis in the alcoholic: differential diagnosis and management. *Hum Exp Toxicol* **15**:482–8.
Iten PX, Meier M. (2000) Beta-hydroxybutyric acid—an indicator for an alcoholic ketoacidosis as cause of death in deceased alcohol abusers. *J Forensic Sci* **45**:624–32.
Jenkins DW, Ecke RE, Craig JW. (1971) Alcoholic ketoacidosis. *JAMA* **217**:177–83.
Kadiš P, Balažic J, Ferlan-Marolt V. (1999) Alcoholic ketoacidiosis: a cause of sudden death of chronic alcoholics. *Forensic Sci Int* **103**(Suppl):s53–9.
Kanetake J, Kanawaku Y, Mimasaka S. *et al*. (2005) The relationship of a high level of serum beta-hydroxybutyrate to cause of death. *Leg Med (Tokyo)* **7**:169–74.
Kuller LH, Perper JA, Cooper M. *et al*. (1974) An epidemic of death attributed to fatty liver in Baltimore. *Prev Med* **3**:61–79.
Levy LJ, Duga J, Girgis M. *et al*. (1973) Ketoacidosis associated with alcoholism in nondiabetic subjects. *Ann Intern Med* **78**:213–19.
Lindroos-Jokinen K, Keltanen T, Vanhala T. *et al*. (2012) Postmortem measurement of C-reactive protein and interpretation of results in ketoacidosis. *Leg Med (Tokyo)* **14**:140–6.
Lu WT, Chen KW, Lin JD. *et al*. (1997) Ketoacidosis with hyperglycemia in heavy drinkers: a report of 12 cases. *Changgeng Yi Xue Za Zhi* **20**:34–8.
McGuire LC, Cruickshank AM, Munro PT. (2006) Alcoholic ketoacidosis. *Emerg Med J* **23**:417–20.
Michiue T, Ishikawa T, Kawamoto O. *et al*. (2013) Postmortem serum levels of amylase and gamma glutamyl transferase (GGT) as markers of systemic tissue damage in forensic autopsy. *Leg Med (Tokyo)* **15**:79–84.
Molina DK. (2010) A characterization of sources of isopropanol detected on postmortem toxicologic analysis. *J Forensic Sci* **55**:998–1002.
Palmer JP. (1983) Alcoholic ketoacidosis: clinical and laboratory presentation, pathophysiology and treatment. *Clin Endocrinol Metab* **12**:318–9.
Palmiere C, Mangin P. (2012a) Postmortem chemistry update: Part I. *Int J Legal Med* **126**:187–98.
Palmiere C, Mangin P. (2012b) Postmortem chemistry update: Part II. *Int J Legal Med* **126**:199–215.
Palmiere C, Sporkert F, Werner D. *et al*. (2012) Blood, urine and vitreous isopropyl alcohol as biochemical markers in forensic investigations. *Leg Med (Tokyo)* **14**:17–20.
Palmiere C, Mangin P, Werner D. (2013a) Postmortem distribution of 3-beta-hydroxybutyrate. *J Forensic Sci*. doi: 10.1111/1556–4029.12265.
Palmiere C, Bardy D, Mangin P. *et al*. (2013b) Postmortem diagnosis of unsuspected diabetes mellitus. *Forensic Sci Int* **226**:160–7.
Palmiere C, Bardy D, Letovanec I. *et al*. (2013c) Biochemical markers of fatal hypothermia. *Forensic Sci Int* **226**:54–61.
Parai JL, Kodikara S, Milroy CM. *et al*. (2012) Alcoholism and the Armanni-Ebstein lesion. *Forensic Sci Med Pathol* **8**:19–22.
Petersen TH, Williams T, Nuwayhid N. *et al*. (2012) Postmortem detection of isopropanol in ketoacidosis. *J Forensic Sci* **57**:674–8.
Petersson B. (1988) Analysis of the role of alcohol in mortality, particularly sudden unwitnessed death, in middle-aged men in Malmö, Sweden. *Alcohol Alcohol* **23**:259–63.
Platia EW, Hsu TH. (1979) Hypoglycemic coma with ketoacidosis in nondiabetic alcoholics. *West J Med* **131**:270–6.
Pounder DJ, Stevenson RJ, Taylor KK. (1998) Alcoholic ketoacidosis at autopsy. *J Forensic Sci* **43**:812–6.
Randall B. (1980a) Fatty liver and sudden death. A review. *Hum Pathol* **11**:147–53.
Randall B. (1980b) Sudden death and hepatic fatty metamorphosis. A North Carolina Survey. *JAMA* **243**:1723–5.
Rehman HU. (2012) A woman with ketoacidosis but not diabetes. *BMJ* **344**:e1535.
Ruell PA, Gass GC. (1991) Enzymatic measurement of 3-hydroxybutyrate in extracts of blood without neutralization. *Ann Clin Biomed* **28**:183–4.
Sibaï K, Eggiman P. (2005) [Alcoholic ketoacidosis: not rare cause of metabolic acidosis]. *Rev Med Suisse* **1**:2106–15.
Smith D, Kelly D, Daly A. *et al*. (1999) Alcoholic ketoacidosis presenting as diabetic ketoacidosis. *Ir J Med Sci* **168**:186–8.
Soffer A, Hamburger S. (1982) Alcoholic ketoacidosis: a review of 30 cases. *J Am Med Womens Assoc* **37**:106–10.
Tanaka M, Miyazaki Y, Ishikawa S. *et al*. (2004) Alcoholic ketoacidosis associated with multiple complications: report of 3 cases. *Intern Med* **43**:955–9.
Teresiński G, Buszewicz G, M□dro R. (2009) Acetonaemia as an initial criterion of evaluation of a probable cause of sudden death. *Leg Med (Tokyo)* **11**:18–24.
Thomsen JL. (1996) Causes and manners of death in alcoholic. *Med Sci Law* **36**:209–16.
Thomsen JL, Frohlich B. (1995) Drug abuse and intoxication in alcoholics. *Alcohol Alcohol* **30**:379–83.
Thomsen JL, Theilade P, Felby S. *et al*. (1993) Alcoholism and ketoacidosis. *Forensic Sci Int* **60**:3–4.
Thomsen JL, Felby S, Theilade P. *et al*. (1995) Alcoholic ketoacidosis as a cause of death in forensic cases. *Forensic Sci Int* **75**:163–71.
Thomsen JL, Simonsen KW, Felby S. *et al*. (1997) A prospective toxicology analysis in alcoholics. *Forensic Sci Int* **90**:33–40.
Umpierrez GE, DiGirolamo M, Tuvlin JA. *et al*. (2000) Differences in metabolic and hormonal milieu in diabetic- and alcohol-induced ketoacidosis. *J Crit Care* **15**:52–9.
Wrenn KD, Slovis CM, Minion GE. *et al*. (1991) The syndrome of alcoholic ketoacidosis. *Am J Med* **91**:119–28.
Yuzuriha T, Nakamura T, Shoji M. *et al*. (1993) Alcohol and sudden death: a survey on alcohol-related deaths at Tokyo Metropolitan Medical Examiner's Office (1989). *Arukoru Kenkyuto Yakubutsu Ison* **28**:95–119.
Yuzuriha T, Okudaira M, Tominaga I. *et al*. (1997) Alcohol-related sudden death with hepatic fatty metamorphosis: a comprehensive clinicopathological inquiry and its pathogenesis. *Alcohol Alcohol* **32**:745–52.
Zhou C, Gilbert JD, Yool A. *et al*. (2013) Basal epithelial formalin pigment deposition in the kidney—a useful marker for ketoacidosis at autopsy. *J Forensic Leg Med* **20**:305–7.