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Review

Postmortem biochemistry: Current applications

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ABSTRACT

The results of biochemical analyses in specimens obtained postmortem may aid death investigation when diabetic and alcoholic ketoacidosis is suspected, when death may have been the result of drowning, anaphylaxis, or involved a prolonged stress response such as hypothermia, and in the diagnosis of disease processes such as inflammation, early myocardial infarction, or sepsis. There is often cross-over with different disciplines, in particular with clinical and forensic toxicology, since some endogenous substances such as sodium chloride, potassium chloride, and insulin can be used as poisons. The interpretation of results is often complicated because of the likelihood of postmortem change in analyte concentration or activity, and proper interpretation must take into account all the available evidence. The unpredictability of postmortem changes means that use of biochemical measurements in time of death estimation has little value.

The use of vitreous humour is beneficial for many analytes as the eye is in a physically protected environment, this medium may be less affected by autolysis or microbial metabolism than blood, and the assays can be performed with due precaution using standard clinical chemistry analysers. However, interpretation of results may not be straightforward because (i) defined reference ranges in life are often lacking, (ii) there is a dearth of knowledge regarding, for example, the speed of equilibration of many analytes between blood, vitreous humour, and other fluids that may be sampled, and (iii) the effects of post-mortem change are difficult to quantify because of the lack of control data. A major limitation is that postmortem vitreous glucose measurements are of no help in diagnosing antemortem hypoglycaemia.

1. Introduction

Postmortem biochemistry[c] has a role in investigating some apparently natural deaths, including diabetes- and alcoholic ketoacidosis-related deaths, anaphylaxis-related deaths, deaths that may have involved a prolonged stress response such as hypothermia, and in the diagnosis of disease processes such as early myocardial infarction. There is also clearly much overlap with clinical and forensic toxicology in that some endogenous substances can be used as poisons. Sodium chloride, potassium chloride, and insulin are obvious examples. Indeed, in some instances suspicion of poisoning may be aroused by abnormal biochemical results (Table 1).

Postmortem biochemistry has been an active area of research for many years. Detection of acetone and measurement of β-hydroxybutyrate (3-hydroxybutyrate; BHB) in blood, or in vitreous humour, urine, pericardial fluid, or cerebrospinal fluid (CSF) may be valuable in the diagnosis of alcohol- or diabetes-related deaths, for example.[1] Likewise, measurement of D-glucose,[2] and of urea and creatinine in vitreous humour and other specimens,[3] may give information on the presence of hyperglycaemia and of renal impairment, respectively, before death. Vitreous humour is an important specimen in this context as in life the concentrations of low molecular weight, non-protein bound solutes such as ethanol and electrolytes in blood equilibrate with the respective concentrations in vitreous humour. However, at present few other measurements are undertaken routinely (Table 2), in part because the necessary samples, not only vitreous humour, but also CSF, and pericardial and synovial fluids (Table 3), may not be collected. The aim of this review is to summarise considerations important in undertaking

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[c] Postmortem biochemistry is sometimes termed thanatochemistry (from the Greek θάνατος, Thanatos, the personification of death in Greek mythology).

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Table 1
Some laboratory investigations in life that may arouse or confirm a suspicion of poisoning.

Investigation	Fluid	Possible cause of increase	Possible cause of decrease
Anion gap ($[Na^+] + [K^+]$) − ($[HCO_3^-] + [Cl^-]$)	Plasma	Ethanol, ethylene glycol, iron salts, isoniazid, methanol, metformin, paraldehyde, salicylates, toluene (chronic)	–
Calcium	Plasma or serum	–	Ethylene glycol, fluorides, magnesium salts
Chloride	Plasma	Bromide or organobromines (actually interference in method)	–
D-Glucose	Blood or fluoride/oxalate plasma	Salicylates, theophylline	Ethanol (especially children), insulin, salicylates, sulfonylureas, valproate
International normalised ratio (INR, prothrombin time)	Blood	Anticoagulant rodenticides (warfarin, brodifacoum), paracetamol (early marker of hepatic damage)	–
Lactate	Plasma	Glycolate (from ethylene glycol, artefact on some blood gas and other analysers)	–
Magnesium	Plasma or serum	Magnesium salts	–
Osmolar gap[b]	Plasma	Acetone, ethanol, ethylene glycol, methanol, 2-propanol, hypertonic i.v. solutions (e.g. mannitol)	–
Potassium	Plasma	Digoxin, potassium salts	Diuretics, laxatives (both chronic), insulin, salbutamol, sulfonylureas, theophylline
Sodium	Plasma or serum	MDMA (malignant hyperthermia),[a] sodium salts	Diuretics, water intoxication (acute and chronic), MDMA (very rare)

[a] *Methylenedioxymethamphetamine.*
[b] Measured osmolality (freezing point depression) − calculated osmolality. Calculated osmolality = $2([Na^+] + [K^+])$ + urea + glucose (all mmol/L).

Table 2
Postmortem biochemistry: Some little used/unvalidated tests.

Analyte	Matrix	Suggested diagnostic role [references]
Adrenaline:noradrenaline ratio	Urine	Hypothermia[4]
Ammonia	Vitreous humour	Liver failure; time of death estimation[5]
Calcium	Vitreous humour	No clear role; does not relate to antemortem serum calcium
Carbohydrate-deficient transferrin (CDT)	Blood, vitreous humour	Chronic alcohol use[6,7]
Chymase activity	Blood	Anaphylactic shock[8]
Chromogranin A	Serum, cerebrospinal fluid	Hypothermia[9]
C-Reactive protein (CRP)	EDTA blood, liver	Recent infection (bacterial, viral, fungal, mycobacterial), trauma, burns, ketoacidosis, tissue necrosis (myocardial infarction, pancreatitis), inflammatory diseases, malignancy. Suggested role in diagnosis of sepsis if measured soon after death[10,11]
Creatine kinase brain (CK-BB)	CSF	Diffuse brain injury/cerebral hypoxia
Creatine kinase-muscle brain (CK-MB)	Pericardial fluid	Myocardial damage
Ethyl glucuronide (EtG) and ethyl sulphate (EtS)	Vitreous humour, urine	Antemortem ingestion of ethanol[12,13]
Free fatty acids	Blood	Hypothermia[14]
Fructosamine	Vitreous humour	Diabetic ketoacidosis[15,16]
Glucocorticoids	Blood	Hypothermia[17]
Hypoxanthine	Vitreous humour	Time of death[18]
L-Lactate	Vitreous humour	If very high may indicate lactic acidaemia, but may be formed perimortem[19] and increases after death
Myoglobin	Blood, urine	Hyperthermia[20]
Neurone specific enolase	CSF	Diffuse brain injury/cerebral hypoxia
Osmolality	Vitreous humour	No clear role; increases with postmortem interval
Thyroglobulin, free triiodothyronine (T_3)	Blood	Neck trauma, e.g. strangulation[21,22]
Troponin	Pericardial fluid	Myocardial damage present prior to microscopic changes[23]

postmortem biochemical analyses, including sample collection and the interpretation of results. In all cases of course the results can only be properly interpreted in the light of all the available information as to the case under investigation.[2]

In death investigations, although specimens collected postmortem are all that are usually available for biochemical and toxicological analysis, in general it is information on analyte concentration or other physiological parameter prior to, or at the time of, death that is required. Gradients that are maintained by active processes in life such as that between intra- and extra-cellular potassium begin to break down soon after the occurrence of hypoxic or anoxic damage. Thus, the possibility of both terminal and postmortem change has to be evaluated when interpreting results. Since most deaths that become the subject of further investigation occur outside hospital, it may be some days before a body is found. Therefore blood samples are often haemolysed, there is the possibility of sample contamination during collection, and the likelihood of other changes such as loss of labile analytes (for example glucose, insulin) is high. It should be remembered that most clinical reference values are established for plasma or serum, and not haemolysed whole blood even for stable analytes such as many drugs. Moreover, since samples such as vitreous humour are, for practical purposes, rarely available during life except from laboratory animals, reference ranges are by definition difficult to establish. Method validation is also compromised by this same lack of reference material. Furthermore, the time needed for analyte equilibration between plasma and, for example, vitreous humour during life remains unknown.

Table 3
Sample requirements: General postmortem biochemistry and toxicology.[a]

Sample	Notes[b]
Heart whole blood (right ventricle)	20 mL unpreserved (qualitative toxicology only)
Jugular vein whole blood	20 mL unpreserved (qualitative toxicology only)
Peripheral whole blood	20 mL from femoral or other peripheral site ensuring no contamination from urine or from central or cavity blood. Collect one portion into 2% w/v sodium fluoride and another into a plain tube
Urine	20–50 mL if available (plain tube, no preservative unless a portion required for ethanol measurement)
Gastric contents[c]	25–50 mL (plain bottle, no preservative)
Vitreous humour	Maximum available, plain tube, separate specimens from both eyes if feasible. Collect one portion into 2% w/v sodium fluoride if for ethanol measurement
Cerebrospinal fluid	5–10 mL, plain tube
Pericardial fluid	Maximum available, plain tube
Synovial fluid[d]	Maximum available, plain tube
Liver and other tissues	Liver 10 g (deep inside right lobe), other tissues 10 g as appropriate
Scene residues[e]	As appropriate

[a] See Dinis-Oliveira et al.[24] for detailed discussion on samples and sampling with especial reference to forensic toxicology.
[b] Smaller volumes may often be acceptable, for example in the case of young children.
[c] Includes vomit, gastric lavage (stomach washout, first sample), etc.
[d] Alternative if vitreous humour not available.
[e] Tablet bottles, drinks containers, aerosol canisters, etc. – pack entirely separately from biological samples, especially if poisoning with volatiles is a possibility.

1.1. Use of vitreous humour

The above considerations notwithstanding, vitreous humour is preferred to blood for most postmortem biochemistry (Table 4) since it is thought far less susceptible to autolytic change, is less likely to be subject to postmortem contamination by diffusion of microbes or of drugs or other poisons that may be present at high concentration in the thorax or abdomen at death, and lies within the relatively protected environment of the eye socket.[25,26] A further practical point is that the sample if uncontaminated with blood is amenable to analysis using standard clinical chemistry systems and as such the cost of routine measurements (Table 4) is minimal.[2] Note however that vitreous humour is viscous, hence may require pre-treatment such as centrifugation, heating, dilution, or addition of hyaluronidase to facilitate accurate pipetting.[16,27,28] There is also the likelihood of loss of water from the eye with time since death and body storage conditions, leading to not only increased vitreous humour viscosity, but also increased analyte concentrations.

Vitreous samples should be collected without preservative unless for a specific requirement such as ethanol measurement since even dipotassium EDTA contains sufficient sodium to invalidate vitreous sodium measurement. Vitreous humour may not be available if the body has suffered severe trauma, and the possibility of concurrent vitreous disease confounding the results must be remembered.[29] In cases where bodies have been immersed in water then either dilution (fresh water), or concentration (salt water) as well as microbial contamination are possible. Synovial fluid may represent an alternative if vitreous humour is not available.[30]

When collecting vitreous humour, ideally both eyes should be sampled independently and the results reported separately,

Table 4
Postmortem biochemistry: Interpretation of results.

Analyte	Matrix	Typical reference range	Interpretation of raised concentration
Acetone[a]	Blood	<2.5 mg/L	Fasting, prolonged alcohol abuse, diabetic ketoacidosis, stress response (e.g. hypothermia)
Chloride	Vitreous humour[b]	105–135 mmol/L	Sodium chloride poisoning; salt water drowning; dehydration (interpret in conjunction with creatinine and urea)
Creatinine	Vitreous humour[b]	<115 μmol/L	Poor renal function; high protein intake; large muscle mass; heat shock
D-Glucose	Vitreous humour[b]	After death vitreous humour (and CSF) glucose falls rapidly therefore any detectable glucose requires investigation	(Drug induced) hyperglycaemia, diabetic ketoacidosis, stress response (interpret in conjunction with lactate)
Haemoglobin A1c (HbA1c)	Blood	<39 mmol/mol Hb	Poor long term (8–12 weeks) blood glucose control
β-Hydroxybutyrate (BHB)	Blood, vitreous humour[b]	0.1–1.0 mmol/L (pathologically significant >2.5 mmol/L)	Fasting, prolonged alcohol abuse, diabetic ketoacidosis, stress response (e.g. hypothermia)
L-Lactate	Vitreous humour[b]	<10 mmol/L	Interpret in conjunction with vitreous glucose and postmortem interval
Potassium	Vitreous humour[b]	After death vitreous potassium increases rapidly. Concentrations > 15 mmol/L suggest postmortem decomposition	Postmortem decomposition, little interpretative value
Sodium	Vitreous humour[b]	135–145 mmol/L	Sodium chloride poisoning; salt water drowning; dehydration (interpret in conjunction with vitreous creatinine and urea)
Tryptase	Blood	<100 μmol/L	Anaphylactic shock
Urea	Vitreous humour[b]	<10 mmol/L	Renal function; upper GI haemorrhage

[a] Acetone not often measured; normally detected sometimes with other ketones and alcohols such as butanone and propanol during blood/urine ethanol analysis by gas chromatography.
[b] See Rose and Collins.[31]

although this may not always be possible, for example in the case of very young children. Potassium concentrations have been said to differ by up to 2.34 mmol/L between the two eyes in samples from non-putrefied bodies.[32] However, these and other reported differences may be simply due to problems in sample collection and handling.[33] More importantly, after death potassium quickly leaks from the retina and hence vitreous potassium is not a reliable indicator of antemortem plasma potassium and is of minimal value in the diagnosis of exogenous potassium administration. Specimen contamination with retinal cells is also a recognised source of falsely raised vitreous potassium concentrations.[29] Hence aspiration must be as gentle as possible to minimise the risk of contamination with retinal fragments. Measurement of uric acid in vitreous humour may offer a criterion for identifying blood contamination before colouration of the fluid is apparent.[34]

Vitreous sodium and chloride concentrations may fall after death at rates of up to 1 mmol L^{-1} h^{-1}, whereas potassium increases at a rate of 0.14–0.19 mmol L^{-1} h^{-1}. Nevertheless, if the potassium concentration is < 15 mmol/L, then the sodium and chloride concentrations are thought likely to reflect the situation at death. Urea and creatinine, on the other hand, are relatively stable in postmortem specimens such as vitreous humour and pericardial fluid.[3] If vitreous sodium, chloride, and urea are >155, >115, and >10 mmol/L, respectively, this may indicate antemortem dehydration. If the urea concentration is >20 mmol/L and creatinine >200 μmol/L with sodium and chloride being within the normally accepted range, this indicates uraemia may have been present before death depending on other factors (age, sex, muscle mass, etc.). An especially difficult area is attempting to distinguish between hypernatraemic dehydration and sodium chloride poisoning in infants and children and due caution must be exercised in interpreting results.[35]

2. Specific diagnostic problems

2.1. Anaphylaxis/anaphylactoid reactions

Death from anaphylaxis is rare (0.12–1.06 deaths per million person-years) and more likely in older individuals in the case of drug- and *Hymenoptera*-induced anaphylaxis.[36] Drug-induced anaphylaxis is a common cause of anaphylaxis and a leading cause of fatal anaphylaxis. Antibiotics, radiocontrast media, and nonsteroidal anti-inflammatory drugs are commonly implicated compounds.[37] Mast-cell tryptase is an indicator of mast-cell activation.[38] Sampling from the femoral vein is recommended postmortem as aggressive attempts at resuscitation and defibrillation can release tryptase from the heart, but this is thought not to affect femoral blood tryptase concentrations.[39] Measurement of blood chymase[40] and of blood histamine and blood diamine oxidase[41] has also been suggested in this context. Although a raised serum or plasma tryptase can supply important supporting evidence in the diagnosis of anaphylaxis,[38,42] it cannot be used as the sole criterion for the postmortem diagnosis of anaphylaxis even if an appropriate sample is available because elevated tryptase concentrations have also been reported in deaths unrelated to anaphylaxis.[43–48]

Immunoglobulin E (IgE) measurement has been suggested as a confirmatory test if serum tryptase is raised. Specific IgEs produced in response to venoms and to many foods, including those commonly causing anaphylaxis, can be measured. IgEs produced in response to some penicillins and a thiocholine epitope that in many cases cross-reacts with muscle relaxants, which are possibly the most common cause of fatal iatrogenic anaphylaxis, can also be assayed.[49] However, IgE elevation can only be assessed if the offending allergen is either known, or suspected. As many deaths are unwitnessed and possible allergens go unrecognised, the utility of IgE measurement is limited. A screening approach, utilizing an array of commonly encountered allergens, has been suggested.[50] However, there is wide inter-individual variability in serum IgE concentration. Serum IgE may be either seasonally, or chronically elevated in the absence of disease, and thus establishing 'normal' and 'elevated' IgE concentrations is difficult.[41] Increased IgE concentrations have also been reported in association with trauma and with sepsis.[51] IgE remains relatively unused, and further studies are required to confirm its postmortem stability.[50]

2.2. Disorders of glucose metabolism

Lundquist and Osterlin[52] assayed D-glucose in blood and vitreous humour from three patient groups undergoing vitrectomy: those classified as nondiabetic (ND), those with Type 1 diabetes (diabetes mellitus Type 1, T1D), and patients with Type 2 diabetes (diabetes mellitus Type 2, T2D). In the ND group the vitreous glucose concentration (3.5 ± 1.8 mmol/L) was always lower than the blood glucose (9.1 ± 3.5 mmol/L). In the diabetic groups the vitreous glucose was generally lower than the blood glucose, but was generally higher (T1D 9.4 ± 3.3 mmol/L, T2D 7.2 ± 3.9 mmol/L) than in the ND group, and in 15 specimens exceeded 11 mmol/L. However, blood and vitreous humour glucose concentrations generally fall rapidly after death and are thus an unreliable guide to antemortem glucose concentration. However, any measurable glucose suggests antemortem hyperglycaemia. Collecting samples into fluoride preservative does not make any difference to vitreous glucose concentrations.[16]

D-Glucose undergoes anaerobic glycolysis to L-lactate, and some investigators have suggested that measuring the sum of vitreous humour glucose and lactate may give a better estimation of the glucose concentration at the time of death.[19,53–55] However, vitreous lactate may continue to increase for several days after death and interpretation in relation to vitreous glucose is equivocal at best.[56] Some drugs that are themselves unstable in biological systems, for example ethanol and insulin, may cause fatal hypoglycaemia, hence compounding the difficulties that may be encountered in establishing a cause of death. A further complication is that death may be accompanied by attempted resuscitation and agonal processes resulting in administration or secretion of catecholamines, leading to the breakdown of glycogen and formation of glucose in a counter-balancing phenomenon.[57]

An elevated postmortem blood haemoglobin A1c (HbA1c) concentration might indicate poor glucose control during life,[58] but note that this measurement is unreliable in decomposed/degraded samples. Measurement of fructosamine postmortem has been suggested as an indicator for confirming the presence of antemortem hyperglycaemia,[59] but is not commonly measured. Note that fructosamine can be measured in postmortem blood and in vitreous humour, but HbA1c cannot be measured in vitreous humour as this matrix is not vascularized.[16]

In patients with T1D the presence of acetone in blood, urine, and/or vitreous humour may indicate the occurrence of diabetic ketoacidosis (DKA) prior to death especially if considered together with the blood, urine or vitreous BHB concentration.[60–62] Detection of acetoacetate is not normally possible postmortem since the prevailing acidic conditions favour transformation to acetate.[16] BHB concentrations > 2.4 mmol/L (>250 mg/L) are thought to be pathologically significant.[63] In contrast, hyperosmolar hyperglycaemic syndrome [HHS, also known as hyperosmolar non-ketotic hyperglycaemia (HONK)] normally occurs in patients with T2D who are often nursing home residents aged 55–70 yr. Most who develop HHS do so over days or weeks and have polyuria, polydipsia, and a progressive decline in consciousness.[64] The most common clinical presentation is said to be altered perception of surroundings. HHS

patients do not usually produce acetone or other biochemical markers that can be tested for postmortem, and the only clue as to the presence of hyperglycaemia in life may be a raised vitreous glucose concentration (Fig. 1).

Measurement of ketones in blood or vitreous humour is also recommended in unexplained deaths in chronic alcoholics as well as in diabetics.[65] Prolonged use of alcohol and poor nutrition (common in chronic alcoholics) promote the accumulation of ketones (acetone, butanone) and BHB, and often elevated blood ketone concentrations (acetone > 20 mg/L, BHB > 2.5 mmol/L) are the only notable postmortem findings. It has been suggested that blood acetone concentrations > 90 mg/L can indicate a fatal ketoacidotic coma, but interpretation of an acetone concentration alone is unwise as endogenous acetone accounts for only 2% of total ketone bodies and there are extrinsic sources of acetone such as ingestion of either acetone itself, or of 2-propanol.[66]

Blood ethanol measurement postmortem may aid in distinguishing alcoholic ketoacidosis from starvation or diabetic ketoacidosis. However postmortem production, or indeed loss, of ethanol can complicate interpretation of results. There is said to be a lower likelihood of ethanol production by microbes in vitreous humour as compared to blood. In this context, it has been suggested that measurement of ethyl glucuronide (EtG) and of ethyl sulfate (EtS) in vitreous humour may help distinguish between antemortem ethanol ingestion and postmortem ethanol production.[12,13] EtG and EtS are reported to have high sensitivity and specificity in corpses without signs of putrefaction. EtS may be more stable than EtG in putrefied corpses and measurement of these compounds in urine rather than in vitreous humour may also help as urinary concentrations will usually be higher.[13,67,68]

An additional complication when diagnosing alcoholic ketoacidosis is that some 50% of all alcoholics die with a negligible blood alcohol concentration.[69] In these cases, use of chronic alcohol markers, in particular carbohydrate-deficient transferrin (CDT), may aid a diagnosis. Measurement of CDT using capillary zone electrophoresis or high-performance liquid chromatography is recommended due to the low specificity of some immunoassay methods. CDT is reported to be stable in postmortem blood for up to 7 days after death, and does not appear to be subject to redistribution after death.[7] However, haemolysed samples are unsuitable for CDT analysis at present, and thus investigation into measurement of CDT in other matrices less prone to postmortem change is needed. Preliminary studies measuring CDT in vitreous humour have been conducted,[6,70,71] but further research is required.

Diagnosis of hypoglycaemia may involve detection of drugs that may cause hypoglycaemia such as insulin or ethanol. Calculation of the insulin to C-peptide ratio in blood has been used to differentiate endogenous production from exogenous administration of insulin, which lacks C-peptide.[72] However, murder or suicide by means of insulin, even if suspected, is difficult to prove. Immunohistochemical demonstration or measurement of an elevated insulin concentration in tissue around an injection site compared to a control site can support the diagnosis.[73]

Haemolysed postmortem blood is not the ideal matrix for insulin measurement since release of insulin-degrading enzyme (EC 3.4.24.56) from erythrocytes may give falsely low insulin concentrations.[74] On the other hand, haemolysis could in theory increase plasma insulin and hence falsely elevated insulin concentrations could also be encountered. The use of vitreous humour, bile, and cerebrospinal and pericardial fluids as alternative matrices for insulin measurement has been suggested.[75,76] Finally, analytical methods for insulin used clinically may give different results with the same sample. Many different insulin analogues are available and different analogues may cross-react differently to the antibody used in an immunoassay. There may also be interference from

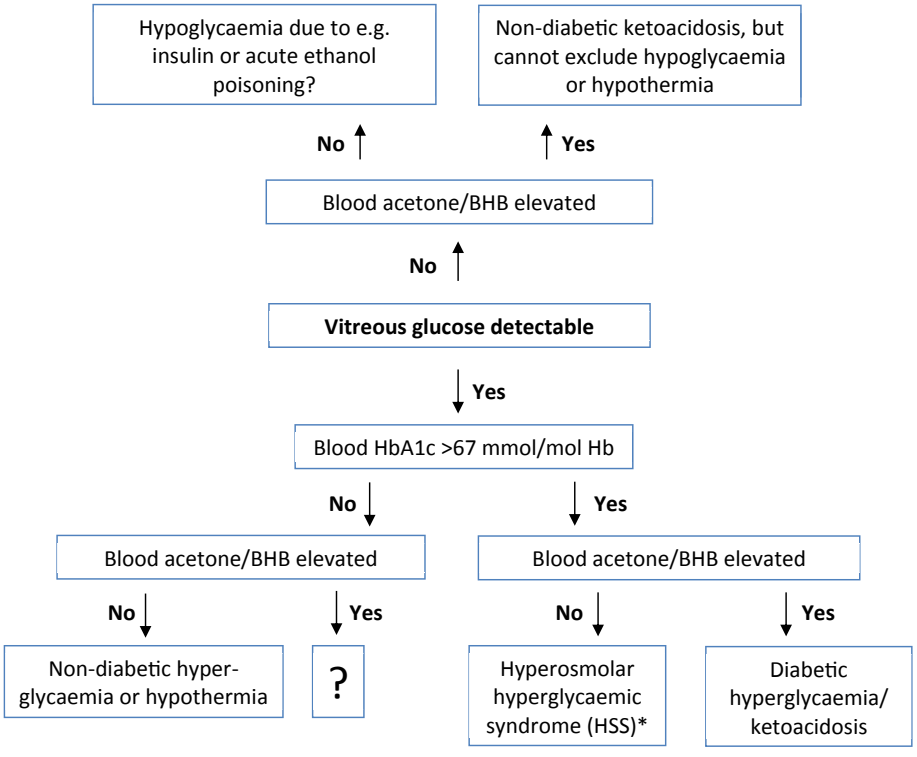

Fig. 1. Interpretation of raised vitreous humour glucose and blood β-hydroxybutyrate (BHB) concentrations.

endogenous anti-insulin antibodies. A two-fold variance in insulin measurements between different commercial immunoassays and between different laboratories using the same immunoassay has been reported.[77,78] Thus, interpretation of postmortem insulin:C-peptide results must always be performed with due caution.[73]

2.3. Drowning

Establishing drowning as a cause of death can be difficult. Evidence showing aspiration of the immersion medium and the subsequent mechanism of death should be forthcoming.[79] Lung weight and diatom analysis help assess the cause of death. However, the use of biochemical markers has been suggested as an additional parameter of study because of the lack of specific morphological findings associated with lung weight in drowning and contamination problems associated with diatom detection, e.g. water filtered through diatomaceous earth. Immunohistochemical measurement of increased pulmonary surfactant-associated protein A (SP-A) concentration has been used as another confirmatory test, but the test is non-specific with increased concentration noted in cases of acute myocardial infarction (AMI).[80] This lack of specificity limits the use of SP-A in diagnosis of drowning, but it remains a good marker of alveolar injury.[81–83]

Haemodilution, as suggested by lowered vitreous sodium concentrations, is likely in victims of fresh water drowning, but not in those drowned in salt water where raised vitreous sodium may be expected.[80,82] Thus concentrations of ethanol, drugs or other analytes measured in the blood of victims of fresh water drowning may be misleadingly low. Conversely, haemoconcentration is likely if a cadaver has been dehydrated, for example by heat or by mummification. In all such cases measurement of blood haemoglobin may give an estimate of the magnitude of haemodilution/concentration if it has not been degraded by heat or by prolonged storage.

It was suggested that haemodilution in fresh water drowning produces a lower chloride concentration in left heart blood as compared to right heart blood, while in salt water drowning haemoconcentration and chloride ion absorption was said to produce the opposite result. It was also suggested that high concentrations of magnesium in left heart blood compared to right heart blood may reflect magnesium absorption from salt water.[80] However, there is no clear consensus as to the predictive value of such measures in blood or in pericardial fluid, for example.[79]

More recently, trace element analysis, in particular measurement of strontium, has been proposed as an aid to diagnose drowning.[84] It has been postulated that transfer of strontium from water into blood causes raises the strontium concentration in left ventricular blood, which can either be interpreted in its own right, or as a ratio to the strontium concentration in right ventricular blood.[85] In the case of fresh water drowning, the concentration of strontium in the water must be higher than the plasma concentration for the investigation to be of diagnostic utility.[86] Measurement of chlorine and bromine in liquid taken from the sphenoid sinus has also been suggested as a means of distinguishing between fresh water and sea water drowning victims.[87]

2.4. Hypothermia/hyperthermia

Cold may be a significant factor in a death, but there are no specific biochemical markers that can be used to confirm a diagnosis of fatal hypothermia.[88] Diagnosis of death due to hypothermia is usually reliant on signs such as frost-erythema and Wischnewski's spots, which are present in approximately two thirds of cases.[89] Biochemical indications of antemortem cold stress may include raised urinary adrenaline and noradrenaline, and/or metanephrine (metadrenaline) concentrations.[4,90,91] Urinary catecholamine measurement has been recommended in preference to blood analysis because of postmortem catecholamine release from sympathetic nerve endings and the adrenal glands,[92] but catecholamine instability is a major issue as with clinical catecholamine measurement and of course urine may not be available postmortem. Vitreous humour glucose and ketones such as acetone may also be elevated, and in persistent hypothermia there may be electrolyte disturbances and metabolic acidosis.[93] A role for chromogranin A measurement in the postmortem diagnosis of hypothermia has been postulated because it is co-secreted with catecholamines.[9] The possible role of analytes such as adrenocorticotropic hormone and thyroid stimulating hormone have also been investigated, but have found limited use.[89,94]

Measurement of dopamine, adrenaline, and noradrenaline in postmortem blood has also been claimed to be suggestive of hyperthermia.[95] However, as with attempts to assess hypothermia, similar practical issues, not least the marked instability of catecholamines in biological samples, complicate attempts to assess hyperthermia from postmortem measurements. Fatal hyperthermia (heat stroke) often involves multiple organ dysfunction, and may include skeletal muscle damage without marked inflammatory responses.[95] Myoglobin has been suggested as a possible indicator of antemortem massive skeletal muscle damage, with concentrations > 330 and >1 mg/L observed in serum and in blood-contamination free urine, respectively, in fatal hyperthermia.[20] However, blood myoglobin may increase after death hence sampling should be within 48 h of death and of course the availability of serum postmortem and the instability of myoglobin in urine[96] are also issues. The isolated elevation of serum creatinine has likewise been suggested as a diagnostic marker for heat stroke,[97] but again is in the main impractical.

2.5. Inflammation

Blood CRP concentration peaks within 6 h of a stimulus and CRP is stable in postmortem samples. Liver is said to be a good alternative specimen if blood is not available,[98–100] but there are practical issues here in using standard clinical chemistry autoanalysers for tissue analysis. Interpretation of results can be difficult, however, as there are many causes of a raised blood CRP in addition to inflammation (Table 2).

2.6. Myocardial infarction

It may be difficult to diagnose AMI from anatomical and histological investigation alone, particularly if there was only a short survival period. The measurement of markers of heart muscle damage (e.g. myoglobin, creatine kinase, troponin) as an adjunct to searching for evidence of microscopic changes in samples of myocardium has thus been investigated.

Cardiac troponin I (cTnI) has high specificity as a marker for myocardial damage as it is found exclusively in cardiac muscle, unlike other markers such as myoglobin and the MB isoenzyme of creatine kinase (CK-MB). Measurement of cTnI in pericardial fluid and in serum obtained postmortem has shown that cTnI is significantly elevated in the pericardial fluid of individuals who have experienced AMI when compared to controls.[101] Cardiac troponin T (cTnT) is also used as a marker of cardiac injury and a raised cTnT concentration in postmortem blood may be associated with individuals who have suffered AMI and after death from electrocution owing to passage of an electric current through the heart damaging the myocardium.[102] Finally, measurement of cTnT and cTnI in CSF has been assessed and could be of use in investigating the progress and duration of myocardial damage,[103] but assay validation is an issue here since these assays are normally only validated in blood.

Table 5
Some biological samples required when investigating Sudden Unexpected Death in Infancy.[120]

Sample (volume)	Handling	Test
Blood or serum (1–2 mL)	Centrifuge, store serum at −20 °C	Toxicology
Urine (20 mL if possible)	Store −20 °C	Toxicology and specialised tests for inherited metabolic diseases
Blood from Guthrie card	Normal (ensure circle is filled). Do not put in plastic bag	Tests for inherited metabolic diseases
Skin biopsy	After discussion with paediatrician	Tests for inherited metabolic disease, e.g. fibroblast enzyme activity
Muscle biopsy	After discussion with paediatrician	If history suggests mitochondrial disorder

CK-MB activity has been said to be independent of the morphological severity of myocardial damage and not to be raised significantly after acute or recurrent myocardial infarction,[22] although this assay is no longer widely available after being superseded by other markers of myocardial damage. Elevated CK-MB activity has been reported after asphyxia, carbon monoxide poisoning, and metamfetamine abuse. CK-MB may be used as a marker of persistent hypoxic myocardial damage before death, whereas cTnT and cTnI are said to be more useful in assessing the progress and duration of myocardial damage.[104]

2.7. Time of death estimation

Attempts to employ the rate of rise of vitreous humour potassium in order to calculate the time of death have largely been abandoned because of the inherent uncertainty of this method even when vitreous humour hypoxanthine and urea are also analysed.[105–107] Use of CSF[98] and of synovial fluid[108] as alternatives to vitreous humour for potassium measurement have also been suggested, but to no avail. There are reports of immunochemical detection of glucagon in pancreatic cells and of calcitonin in thyroid c-cells being used to indicate time of death, but these methods are experimental at best.[109,110] The rate of loss of albumin from CSF has also been suggested for use in time of death estimation,[111] but this approach would seem impractical.

2.8. Sepsis

Sepsis is complex condition that is often difficult to diagnose and treat, reflecting in part the lack of universally-applicable biomarkers for early diagnosis.[112] After death the clinical history and analysis of any specimens obtained in life is of course important in establishing the diagnosis. However, such information may be either incomplete, or unavailable. In such cases serial monitoring of serum C-reactive protein (CRP), procalcitonin, interleukin-6 (IL-6), interleukin-1β, soluble interleukin-2 receptor (sIL-2R), and lipopolysaccharide binding protein in the hours after death has been suggested as an aid to the postmortem diagnosis of sepsis, but has not been widely adopted.[113,114] Procalcitonin has a longer plasma half-life (25–30 h) and is more stable when compared to pro-inflammatory cytokines.[115] Recent preliminary studies have suggested that endocan (endothelial cell-specific molecule-1) and soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) in serum derived postmortem from femoral blood and in pericardial and pleural fluid could be used in conjunction with procalcitonin, CRP, and sIL-2R as additional sepsis markers.[116,117]

2.9. Sudden unexplained/unexpected death

Sudden Unexpected Death in Infancy [SUDI, also known as Sudden Infant Death Syndrome (SIDS)], Sudden Unexplained Death in Epilepsy (SUDEP), and Sudden Arrhythmic Death Syndrome (SADS, also known as Sudden Adult Death Syndrome) are all recognised occurrences. In SUDI, an acute manifestation of an inborn error of metabolism, particularly a fatty acid oxidation defect, has to be excluded. Guidance on biological samples required when investigating such deaths is given in Table 5. Markers of inflammatory response, including CRP, IL-6, and intercellular adhesion molecule-1 (ICAM-1) may be elevated in certain tissues after infections.[118]

SADS cases are thought likely often to have a cardiac origin such as a fatal arrhythmia,[119] but a full toxicological analysis is required to exclude recent use of not only illicit drugs such as cocaine and other stimulants, but also drugs given in therapy, for example antipsychotics and antidepressants, that may increase the risk of a fatal arrhythmia. Even if this is done, however, it is often impossible to state a precise cause of death with any degree of certainty.

SUDEP is the leading cause of seizure-related mortality in people with epilepsy,[121] but is not readily diagnosable except by a process of elimination. Factors may be non-adherence with anticonvulsant therapy (antiepileptic drugs, AEDs) or co-prescription of drugs such as tricyclic antidepressants and clozapine that may lower the seizure threshold. It has been suggested that plasma prolactin could indicate recent seizure activity/status epilepticus, but it has been found that this is not a reliable diagnostic marker.[122]

3. Conclusions

Biochemical analyses using primarily postmortem blood (or serum if available) and vitreous humour are now an accepted part of the investigation of possible alcohol- and diabetes-related deaths, and deaths that may have resulted from anaphylactic shock, from drowning, and from hypothermia. In large part this is because not only is vitreous humour less affected by postmortem changes than blood, but also the sample obtained is amenable to analysis using standard clinical chemistry methods designed for use with plasma or serum. Nevertheless many problems remain, such as assessing the role of exogenous potassium or insulin in a death, and estimating the time of death simply from postmortem changes in body biochemistry. For additional tests to gain widespread acceptance not only will there need to be extensive research and evaluation of the proposed test, but also the necessary methodology will need to be readily usable in clinical and/or forensic laboratories.

Conflict of interest
None declared.

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