

Usefulness of postmortem biochemistry in identification of ketosis : Diagnosis of ketoacidosis at the onset of autoimmune type 1 diabetes in an autopsy case with cold exposure and malnutrition

メタデータ	言語: English 出版者: Elsevier 公開日: 2018-11-01 キーワード (Ja): ケトン体, グルコース キーワード (En): Autoimmune type 1 diabetes, Postmortem biochemistry, Immunohistochemistry, Ketone body, Glucose 作成者: 谷, 直人, 道上, 知美, Chen, Jian-Hua, 織谷, 茂樹, 石川, 隆紀 メールアドレス: 所属: Osaka City University, Osaka City University, Osaka City University, Osaka City University, Osaka City University
URL	https://ocu-omu.repo.nii.ac.jp/records/2020053

Usefulness of postmortem biochemistry in identification of ketosis: Diagnosis of ketoacidosis at the onset of autoimmune type 1 diabetes in an autopsy case with cold exposure and malnutrition

Naoto Tani, Tomomi Michiue, Jian-Hua Chen, Shigeki Oritani,
Takaki Ishikawa

Citation	Legal Medicine, 22; 23-29
Issue Date	2016-09
Type	Journal Article
Textversion	Author
Rights	© 2016 Elsevier Ireland Ltd. This manuscript version is made available under the CC-BY-NC-ND 4.0 License. http://creativecommons.org/licenses/by-nc-nd/4.0/ . The article has been published in final form at https://doi.org/10.1016/j.legalmed.2016.07.006
DOI	10.1016/j.legalmed.2016.07.006

Self-Archiving by Author(s)
Placed on: Osaka City University

Usefulness of postmortem biochemistry in identification of ketosis: diagnosis of ketoacidosis at the onset of autoimmune type 1 diabetes in an autopsy case with cold exposure and malnutrition

Naoto Tani^{1,2}, Tomomi Michiue^{1,2}, Jian-Hua Chen^{1,2}, Shigeki Oritani¹,
Takaki Ishikawa^{1,2}

¹ Department of Legal Medicine, Osaka City University Medical School, Asahi-machi
1-4-3, Abeno, 545-8585 Osaka, Japan

² Forensic Autopsy Section, Medico-legal Consultation and Postmortem Investigation
Support Center, c/o Department of Legal Medicine, Osaka City University Medical
School, Asahi-machi 1-4-3, Abeno, 545-8585 Osaka, Japan

Corresponding author: Takaki Ishikawa

Department of Legal Medicine, Osaka City University Medical School, Asahi-machi
1-4-3, Abeno, 545-8585 Osaka, Japan

Tel.: 81-6-6645-3765; fax: 81-6-6634-3871

E-mail address: takaki@med.osaka-cu.ac.jp

Abstract

A severely malnourished, Japanese female in her twenties was found dead in her apartment. On autopsy, most of the findings from the internal examination were suggestive of hypothermia. Postmortem biochemistry, however, showed severely increased levels of glycated hemoglobin (HbA1c) and blood and urine glucose levels. Levels of acetone, 3-hydroxybutyric acid, and acetoacetate in various body fluids were also highly increased, indicating ketosis. The serum insulin and c-peptide levels were severely low, and subsequent testing was positive for anti-GAD antibodies. Immunohistochemical examination of the pancreatic islet cells revealed few insulin-positive cells but many glucagon-positive cells on staining. Furthermore, slight invasion of CD8-positive lymphocytes in the pancreatic islets of Langerhans was observed. Results of immunostaining of the pancreatic and bronchial epithelial tissues were partly positive for the Influenza A virus. We concluded that severe ketoacidosis associated with rapid-onset hyperglycemia due to autoimmune type 1 diabetes (AT1D) had occurred shortly before death. However, the ketosis was accompanied by hypothermia and malnutrition as well as diabetic ketoacidosis (DKA). Therefore, we retrospectively collected biochemical data on cases of hypothermia and malnutrition and compared them with the present case. Serum glucose, acetone, 3-hydroxybutyric acid, and acetoacetic acid can be used for screening and diagnosis to distinguish DKA from ketosis due to hypothermia and malnutrition. Therefore, in the present case, we diagnosed that the natural cause of death was due to AT1D. In conclusion, screening investigations for relevant biochemical markers can provide essential information for the diagnosis of metabolic disturbances, which fail to demonstrate characteristic autopsy findings.

Keywords: Autoimmune type 1 diabetes, Postmortem biochemistry, Immunohistochemistry, Ketone body, Glucose

Highlights

- Usefulness of postmortem biochemistry in the forensic diagnosis of the cause of death.
- We reported a case of autoimmune type 1 diabetes accompanied with by cold exposure and malnutrition.
- Importance of differentiating diseases associated with ketosis.
- Levels of blood glucose, acetone, total ketone bodies, etc. were used for the diagnosis of diabetic ketoacidosis.
- CRP, BUN, and creatinine were not useful for the pathological differentiation of ketosis.

1. Introduction

Diabetes mellitus (DM) may cause severe metabolic disturbances, which that can be fatal. These can be difficult to diagnose based on macroscopic and histological findings alone at autopsy [1-3]. In such cases, postmortem biochemical markers can provide supporting information [1]. When investigating on metabolic and endocrin disorders, a number of postmortem biochemical laboratory procedures have been reported to be useful for autopsy diagnoses [4-6]. Particularly in the postmortem diagnosis of DM, a common metabolic disease, the main evidence for the diagnosis can be derived from postmortem biochemical parameters [7-9].

Diabetic ketoacidosis (DKA) at the onset of autoimmune type 1 diabetes (AT1D) is a complication of DM characterized by extreme and rapid progression of hyperglycemia and ketoacidosis due to the destruction of the pancreatic β cells [10,11]. The clinical diagnosis of DKA at the onset of AT1D can be made when the following biochemical parameters are found: (1) elevation of urinary and/or serum ketone bodies; (2) high plasma and urinary glucose levels (>250 mg/dL and >100 mg/dL, respectively); (3) an anion gap of >12 mEq/L and arterial blood pH of <7.3; and (4) elevation of plasma blood urea nitrogen (BUN) and creatinine levels [10]. Patients diagnosed with DKA at the onset of AT1D are likely to die within 24 h of the onset of hyperglycemia unless they receive immediate treatment for DKA [10]. In forensics, DKA at the onset of AT1D has become more widely recognized as a cause of sudden death [2, 12]. However, methods for the postmortem diagnosis of DKA at the onset of AT1D have not yet been fully established because there are no characteristic anatomical findings.

Here, we report on a postmortem biochemical diagnosis of DKA at the onset of AT1D, which was made in spite of considerable autopsy findings that were suggestive of hypothermia as being the cause of death. We compared these findings to postmortem cases of other ketotic conditions, namely hypothermia and malnutrition. We also considered whether it was possible to use the postmortem biochemical profile for DKA diagnosis and compare it to other ketotic conditions, such as hypothermia and malnutrition.

2. Case report

2.1. Case history

A Japanese female in her twenties was found dead in her apartment bathroom by her boyfriend at 7:30 a.m. in early February morning.

Five days earlier, she had had a bicycle accident and received a blow to the right inguinal region, after which she experienced nausea and general malaise. She was absent from work for the following 3 days. On the 4th day, she was found dead in her apartment bathroom. She was nude from the waist down and had fallen while taking a cold-water shower. A gas safety device was present, so that hot water would not flow out of the shower and its temperature reduced. The gas safety device seems to work in around five hours. According to her colleagues, she had reported losing weight approximately 2 months prior to her death. However, she did not consult a doctor and therefore she was not evaluated for diabetes including the serum glucose and HbA1c levels. In addition, her medical history was unremarkable.

Her room did not have a heater except for a foot-warmer frame (known as kotatsu in Japan). Her rectal temperature at 4:30 p.m. on the day she was found was 13°C, and the room temperature was 10.5°C.

A forensic autopsy was performed approximately 1 day after death.

2.2. *Postmortem imaging*

Post-mortem computed tomography (CT) showed no subcutaneous or visceral fat areas (Fig. 1a and b), and the lung fields showed the presence of pulmonary emphysema (Fig. 2a and b) [13].

2.3. *Autopsy findings*

The subject's height was 156 cm, and her weight was 37.3 kg [body mass index (BMI) 15.3]. She was pale with a dark, reddish-purple hypostasis on the back. A large liquefied obsolete subcutaneous hematoma (5 × 8 cm) was found in the right inguinal region from the bicycle accident. The heart weighed 205 g, with a striking difference between the color of the blood bilaterally (bright red in the left side of the heart and dark red in the right side of the heart) (Fig. 3a). The blood in the heart contained a small number of soft lard-like clots. Both lungs were considerably emphysematous and had a cherry-red color (Fig. 3b). The left lung weighed 195 g, and the right lung weighed 205 g. The pancreas weighed 60 g, and there was no evidence of fatty degeneration or hemorrhage; however, mild edema was present. The liver weighed 1,355 g, had a smooth surface, and was congested. The gastric mucosa contained a few dark-brownish punctate hemorrhages and erosions. The laryngopharynx mucosa contained congestion and edema; however, there was no enlargement of the lymph nodes. The brain weighed 1,250 g. The right kidney weighed 90 g, and the left kidney weighed 150 g. Significant macroscopic changes were not observed in either kidney. The urinary bladder contained

100 mL of urine. There was no evidence of other pathology or trauma.

2.4. Histopathological findings

Examination of the pancreas revealed mild fatty degeneration and lymphocyte invasion of the islets of Langerhans (yellow arrow head in Fig. 4a). Diffuse fatty changes in the renal tubules and scattered mild fatty changes were also observed in the liver cells [2, 14, 15]. In the present case, no signs of viral or bacterial pneumonia were observed in the lung tissue.

Immunohistochemical examination of the lymphocytes in the inflammatory sites of the pancreatic islets of Langerhans and the submandibular gland revealed positivity for the cytotoxic T-cell marker CD8 (red arrow head in Fig. 4b). T-cell helper (CD4)-positive lymphocytes were not found. Using anti-glucagon and anti-insulin antibodies, the pancreas revealed a paucity of the islets of Langerhans, and few insulin-positive cells were observed (Fig. 4c); however, abundant glucagon-positive cells were seen on staining (Fig. 4d). Immunostaining of the lung and pancreatic tissues was performed using anti-Influenza A (abcam, Japan) and B (Takara Bio Inc, Japan), Adeno (Novus, USA), Respiratory syncytial (RS) (abcam, Japan), and Cytomegalo (Dako, Denmark) virus. Influenza A virus was the sole partly immuno-positive reaction in the pancreatic cells, bronchial epithelial cells, and bronchiolar macrophages (Fig. 5 a, b).

2.5. Biochemical findings

There was a mild elevation in serum C-reactive protein (CRP: 1.19 mg/ml); however, this was lower than the standard forensic cut-off level [16, 17]. The serum neopterin level (15 pmol/ml), which is a systemic inflammatory marker, was within the standard forensic level [17]. In the blood of the right heart, comparisons of the concentrations of acetone, 3-hydroxybutyric acid and acetoacetate as total ketone bodies were made between the present DKA case and cases of hypothermia and malnutrition, as summarized in Fig. 6 and Table 1. The urine dipstick was positive for ketone bodies (approximately 5 mg/dl) and sugar was +3 (approximately 600 mg/dl). The cardiac blood glucose and HbA1c levels were 597 mg/dl and 16.4%, respectively. Glucose and lactate in the right and left vitreous humor showed a high concentration (left: 481.0 mg/dl and 83.80 mg/dl; right: 496.0 mg/dl and 88.6 mg/dl, respectively) in comparison with forensic references [18-21]. The plasma insulin level was 0.61 μ IU/ml, and the plasma c-peptide level was severely low (<0.3 ng/ml). Plasma concentrations of autoantibodies associated with the pancreatic islets of Langerhans, specifically anti-GAD antibody, anti-IA2 antibody, and anti-insulin antibody were 19.9 U/mL

(normal range <1.5 U/mL), 0.4 U/mL (normal range <0.4 U/mL), and <125 nU/mL (normal range <125 nU/mL), respectively. The GH level (32 ng/ml) fell within the standard forensic level [22], and the immunoglobulin (Ig) G level (661 mg/ml) was slightly lower than the clinical standard level.

Retrospective data was collected on 11 hypothermia and 8 malnutrition forensic autopsy cases from our department, and they were compared with the present case in which glucose, CRP, blood urea nitrogen (BUN), creatinine, and acetone, acetoacetic acid, and 3-hydroxybutyric acid as total ketone bodies were analyzed during the process of defining the cause of death. In the present case, glucose, acetoacetic acid, 3-hydroxybutyric acid, acetone, and total ketone body levels were all extremely elevated compared with the cases of hypothermia and malnutrition (Fig. 6 and Table 1) [23-26].

2.6. Blood gas analysis

Postmortem left and right blood gases revealed oxygen partial pressures (P_{O2}) of 93.9% and 41.4%, respectively. It is thought that she was placed under low temperature exposure as for this result [27]

2.7. Postmortem microbiology

The mitochondrial (mt) DNA 3243 mutation [28] was not detected in the whole blood.

2.8. Toxicology findings

No blood alcohol was detected in several body fluids. Drug screening, including blood screening for amphetamines and psychotropic drugs using immunoassay and gas chromatography-mass spectrometry (GC-MS), was negative [29].

3. Discussion

In the present case, most of the macropathological findings on internal examination suggested possible hypothermia (cold exposure) as the cause of death. However, according to the postmortem biochemistry, postmortem HbA1c levels were markedly increased and the glucose levels in the blood of the right heart and urine glucose levels were extremely elevated. HbA1c levels have been reported to stabilize within 72 h postmortem [30], while blood glucose concentrations have been reported to decrease after death and should not be underestimated [7]. HbA1c is formed when glucose is non-enzymatically added to hemoglobin. The proportion of HbA1c compared with total

hemoglobin reflects the mean blood glucose level for the previous 1–2 months, which is the lifespan of red blood cells. However, in a postmortem context, HbA1c levels are useful for distinguishing diabetic ketoacidosis (DKA) from alcoholic ketoacidosis (AKA) and for revealing undiagnosed or poorly managed diabetes [31-33].

Furthermore, in the present case, glucose and lactate in both sides of the vitreous humor showed a high concentration in comparison with forensic references. Karlovsek [19, 20] compared several biochemical parameters (glycated hemoglobin, glucose, lactate, and combined glucose) in vitreous and cerebrospinal fluids in 112 forensic cases divided into two diagnostic groups. The author proposed that vitreous glucose levels greater than 13 mmol/l (corresponding to 234 mg/dl) or combined glucose and lactate values in vitreous or cerebrospinal fluid greater than the threshold values of 23.7 mmol/l (427 mg/dl) and 23.4 mmol/l (422 mg/dl), respectively, could indicate antemortem hyperglycemia with a fatal outcome. Therefore, in the present case, taken together, the Hb1Ac and glucose levels suggested that hyperglycemia occurred rapidly and shortly before death [23].

We retrospectively collected data on 11 hypothermia (cold exposure) and 8 malnutrition forensic autopsy cases from our department and compared them with the data on the present case, in which the levels of glucose, acetone, total ketone bodies, 3-hydroxybutyric acid, acetoacetic acid, BUN, creatinine, and CRP were assessed for the determination of the cause of death. Our analysis suggests that glucose, acetone, total ketone bodies, 3-hydroxybutyric acid, and acetoacetic acid may be useful for the screening and diagnosis of diabetic ketoacidosis and distinguishing it from ketosis caused by hypothermia and malnutrition. BUN, creatinine, and CRP are not ideal markers of DKA because these are elevated only in the most severe cases of hypothermia and malnutrition; however, they and other metabolic markers such as HbA1c can aid a forensic pathologist in the diagnosis of diabetes-related death [34]. An increase in blood glucose levels has been observed in hypothermia cases, likely caused by the enhanced secretion of adrenaline and corticosterone [35]. An elevation of BUN, creatinine and CRP was also associated with gastrointestinal bleeding, pneumonia, and fatal hypothermia, in which known dehydration/hemoconcentration and elevated protein catabolism may have caused the elevation [36-39]. In the present case, BUN, creatinine, and CRP of biochemical markers were belonged to a category of hypothermia and malnutrition due to protein catabolism, respectively. Therefore, based on previous reports [8, 40, 41], the subject must have been in a state of severe ketoacidosis due to hyperglycemia at the time of death.

Subsequent testing was positive for anti-GAD antibodies in the serum; however, anti-IA2 and anti-insulin antibodies were not detected. Furthermore, mtDNA 3243 mutations were not detected. Among Japanese patients with diabetes, mtDNA 3243 mutations are related to the development of diabetes, and these mutations are associated with not only a decrease in insulin secretion but with also advanced diabetic microvascular complications [42]. Although mtDNA 3243 was negative in this case, this is not unexpected because the incidence of mtDNA3243 mutation is very low, at approximately 0.5-2.8 % [43]. From these postmortem biochemical findings, we concluded that severe ketoacidosis associated with a rapid onset of hyperglycemia occurred shortly before death due to autoimmune type 1 diabetes mellitus [10]. Although the survival period in this case was short, fulminant type 1 diabetes mellitus was not diagnosed because the findings did not correspond with the diagnostic criteria for fulminant type 1 diabetes mellitus (such as >8.7% HbA1c and positive anti-GAD antibodies) [44].

In addition, in the present case, CD8-positive T-cells were observed in the pancreas and submandibular gland. In type 1 diabetes mellitus, it is assumed that CD8-positive T-cells in the pancreas primarily contribute to selective pancreatic β -cell destruction [45, 46]. Only a few previous reports have suggested that immunostaining of the pancreas can be useful in forensic autopsy cases in which CD8-positive lymphocytes suggest viral infection [47]. In the present case, immunopositivity to Influenza A virus was observed in the pancreatic and bronchial epithelial cells and bronchiolar macrophages, although there were no clear signs of viral pneumonia. Therefore, we assume that the Influenza A virus infection had induced the development of diabetic ketoacidosis.

With respect to the blood biochemistry results in the present case, a high HbA1c level, and high levels of serum glucose, acetone, total ketone body, 3-hydroxybutyric acid, and acetoacetic acid may be useful in distinguishing DKA from the ketosis associated with hypothermia and malnutrition cases. In addition, diabetes-related autoantibody was positive. It did not contradict it for the immunohistochemistry in DKA due to AT1D either. On the basis of our analyses, we concluded that in the present case, DKA due to AT1D was the cause of death and that cold exposure and malnutrition contributed to the death process; the and manner of death was regarded as natural death. These conclusions are supported by both the autopsy findings and the biochemical results.

The present case can be considered to be a strong example not only of the usefulness of postmortem biochemical investigations but also of the importance of performing these analyses to determine the manner of death.

Conflict of interest statement

The authors declare that they have no proprietary, financial, professional, or other personal interest of any kind in any product, service, and/or company that could be construed as influencing this current manuscript entitled “Usefulness of postmortem biochemistry in forensic autopsy: diagnosis of ketoacidosis at the onset of autoimmune type 1 diabetes in an autopsy case with cold exposure and malnutrition.”

References

- [1] C. Palmiere, M. Lesta Mdel, S. Sabatasso, P. Mangin, M. Augsburger, F. Sporkert, Usefulness of postmortem biochemistry in forensic pathology: illustrative case reports, *Leg. Med. (Tokyo)* 14 (2012) 27-35.
- [2] T. Mizutani, T. Yoshimoto, R. Kaneko, A. Ishii, Diagnosis of fulminant type 1 diabetes mellitus in an autopsy case with postmortem changes, *Leg. Med. (Tokyo)* 13 (2011) 250-3.
- [3] B. Zilg, K. Alkass, S. Berg, H. Druid, Postmortem identification of hyperglycemia, *Forensic Sci. Int.* 185 (2009) 89-95.
- [4] C. Palmiere, M. Lesta Mdel, S. Sabatasso, P. Mangin, M. Augsburger, F. Sporkert, Usefulness of postmortem biochemistry in forensic pathology: illustrative case reports, *Leg. Med. (Tokyo)* 14 (2012) 27-35.
- [5] C. Palmiere, P. Mangin, Postmortem chemistry update part I, *Int. J. Leg. Med.* 126 (2012) 187-98.
- [6] S.A. Blana, F. Musshoff, T. Hoeller, R. Fimmers, B. Madea, Variations in vitreous humor chemical values as a result of pre-analytical treatment, *Forensic Sci. Int.* 210 (2011) 263-70.
- [7] C. Hess, F. Musshoff, B. Madea, Disorders of glucose metabolism-post mortem analyses in forensic cases: part 1, *Int. J. Leg. Med.* 125 (2011) 163-70.

- [8] C. Hess, F. Musshoff, B. Madea, Disorders of glucose metabolism-postmortem analyses in forensic cases: part2, *Int. J. Leg. Med.* 125 (2011) 171-80.
- [9] B. Zilg, K. Alkass, S. Berg, H. Druid, Postmortem identification of hyperglycemia, *Forensic Sci. Int.* 185 (2009) 89-95.
- [10] T. Hanafusa, A. Imagawa, Fulminant type 1 diabetes: a novel clinical entity requiring special attention by all medical practitioners, *Nat Clin Pract Endocrinol Metab.* 3 (2007) 36-45.
- [11] J. Lu, J. Zhou, Y. Bao, T. Chen, Y. Zhang, A. Zhao, Y. Qiu, G. Xie, C. Wang, W. Jia, W. Jia, Serum metabolic signatures of fulminant type 1 diabetes, *J. Proteome. Res.* 11 (2012) 4705-11.
- [12] S. Goto, H. Abiru, M. Iino, K. Tamaki, An autopsy of fulminant type 1 diabetes mellitus, *Jpn. J. Legal Med.* 59 (2005) 201 [in Japanese].
- [13] N. Sogawa, T. Michiue, T. Ishikawa, O. Kawamoto, S. Oritani, Maeda H, postmortem volumetric CT data analysis of pulmonary air/gas content with regard to the cause of death for investigating terminal respiratory function in forensic autopsy, *Forensic Sci. Int.* 241 (2014) 112-7.
- [14] C. Zhou, L. Moore, A. Yool, A. Jaunzems, R.W. Byard, Renal tubular epithelial vacuoles-a marker for both hyperlipidemia and ketoacidosis at autopsy, *J. Forensic Sci.* 60 (2015) 638-41.
- [15] S. Kodikara, P. Paranitharan, M.S. Pollanen, The role of the Armanni-Ebstein lesion, hepatic steatosis, biochemical analysis and second generation anti-psychotic drugs in fatal diabetic ketoacidosis, *J. Forensic. Leg. Med. (Tokyo)* 20 (2013) 108-11.
- [16] M.Q. Fujita, B.L. Zhu, K. Ishida, L. Quan, S. Oritani, H. Maeda, Serum C-reactive protein levels in postmortem blood--an analysis with special reference to the cause of death and survival time, *Forensic Sci. Int.* 130 (2002) 160-6.
- [17] T. Ishikawa, M. Hamel, B.L. Zhu, D.R. Li, D. Zhao, T. Michiue, H. Maeda, Comparative evaluation of postmortem serum concentrations of neopterin and

C-reactive protein, *Forensic Sci. Int.* 179 (2008) 135-43.

[18] C. Palmiere, P. Mangin, Postmortem chemistry update part I, *Int. J. Legal. Med.* 126 (2012) 187-98.

[19] M.Z. Karlovsek, Diagnostic values of combined glucose and lactate values in cerebrospinal fluid and vitreous humour-our experiences, *Forensic. Sci. Int.* 146 (2004) S19-23.

[20] B. Jacob, W. Bonte, *Advances in Forensic Sciences: Forensic Criminalistic 2.*, Verlag Dr Kösrner, Berlin 1995.

[21] V.J. DiMaio VJ., D.D. DiMaio, *Forensic Pathology*, Second ed., CRC Press, New York, 2001.

[22] T. Ishikawa, T. Michiue, H. Maeda, Evaluation of postmortem serum and cerebrospinal fluid growth hormone levels in relation to the cause of death in forensic autopsy, *Hum Cell.* 24 (2011) 74-7.

[23] J.H. Chen, T. Michiue, O. Inamori-Kawamoto, S. Ikeda, T. Ishikawa H. Maeda, Comprehensive investigation of postmortem glucose levels in blood and body fluids with regard to the cause of death in forensic autopsy cases, *Leg. Med. (Tokyo)* 17 (2015) 475-82.

[24] M. Tominaga, T. Ishikawa, T. Michiue, S. Oritani, I. Koide, Y. Kuramoto, M. Ogawa, H. Maeda, Postmortem analyses of gaseous and volatile substances in pericardial fluid and bone marrow aspirate, *J. Anal. Toxicol.* 37 (2013) 147-51.

[25] B.L. Zhu, K. Ishida, L. Quan, M. Taniguchi, S. Oritani, D.R. Li, M.Q. Fujita, H. Maeda, Postmortem serum uric acid and creatinine levels in relation to the causes of death, *Forensic. Sci. Int.* 125 (2002) 59-66.

[26] T. Ishikawa, M. Hamel, B.L. Zhu, D.R. Li, D. Zhao, T. Michiue, H. Maeda, Comparative evaluation of postmortem serum concentrations of neopterin and C-reactive protein, *Forensic. Sci. Int.* 179 (2008) 135-43.

- [27] K. Shimizu, H. Mizukami, T. Fukushima, M. Sasaki, H. Shiono, Use of a CO-oximeter for forensic diagnosis of hypothermia, *Nihon. Hoigaku. Zasshi.* 52 (1998) 196-201. (Japanese)
- [28] M. Fukuda, S. Nakano, N. Imaizumi, M. Kitazawa, M. Nishizawa, T. Kigoshi, K. Uchida, Mitochondrial DNA mutations are associated with both decreased insulin secretion and advanced microvascular complications in Japanese diabetic subjects, *J. Diabetes. Complications.* 13 (1999) 277-83.
- [29] M. Tominaga, T. Michiue, T. Ishikawa, O. Inamori-Kawamoto, S. Oritani, H. Maeda, Evaluation of postmortem drug concentrations in cerebrospinal fluid compared with blood and pericardial fluid, *Forensic Sci. Int.* 254 (2015) 118-25.
- [30] K. Uemura, K. Shintani-Ishida, K. Saka, M. Nakajima, H. Ikegaya, Y. Kikuchi, K. Yoshida, Biochemical blood markers and sampling sites in forensic autopsy, *J. Forensic. Leg. Med.* 15 (2008) 312-7.
- [31] J. Hockenhull, W. Dhillon, R. Andrews, S. Paterson, Investigation of markers to indicate and distinguish death due to alcoholic ketoacidosis, diabetic ketoacidosis and hyperosmolar hyperglycemic state using post-mortem samples, *Forensic. Sci. Int.* 214 (2012) 142-7.
- [32] C. Palmiere, D. Bardy, P. Mangin, D. Werner, Postmortem diagnosis of unsuspected diabetes mellitus, *Forensic. Sci. Int.* 226 (2013) 160-7.
- [33] T. Keltanen, A. Sajantila, T. Valonen, T. Vanhala, K. Lindroos, Measuring postmortem glycated hemoglobin-A comparison of three methods, *Leg. Med. (Tokyo)* 15 (2013) 72-8.
- [34] Keltanen, A. Sajantila, T. Valonen, T. Vanhala, K. Lindroos. Measuring postmortem glycated hemoglobin – A comparison of three methods, *Leg. Med. (Tokyo)* 15 (2013) 72-8.
- [35] C. Palmiere, P. Mangin, Postmortem biochemical investigations in hypothermia fatalities. *Int. J. Legal. Med.* 127 (2013) 267-31.

- [36] D.F. Danzl, J.R. Hedges, R.S. Pozos, Hypothermia outcome score: development and implications, *Crit. Care. Med.* 17 (1989) 227-31.
- [37] R.H. Danzl, W.W. Lawrence, *Surgical diagnosis and treatment*, nine ed., Appleton and Lange, CT, 1991.
- [38] B.L. Zhu, K. Ishida, L. Quan, M. Taniguchi, S. Oritani, D.R. Li, M.Q. Fujita, H. Maeda, Postmortem serum uric acid and creatinine levels in relation to the causes of death, *Forensic. Sci. Int.* 125 (2002) 59-66.
- [39] H. Maeda, B.L. Zhu, Bessho, T. Ishikawa, L. Quan, T. Michiue, D. Zhao, D.R. Li, A. Komatsu, Postmortem serum nitrogen compounds and C-reactive protein levels with special regard to investigation of fatal hyperthermia, *Forensic. Sci. Med. Pathol.* 4 (2008) 175-80.
- [40] J. Kanetake, Y. Kanawaku, S. Mimasaka, J. Sakai, M. Hashiyada, M. Nata, M. Funayama, The relationship of a high level of serum beta-hydroxybutyrate to cause of death, *Leg. Med. (Tokyo)* 7 (2005) 169-74.
- [41] E. Osuna, G. Vivero, J. Conejero, J.M. Abenza, P. Martínez, A. Luna, M.D. Pérez-Cárceles, Postmortem vitreous humor beta-hydroxybutyrate: its utility for the postmortem interpretation of diabetes mellitus, *Forensic Sci. Int.* 153 (2005) 189-95.
- [42] M. Inada, M. Oishi, M. Nishikawa, S. Kurata, H. Imura, Clinical evaluation of measuring glycosylated hemoglobin levels for assessing the long-term blood glucose control in diabetics, *Endocrinol. Jpn.* 27 (1980) 411-5.
- [43] S. Suzuki, Y. Okada, T. Kadowaki, A. Kanatsuka, T. Kuzuya, M. Kobayashi, T. Sanke, Y. Seino, K. Nanjo, Research committee on specific types of diabetes mellitus with gene mutations of the Japan diabetes society. Clinical features of diabetes mellitus with the mitochondrial DNA 3243 (A-G) mutation in Japanese: maternal inheritance and mitochondria-related complications, *Diabetes. Res. Clin. Pract.* 59 (2003) 207-11.
- [44] S. Shibasaki, A. Imagawa, T. Hanafusa, Fulminant type 1 diabetes mellitus: a new class of type 1 diabetes, *Adv. Exp. Med. Biol.* 771 (2012) 20-3.

[45] M. Nagata, H. Moriyama, R. Kotani, H. Yasuda, M. Kishi, M. Kurohara, K. Hara, K. Yokono, Immunological aspects of 'fulminant type 1 diabetes', *Diabetes. Res. Clin. Pract.* 77 (2007) S99-103.

[46] T. Hanafusa, A. Imagawa, Insulinitis in human type 1 diabetes. *Ann. NY. Acad. Sci.* 1150 (2008) 297-9.

[47] R.S. Fujinami, M.B. Oldstone, Z. Wroblewska, M.E. Frankel, H. Koprowski, Molecular mimicry in virus infection: crossreaction of measles virus phosphoprotein or of herpes simplex virus protein with human intermediate filaments, *Proc. Natl. Acad. Sci. U S A.* 80 (1983) 2346-50.

Figure legends

Fig. 1 Postmortem CT morphology of the abdominal sections: renal level (a) and umbilical level (b) using fat Pointer® (Hitachi Medical Co., Ltd., Tokyo, Japan). The red and blue parts show visceral fat and subcutaneous fat, respectively [distribution of CT attenuation (HU) values in the fat tissue: from -50 to -201 HU]. Renal level: visceral fat area, 1.2 cm^2 ; subcutaneous fat area, 0.0 cm^2 , Umbilical level: visceral fat area, 1.8 cm^2 ; subcutaneous fat area, 0.1 cm^2 .

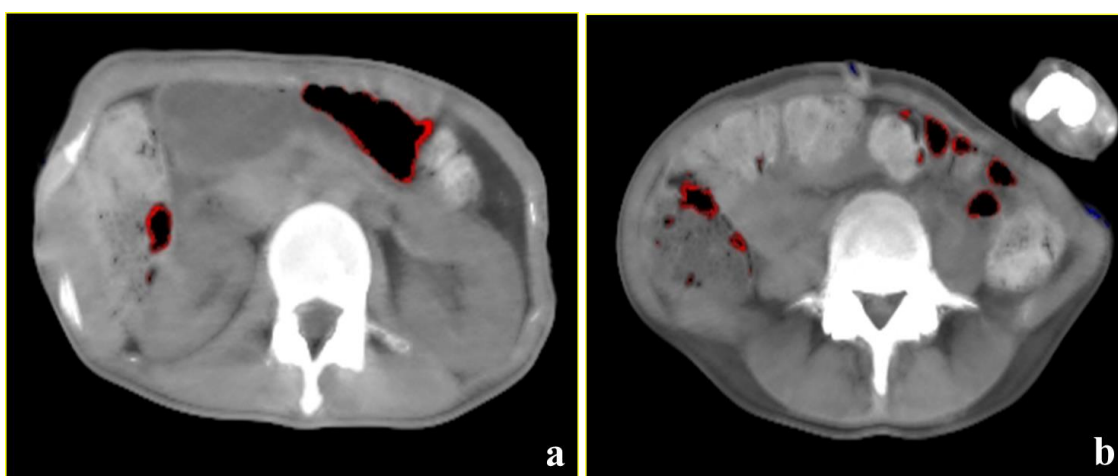


Fig. 2 Plain CT images of the lungs in Case 1. (a) HU values in the reconstructed axial two-dimensional (2D) images of the bilateral lungs, as demonstrated by color contrasting using risk Pointer[®] (Hitachi Medical Co., Ltd., Tokyo, Japan). (b) The colors indicating these areas as follows: $-2000 < \text{HU} < -800$ in yellow, $-799 < \text{HU} < -500$ in blue, and $-499 < \text{HU} < -400$ in red. In this case, the blue image indicating the area of edema was 80.1 cm^2 , the yellow image indicating the gas/air area was 62.8 cm^2 , and the red image indicating the remaining area (area with/without aeration or edema) was 6.2 cm^2 .

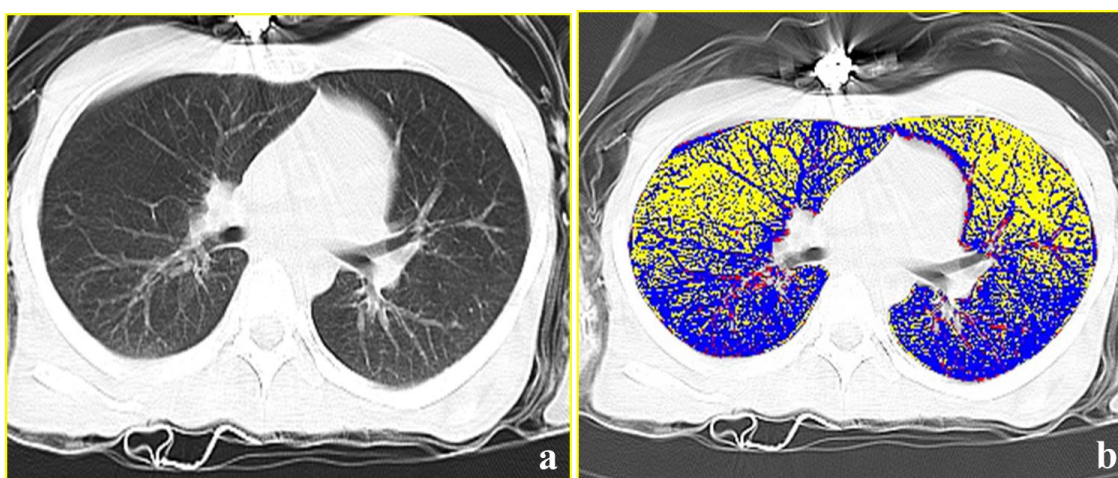


Fig. 3 This figure shows the difference between the color of the blood in the bilateral parts of the heart (bright red in the left side of the heart and dark red in the right side of the heart) depending on the blood oxyhemoglobin saturation (a) and cherry-red emphysematous lungs with mild edema suggesting cold air inhalation (b).

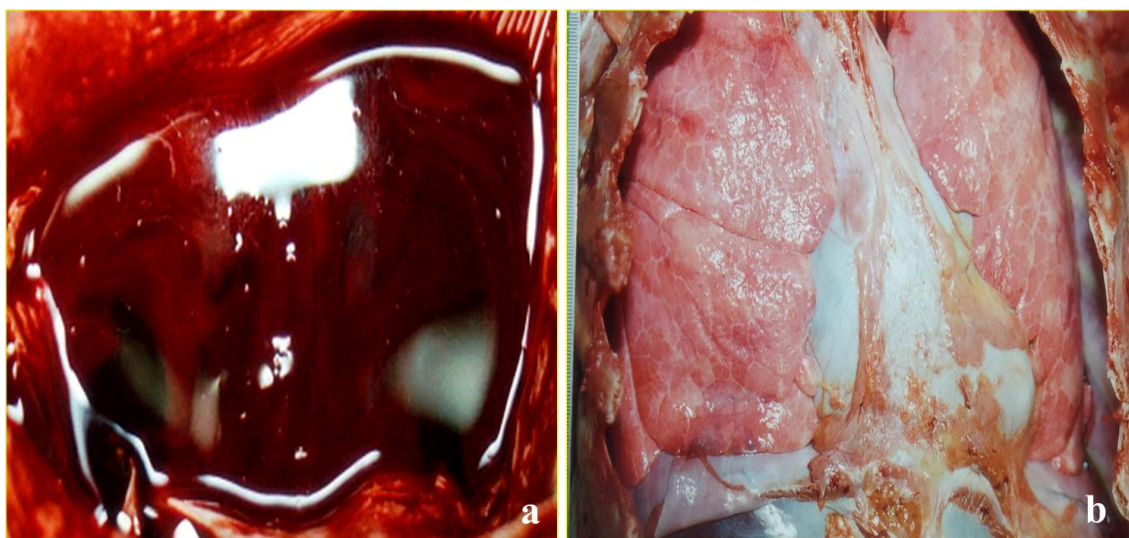


Fig. 4 (a) Histopathological findings in the pancreas (peri-islets): yellow arrow head shows lymphocytic infiltration (hematoxylin eosin staining, magnification $\times 100$). (b) Red arrow head shows anti-human CD-8 positive T-cells on immunostaining in the peri-islets (magnification $\times 200$). Immunopositivity findings of insulin (c) into islets was almost absent, and glucagon (d) was barely present (magnification $\times 200$).

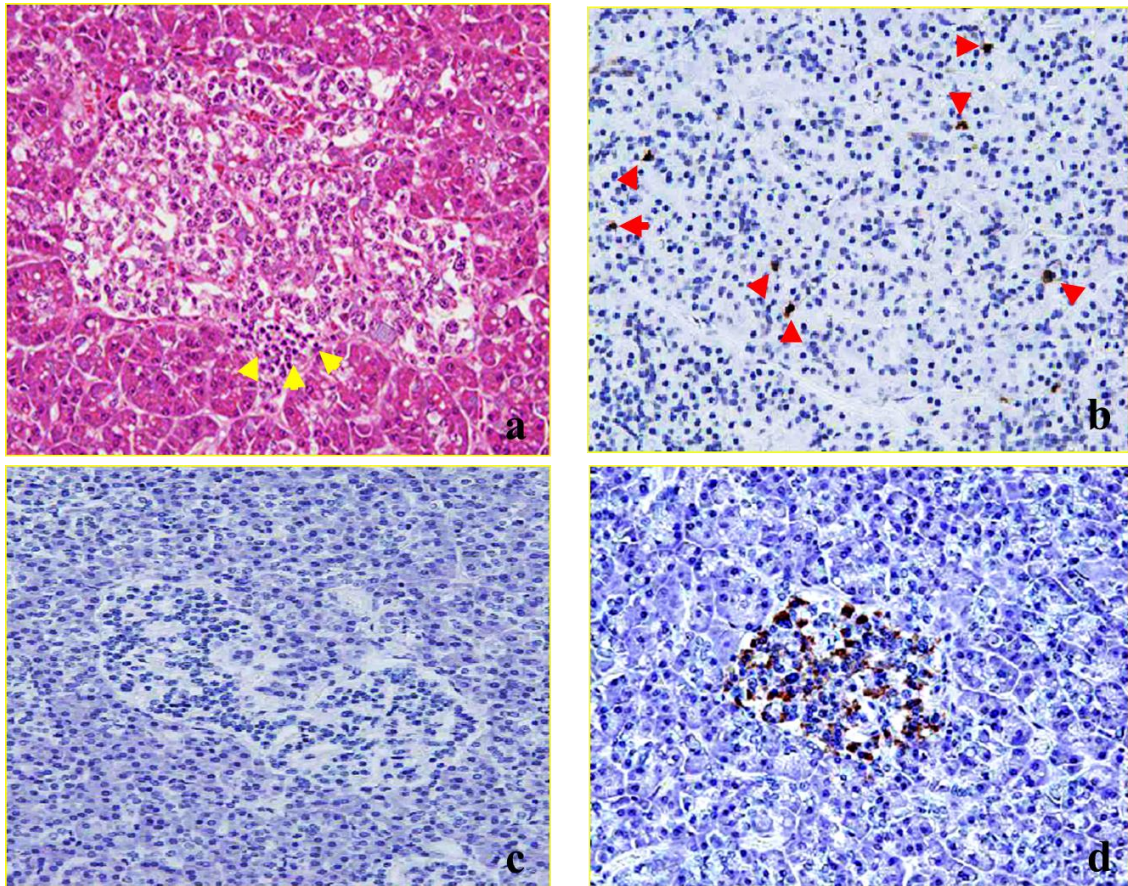


Fig. 5 Immunostaining for Influenza A virus showed positive reaction in pancreatic cells (a), bronchial epithelial cells, and bronchiolar macrophages (b).

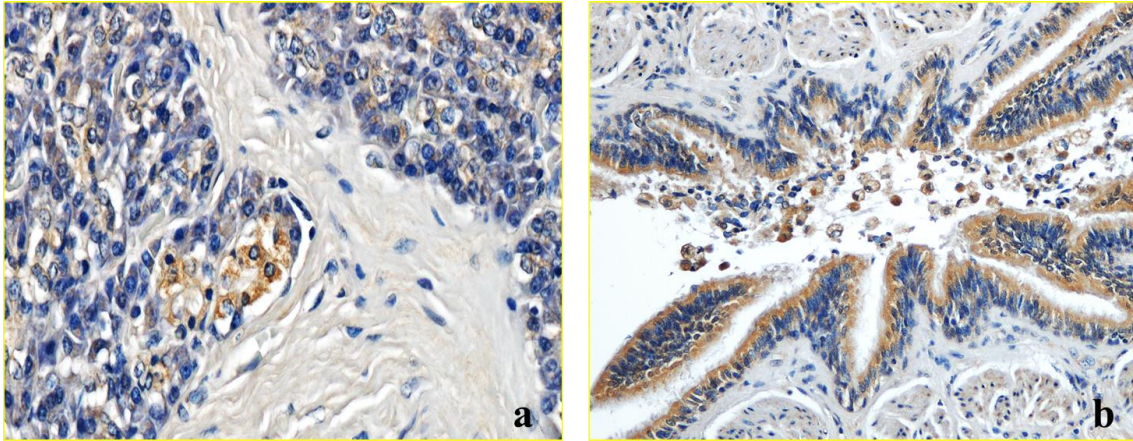


Fig. 6 We collected retrospective data on 11 hypothermia (cold exposure) and 8 malnutrition forensic autopsy cases and compared them with the present case (red-circle) in which glucose, acetone, total ketone bodies, acetoacetic acid, 3-hydroxybutyric acid, BUN, creatinine, and CRP were analyzed during the process of defining the cause of death. Glucose, acetone, total ketone bodies, 3-hydroxybutyric acid, and acetoacetic acid (red color in the graph) may be useful for the screening and diagnosis of diabetic ketoacidosis in order to distinguish it from ketosis due to hypothermia and malnutrition.

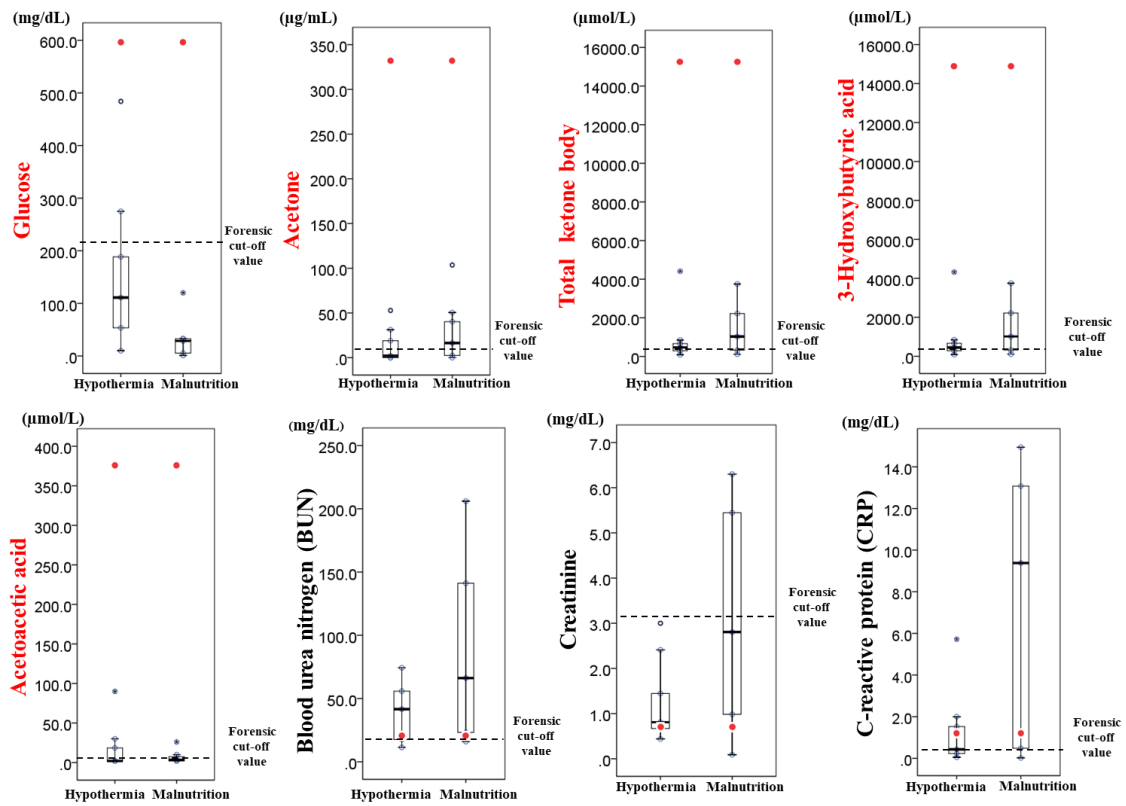


Table legend

Table 1. Summary of the ketosis-related markers in the present case compared with cases of hypothermia and malnutrition.

Right heart blood	Hypothermia (n = 11) (Male: n = 6, Female: n =5) (BMI: 12.7-25.3 Kg/m ² , median: 19.1 Kg/m ²)	Malnutrition (n =8) (Male: n = 3, Female: n =5) (BMI: 9.8-18.4 Kg/m ² , median: 14.0 Kg/m ²)	Present case	Forensic Cut-off value	Reference No.
Glucose	10.0-484.0 mg/dL (median: 111.0 mg/dL)	1.0-120.0 mg/dL (median: 28.5 mg/dL)	597.0 mg/dL	227.5 mg/dL	J-H. Chen 2015 ¹⁸⁾
Acetone	0.0-52.8 µg/mL (median: 2.1 µg/mL)	0.0-103.6 µg/mL (median: 16.4 µg/mL)	332.4 µg/mL	<50 µg/mL	M.Tominaga 2013 ¹⁹⁾
Total ketone bodies	86.0-4410.0 µmol/L (median: 465.0 µmol/L)	115.0-3750.0 µmol/L (median: 1027.5 µmol/L)	15269.0 µmol/L	217.5 µmol/L	J-H. Chen 2015 ¹⁸⁾
Acetoacetic acid	2.0-90.0 µmol/L (median: 2.0µmol/L)	2.0-26.0 µmol/L (median: 3.5 µmol/L)	376.0 µmol/L	12.0 µmol/L	No published data
3-Hydroxybutyric acid	86.0-4320.0 µmol/L (median: 449.0 µmol/L)	110.0-3750.0 µmol/L (median: 1014.5 µmol/L)	14893.0 µmol/L	260.0 µmol/L	No published data
Blood urea nitrogen (BUN)	11.4-74.3 mg/dL (median: 41.6 mg/dL)	16.0-206.0 mg/dL (median: 66.2 mg/dL)	20.5 mg/dL	38.48 mg/dL	B-L. Zhu 2002 ²⁰⁾
Creatinine	0.5-2.4 mg/dL (median: 0.8 mg/dL)	0.09-5.5 mg/dL (median: 2.8 mg/dL)	0.7 mg/dL	3.32 mg/dL	B-L. Zhu 2002 ²⁰⁾
C-reactive protein (CRP)	0.05-5.72 mg/dL (median: 0.4 mg/dL)	0.02-14.9 mg/dL (median: 9.4 mg/dL)	1.19 mg/dL	<0.1 mg/dL	T. Ishikawa 2002 ²¹⁾