applied sciences

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Postmortem Diagnosis of Ketoacidosis by Determining Beta-Hydroxybutyrate Levels in Three Types of Body Fluids by Two Different Methods

Diana Bulgaru Iliescu [1,2], Cristina Furnica [1,2,*], Nona Girlescu [1,2,*], Raluca Ozana Chistol [1,3], Lacramioara Perianu [1,4], Madalina Diac [1,2], Andrei Daniel Timofte [1], Anton Knieling [1,2] and Ioan-Adrian Ciureanu [1]

1 Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, 700115 Iasi, Romania; diana.bulgaru@umfiasi.ro (D.B.I.); raluca-ozana.chistol@umfiasi.ro (R.O.C.); lacramioara.perianu@umfiasi.ro (L.P.); madalina-maria.diac@umfiasi.ro (M.D.); andrei-daniel.timofte@umfiasi.ro (A.D.T.); tony_knieling@yahoo.com (A.K.); adrian.ciureanu@umfiasi.ro (I.-A.C.)
2 Institute of Forensic Medicine, 700455 Iasi, Romania
3 "Prof. Dr. George I.M. Georgescu" Cardiovascular Diseases Institute, 700503 Iasi, Romania
4 "Sf. Spiridon" County Hospital, 700111 Iasi, Romania
* Correspondence: cristina.furnica@umfiasi.ro (C.F.); nona-girlescu@umfiasi.ro (N.G.)

Abstract: Background: Postmortem assessment of endogenous ketoacidosis is primarily focused on the determination of 3-beta-hydroxybutyrate (BHB). The aim of our study was to identify the most adequate body fluid and postmortem quantification method for assessing ketoacidosis status immediately prior to death. Material and method: We performed a prospective study on 53 cases of sudden death or in-hospital death that were considered forensic cases and could present a state of ketoacidosis prior to death, the autopsies being performed at a post-mortem interval of 24–72 h. BHB analysis was performed by Multi-Functional Monitoring System XPER Technology analyzer (method A—portable analyzer) for peripheral blood, and by BHB Assay MAK041 Kit (method B) for vitreous humor (VH) and cerebrospinal fluid (CSF). Results: We identified 11 ketoacidosis cases using method A and 9 ketoacidosis cases using method B. All nine cases of ketoacidosis identified using the MAK041 kit were confirmed with the portable analyzer. For the 2 cases of ketoacidosis identified only with the portable analyzer, the values obtained by method B were at the diagnostic limit. BHB concentrations determined in VH and CSF by method B were statistically significantly correlated with each other and with peripheral blood BHB concentration. Conclusion: BHB, a marker of ketoacidosis, should be determined post-mortem whenever a metabolic imbalance is suspected irrespective of known risk factors or obvious morphological substrate to help establish the thanatogenic mechanism. BHB quantification can easily be performed using a handheld automatic analyzer and a sample of peripheral blood as BHB levels in various body fluids correlate with each other.

Keywords: 3-beta-hydroxybutyrate; ketoacidosis; vitreous humor; cerebrospinal fluid; blood; forensic

Citation: Iliescu, D.B.; Furnica, C.; Girlescu, N.; Chistol, R.O.; Perianu, L.; Diac, M.; Timofte, A.D.; Knieling, A.; Ciureanu, I.-A. Postmortem Diagnosis of Ketoacidosis by Determining Beta-Hydroxybutyrate Levels in Three Types of Body Fluids by Two Different Methods. *Appl. Sci.* **2022**, *12*, 5541. https://doi.org/10.3390/app12115541

Academic Editors: Francesco Garzotto and Hiroshi Ikegaya

Received: 2 April 2022
Accepted: 27 May 2022
Published: 30 May 2022

1. Introduction

Ketone bodies (acetoacetate = AcAc, 3-beta-hydroxybutyrate = BHB, acetone = Ac) are produced in the liver by breaking down free fatty acids and are used as an alternative energetic substrate in case of glucose deprivation. Massive accumulation of ketone bodies leads to a drop in blood pH, with the onset of a state of ketoacidosis that can ultimately lead to death [1,2].

A mild to moderate physiological increase in ketone bodies, without clinical effect, may be found in association with prolonged fasting or sustained physical exertion [3]. Clinically, a marked serum increase of ketone bodies (ketosis/ketonemia) can

be easily noticed in cases of decompensated diabetes (diabetic ketoacidosis-DKA), starvation/malnutrition, chronic alcoholism (alcoholic ketoacidosis), infections, trauma, hypothermia, or certain intoxications [2,3].

Serum levels of circulating ketone bodies vary in a not metabolically ill individual depending on the rate of basal metabolism, hepatic glycogen stores and mobilization of amino acids of muscle proteins [3]. Normal serum level of total ketone bodies is generally considered <0.5 mM (<500 µmol/L); hyperketonemia being defined by values >1 mM (>1000 µmol/L), and the state of ketoacidosis is characterized by values exceeding 2.5–3 mM [3]. For each form of ketone bodies, plasma values in a healthy adult vary according to the type and time since food intake, most studies considering the following serum normal values: AcAc < 56 µmol/L, BHB < 86 µmol/L, Ac < 5 µmol/L [4,5]. Mitchell et al. and Laun et al. state that in the absence of prolonged periods of food deprivation, normal serum of BHB levels range from 0 to 160 µmol/L and AcAc from 0 to 70 µmol/L [6,7]. Analyzing these data and considering that in an acidic environment, AcAc is rapidly converted to Ac, which is rapidly eliminated by respiration, it can be deduced that BHB is the main serum exponent of total ketone bodies [3,5,6].

Diabetic ketoacidosis develops because of insulin deficiency or insulin resistance, with increased production of free fatty acids, decreased utilization in tissues and accelerated transformation into ketone bodies in hepatocytic mitochondria. During starvation, the glucagon/insulin ratio is >1 and fatty acids are mobilized from triglycerides in adipose tissue and converted to ketone bodies [8]. Disease and trauma can also be associated with rapid protein and lipid depletion, with potential significant metabolic imbalances and endogenous production of ketone bodies. Alcoholic ketoacidosis (AKA) can occur because of glycogen and insulin depletion, reduced extracellular fluid volume, increased NADH/NAD+ ratio secondary to ethanol metabolization, but can also be associated with hypoglycemia because of starvation and lipolysis [9,10]. Increased ketone body values have been identified in hypothermia, a phenomenon explained by increased levels of glucagon, cortisol, and growth hormones with catabolic action on fatty acids [11,12].

The first thanatochemical studies to prove ketoacidosis post-mortem involved simple qualitative and/or semi-quantitative determination of total ketone bodies and qualitative and/or quantitative determination of acetone, respectively. Over time, ketoacidosis detection has undergone a series of improvements both in a clinical and forensic setting [13–15]. Currently, postmortem assessment of endogenous ketoacidosis is primarily focused on the determination of BHB, the biochemical marker that most accurately reflects the level of ketone bodies relative to that present immediately prior to death [16,17], as BHB is the most stable ketone body that can be identified in a state of ketoacidosis. The other two ketone bodies, AcAc and Ac, are unreliable due to their unstable nature. AcAc is converted to Ac under acidic conditions and Ac is eliminated by respiration. The postmortem diagnosis of endogenous ketoacidosis can only be performed by BHB determination, as it belongs to the category of sudden deaths without an obvious macro/microscopic morphological substrate. The autopsy performed in these cases does not detect the exact cause of death when no thanatochemical determinations are performed. The postmortem demonstration of this diagnosis is extremely important because it clearly differentiates between endogenous and exogenous ketoacidosis, the latter being detected exclusively/predominantly by elevated acetone values [18,19]. Moreover, it has been shown that Ac and isopropyl alcohol (IPA), another biochemical compound associated with the ketosis state, can be produced during degradation processes occurring post-mortem and thus do not reflect accurately the level of ante-mortem ketosis [17,19].

Even though extensive studies on the importance of post-mortem identification of BHB are reported in the literature, the actual determination of this biomarker in daily forensic medicine practice is rather limited. Such tests should be carried out in all forensic services, as BHB determination could contribute to diagnosing the cause of death.

To date, BHB values in multiple body fluids and collected through multiple techniques were not compared, and the optimal collecting technique and determination method were not identified.

The aim of our study was to determine the most adequate postmortem body fluid for assessing ketoacidosis status (BHB levels) immediately prior to death using different analysis methods.

2. Materials and Methods

2.1. Study Design

We performed a prospective study on 53 cases of sudden death or in-hospital death that were considered forensic cases and could present a state of ketoacidosis prior to death, the autopsies being performed by forensic pathologists at the Institute of Forensic Medicine (Iasi, Romania) at a post-mortem interval of 24–72 h. Fifteen control cases (violent deaths due to mechanical asphyxia) were used for reporting BHB in vitreous humor (VH) and cerebrospinal fluid (CSF), for which reference values are not known.

According to the Romanian legislation, a forensic autopsy is ordered in case of violent death, when the cause of death is not known or when there is a reasonable suspicion that a crime might have been committed [20].

2.2. Biological Fluids Collected

Blood samples, VH and CSF were all collected at the time of autopsy. Blood samples were analysed on site immediately after the autopsy. VH and CSF were stored in the refrigerator (4 °C) if analysed the same day or in the freezer (−18 °C) if they were preserved for more than 24 h until analysis, which was performed for 2 batches of 27 and 26 samples, respectively.

2.2.1. Vitreous Humor (VH)

The entire VH was collected from both eyes using sterile 7 mL vacutainers with 3% sodium fluoride (NaF) preservative and 20 gauge thin needles. The harvesting technique was the classical one, which involved inserting the needle into the globe of the eye at the level of the lateral canthus and aspiring the fluid. The fluid was visually inspected and considered adequate for analysis when colorless and quasi-viscous. Contaminated samples (VH with reddish/bloody streaks, dirty appearance, various foreign bodies) were excluded. Before storage, all samples were centrifuged at 5000 rpm for 10 min. For each individual case, the fluid collected was replaced with saline solution.

2.2.2. Cerebrospinal Fluid (CSF)

Cerebrospinal fluid was collected by two types of puncture: suboccipital, to extract CSF from cisterna magna (CSF occ), and lumbar puncture, to extract CSF from the lumbar subarachnoid space (CSF L). CSF was collected by using 20G stainless steel Quincke beveled spinal needles with a diameter of 0.9 mm and a length of 90 mm. Samples were collected in sterile 7 mL vacutainers with 3% sodium fluoride (NaF) preservative.

2.2.3. Lumbar Puncture Technique

Drawing a line joining the two upper edges of the iliac crests, the point on this line which intersects the posterior midline corresponds to the body of the fourth lumbar vertebra. Once the point of insertion of the needle had been identified, the needle was advanced slowly at an angle to the cranium, towards the umbilicus. The approximate distance from the tegument to the epidural space is 45–50 mm, and may be greater in obese individuals. The stylet of the spinal needle was then removed, allowing the CSF to progress.

2.2.4. Cisterna Magna Puncture Technique

The needle was advanced by puncturing the skin of the posterior cephalic region on the midline, just above the process of the first cervical vertebra, in a slightly upward

direction towards the nazion. Advancement was made until increased resistance was encountered, usually to a depth of about 2.5–3.5 cm, at which point the atlanto-occipital membrane was penetrated. As soon as this point was passed, there was a sudden drop in resistance given by the needle entering the cistern.

2.2.5. Blood

Blood was collected from femoral vessels (artery/vein) using the same sterile 7 mL vacutainers.

2.2.6. Femoral Blood Collection Technique

The pelvic limb from which the blood is collected should be kept in a slightly elevated position to obtain a greater quantity of blood, and excessive mobilisation of the limb is not recommended. The following anatomical landmarks shall be identified and marked: the anterosuperior iliac spine, the pubic tubercle, the inguinal ligament, and medial border of the cruciate muscle. The region was then dissected layer by layer, showing the vascular formations at this level: femoral artery, femoral vein and saphenous vein. The vessels were clamped proximally, close to the inguinal ligament, thus avoiding blood leakage from the large vessels. The externalized mixed blood was collected in a threaded tube.

2.3. BHB Analysis

BHB analysis was performed by two methods:

2.3.1. Method A

For peripheral blood, we used a commercially available Multi-Functional Monitoring System XPER Technology I1 analyzer (TaiDoc Tech Corporation, New Taipei City, Taiwan), with its specific test strips for BHB, a limit of detection (LOD) of 0.1 mmol/L, and an analysis range of 0.1–8 mmol/L. Before analyzing the samples, a control test was performed according to the manufacturer's instructions. The blood BHB concentration was determined by measuring the electrical current generated by the reaction of blood BHB with the reagent on the strip. The amount of blood sample required for determination was 0.8 µL. The measurement time was 10 s.

2.3.2. Method B

For VH and CSF, we used a Beta-Hydroxybutyrate Assay Kit (MAK041, Sigma-Aldrich, Saint Louis, MO, USA) based on beta HB Dehydrogenase and on an enzymatic cycling reaction in which the reduced cofactor NADH reacts with a colorimetric reagent producing a colored compound measurable at 450 nm. The end-point reading was performed using a Platos microplate reader (AMP Diagnostics, Graz, Austria). The vials were centrifuged shortly before opening. Ultra-pure water was used for reagent preparation and to maintain reagent integrity, and repeated freeze/thaw cycles were avoided. BHB standard solution was reconstituted with 100 mL of water to generate a 10 mM stock solution. Homogenization was performed by pipetting; the next step was aliquotation and storage in the dark at −20 °C.

2.3.3. Preparation of BHB Standard Solutions for Colorimetric Detection

We diluted 10 µL stock standard solution of BHB with 90 µL buffer solution to prepare a 1 mM (1 nmol/µL) working standard solution. We added 0, 2, 4, 6, 8 and 10 µL of 1 mM BHB standard solution to a 96 well plate, generating final concentrations of 0, 2, 4, 6, 8 and 10 nmol/well. A BHB buffer was added in such a way that a final volume of 50 µL was reached in all wells with standard solutions.

Calibration curves resulting from each set of determinations were used to calculate and convert the results.

BHB concentration was calculated according to the formula:

$$Sa/Sv = C$$

Sa = Amount of BHB in the resulting sample, deduced from the standard curve
Sv = Volume of sample (µL) added to the wells
C = BHB concentration in the sample
Molecular weight of BHB: 104.1 g/mol

The LOD for this method was 0.01 mmol/L with no upper limit as we used a research kit.

Ketoacidosis was affirmed for BHB values $\geq$ 2.5 mmol/L when using method A and for BHB values $\geq$ 0.5 mmol/L when using method B. Clinical reference values were used as they also apply to post-mortem studies [9].

2.4. Statistical Analysis

Obtained values were analyzed using SPSS 26 (IBM, Armonk, NY, USA) and Microsoft Excel 16.0 (Microsoft, Redmond, WA, USA). Continuous variables are expressed as mean $\pm$ standard deviation, median, maximum/minimum values and inter-quartile range. Variables were compared using a Student's independent t-test and association between variables was tested using Pearson product-moment correlation and Goodman and Kruskal's gamma. A *p*-value of 5% or lower was considered to be statistically significant.

The study was approved by the Ethics Committees of the Institute of Forensic Medicine Iasi and of the Grigore T. Popa University of Medicine and Pharmacy Iasi, Romania (no. 7244/12 April 2018).

3. Results

The mean age of the decedents was 63.24 $\pm$ 15.82 years with a range between 31–99 years, and 36 (67.92%) were males with an average age of 60.5 years, respectively. Seventeen (32.07%) were females with an average age of 69.06 years. One case had a history of type 1 diabetes mellitus (1.89%), 11 cases had a history of type 2 diabetes mellitus (20.75%), and 16 (30.19%) had a blood alcohol level ranging from 0.1–2.87 g/L.

The BHB biomarker was determined from VH and CSF collected from the occipital site using the method B kit in all 53 cases included in the study. In 24 cases (45.28%), BHB was also determined from CSF collected from the lumbar site using the same method B and in 35 cases (66.03%) BHB was also determined from peripheral blood using the method A portable multifunctional monitoring system.

There were no statistically significant differences between mean values obtained at different PMI (Table 1), and there was no correlation of BHB values with the PMI ($p = 0.43$).

The BHB concentration in various fluids irrespective of the PMI is detailed in Table 2.

According to the obtained BHB values and using the diagnostic thresholds, we identified 11 ketoacidosis cases using method A and 9 ketoacidosis cases using method B. All nine cases of ketoacidosis identified using method B (values above the threshold for both VH and CSF occ) were confirmed with the portable analyzer (method A). For the two cases of ketoacidosis identified only by method A, the values obtained using the MAK041 kit were at the diagnostic limit (0.42–0.47) (Table 3).

BHB concentrations determined in VH and CSF occ using method B correlated with each other and with peripheral blood BHB concentration determined using method A ($p < 0.05$) (Table 4). CSF L BHB concentrations correlated the least with the other values.

Table 1. Comparison of BHB values by PMI.

Variable	PMI	No. of Cases	Mean	Std. Deviation	Minimum	Maximum	*p*
BHB VH	24 h	36	0.39	0.12	0.13	0.57	0.86
	36 h	7	0.39	0.14	0.23	0.57	
	48 h	8	0.36	0.09	0.23	0.46	
	72 h	2	0.35	0.07	0.30	0.40	
	Total	53	0.39	0.12	0.13	0.57	
BHB CSF occ	24 h	36	0.35	0.13	0.10	0.55	0.71
	36 h	7	0.36	0.15	0.17	0.55	
	48 h	8	0.29	0.13	0.15	0.45	
	72 h	2	0.31	0.06	0.27	0.35	
	Total	53	0.34	0.13	0.10	0.55	
BHB CSF L	24 h	15	0.19	0.06	0.11	0.30	0.58
	36 h	3	0.22	0.08	0.13	0.30	
	48 h	4	0.18	0.04	0.15	0.24	
	72 h	2	0.24	0.02	0.22	0.26	
	Total	24	0.19	0.06	0.11	0.30	
BHB peripheral blood	24 h	25	2.66	2.22	0.30	7.10	0.38
	36 h	4	3.58	3.49	0.60	7.30	
	48 h	4	1.33	0.63	0.70	2.20	
	72 h	2	0.85	0.07	0.80	0.90	
	Total	35	2.51	2.25	0.30	7.30	

VH, CSF occ, CSF L were analyzed by method B, peripheral blood using method A.

Table 2. Statistical description of BHB values.

Variable	N	Mean	Median	Minimum	Maximum	Q1	Q4	Q Range	SD
VH BHB (mmol/L)	53	0.38	0.40	0.13	0.57	0.28	0.47	0.44	0.12
CSF occ BHB (mmol/L)	53	0.34	0.33	0.09	0.55	0.24	0.45	0.45	0.13
CSF L BHB (mmol/L)	24	0.19	0.19	0.11	0.30	0.14	0.25	0.19	0.06
Peripheral blood BHB (mmol/L)	35	2.50	1.90	0.30	7.30	0.80	2.80	7.00	2.25

N = number of cases, SD = standard deviation; VH, CSF occ, CSF L were analyzed by method B, peripheral blood using method A.

Table 3. Frequency of BHB values according to threshold values.

BHB Values	(1) VH BHB (53 Cases)		(2) CSF occ BHB (53 Cases)		(3) CSF L BHB (24 Cases)		(4) Peripheral Blood BHB (35 Cases)	
	N	%	N	%	N	%	N	%
Normal	0	0	1	1.89	0	4.17	2	5.71
Increased	44	83.02	43	81.13	24	100	22	62.86
Ketoacidosis	9	16.98	9	16.98	0	0	11	31.43
Total	53	100	53	100	24	100	35	100

N = number of cases, % = percentage; VH, CSF occ, CSF L were analyzed by method B, peripheral blood using method A.

Table 4. Correlations between VH BHB, CSF occ BHB, CSF L BHB and peripheral blood BHB.

Variable	Pearson r	r^2	t	*p*	No of Cases
VH BHB-CSF occ BHB	0.945	0.89	20.78	<0.0000001	53
VH BHB-CSF L BHB	0.55	0.29	3.07	0.0056	24
CSF occ BHB-CSF L BHB	0.56	0.32	3.21	0.004	24
VH BHB–peripheral blood BHB	0.76	0.58	6.75	<0.0000001	35
CSF occ BHB-peripheral blood BHB	0.81	0.66	8.01	<0.0000001	35
CSF L BHB-peripheral blood BHB	0.54	0.29	2.99	0.0067	24

VH, CSF occ, CSF L were analyzed by method B, and peripheral blood using method A.

Mean BHB concentration in various fluids did not significantly differ according to prior diabetes mellitus diagnosis or to glycosylated hemoglobin level (<7% or ≥7%). A Chi-squared test confirmed the results by showing no association of ketoacidosis diagnosed by BHB concentration with prior diabetes mellitus diagnosis, glycosylated hemoglobin ≥7% or with the presence of alcohol in peripheral blood, thus suggesting that none of the deaths occurred due to alcoholic or diabetic ketoacidosis and that ketoacidosis could be attributed to other causes (starvation, medication, intoxication, etc.) and should prompt the investigation of another cause of death (Table 5) within the principles of toxicological analysis for forensic purposes [21].

Table 5. BHB according to presence/absence of known DM antemportem/postmortem.

BHB from:	Mean 1	Mean 2	Test t	gl	*p*	N1	N2	SD 1	SD 2
VH	0.3687	0.3906	0.58	51	0.56	12	41	0.1345	0.1130
CSF occ	0.3245	0.3451	0.50	51	0.62	12	41	0.1415	0.1255
CSF L	0.2103	0.1831	1.11	22	0.28	10	14	0.0636	0.0559
Peripheral blood	2.1077	2.7409	0.80	33	0.43	12	23	1.7495	2.5126

1 = with DM, 2 = without DM, N = number of cases, SD = standard deviation.

4. Discussion

Ketone bodies (AcAc, BHB and Ac) are secondary compounds of lipid metabolism and are used as an energy substrate when glucose is insufficient or inaccessible. Thus, when blood glucose levels are low, free fatty acids are used as an alternative source of energy through the process of ketogenesis which takes place exclusively in the liver. AcAc is formed by the beta-oxidation of fatty acids and can be reduced to BHB by the enzyme 3-hydroxybutyrate dehydrogenase in a reversible reaction depending on the NADH/NAD+ ratio [13,15]. Ac is formed by spontaneous decarboxylation of AcAc and is generally considered to be of minor metabolic importance [15–17]. IPA is another compound related to the ketosis state, and it is observed in many conditions characterized by ketosis and increased NADH/NAD+ ratio [17,18]. Ketone bodies are converted to acetyl-CoA in hepatocytic mitochondria, especially the perivenous hepatocytes, and thus used as an energy substrate for the Krebs cycle [13].

In the present study, we determined BHB concentration in various body fluids using two methods with different principles and applicability. The obtained results were similar to those reported in the literature [6,9,22–24]. We chose two different threshold values for affirming ketoacidosis depending on the method of BHB determination. When using the handheld multifunctional β-analyzer we applied the threshold values most used in the literature [9,19,25–27]. For evaluating ketone bodies with an MAK041 kit we chose lower diagnostic values according to the results obtained by other researchers using the same diagnostic kit [28].

The BHB values determined were not similar in the four collection sites, but the concentrations quantified in VH, CSF occ and peripheral blood significantly correlated with each other ($p < 0.001$). The correlation coefficients we obtained demonstrate the reliability of

both methods as well as the stability of the biochemical marker in all three types of collected fluids. Therefore, our results are consistent with other research and allow us to affirm that any of the three types of body fluids can be used for post-mortem BHB determination for the diagnosis of ketoacidosis [5,29].

The few determinations of BHB levels in CSF performed over time have led to divergent opinions, with some researchers suggesting that BHB should be quantified post-mortem only in VH and peripheral blood [30]. In contrast, other investigators claimed that the post-mortem value of BHB in CSF would be closer to the true value compared to that identified in VH [23] without stating the collection site, whether suboccipital or lumbar. Two studies published by Garland et al. analyzed BHB values in VH and CSF collected from the cerebral ventricular system and lumbar region in 20 autopsies [31,32]. Even though no significant differences were found between BHB values in fluids collected from the three sites, Garland et al. made no suggestion concerning the optimal site for BHB determination, as all values were within physiological limits [31,32].

When analyzing individual values obtained in our research, we observed that the lowest BHB values were obtained from lumbar CSF. Unlike the values quantified in the cisterna magna CSF, those obtained from the lumbar CSF correlated less with the values obtained from the other body fluids. Thus, we consider that determination of BHB in CSF collected by lumbar puncture should be avoided in favor of that collected from cisterna magna. We were unable to identify any other study that compared BHB values in CSF collected from the two sites.

Detection of abnormal BHB values above the threshold led to the identification of nine cases of ketoacidosis using the MAK041 test kit and 11 cases using the handheld multi-purpose β-analyzer. All nine cases of ketoacidosis identified using the MAK041 kit were also confirmed with the portable β-analyzer. These results demonstrate once again the reliability of the methods used in our study, especially the determinations performed using the MAK041 kit. For the 2 cases of ketoacidosis identified only by using the portable β-analyzer, the values obtained from the MAK041 kit were at the upper limit of the normal range (0.42–0.47). In these 2 cases, we cannot exclude a possible overestimation of ketoacidosis when only using the rapid test.

A recent study published by Peyron et al. highlights the excellent specificity and sensitivity of BHB values obtained with a portable analyzer operating according to the same principle as the one used in our research [27]. Moreover, Peyron et al. recommend the routine use of a portable analyzer in forensic practice, especially for cases of suspected metabolic imbalance [27]. Our results support this suggestion, as the nine ketoacidosis cases did not occur secondary to diabetes or alcohol abuse and require the investigation of other causes of death.

A significant advantage of BHB determination is the post-mortem stability of this biochemical marker for 24–72 h. Our results are consistent with those published in the literature, which emphasize the post-mortem stability of BHB compared to Ac or other biochemical markers as mean values obtained at various PMI did not significantly differ [1,6,9,19].

In terms of the applicability, the usage of the MAK041 test kit is more laborious, as more samples need to be collected to perform the actual determinations. Also, it has been developed for research purposes and our study is the first to use it for forensic analysis. At the opposite, the portable multifunctional monitoring system-XPER-Technology I1, is easy to use and fast, the results being obtained while performing the autopsy or immediately after.

The major limitation of this research is the lack of baseline control values, the relative small number of cases included, as well as the fact that one of the applied methods for determining BHB is experimental and used for the first time in the forensic field. Even though in our study we did not determine BHB from VH using the portable multifunctional analyzer, it is worth pointing out the controversies in the literature: Peyron et al. identified excellent sensitivity and low specificity, while Walta et al. identified the same sensitivity but much better specificity (0.72 vs. 0.50) [27,33]. Of course, further studies employing similar methods are needed to provide even greater credibility.

Given the limitation underlined above, we plan to continue this study direction and validate the multiple analyzer for use in forensic determination of BHB on a large sample of cases with and without antemortem causes of ketoacidosis.

The results obtained in this study complement existing forensic research by emphasizing the importance of the BHB determination site.

5. Conclusions

Beta-hydroxybutyrate, a marker of ketoacidosis, should be determined post-mortem whenever a metabolic imbalance is suspected irrespective of known risk factors (diabetes, alcoholism) or obvious morphological substrate to help establish the thanatogenic mechanism. BHB quantification can easily be performed using a handheld automatic analyzer and a sample of peripheral blood as BHB levels in various body fluids (VH, CSF, peripheral blood) correlate with each other.

Author Contributions: Conceptualization, D.B.I. and N.G.; methodology, C.F. and R.O.C.; software, A.D.T.; formal analysis, C.F. and A.K.; investigation, M.D. and L.P.; data curation, N.G. and I.-A.C.; writing-original draft preparation, N.G. and R.O.C.; writing-review and editing, D.B.I.; supervision, D.B.I. and I.-A.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This study was conducted according to the guidelines of the Declaration of Helsinki. The study was approved by the Research Ethics Commission of the University of Medicine and Pharmacy "Grigore T. Popa", Iași, Romania (no. 7244/12 April 2018).

Informed Consent Statement: Informed Consent for the autopsy is not required, the autopsy is mandatory under Romanian Law (Romanian Criminal Procedure Code Law no. 135/2010, chapter VII, article 185).

Conflicts of Interest: The authors declare that they have no conflict of interest.

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