J Vet Intern Med 2002;16:411–417

Accuracy of Serum β-Hydroxybutyrate Measurements for the Diagnosis of Diabetic Ketoacidosis in 116 Dogs

Ricardo Duarte, Denise M.N. Simoes, Maria Luisa Franchini, Maurício L. Marquezi, Julia H. Ikesaki, and Marcia M. Kogika

The aim of this study was to evaluate the accuracy of serum β-hydroxybutyrate (β-OHB) measurements for the diagnosis of diabetic ketoacidosis (DKA) in dogs. One hundred sixteen diabetic dogs were prospectively enrolled in the study: 18 insulin-treated (IT) diabetic dogs that had a positive urine ketone test and 88 untreated, newly diagnosed diabetic dogs. Venous blood gas tensions and pH, serum glucose and urea nitrogen (SUN), and electrolyte (Na^+, Cl^-, and K^+) and urine acetoacetate (AA) concentrations were measured concurrently with serum β-OHB concentrations. On the basis of laboratory findings, the patients were assigned to 1 of 3 groups: diabetic ketoacidosis (n = 43); diabetic ketosis (DK, n = 41); and nonketotic diabetes (NDK, n = 31). Serum β-OHB concentrations differed significantly ($P < .001$) among the study groups. Although marked differences in β-OHB concentrations were found, a considerable overlap exists between the distributions of dogs with DK and those with DKA. The overall accuracy of β-OHB determination as a diagnostic test for DKA, determined by the area under the receiver operating characteristic (ROC) curve, was 0.92. In the 1.9- to 4.8-mmol/L range, serum β-OHB determination sensitivity varied from 100 to 35.7%, whereas specificity varied from 39 to 100%. The cutoff value of 3.8 mmol/L showed the best equilibrium between specificity (95%), sensitivity (72%), and likelihood ratio (14.8). We concluded that the quantitative measurement of serum β-OHB may be a potential tool for diagnosing and monitoring ketosis and ketoacidosis in diabetic dogs.

Key words: Canine; Diabetes mellitus; Ketone bodies; Receiver operating characteristic analysis.

In small animal practice, the degree of ketonemia is usually assessed indirectly with strip tests that provide a semiquantitative estimate of acetoacetate (AA) in the urine. Such tests, based on the nitroprusside reaction, do not detect the presence of β-hydroxybutyrate (β-OHB), the predominant ketone body produced during diabetic ketoacidosis (DKA). The β-OHB : AA ratio in DKA is 3 : 1.[1] When concomitant severe hypovolemia, tissue hypoxia, and lactic acidosis are present, the β-OHB : AA ratio can rise to 20 : 1.[2] In these conditions, severe hyperketonemia could be underestimated or even undetected by the nitroprusside reaction.[3]

The concentration of ketone bodies in urine is proportional to the plasma concentration, but it is also dependent on the volume and urine concentration. Although ketone bodies are reabsorbed from the renal tubules, final concentrations in urine are usually much higher than those in plasma. Therefore, the presence of ketone bodies in urine cannot be considered diagnostic for DKA.[1,4] Dogs with DKA require an intensive therapeutic plan involving frequent clinical assessments and laboratory monitoring. Dogs with ketosis, but not acidosis (also referred as ''healthy ketoacidotics''),[5] generally are not clinically ill, are not in need of aggressive therapy, and can be managed on an outpatient basis.[6] The absence of ketonuria does not rule out the possibility of hyperosmolar nonketotic variants of diabetes mellitus (DM). This common scenario represents the variable spectrum of uncontrolled hyperglycemic states, ranging from pure ketoacidosis to pure hyperosmolar nonketotic syndrome.[7]

The limitations of the nitroprusside test prompted the development of assays that quantitatively measure β-OHB in blood.[8–11] β-OHB measurements are considered reliable for diagnosing and monitoring the treatment of DKA[12] and have been used as an alternative to the urine ketone test for people in many diabetic care facilities.[13,14] The purpose of this study was to evaluate the accuracy of serum β-OHB measurements for the diagnosis of DKA in dogs with DM.

Materials and Methods

Dogs

The study group consisted of 116 diabetic dogs successively recruited from the medical service of the Veterinary Teaching Hospital, University of Sao Paulo, from January 1998 to September 2000. All dogs included in the study were newly diagnosed as diabetic (without previous insulin therapy) or had a positive urine ketone test. For dogs with more than 1 admission to the hospital, data were collected only from the 1st admission. Dogs were assigned to 1 of 3 groups on the basis of laboratory findings: (1) diabetic ketoacidosis (DKA; n = 43): (a) hyperglycemia (serum glucose > 250 mg/dL) and glycosuria, (b) acidosis (plasma bicarbonate [HCO_3] < 15 mmol/L) or acidemia (pH < 7.3), and (c) positive urine ketones; (2) diabetic ketosis (DK; n = 41): (a) hyperglycemia (serum glucose > 250 mg/dL) and glycosuria, (b) lack of acidosis or acidemia ($HCO_3 \geq 15$ mmol/L and pH ≥ 7.3), and (c) positive urine ketones; and (3) nonketotic diabetes (NKD; n = 32): (a) hyperglycemia (serum glucose > 250 mg/dL) and glycosuria, (b) lack of acidosis or acidemia as described above, and (c) negative urine ketones. Fifty healthy dogs were selected for the reference range study. Dogs were of assorted breeds; 25 were male, and 25 were female, with ages ranging from 1 to 15 years. All healthy dogs had serum glucose values within the reference range (80–120 mg/dL) and negative urine glucose and ketones. The study design was approved by the institutional review board of our institution.

From the Department of Medical Clinics, School of Veterinary Medicine, University of Sao Paulo, Sao Paulo, SP, Brazil. Dr Duarte is presently affiliated with the Veterinary Teaching Hospital of CREUPI, Espirito Santo do Pinhal, SP, Brazil. *Preliminary findings of this work were presented at the 18th American College of Veterinary Internal Medicine Forum, Seattle, WA, May 2000.*

Reprint requests: Ricardo Duarte, DVM, MSci, rua Francisco O. Alves, 51—Atibaia—SP 12941-630, Brazil; e-mail: ricardoduarte@diabetes.vet.br.

Submitted May 9, 2001; Revised September 24, 2001 and January 2, 2002; Accepted February 4, 2002.

0891-6640/02/1604-0000/$3.00/0

Sample Collection

Blood samples were collected, transferred to serum separator tubes,[a] and centrifuged. Separated serum was aliquoted and, according to medical judgment, 1 aliquot was processed immediately for routine biochemical determinations, and the remaining aliquots were stored at −20°C until analyzed (within 1 month). For blood gas analysis, heparinized venous blood samples were collected from jugular or cephalic veins and handled under anaerobic conditions until analysis within 15 minutes after collection. Urine glucose and ketone concentrations were measured. Blood and urine samples were obtained simultaneously before treatment with short-acting insulin or IV fluids. Blood and urine samples from healthy control dogs were obtained before 10:00 AM on the morning of study after an overnight fasting.

Analytic Procedures

Serum β-OHB, glucose, urea nitrogen (SUN), sodium (Na^+), chloride (Cl^-), and potassium (K^+) concentrations, venous blood gas tensions and pH, and the presence of glucose or ketones in urine were determined for all diabetic dogs included in the study. The concentration of β-OHB in serum was measured with an automatic analyzer,[b] with the use of a standard liquid reagent.[c] This method is based on the oxidation of β-OHB to AA by the enzyme β-hydroxybutyrate dehydrogenase. During this reaction, an equimolar amount of nicotamide adenine nucleotide (NAD) is reduced to $NADH^+$. The $NADH^+$ absorbs the light at 340 nm, and the increase in absorbance is directly proportional to the β-OHB concentration in the sample.

Analytic evaluation of the method was performed with serum samples obtained from dogs with DM, presented to our hospital, whose weight and health condition allowed removal of a larger volume of blood. The intra-assay coefficient of variation for the β-OHB assay was calculated by assaying 15 replicates of 3 serum samples with ''low'' (0.90 ± 0.07 mmol/L; mean ± 1 SD), ''median'' (2.4 ± 0.08 mmol/L), and ''high'' (6.4 ± 0.05 mmol/L) β-OHB concentrations and ranged from 0.82 to 8% (greater variation at the lowest value) with a mean coefficient of variation of 4.1%. The interassay coefficient of variation of β-OHB measurements (n = 10) of the same specimens ranged from 3.4 to 5.1% (mean, 4.1%). The detection limit of the test was 0.02 mmol/L, calculated as 2 standard deviations from the mean of the apparent β-OHB concentrations in 15 replicates of distilled, deionized water. For statistical purposes, β-OHB concentrations below the detection limit were arbitrarily set at a value of 0.01 mmol/L. The standard curve was linear up to at least 5 mmol/L.

Venous blood pH, partial pressure of Co_2 (Pco_2), and O_2 (Po_2) determinations were performed with blood gas analyzers.[d,e] The HCO_3 and base excess (BE) values were calculated by nomograms within the instruments on the basis of measured pH and Pco_2. Electrolytes were determined by an ion-specific method.[e] Other serum chemistry tests were performed by standard methods. The anion gap (AG) was defined as being equal to serum $Na^+ - (HCO_3 + Cl^-)$, and the effective osmolality (E_{OSM}) was calculated as equal to $2(Na^+ + K^+)$ + glucose/18.[15,16] Urine ketones and glucose were assessed by urine strip tests.[f]

Statistical Analysis

Results of data analysis are expressed as medians and ranges, unless otherwise stated. Because data were non-normally distributed, statistical analyses were performed by use of nonparametric tests, except for gender, weight, and age data (normally distributed). For these data, the one-way analysis of variance test was used to analyze continuous data, and the chi-square test was used for categorical data. For nonparametric data, the Kruskal-Wallis test was employed for comparisons among groups (followed by the Dunn's multiple comparisons posttest), and the Mann-Whitney *U*-test was employed for pairwise comparisons. We assessed the performance of different cutoff points of β-OHB concentrations for specificity and sensitivity for the diagnosis of DKA by comparing the DKA with the DK group, by means of the receiver operating characteristic (ROC) curve approach.[17] In brief, the ROC curve is constructed by plotting the true-positive rate (TPR or sensitivity) as a function of the false-positive rate (FPR or 1 specificity) for all possible cutoff values of a diagnostic test. The TPR is the proportion of patients with a disease that have a positive test (represented in this study by the DKA group), and the FPR is the proportion of patients without a disease that have a positive test (represented by the DK group). The diagnostic accuracy (ie, the ability to correctly classify subjects into clinically relevant subgroups) was accessed by the area under the ROC curve: an area of 0.5 corresponds to no discrimination between the groups, and an area of 1.0 corresponds to perfect separation.[18] The reference range for β-OHB concentrations was established by the interval between the 2.5th and 97.5th percentiles with results from the 50 clinically healthy dogs.[19] Data were analyzed with computer software.[g,h] For all statistical analysis, a P value $< .05$ was considered significant.

Results

Characteristics of the Diabetic Dogs

The age of diabetic dogs ranged from 3 months to 14 years (median, 9 years). There was no difference in the age distribution among the 3 study groups. There was also no difference in the body weight of the dogs in the 3 study groups (median, 13 kg; range, 3–41 kg). A predominance of females was observed in all study groups. Of the 116 dogs enrolled, 105 were females, and only 10 of these were spayed. Eleven dogs were male (only 1 neutered). There was no difference regarding gender distribution among the 3 groups. Table 1 presents the clinicopathologic data of the dogs included in each group.

The DKA group was composed of 29 newly diagnosed diabetic dogs and 14 insulin-treated (IT) dogs. There was no statistical difference between IT and untreated dogs in any study variables, with the exception of the AG, whose median was higher in IT dogs (28.0 mEq/L) than in untreated dogs (23.0 mEq/L, $P = .008$) and SUN (80 and 49 mg/dL, respectively, $P = .047$). Suspected comorbid conditions or precipitant factors for DKA in IT dogs included diestrus (n = 4); renal failure (2); mammary neoplasia (2); urinary tract infection (2); omission of insulin therapy (1); pancreatitis (1); pyoderma (1); and pyometra (1). The DK group was composed of 37 diabetic dogs without previous treatment and 4 IT dogs. Diestrus was the probable reason for decompensation in 2 of the IT dogs; in the other 2 dogs, a reason for metabolic decompensation was not identified.

Serum β-Hydroxybutyrate Measurements

Serum β-OHB concentrations differed significantly ($P < .001$) among the study groups (Fig 1). All dogs from the DKA and DK groups and 21 dogs from the NKD group had serum β-OHB concentrations above the upper limit of the reference values stated for this study (0.15 mmol/L; Fig 1B). Serum β-OHB concentrations were higher in dogs from the NKD group in relation to healthy dogs ($P < .001$).

Evaluation of the Performance of the Test and Cutoff Values

The general accuracy of the test, determined by the area under the ROC curve (Fig 2), was 0.92 ± 0.03 (±SEM).

Table 1. Laboratory values of the diabetic dogs on admission.[a]

	DKA (n = 43)	DK (n = 41)	NKD (n = 32)	Reference Values
β-OHB (mmol/L)	4.7 (2.0 to 20.2)[b]	2.1 (0.31 to 4.8)[b]	0.30 (0.01 to 1.3)	0.02 to 0.15
Venous blood pH[c]	7.228 (6.979 to 7.374)[b]	7.360 (7.300 to 7.435)	7.364 (7.301 to 7.452)	7.302 to 7.454
P_{CO_2} (mmHg)[c]	27.9 (16.0 to 43.9)[b]	36.0 (26.6 to 44.8)	38.6 (28.0 to 48.2)	29.0 to 51.6
HCO_3 (mEq/L)[c]	10.1 (4.0 to 19.3)[b]	20.3 (15.1 to 27.0)	20.5 (16.0 to 26.1)	18 to 27
BE (mEq/L)[c]	−15.1 (−26.0 to −6.5)[b]	−3.7 (−9.0 to 3.1)	−3.2 (−7.8 to 1.0)	−5 to 2
Glucose (mg/dL)	493 (307 to 742)	502 (336 to 768)	536 (333 to 612)[d]	80 to 120
SUN (mg/dL)	58 (17 to 343)[e]	31 (15 to 71)	41 (13 to 219)[d]	17 to 48
AG (mEq/L)	25 (12 to 44)[b]	17.1 (10.2 to 26.4)	15 (4.9 to 22.9)	8 to 17
E_{OSM} (mOsm/kg)	316 (281.9 to 347.8)	321 (287.7 to 345.9)	320 (279.1 to 348.7)	300 to 315

DKA, diabetic ketoacidosis; DK, diabetic ketosis; NKD, nonketotic diabetes; β-OHB, β-hydroxybutyrate; P_{CO_2}, partial pressure of C_{O_2}; HCO_3, plasma bicarbonate; BE, base excess; SUN, serum urea nitrogen; AG, anion gap; E_{OSM}, effective osmolality.

[a] Data are median (range).

[b] Significantly different ($P < .001$) from other groups.

[c] Grouping variables.

[d] Significantly different ($P < .05$) from DKA group.

[e] Significantly different ($P < .001$) from DK group.

In the 1.9- to 4.8-mmol/L range, serum β-OHB determination sensitivity varied from 100 to 35.7%, whereas specificity varied from 39 to 100%. The cutoff value of 3.8 mmol/L showed the best equilibrium between specificity (95%), sensitivity (72%), and likelihood ratio (14.8). The characteristics of several cutoff values of β-OHB for the diagnosis of DKA are shown in the Appendix, along with respective 95% confidence intervals and positive and negative likelihood ratios.

Discussion

Serum β-OHB concentrations observed in dogs with DKA in this series (range, 1.9–20.2 mmol/L) are similar to those reported in humans and cats with DKA.[9,11,14,20,21] Although differences were found in relation to the distribution of serum β-OHB concentrations among the groups, partial interpolation of values has always existed (Fig 1). No optimal dichotomization occurred that would enable the selection of a cutoff value to accurately discriminate the several stages of the disease. This overlap was not unexpected, because the metabolic abnormalities of hyperglycemic states can be viewed as a continuum progressing from the uncomplicated diabetes to overt DKA.[22] This implies that the selection of a given cutoff value should take into account the trade-offs between sensitivity and specificity (in other words, as one ''achieves'' sensitivity, one inevitably ''loses'' specificity and vice versa).

In humans, β-OHB values greater than 1.1–1.3 mmol/L are considered suggestive of DKA.[23] This is because of the observation that, in a study involving 21 patients with DKA, no patient whose serum β-OHB concentration was greater than 1.1 mmol/L recovered from acidosis.[8] Such

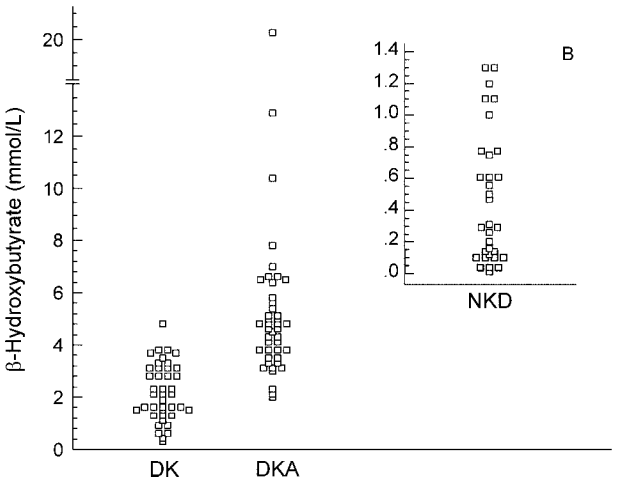

Fig 1. Serum β-hydroxybutyrate (β-OHB) concentrations from dogs with diabetic ketoacidosis (DKA) and diabetic ketosis (DK). The smaller plot **(B)** is a graphic depiction of serum β-OHB concentrations from dogs with nonketotic diabetes mellitus (NKD) compared to the reference interval (shaded area).

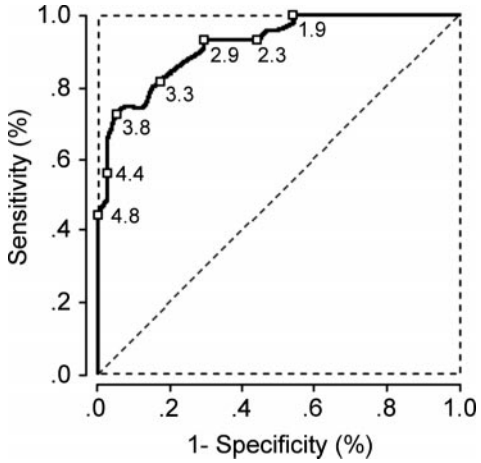

Fig 2. Receiver operating characteristic (ROC) curve of serum β-hydroxybutyrate (β-OHB) measurements for the diagnosis of diabetic ketoacidosis (DKA). Each point on the ROC curve represents a sensitivity/specificity pair corresponding to a particular cutoff value. Selected cutoff values (in mmol/L) are displayed in the plot. A test that perfectly discriminates between the 2 groups would yield a ''curve'' that coincides with the upper left corner of the plot. The diagonal, traced line represents a hypothetical test with no discriminatory value.

extrapolation may not be strictly valid, because a large number of patients develop some degree of hyperchloremic acidosis after the institution of therapy.[24] Therefore, acidosis during the remission period of DKA may not arise exclusively from ketonemia.

In this study, serum β-OHB performance as a diagnostic test for DKA was evaluated by means of the ROC curve approach. Thus, one can analyze the test performance in terms of specificity and sensitivity within a broad and ongoing range of possible cutoff values. The overall accuracy of the test was high (0.92). This means that a randomly selected individual from the DKA group will have a serum β-OHB value greater than that of a randomly chosen animal from the DK group 92% of the time.[18] Because DKA is a potentially fatal disease, the clinician may select a high sensitivity cutoff value, with β-OHB determination as a screening test for DKA (or ''possible DKA''), from which value complementary tests would be requested either to confirm or rule out the presence of ketoacidosis. The clinician may furthermore choose a high specificity cutoff value (or ''definitely DKA''), from which he or she could confidently request the relevant laboratory tests and almost simultaneously start the adequate therapy. In this series, the cutoff value of 1.9 mmol/L showed the best sensitivity (100%, with specificity = 45%), whereas the cutoff value of 3.8 mmol/L presented high specificity (95%, sensitivity = 72%) and a greater than 14 positive likelihood ratio (see Appendix for further explanation about likelihood ratios).

Sensitivity and specificity are properties inherent to the test and are independent of the prevalence of the disease.[18] Nevertheless, such properties depend on the relevance of the individuals selected for the test evaluation as well as on the criteria used to classify them into ill and not ill (the ''gold standard''). Should the criteria that comprise the gold standard for the diagnosis of DKA defined for this study be changed, the cutoff value characteristics would be changed as well. Likewise, if we look at specific strata of the sample and consider the influence of other variables, the precision of the test would be affected. For example, in the DKA group, the IT dogs have significantly higher values of AG and SUN than the untreated diabetic dogs. There was no difference, however, between IT and untreated dogs regarding serum β-OHB concentrations. Albeit speculative, it could be inferred that IT dogs have more severe acidosis, possibly due to uremic acidosis and other associated disorders. Thus, individual characteristics can influence the general accuracy of the test.[18] Moreover, we could not evaluate the influence of gender or age on the test performance because of the composition of this study population.

It might be argued that it is also important to detect DK. This is a valid concern, because DK can rapidly progress to DKA and, particularly in a previously insulin-controlled diabetic, DK may represent an early sign of metabolic deterioration.[5] But to decide above what value of β-OHB should be interpreted as impending DKA, and not merely lack of adequate insulin, is not a simple task. Part of the nonketonuric dogs (64%) in this series exhibited serum β-OHB concentrations above the upper limit of the reference values stated for this study (0.15 mmol/L). Hyperketonemia is a common finding in type 1 diabetic subjects despite insulin treatment.[25] In an early study,[26] high β-OHB values were observed in 73% of plasma specimens from human patients with poorly controlled type 1 DM. In that study, only 43% of abnormal β-OHB values were associated with simultaneous ketonuria. Supranormal β-OHB values were also found in type 1 diabetic subjects with near-normal fasting plasma glucose concentrations (<150 mg/dL).[27] If we use the ROC curve approach, comparing the DK with the NKD group (data not shown), the cutoff value of 0.30 mmol/L showed the higher sensitivity for the diagnosis of DK (100%, with specificity = 24%), whereas the cutoff value of 1.3 mmol/L showed the higher specificity (100%, with sensitivity = 81%). The overall accuracy of the β-OHB determination as a diagnostic test for DK was high (0.93 ± 0.03).

The discussion above also highlights another perspective on the clinical use of β-OHB determination. Evidence exists that β-OHB can serve as a sensitive marker of ketosis and insulin deficiency in human patients with type 1 diabetes.[23] Diurnal changes of β-OHB are strongly associated with plasma-free insulin in human IT patients.[25,27,28] More recently, Laffel et al[29] evaluated β-OHB determination in children and adults with type 1 DM and found that high β-OHB concentrations (>1 mmol/L) occurred more frequently in individuals with poor glycemic control. These investigators concluded that home monitoring of β-OHB can help patients and physicians optimize glycemic control and avert DKA. Urine ketone testing doesn't seem to be a sensitive method of assessment of poor glycemic control in dogs.[30] Therefore, the evaluation of blood β-OHB can be an additional tool in the monitoring of insulin therapy in dogs and cats; however, this issue ought to be addressed in an appropriate study design.

One of the primary limitations in this study refers to the criteria adopted to define DKA. Although DKA is normally defined as a triad composed of hyperglycemia, acidosis, and ketosis,[31] specific values for glucose, bicarbonate, and blood pH vary in the veterinary literature.[5,7] Criteria in this study are quite similar to the criteria used in other studies in cats[32,33] and those recommended for human patients.[34]

We decided to limit our criteria for diagnosis of DKA, DK, and NDK to laboratory variables, in order to generate dichotomous groups based on objective, numeric data. On the other hand, dogs with DKA frequently present conditions such as protracted vomiting, diarrhea, and dehydration that may cause mixed acid-base disturbances.[35] Therefore, because we relied on stringent laboratory indices, some dogs might have been misclassified. This could be an additional explanation for the partial overlap in β-OHB concentrations among groups. These specific subsets may be more clearly differentiated by clinical judgment, but this could be an important source of bias.

It should be noted, however, that serum biochemical and blood gas abnormalities observed in the dogs with DKA were similar to those found by other authors.[5,36] Furthermore, the diabetic dogs in NKD and DK groups showed blood pH, HCO_3, BE, and AG medians within reference ranges, which reinforces the idea that, as a rule, such animals do not have acid-base abnormalities.

Also, optimal acid-base analysis requires examination of arterial blood gas. We decided to use venous blood because sampling arterial blood is sometimes technically difficult,

demanding multiple attempts, and this could be associated with pain and hematoma formation. Although such risks are justified in the severely ill diabetic dog, they may not be so in dogs with normal circulatory status (as part of the diabetic dogs in the NKD and DK groups). Venous blood gases accurately reflect the acid-base status in dogs with normal circulatory status[37] and are considered suitable to access the degree of acidosis in human beings presenting with DKA.[38,39]

The rationale to include only IT diabetic dogs admitted with ketonuria and dogs newly diagnosed as diabetics, without previous insulin therapy, was made to simulate a population with a wide range of hyperglycemic decompensation, in which tests for ketonemia and acid-base disturbances would be warranted. We decided to exclude ''recurrence'' episodes, because this could potentially bias results for certain individual-specific characteristics. Thus, only 1 admission was considered for each dog.

The predominance of female dogs in the present series was remarkable, probably because both intact females and males are overrepresented in this study population (90%) as opposed to other series.[40–42] Intact bitches are at increased risk for the development of DM (whereas intact males are at low risk).[41] Also, DKA is believed to be diagnosed more often in female dogs, presumably due to diestrus-induced insulin resistance.[5] In a series of 21 dogs with DKA, 17 (81%) were females.[36] In our study, diestrus constituted the precipitating factor suspected in 33% of the IT dogs presenting with DKA.

Finally, it was not possible to make any statement with regard to the initial serum β-OHB concentration and its relation to the outcome of diabetic dogs, because the treatment had no systematic follow-up. Evidence exists that β-OHB measurements in human patients can provide additional information to predict hospitalization time and the occurrence of in-hospital complications.[43]

The diagnosis of DKA in the ill-appearing diabetic dog is usually straightforward. Dogs with DKA usually present with dramatic clinical manifestations, such as anorexia, vomiting, and lethargy.[7] Within such a setting, detection of ketonuria can rapidly confirm the suspicion of DKA, but it has few implications in the subsequent approach.[22,44] Practical issues arise when a diabetic dog presents with vague clinical signs, such as reduced appetite, occasional vomiting, and hyperglycemia. Should the urine ketone bodies test be positive, additional tests will be required, either to confirm or rule out the diagnosis of DKA.[5]

Ideally, initial laboratory evaluation of the dog with DKA should include urinalysis, blood glucose, CBC, urea, creatinine, serum potassium, and determination of blood gas or total Co_2.[5,31] No doubt, the decision to request such tests is burdened by the increased animal cost both to the clinician and the owner (considering the cost for tests, care-facility stay time, and the consequences, not only financial ones, of the delay to diagnose a dog with DKA).

The method herein employed proved to have good analytic performance; it is easily adaptable to automated analyzers, at a relatively low cost. Besides, the analyte has good stability in storage (β-OHB in serum is stable for 2 weeks at 4°C and for up to 2 months at −20°C),[45,46] rendering it convenient for research institutions and large hospitals. However, this method requires reagent preparation and is relatively time- and labor-consuming (eg, blood gas determination results are made available within a few minutes, with minimum technical complexity). Currently, at least 2 manufacturers provide hand-held devices for a rapid and quantitative measurement of blood β-OHB.[8,47] Such devices can be more suitable for medium- to small-size medical practice facilities and critical care services.

Our findings suggest that β-OHB determination may be a potential tool for diagnosing and monitoring ketoacidosis in diabetic dogs and therefore merits further study in the clinical setting. The considerable overlap between the DKA and DK groups may limit its use as a single laboratory index for the diagnosis of DKA with a simple cutoff value. But it is important to note that there is no single test that could be used alone for the diagnosis of DKA and that a set of values may improve the performance of the test. We cannot make conclusions about the practical value of the information we have gathered, because this study was not designed to address the clinical usefulness of the test. Therefore, the role of β-OHB determinations in the management of the diabetic dog and in the validity of these decision thresholds should be evaluated from an extensive clinical investigation.

Footnotes

[a] Vacutainer blood collection tubes SST, Becton Dickinson UK Ltd, Plymouth, UK
[b] RA-100 Clinical Analyzer, Technicon, Tarrytown, NY
[c] Procedure 310A, Sigma Chemical Co, St Louis, MO
[d] ABL 330, Radiometer, Kopenhagen, Germany
[e] OMNI 4, AVL Medical Instruments, Graz, Austria
[f] Gluketur test or Combur, Roche Diagnostics, Mannhein, Germany
[g] Microsoft Excel 97, Microsoft Corp, Redmond, CA
[h] SPSS for Windows 8.0, SPSS Inc, Chicago, IL

Acknowledgments

We wish to express our appreciation to Dr Thomas Schermerhorn (Kansas State University) for his contribution in this study. This study was supported by a grant (98/04638-8) from FAPESP.

References

1. Goldstein DE. Tests of glycemia in diabetes. Diabetes Care 1995; 18:896–909.

2. Li PK, Lee JT, Macgillivray MH, et al. Direct, fixed-time kinetic assays for β-hydroxybutyrate and acetoacetate with a centrifugal analyzer or a computer-baked spectrophotometer. Clin Chem 1980;26: 1713–1717.

3. Kinoshita O, Masuda I, Suzuki M, et al. A case of diabetic nonketotic hyperosmolar coma with an increase with plasma 3-hydroxybutyrate. Endocrinol Jpn 1991;38:465–470.

4. Adrogué HJ, Eknoyan G, Suki WK. Diabetic ketoacidosis: Role of the kidney in the acid-base homeostasis re-evaluated. Kidney Int 1984;25:591–598.

5. Feldman EC, Nelson RW. Diabetic ketoacidosis. In: Feldman EC, ed. Canine and Feline Endocrinology and Reproduction, 2nd ed. Philadelphia, PA: WB Saunders; 1996:392–421.

6. Labato MA, Manning AM. Management of the uncomplicated canine diabetic. Semin Vet Med Surg (Small Anim) 1997;12:248–258.

7. Crenshaw KL, Nichols R. Complications and concurrent disease associated with diabetic ketoacidosis and other severe forms of diabetes mellitus. Vet Clin North Am (Small Anim Pract) 1995;25:617–624.

8. Umpierrez GE, Watts NB, Phillips LS. Clinical utility of beta-hydroxybutyrate determined by reflectance meter in the management of diabetic ketoacidosis. Diabetes Care 1995;18:137–138.

9. Mercer DW, Losos FJ, Mason L, et al. Monitoring therapy with insulin in ketoacidotic patients by quantifying 3-hydroxybutyrate with a commercial kit. Clin Chem 1986;32:224–225.

10. Koch DD, Feldbruegge DH. Optimized kinetic method for automated determination of β-hydroxybutyrate. Clin Chem 1987;33: 1761–1766.

11. Nuwayhid NF, Johnson GF, Feld RD. Kinetic measurement of the combined concentrations of acetoacetate and β-hydroxybutyrate in serum. Clin Chem 1988;34:1790–1793.

12. American Diabetes Association. Tests of glycemia in diabetes. Diabetes Care 2001;24(Suppl 1):S80–S82.

13. Foreback CC. β-Hydroxybutyrate and acetoacetate levels. Am J Clin Pathol 1997;108:602–603.

14. Fulop MD, Murthy V, Michili A, et al. Serum β-hydroxybutyrate measurement in patients with uncontrolled diabetes mellitus. Arch Intern Med 1999;159:381–384.

15. Dibartola SP. Introduction to acid-base disorders acidosis. In: Dibartola SP, ed. Fluid Therapy in Small Animal Practice, 2nd ed. Philadelphia, PA: WB Saunders; 1999:189–210.

16. Schermerhorn T. Re-evaluation of the treatment of hyperosmolality in patients with diabetic ketoacidosis. In: Proceedings of the 18th American College of Veterinary Internal Medicine Forum. Seattle, WA, 2000.

17. Campbell G. Advances in statistical methodology for the evaluation of diagnostic and laboratory tests. Stat Med 1994;13:499–508.

18. Zweig MH, Campbell G. Receiver operating characteristic (ROC) plots: A fundamental evaluation tool in clinical medicine. Clin Chem 1993;39:561–577.

19. Farver TB. Concepts of normality in clinical biochemistry. In: Kaneko JJ, ed. Clinical Biochemistry of Domestic Animals, 4th ed. San Diego, CA: Academic Press; 1989:1–20.

20. Porter WP, Yao HH, Karounos DG. Laboratory and clinical evaluation of assays for β-hydroxybutyrate. Am J Clin Pathol 1997; 107:353–358.

21. Christopher MM, Broussard JD, Peterson ME. Heinz body formation associated with ketoacidosis in diabetic cats. J Vet Intern Med 1995;9:24–31.

22. Schwab TM, Hendey GW, Soliz TC. Screening for ketonemia in patients with diabetes. Ann Emerg Med 1999;34:342–346.

23. Laffel L. Ketone bodies: A review of physiology, pathophysiology and application of monitoring to diabetes. Diabetes Metab Res Rev 1999;15:412–426.

24. Okuda Y, Adrogue HJ, Field JB, et al. Counterproductive effects of sodium bicarbonate in diabetic ketoacidosis. J Clin Endocrinol Metab 1996;81:314–320.

25. Harano Y, Kosugi K, Hyosu T, et al. Ketone bodies as markers for type 1 (insulin-dependent) diabetes and their value in the monitoring of diabetic control. Diabetologia 1984;26:343–348.

26. Macgillivray MH, Li PK, Lee JT, et al. Elevated plasma β-hydroxybutyrate concentrations without ketonuria in healthy insulin-dependent diabetic patients. J Clin Endocrinol Metab 1982;54:665–668.

27. Ubukata E. Diurnal variation of blood ketone bodies in insulin-dependent diabetes mellitus and noninsulin-dependent diabetes mellitus patients: The relationship to C-peptide immunoreactivity and free insulin. Ann Nutr Metab 1991;34:333–342.

28. Edge JA, Harrys DA, Phillips PE, et al. Evidence for a role for insulin and growth hormone in overnight regulation of 3-hydroxybutyrate in normal and diabetic adolescents. Diabetes Care 1993;16: 1011–1018.

29. Laffel L, Brink S, Kaufman F, et al. Frequency of elevations in blood B-hydroxybutyrate (B-OHB) during home monitoring and association with glycemia in insulin-treated children and adults. Diabetes 2000;49(Suppl 1):A92 (abstract).

30. Briggs CE, Nelson RW, Feldman EC, et al. Reliability of history and physical examination findings for assessing control of glycemia in dogs with diabetes mellitus: 53 cases (1995–1998). J Am Vet Med Assoc 2000;217:48–53.

31. Macintire DK. Emergency therapy of diabetic crisis: Insulin overdose, diabetic ketoacidosis, and hyperosmolar coma. Vet Clin North Am (Small Anim Pract) 1995;25:639–650.

32. Crenshaw KL, Peterson ME. Pretreatment clinical and laboratory evaluation of cats with diabetes mellitus: 104 cases (1992–1994). J Am Vet Med Assoc 1996;209:943–949.

33. Bruskiewicz KA, Nelson RW, Feldman EC, Griffey SM. Diabetic ketosis and ketoacidosis in cats: 42 cases. J Am Vet Med Assoc 1997;211:188–192.

34. American Diabetes Association. Hospital admission guidelines for diabetes mellitus. Diabetes Care 2001;24(Suppl 1):S91.

35. Morais HA. Mixed acid-base disorders. In: Dibartola SP, ed. Fluid Therapy in Small Animal Practice, 2nd ed. Philadelphia, PA: WB Saunders; 1999:251–261.

36. Macintire DK. Treatment of diabetic ketoacidosis in dogs by continuous low-dose intravenous infusion of insulin. J Am Vet Med Assoc 1993;202:1266–1272.

37. Ilkiw JE, Rose RJ, Martin ICA. A comparison of simultaneously collected arterial, mixed venous, jugular venous and cephalic venous blood samples in the assessment of blood gas and acid-base status in the dog. J Vet Intern Med 1991;5:294–298.

38. Branderburg MA, Dire DJ. Comparison of arterial and venous blood gas values in the initial emergency department evaluation of patients with diabetic ketoacidosis. Ann Emerg Med 1998;31:459–465.

39. Gokel Y, Paydas S, Koseoglu Z, et al. Comparison of blood gas and acid-base measurements in arterial and venous blood samples in patients with uremic acidosis and diabetic ketoacidosis in the emergency room. Am J Nephrol 2000;20:319–323.

40. Ling GV, Lowenstine LJ, Pulley LT, et al. Diabetes mellitus in dogs: A review of initial evaluation, immediate and long-term management, and outcome. J Am Vet Med Assoc 1977;170:521–530.

41. Marmor M, Willeberg P, Glickman LT, et al. Epizootiologic patterns of diabetes mellitus in dogs. Am J Vet Res 1982;43:465–470.

42. Hess RS, Saunders HM, Van Winkle TJ, Ward CR. Concurrent disorders in dogs with diabetes mellitus: 221 cases (1993–1998). J Am Vet Med Assoc 2000;217:1166–1173.

43. Timmons JA, Myer P, Maturen A, et al. Use of beta-hydroxybutyric acid levels in the emergency department. Am J Ther 1998;5: 159–163.

44. Kitabchi AE, Wall BM. Diabetic ketoacidosis. Med Clin North Am 1995;79:9–38.

45. Christopher M, Periera J, Brigmon R, Schaer M. Automated determination of β-hydroxybutyrate for the assessment of ketoacidosis. J Vet Intern Med 1991;5:147 (abstract).

46. Hsu WS, Kao JT, Tsai KS. Fully automated assay of blood D-3-hydroxybutyrate for ketosis. J Formos Med Assoc 1993;92:336–340.

47. Byrne HA, Tieszen KL, Hollis S, et al. Evaluation of an electrochemical sensor for measuring blood ketones. Diabetes Care 2000; 23:500–503.

Appendix. Characteristics of different cutoff values of β-OHB as a diagnostic test for DKA.[a]

Criterion (mmol/L)	Sensitivity (95% CI)	Specificity (95% CI)	+LR	−LR
1.9	100.0 (100.0–100.0)	46.3 (30.7–62.6)	1.86	0.00
2.0	97.7 (87.7–99.6)	46.3 (30.7–62.6)	1.82	0.05
2.2	95.3 (84.2–99.3)	53.7 (37.4–69.3)	2.06	0.09
2.4	93.0 (80.9–98.5)	61.0 (44.5–75.8)	2.38	0.11
2.8	93.0 (80.9–98.5)	65.9 (49.4–79.9)	2.72	0.11
3.0	90.7 (77.8–97.3)	70.7 (54.5–83.9)	3.10	0.13
3.2	83.7 (69.3–93.2)	80.5 (65.1–91.2)	4.29	0.20
3.4	79.1 (64.0–89.9)	85.4 (70.8–94.4)	5.40	0.25
3.5	74.4 (58.8–86.5)	87.8 (73.8–95.9)	6.10	0.29
3.8	72.1 (56.3–84.7)	95.1 (83.4–99.3)	14.78	0.29
3.9	65.1 (49.1–79.0)	97.6 (87.1–99.6)	26.70	0.36
4.1	62.8 (46.7–77.0)	97.6 (87.1–99.6)	25.74	0.38
4.2	60.5 (44.4–75.0)	97.6 (87.1–99.6)	24.79	0.41
4.7	48.8 (33.3–64.5)	97.6 (87.1–99.6)	20.02	0.52
4.8	44.2 (29.1–60.1)	100.0 (100.0–100.0)	—	0.56

+LR, positive likelihood ratio; −LR, negative likelihood ratio.

[a] The likelihood ratio (LR) is defined as the ratio between the probability of a defined test result, given the presence of the disease, and the probability of the same test result, given the absence of the disease. By means of a specific criterion for positivity, 2 LR can be defined: (1) +LR = probability of a positive test among the diseased individuals/probability of a positive test among the nondiseased individuals; and (2) −LR = probability of a negative test among the diseased individuals/probability of a negative test among the nondiseased individuals. (Choi BC. Slopes of a receiver operating characteristic curve and likelihood ratios for a diagnostic test. Am J Epidemiol 1998;148:1127–1132.)