Evaluation of total and ionized calcium status in dogs with blastomycosis: 38 cases (1997–2006)

Laura J. Crews, DVM, MS; Leslie C. Sharkey, DVM, PhD, DACVP; Daniel A. Feeney, DVM, MS, DACVR; Carl R. Jessen, DVM, PhD, DACVR; Tammy Ruska

Objective—To determine blood ionized calcium (iCa) and serum total calcium (tCa) concentrations in dogs with blastomycosis and to evaluate whether serum tCa concentration, albumin-adjusted serum calcium concentration (AdjCa-Alb), and total protein–adjusted serum calcium concentration (AdjCa-TP) accurately predict iCa status.

Design—Retrospective case series.

Animals—38 client-owned dogs with a cytologic diagnosis of blastomycosis.

Procedures—Dogs were classified as hypocalcemic, normocalcemic, or hypercalcemic on the basis of blood iCa concentration, serum tCa concentration, AdjCa-Alb, and AdjCa-TP; classification on the basis of serum tCa concentration, AdjCa-Alb, and AdjCa-TP was compared with blood iCa concentration.

Results—Except for 2 hypercalcemic dogs, all dogs had blood iCa concentrations within the reference interval. Use of serum tCa concentration overestimated hypocalcemia in 57.9% (22/38) of dogs and underestimated hypercalcemia in 1 dog. Use of AdjCa-Alb correctly reclassified all dogs as normocalcemic that were classified as hypocalcemic on the basis of serum tCa concentration, but failed to predict hypercalcemia in 1 dog. Use of AdjCa-TP correctly reclassified all but 2 dogs as normocalcemic that were classified as hypocalcemic on the basis of serum tCa concentration, and failed to predict hypercalcemia in 1 dog. No correlation was found between blood iCa concentration and serum concentrations of tCa, total protein, and albumin; AdjCa-Alb; or AdjCa-TP.

Conclusions and Clinical Relevance—High blood iCa concentration was uncommon in dogs with blastomycosis. Hypercalcemia contributed to a low serum tCa concentration despite a blood iCa concentration within reference interval. The use of serum tCa concentration, AdjCa-Alb, and AdjCa-TP may fail to identify a small number of dogs with high blood iCa concentrations. (J Am Vet Med Assoc 2007;231:1545–1549)

Hypocalcemia may be caused by renal failure, malignancy, hypervitaminosis D, increased protein-bound calcium, and hypoadrenocorticism.1 Granulomatous disease, including blastomycosis, is considered to be a less common cause of hypercalcemia, putatively related to the production of vitamin D by stimulated macrophages.1,3 Results of studies on dogs with blastomycosis indicate that hypercalcemia is a feature of this disease. These studies rely on measurement of serum tCa concentration with or without the application of quantitative correction factors adjusting for the abnormalities of serum protein concentrations that are commonly present in dogs with blastomycosis.1-6

Calcium is present in blood in 3 fractions. Approximately 56% is iCa (unbound) and is biologically active in healthy dogs; protein-bound calcium is approximately 34%, and the fraction of calcium complexed with small anions is approximately 10% of serum tCa.7 Because protein-bound calcium is a substantial fraction of tCa, low serum protein concentrations may result in a decrease in serum tCa concentration with no change in the biologically active form. Despite the fact that the biologically active blood iCa is the most diagnostically important, routine serum biochemical testing includes only a measure of serum tCa concentration. In the absence of a measured blood iCa concentration, clinicians may use correction formulas in an attempt to estimate the impact of low serum total serum protein or albumin concentrations on serum tCa concentrations.8,9 These corrections imply that corrected serum tCa concentrations that are within the reference interval are associated with blood iCa concentrations within reference interval, and those that are outside of the reference interval are associated with an abnormal blood iCa concentration. Unfortunately, use of these correction formulas may not be justified. Results of a large study10 of dogs revealed that the use of correction factors resulted in erroneous prediction of true calcium status in more than a third of all dogs and in more than a half of dogs with renal failure. These findings imply that the previous use of correction factors to predict calcium status in dogs with blastomycosis may have biased the interpretation of results. Results of another study11 indicate that serum tCa

<table>
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<th>Abbreviations</th>
<th>Description</th>
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<tr>
<td>tCa</td>
<td>Total calcium</td>
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<tr>
<td>iCa</td>
<td>Ionized calcium</td>
</tr>
<tr>
<td>AdjCa-TP</td>
<td>Total protein–adjusted serum calcium concentration</td>
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<tr>
<td>AdjCa-Alb</td>
<td>Albumin-adjusted serum calcium concentration</td>
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From the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN 55108. Address correspondence to Dr. Sharkey.
concentration did not accurately predict the iCa status in dogs with chronic renal failure and metabolic acidosis, further demonstrating the importance of verifying abnormalities in calcium metabolism by measurement of blood iCa concentration.

The purpose of the study reported here was to evaluate the calcium status of dogs with blastomycosis by use of measurements of tCa and iCa concentrations. The use of quantitative correction factors to evaluate appropriately patient calcium status by use of serum tCa and protein concentrations, compared with concurrent measurement of blood iCa concentration, was assessed.

Criteria for Selection of Cases

Computer database searches at the University of Minnesota Small Animal Veterinary Medical Center were used to identify retrospectively dogs with a cytologic or histologic diagnosis of blastomycosis between February 1997 to November 2006. Medical records were evaluated for laboratory data and information about patient demographics, treatment, and concurrent diagnoses. Dogs were included in the study if they had measurements of tCa, total protein, and iCa concentrations determined at the University of Minnesota Veterinary Clinical Pathology Laboratory at the first hospital admission or at a subsequent hospital admission for disease progression. It was necessary to perform these measurements on the same day. Dogs were excluded if calcium concentrations were only available after the patient had been treated with an antifungal drug unless disease progression was documented in the medical record as deteriorating clinical signs or radiographic evidence of progressive disease. Records were examined for evidence of previous treatment with corticosteroids. Some dogs in the study did have a history of previous empiric treatment with antimicrobials or antiinfectives and fluid therapy prior to hospital admission. Examination of the medical records did not reveal any concurrent diagnoses that would have been expected to alter calcium status, such as neoplasia, hyper- or hypoparathyroidism, renal failure, hyper- or hypovitaminosis D, malabsorptive enteropathies, or pancreatitis.1

Procedures

Analysis of calcium concentration—Blood iCa concentration was measured by use of ion-sensitive biosensors (coefficients of variation < 3%). Blood samples were collected and handled anaerobically into syringes containing dry heparin. The protocol for use of the syringes requires filling to just below the 1-mL mark; overfilling must be avoided to prevent clotting. Serum tCa, total protein, and albumin concentrations were measured by use of spectrophotometric methods (coefficients of variation ≤ 2%).

Adjusted serum tCa concentrations (mg/dL) were calculated on the basis of serum total protein concentration (mg/dL) or serum albumin concentration (mg/dL) by use of the following formulas:10

\[
\text{AdjCa-TP} = (\text{tCa} - [0.4 \times \text{total protein}]) + 3.3
\]

\[
\text{AdjCa-Alb} = (\text{tCa} - \text{albumin}) + 3.5
\]

Calcium status—If multiple measurements were performed during hospitalization, only the first value was used to minimize the impact of treatment on data. Dogs were classified as hypercalcemic, normocalcemic, or hypocalcemic on the basis of established reference intervals. Blood iCa concentration, the established reference interval was 4.9 to 6.0 mg/dL, which is similar to published ranges.11 Several changes in reagent systems occurred during the study, including replacement of 1 chemistry analyzer with another model. Reference intervals for serum tCa, total protein, and albumin concentrations were updated appropriately with each modification of method, and patient values were categorized as high, low, or normal, compared with the appropriate reference interval for the method used. The AdjCa-Alb and AdjCa-TP were calculated, and dogs were reclassified with the corrected value to determine how many would have a change in calcium status. Classifications based on serum tCa concentration, AdjCa-Alb, AdjCa-TP, and blood iCa concentrations were compared to determine whether serum tCa concentration, AdjCa-Alb, or AdjCa-TP most accurately predicted blood iCa status.

Statistical analysis—Linear regression analysis and correlations were performed by use of statistical analysis software to determine how closely serum tCa concentration, AdjCa-Alb, and AdjCa-TP correlated with blood iCa concentration. Linear regression analysis and correlations were also performed to evaluate the relationship between the classifications of high, low, and normal for concentrations of tCa, iCa, total protein, and albumin. Correlations were performed between tCa, iCa, total protein, and albumin concentrations and survival to discharge. Significance for all analyses was defined as the probability that the null hypothesis (eg, no relationship or no difference) was rejected, although true; values of P < 0.05 were considered significant.

Results

Study population—Breed representation included 15 Labrador Retrievers; 3 mixed-breed dogs; 2 Golden Retrievers; 2 Malamutes; 2 Rottweilers; and 1 of each of the following: Australian Shepherd, Bloodhound, Boxer, Brittany Spaniel, Cocker Spaniel, Doberman Pinscher, German Shepherd Dog, German Shorthair Pointer, Great Dane, Husky, Irish Wolfhound, Sharpei, Springer Spaniel, and Weimeraner. Twenty dogs were neutered males, 6 were sexually intact males, 10 were spayed females, and 2 were sexually intact females. Findings from 22 of these dogs are presented elsewhere as a subset of data on the radiographic manifestations of pulmonary blastomycosis and on their response to medical treatment.12,13 Mean age of dogs at the time of first hospital admission was 4.4 years (median, 3.5 years; range, 7 months to 9.8 years). Mean weight of dogs at hospital admission was 32.22 kg (71 lb; median, 31.6 kg [69.5 lb]; range, 14 to 64 kg [30.8 to 140.8 lb])

Of the 38 dogs in the study, 2 dogs had been treated with antifungal treatment prior to measurement of blood iCa and serum tCa concentrations. One dog had been receiving antifungal treatment for 5 days, and the other had been receiving antifungal treatment for 5 days.
Discussion

The demographic characteristics of the population of dogs with blastomycosis in our study are similar to those in previous reports\(^5,5\) with large-breed dogs predominating. The sex distribution is similar to that in 1 study\(^7\) in which sexually intact male and neutered male dogs were overrepresented; however, results of another study\(^2\) do not demonstrate this difference. Our results indicate that an increase in the biologically active fraction of calcium is not common in dogs with blastomycosis and may occur in approximately 5% of the population admitted to a large referral institution during a 10-year period.

The 2 dogs that had hypercalcemia in our study were 4- to 5-year-old male dogs with remarkable medical histories: 1 neutered Bloodhound and 1 sexually intact Labrador Retriever. The Bloodhound had peripheral lymphadenopathy and an increase in lung sounds. Smears of the affected lymph nodes for cytologic examination revealed pyogranulomatous inflammation with large numbers of blastomycosis organisms. Serologic results of this dog for fungal infections were negative for histoplasmosis, coccidioides, and aspergillus, but positive for blastomycosis. The Labrador Retriever had coughing and enlarged lymph nodes at the time of admission; blastomycosis organisms with pyogranulomatous inflammation were observed on cytologic examination of affected lymph nodes. In both affected dogs, respiratory compromise responded to antifungal treatment and supportive care, and both dogs were discharged. Neither dog had any clinical or laboratory evidence of neoplasia, renal failure, endocrinopathy, or other potential causes of hypercalcemia other than the infection with blastomycosis.

The association between hypercalcemia and granulomatous disease appears to be well documented in the human medical literature.\(^1,13\) It has been shown that proliferating activated macrophages in the lesions of sarcoidosis convert 25-hydroxyvitamin D to the active form 1,25-dihydroxyvitamin D in an unregulated manner.\(^13\) This mechanism has been extrapolated to explain hypercalcemia in other granulomatous diseases in people; however, people with coccidioidomycosis-associated hypercalcemia did not consistently have high serum concentrations of 1,25-dihydroxyvitamin D.\(^13\) The association between hypercalcemia and granulomatous diseases in domestic animal species is not as well documented, and mechanisms have not been thoroughly investigated. In a clinical report\(^16\) of a dog with hypercalcemia associated with granulomatous lymphadenitis of undetermined etiology, serum concentrations of 1,25-dihydroxyvitamin D were high with suppressed serum parathyroid hormone concentrations and serum concentrations of parathyroid related protein were within reference interval. Those findings support the potential for high serum 1,25-dihydroxyvitamin D concentrations to contribute to hypercalcemia in dogs with granulomatous disease, but they do not confirm this mechanism in dogs with fungal disease in general, or blastomycosis in particular.\(^16\)

To our knowledge, blood iCa concentrations of dogs with blastomycosis has not been reported previously. In a study\(^9\) of 47 dogs with blastomycosis, 3 of

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Table 1—Classification of the calcium status of 38 dogs with blastomycosis determined by measurement of serum tCa concentration, blood iCa concentration, AdjCa-Alb, and AdjCa-TP.

<table>
<thead>
<tr>
<th>Classification</th>
<th>iCa (%)</th>
<th>tCa (%)</th>
<th>AdjCa-Alb (%)</th>
<th>AdjCa-TP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypocalcemic</td>
<td>2 (5.3)</td>
<td>1 (2.6)</td>
<td>1 (2.6)</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>Normocalcemic</td>
<td>36 (94.7)</td>
<td>14 (36.8)</td>
<td>37 (97.4)</td>
<td>35 (92.1)</td>
</tr>
<tr>
<td>Hypocalcemic</td>
<td>0</td>
<td>23 (60.5)</td>
<td>0</td>
<td>0</td>
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47 (6.4%) dogs had high serum tCa concentrations, whereas 2 of 40 (2.5%) dogs in our study had high serum tCa concentrations. In another study of 115 dogs with blastomycosis, a larger proportion of dogs had high serum tCa (13.7%) concentrations after the application of quantitative correction formulas for hypoalbuminemia, whereas only 1 of 38 dogs in our study had high corrected serum tCa concentrations. It is difficult to compare our data directly with that of the other study because the formula used to correct serum tCa concentrations in that study was not reported and blood iCa concentrations were not determined. Arceneaux et al reported a similar proportion of dogs with hypoalbuminemia (77% vs 81.6% in our study), but did not directly indicate how many dogs were hypo-, normo-, or hypercalcemic on the basis of serum tCa concentrations or how many had a change in calcium status after the correction formula was applied. In that study, the same reference intervals were used during a 13-year period, whereas our reference intervals were updated several times during the 10-year period and patient data were compared with the reference interval in use at the time of hospital admission.

Our results indicate that serum tCa concentrations in dogs with blastomycosis do not correlate well with blood iCa concentrations and also overestimate the number of dogs with hypocalcemia. In our study, quantitative correction factors appeared to improve the use of serum tCa concentrations to estimate concentrations of the biologically active iCa; however, use of serum tCa concentration, AdjCa-Alb, and AdjCa-TP all appeared to slightly underestimate hypercalcemia in our patient population. Despite the improved qualitative classification of normal calcium status versus hypocalcemia by use of correction factors, results of linear regression analysis did not support a strong quantitative association between the corrected calcium concentrations and blood iCa concentrations. Therefore, measurement of blood iCa concentration should be performed if a clinical rationale exists for accurate determination of calcium status.

Current textbooks present quantitative correction factors that may be used by clinicians to adjust for the potential of low serum albumin or total protein concentrations to negatively influence the serum tCa concentration via decreases in the protein-bound fraction of calcium in the blood. In presenting these formulas, authors qualify that the corrections have been validated only for dogs and that the adjustments may not accurately predict iCa status, particularly in patients with substantial acid-base abnormalities. Results of studies in dogs with chronic renal failure and a variety of other disorders indicate that iCa concentration may vary widely from serum tCa concentration and that the application of quantitative correction factors may actually cause an increase in error in predicting iCa status as high, normal, or low in some patients. To our knowledge, iCa status and the diagnostic usefulness of quantitative correction factors have not been specifically evaluated in dogs with blastomycosis until now. Our findings suggest that qualitatively, hypoalbuminemia may contribute to a low serum tCa concentration despite a blood iCa concentration that is within the reference interval, but the poor correlation between the corrected value and serum tCa concentration does not support a predictable quantitative relationship. Furthermore, the use of serum tCa concentration, AdjCa-Alb, or AdjCa-TP may fail to identify a small number of patients with high blood iCa concentrations. It is likely that the impact of protein abnormalities on the use of serum tCa concentrations to predict iCa status should be evaluated for each disease process that is characterized by altered calcium metabolism and that measurement of blood iCa concentration will usually be the best way to characterize calcium concentrations in clinical patients.

Although our study provides new and clinically relevant information about the calcium status of dogs with blastomycosis, several methodologic issues should be considered when extrapolating our data to other patient populations. First, because the University of Minnesota Veterinary Medical Center is a referral hospital, the potential exists for bias towards sicker patients or patients with more severe pulmonary involvement that may have been referred for support in the intensive care unit. Likewise, much of the iCa data were collected as part of blood gas analysis, so patients with respiratory compromise requiring blood gas monitoring may have been more likely to have blood iCa and serum tCa concentrations determined on the same day. The potential exists for previous corticosteroid treatment in a small subset of patients to have lowered calcium concentrations and decreased numbers of hypercalcemic patients. Two dogs had been treated with antifungal drugs, which may also have lowered calcium concentrations; however, previously treated dogs were only included if strong clinical evidence existed of disease progression despite treatment. None of these potential sources of bias should have substantially affected the assessment of correction factors or the use of serum tCa concentrations to predict iCa status in the study dogs, but they may have influenced the overall incidence of blastomycosis-associated hypercalcemia. Finally, collection of differing amounts of blood into the preheparinized syringes could have resulted in dilutional effects on measurement of blood iCa concentrations.

### References


