Use of a combination of routine hematologic and biochemical test results in a logistic regression model as a diagnostic aid for the diagnosis of hypoadrenocorticism in dogs

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OBJECTIVE
To assess the discriminatory value for corticosteroid-induced alkaline phosphatase (CiALP) activity and other variables that can be measured routinely on a CBC and biochemical analysis for the diagnosis of hypoadrenocorticism in dogs.

SAMPLE
Medical records of 57 dogs with confirmed hypoadrenocorticism and 57 control dogs in which hypoadrenocorticism was suspected but ruled out.

PROCEDURES
A retrospective case-control study was conducted. Dogs were included if a CBC and complete biochemical analysis had been performed. Dogs with iatrogenic hypoadrenocorticism and dogs treated previously with glucocorticoids were excluded. Cortisol concentration for dogs with hypoadrenocorticism was ≤ 2 μg/dL both before and after ACTH administration. Cortisol concentration for control dogs was > 4 μg/dL before or after ACTH administration.

RESULTS
Area under the receiver operating characteristic (ROC) curve for CiALP activity was low (0.646; 95% confidence interval, 0.494 to 0.798). Area under the ROC curve for a model that combined the CiALP activity, Na-to-K ratio, eosinophil count, activity of creatine kinase, and concentrations of SUN and albumin was high (0.994; 95% confidence interval, 0.982 to 1.000). Results for this model could be used to correctly classify all dogs, except for 1 dog with hypoadrenocorticism and no electrolyte abnormalities.

CONCLUSIONS AND CLINICAL RELEVANCE
CiALP activity alone cannot be used as a reliable diagnostic test for hypoadrenocorticism in dogs. Combined results for CiALP activity, Na-to-K ratio, eosinophil count, creatine kinase activity, and concentrations of SUN and albumin provided an excellent means to discriminate between hypoadrenocorticism and diseases that mimic hypoadrenocorticism. (*Am J Vet Res* 2017;78:1171–1181)

Hypoadrenocorticism (Addison disease) is an uncommon disease in dogs and has been referred to as the great pretender because its clinical manifestation often mimics that of other common diseases.[1] Most dogs are affected by primary hypoadrenocorticism caused by immune-mediated destruction of all layers of the adrenal cortex, which results in deficiencies of glucocorticoid (cortisol) and mineralocorticoid (aldosterone).[2] Typically, dogs with hypoadrenocorticism have hyponatremia and hyperkalemia (MGDH). However, dogs with hypoadrenocorticism sometimes have no alterations in Na and K concentrations (GDH). Dogs with GDH represent a minority (4% to 24%) of the cases, but identifying GDH is a major diagnostic challenge.[3–5]

ABBREVIATIONS

AIC	Akaike information criterion
ALP	Alkaline phosphatase
AST	Aspartate aminotransferase
AUC	Area under the curve
CI	Confidence interval
CiALP	Corticosteroid-induced alkaline phosphatase
GDH	Glucocorticoid-deficient hypoadrenocorticism
MGDH	Mineralocorticoid- and glucocorticoid-deficient hypoadrenocorticism
ROC	Receiver operating characteristic

The criterion-referenced standard for the diagnosis of hypoadrenocorticism is the ACTH stimulation test.[6,7] Measurement of the basal cortisol concentration was established as an effective screening test to rule out hypoadrenocorticism in dogs.[6,7] A basal cortisol concentration of < 2 μg/dL has a high sensitivity (100%) and also a high negative-predictive value for ruling out hypoadrenocorticism in dogs that are not receiving corticosteroids, mitotane, or ketoconazole.[6,7] However, measurement of cortisol concen-

trations is not typically conducted unless hypoadrenocorticism is already suspected, which makes it less useful as an initial screening test.

Various routine hematologic and biochemical variables have been evaluated in an attempt to improve the screening process and optimize decision making regarding cortisol measurements. A Na-to-K ratio of < 27 is useful as a screening value for MGDH but not for GDH.[8] Hypocholesterolemia, hypoalbuminemia, and lack of a stress leukogram were suggested as markers for GDH, but their use as screening variables were not assessed.[4] In 1 study,[8] the total lymphocyte count combined with the Na-to-K ratio provided more accurate screening for hypoadrenocorticism, compared with screening by use of the Na-to-K ratio alone or the lymphocyte count alone.

Corticosteroid-induced ALP is one of several isoenzymes of ALP.[9–12] Typically, CiALP is detected in canine serum after dogs have had prolonged exposure to endogenous or exogenous glucocorticoids.[9] Elevations in CiALP activity have been reported for dogs with hyperadrenocortisolism, diabetes mellitus, hypothyroidism, and other chronic diseases that affect the hypothalamic-pituitary-adrenal axis.[10,13–15] Thus, high CiALP activity is a sensitive but not specific test for hyperadrenocortisolism, which restricts its use to that of a screening variable.[10,13,16]

The objective of the study reported here was to assess the diagnostic value of CiALP activity and other variables that are routinely measured on a CBC and biochemical analysis of dogs suspected of having hypoadrenocorticism. Because of the effect of stress during chronic disease on CiALP activity and the fact that dogs affected by hypoadrenocorticism are cortisol deficient, we hypothesized that CiALP activity would be within the reference interval in dogs with hypoadrenocorticism and therefore would be a good means to discriminate between hypoadrenocorticism and the diseases it mimics. The CiALP activity can be measured by automated biochemical analyzers as a part of routine biochemical analysis, which makes it a good candidate to use to screen for hypoadrenocorticism (particularly GDH). We further hypothesized that the combination of CiALP activity with results of other routine hematologic tests (eg, lymphocyte count) would yield an improved discriminatory test for hypoadrenocorticism in dogs.

Materials and Methods

Sample

The study was conducted at The Ohio State University. Medical records of The Ohio State University Veterinary Medical Center from 2005 through 2013 were reviewed. Dogs were excluded from the study if they had a history of corticosteroid administration (any formulation administered via any route), they had a history of treatment with drugs that affect the adrenal cortex (eg, ketoconazole or trilostane), the diagnosis was hyperadrenocortisolism, they had a history of preadmission fluid therapy, or the basal cortisol concentration was < 2 µg/dL but an ACTH stimulation test was not performed.

Hypoadrenocorticism group—Discharge summary reports were searched for a diagnosis of hypoadrenocorticism or Addison disease to create an initial study population. Dogs were included in the hypoadrenocorticism group if their basal serum cortisol concentration and serum cortisol concentration at 1 hour after ACTH administration (cosyntropin; 5 µg/kg, IV) were both ≤ 2 µg/dL and if their record included results for a CBC and biochemical analysis. Cortisol concentrations < 1 µg/dL (below the limit of detection) were recorded as 0.9 µg/dL.

A total of 133 dogs with hypoadrenocorticism were identified. Seventy-six dogs were excluded; reasons for exclusion included iatrogenic hypoadrenocorticism (n = 6 dogs), prior treatment for hyperadrenocortisolism (21), current treatment for hypoadrenocorticism (19), prior treatment with corticosteroids (7), incorrect diagnosis coding (13), or incomplete data (10). Thus, 57 dogs met the inclusion criteria and were subsequently subcategorized as having GDH (n = 20 dogs) or MGDH (37). Dogs with GDH were defined as those in which Na and K concentrations were within the laboratory's reference interval and the Na-to-K ratio was > 27.

Control group—Control dogs were defined as patients for which the attending clinician suspected hypoadrenocorticism (as noted in the case assessment in the medical record), but the diagnosis of hypoadrenocorticism was subsequently refuted. Control dogs had clinical signs (eg, vomiting, diarrhea, weakness, or lethargy) routinely seen in dogs with hypoadrenocorticism. Control dogs were identified through a search of The Ohio State University Veterinary Medical Center Clinical Pathology Laboratory database from 2005 through 2013 to identify dogs for which cortisol concentration was measured. Dogs included in the control group had a basal cortisol concentration or a post-ACTH cortisol concentration (or both) ≥ 4 µg/dL. The more stringent cutoff value of ≥ 4 µg/dL was chosen over the previously reported[17] cutoff value of 2 µg/dL because measurement of cortisol concentration differs among laboratories. Use of a cutoff value of 2 µg/dL (which was generated by another laboratory) might have resulted in misclassification of some dogs, and we wanted to ensure the exclusion of borderline cases.

The medical record search yielded 360 dogs for which a basal cortisol concentration was measured during the study period. The list was sorted alphabetically on the basis of each owner's last name, and the first 57 dogs that met the inclusion criteria for the control group were selected.

Procedures

All medical records were reviewed by 1 author (SB-C). Signalment, body weight, clinical signs (historical as well as those detected during physical ex-

amination), results of a CBC and serum biochemical analysis, and cortisol concentrations were recorded for each dog and tabulated for statistical analysis. Final diagnosis, when available, was recorded for the control group. Laboratory data for both groups were recorded for the day on which the diagnosis was made (as determined on the basis of cortisol concentrations) or the closest measurement obtained within 7 days of that day. Blood samples were analyzed by use of an automated hematology analyzer[a] and biochemical analyzer,[b] and WBC differential counts were performed manually. Cortisol concentrations were measured by use of an automated chemiluminescence analyzer.[c]

Statistical analysis

Categorical variables (clinical signs, age, age group [≤ 6 months, > 6 months to ≤ 3 years, > 3 years to ≤ 9 years, and > 9 years], and sex) were compared by use of a Pearson χ^2 test. To determine the manner in which results for individual biochemical and CBC variables were related to hypoadrenocorticism, each variable was assessed separately. The variables were not normally distributed; therefore, the nonparametric Mann-Whitney *U* test was used to individually compare continuous variables between all dogs with hypoadrenocorticism and the control dogs and between dogs with GDH and the control dogs. Variables that differed significantly ($P < 0.05$) between the hypoadrenocorticism (or GDH) dogs and control dogs in this initial analysis were assessed further by use of an ROC curve analysis, which was performed by calculating the sensitivity and specificity for detection of hypoadrenocorticism at various cutoff values. The AUC and 95% CI were calculated for each ROC curve.

After individual biochemical and CBC variables were assessed, the discriminatory power of combinations of these variables was evaluated in a second analysis. Differences in the likelihood of being a hypoadrenocorticism-positive dog were estimated by use of a logistic regression model.[d] Variables were selected from the CBC and biochemical variables by use of the purposeful selection method.[18] All predictor variables significant in individual analysis were included in the model selection process. Because some variables were not measured for all dogs, 3 control dogs, 3 dogs with MGDH, and 5 dogs with GDH were excluded from the regression analysis.

Logistic regression models were developed in a forward stepwise manner. Each model with only 1 laboratory variable was fit. The best laboratory variable (determined on the basis of the AIC) was retained in the model. All models with 2 laboratory variables that included the initially retained variable were fit, and the best 2-variable model (as determined on the basis of the AIC) was retained. Additional variables were then added to that 2-variable model. Once the addition of additional variables did not improve the model (candidate model), individual variables were removed from that model, and interactions of variables in that model were included and the results compared (by use of the AIC) with the candidate model; thus, it was determined whether the addition or removal of a variable changed the effects of other variables. Variables that improved the model prediction were retained. Regression coefficients and their 95% CIs were reported for each predictor variable in the final model; *P* values for the significance of the individual predictor variables were also reported.

The final model was designed to determine the likelihood of hypoadrenocorticism for a specific dog on a continuum of probabilities. Utility of the model as a binary test was analyzed. A probability of ≥ 50% was defined as a positive result for hypoadrenocorticism, and a probability of < 50% was defined as a negative result for hypoadrenocorticism. By use of this approach, sensitivity and specificity of the model were calculated and an ROC curve was constructed. The final predictive regression model was described and compared with other intuitive perturbations to the model by use of the AICs calculated for the other models.[19] This was followed by calculation of the AUC as well as the optimal cutoff value, sensitivity, specificity, and 95% bootstrap CI for the other models.[20]

Results

Sample

The control group comprised 57 dogs that included Labrador Retrievers (n = 6 dogs), German Shepherd Dogs (3), Golden Retrievers (3), Shih Tzus (3), Rottweilers (2), Boxers (2), Greyhounds (2), and mixed-breed dogs (12). Other breeds were each represented by 1 dog. A total of 57 dogs with hypoadrenocorticism were identified. The hypoadrenocorticism group comprised 57 dogs that included Standard Poodles (n = 7 dogs), Beagles (3), German Shepherd Dogs (2), Golden Retrievers (2), Miniature Pinschers (2), Saint Bernards (2), and mixed-breed dogs (15). Other breeds were each represented by 1 dog. Of the 57 dogs with hypoadrenocorticism, 20 were identified with GDH. The group with GDH included 10 large-breed dogs, 4 of which were Standard Poodles.

Signalment and clinical signs

The final diagnoses (as recorded in the medical record) for the control group were chronic large bowel diarrhea (n = 11 dogs), gastroenteritis (8), hemorrhagic gastroenteritis (5), protein-losing enteropathy (4), idiopathic megaesophagus (2), inflammatory bowel disease (2), gastrointestinal ulceration (2), acute renal failure (2), myasthenia gravis (1), hepatic microvascular dysplasia (1), Fanconi syndrome (1), exocrine pancreatic insufficiency (1), colitis (1), gastritis (1), hydrocephalus (1), and chronic kidney disease (1). Thirteen dogs were examined because of vomiting (10 chronic and 3 acute), and a final diagnosis was not specified.

No significant differences were found between all dogs with hypoadrenocorticism and the control

Table 1—Comparison of age groups for dogs with hypoadrenocorticism (including MGDH and GDH), dogs with GDH (a subset of all dogs with hypoadrenocorticism), and control dogs on the basis of data collected retrospectively from review of medical records of dogs suspected of having hypoadrenocorticism that were examined at The Ohio State University Veterinary Medical Center between 2005 and 2013.

	All dogs with hypoadrenocorticism (n = 57)		Dogs with GDH (n = 20)		Control dogs (n = 57)	
Age (mo)	Number (%)	Median (range)	Number (%)	Median (range)	Number (%)	Median (range)
≤ 6	0 (0)	—	0 (0)	—	3 (5.3)	5 (4–6)
> 6 to ≤ 36	14 (24.6)	23.9 (16–35)	1 (5.0)	31 (—)	12 (21.1)	16.0 (8–24)
> 36 to ≤ 108	35 (61.4)	65.3 (36–104)	13 (65.0)	68.9 (42–96)	28 (49.1)	68.1 (36–96)
> 108	8 (14.0)	136.5 (108–156)	6 (30.0)	136.0 (108–156)	14 (24.6)	132.4 (108–156)

— = Not applicable.

Table 2—Comparison of clinical signs and sex among dogs with hypoadrenocorticism, dogs with GDH, and control dogs on the basis of data collected retrospectively from review of medical records of dogs suspected of having hypoadrenocorticism that were examined at The Ohio State University Veterinary Medical Center between 2005 and 2013.

Variable	All dogs with hypoadrenocorticism (n = 57)	Dogs with GDH (n = 20)	Control dogs (n = 57)
Clinical signs			
Vomiting	40 (70.2)	8 (40.0)	41 (71.9)
Anorexia	40 (70.2)	9 (45.0)	10 (17.5)*†
Lethargy	39 (68.4)	7 (35.0)	20 (35.1)*
Diarrhea	20 (35.1)	4 (20.0)	26 (45.6)†
Weakness	13 (22.8)	3 (15.0)	4 (7.0)*
Adipsia	12 (21.1)	4 (20.0)	3 (5.3)*
Signs of depression	10 (17.5)	2 (10.0)	1 (1.8)*
Regurgitation	3 (5.3)	1 (5.0)	8 (14.0)
Tremors	3 (5.3)	0 (0)	1 (1.8)
Sex			
Spayed female	30 (52.6)	12 (60.0)	27 (47.4)
Castrated male	22 (38.6)	7 (35.0)	24 (42.1)
Sexually intact female	3 (5.3)	1 (5.0)	1 (1.8)
Sexually intact male	2 (3.5)	0 (0)	5 (8.8)

Values reported are number (percentage).

*Value differs significantly ($P < 0.05$) from the value for all dogs with hypoadrenocorticism. †Value differs significantly ($P < 0.05$) from the value for dogs with GDH.

dogs or between dogs with GDH and the control dogs with regard to age **(Table 1)**. Similarly, body weight did not differ significantly between all dogs with hypoadrenocorticism (median, 20.0 kg; range, 3.2 to 54 kg) and the control dogs (median, 21.0 kg; range, 3.2 to 54 kg) or between dogs with GDH (median, 21.3 kg; range, 3.2 to 56 kg) and the control dogs. Sex also did not differ between all dogs with hypoadrenocorticism and the control dogs or between dogs with GDH and the control dogs **(Table 2)**. Historical and physical examination findings did not differ significantly between all dogs with hypoadrenocorticism and the control dogs, except for anorexia ($P < 0.001$), lethargy ($P = 0.001$), signs of depression ($P = 0.002$), weakness ($P = 0.012$), and adipsia ($P = 0.008$), which were significantly more common in dogs with hypoadrenocorticism (combined MGDH and GDH) than in the control dogs.

Initial medical concerns and clinical signs were similar for dogs with GDH, compared with those for control dogs, except for anorexia (which was significantly [$P = 0.008$] more common) and diarrhea (which was significantly [$P = 0.010$] less common) for the GDH group (Table 2). No differences between dogs with GDH and the control dogs were found for age ($P = 0.122$), body weight ($P = 0.349$), and sex ($P = 0.262$).

Clinicopathologic findings

Selected CBC and biochemical variables were compared among all dogs with hypoadrenocorticism, dogs with GDH, and the control dogs **(Tables 3 and 4)**. Variables that were significantly different among groups were further analyzed for their discriminatory value as a single screening variable by use of ROC curves **(Table 5)**.

Sensitivity and specificity of selected variables for diagnosis of hypoadrenocorticism

Three cutoff values (≤ 12, ≤ 36, and ≤ 66 U/L, which corresponded to 2-, 6-, and 11-fold increases

Table 3—Comparison of selected CBC variables among dogs with hypoadrenocorticism, dogs with GDH, and control dogs on the basis of data collected retrospectively from review of medical records of dogs suspected of having hypoadrenocorticism that were examined at The Ohio State University Veterinary Medical Center between 2005 and 2013.

			All dogs with hypoadrenocorticism		Dogs with GDH		Control dogs	
Variable	Refererence interval	Category	n	Median (range)	n	Median (range)	n	Median (range)
Hct (%)	36–54	NA	56	47.0 (27–67)	19	36.5 (27–61)	55	43.2 (18–62)†
		< RI		10 (17.8)		6 (31.6)		10 (18.2)
		= RI		37 (66.1)		12 (63.2)		43 (78.2)
		> RI		9 (16.1)		1 (5.2)		2 (3.6)
WBCs (X 10^3 cells/µL)	4.1–15.2	NA	56	12.1 (5.9–39.1)	19	14.0 (6.1–39.1)	55	10.5 (5.8–47.1)
		< RI		0 (0)		0 (0)		0 (0)
		= RI		40 (71.4)		13 (68.4)		43 (78.2)
		> RI		16 (28.6)		6 (31.6)		12 (21.8)
Neutrophils (X 10^3 cells/µL)	3.0–10.4	NA	55	7.28 (3.4–31.4)	18	8.4 (3.4–31.4)	55	7.1 (3.5–40.0)
		< RI		0 (0)		0 (0)		0 (0)
		= RI		42 (76.4)		11 (61.1)		39 (70.9)
		> RI		13 (23.6)		7 (38.9)		16 (29.1)
Lymphocytes (X 10^3 cells/µL)	1.0–4.6	NA	55	2.9 (0–6.9)	18	2.8 (0–5.3)	55	1.4 (0–5.8)*†
		< RI		8 (14.5)		3 (16.7)		15 (27.3)
		= RI		36 (65.5)		14 (77.8)		38 (69.1)
		> RI		11 (20.0)		1 (5.5)		2 (3.6)
Lymphocyte-to-neutrophil ratio	NA	NA	55	0.37 (0–1.54)	18	0.34 (0.11–0.96)	55	0.17 (0–1.40)*
		< RI		NA		NA		NA
		= RI		NA		NA		NA
		> RI		NA		NA		NA
Monocytes (X 10^3 cells/µL)	0 –1.2	NA	55	0.6 (0–4.89)	18	0.6 (0–4.89)	55	0.6 (0–3.40)
		< RI		0 (0)		0 (0)		0 (0)
		= RI		47 (85.5)		14 (77.8)		49 (89.1)
		> RI		8 (14.5)		4 (22.2)		6 (10.9)
Eosinophils (X 10^3 cells/µL)	0–1.3	NA	55	0.7 (0–10.6)	18	1.0 (0–2.9)	55	0.3 (0–2.6)*†
		< RI		0 (0)		0 (0)		0 (0)
		= RI		45 (81.8)		14 (77.8)		51 (92.7)
		> RI		10 (18.2)		4 (22.2)		4 (7.3)

For each variable, the number (percentage) of dogs with results less than the reference interval (< RI), within the reference interval (= RI), and greater than the reference interval (> RI) is reported.

NA = Not applicable.

See Table 2 for remainder of key.

above the reference interval) were used to calculate sensitivity and specificity of CiALP activity for diagnosis of hypoadrenocorticism. Sensitivity for diagnosis of all dogs with hypoadrenocorticism (MGDH and GDH) was 82%, 94%, and 98%, respectively, for the 3 cutoff values. Sensitivity for diagnosis of GDH was 81%, 100%, and 100%, respectively, for the 3 cutoff values. Specificity (ie, 100 X true negative result/total No. of control dogs) was 40%, 18%, and 14%, respectively, for the 3 cutoff values. No cutoff value yielded a clinically useful combination that had both high sensitivity and high specificity. In contrast, when an abnormally high creatine kinase activity (> 400 U/L) was used for the diagnosis of hypoadrenocorticism, sensitivity was 49% for all dogs with hypoadrenocorticism and 71% for dogs with GDH, and specificity was 91% for both groups.

Basal cortisol concentration ≤ 2.0 µg/dL was an inclusion criterion for dogs with hypoadrenocorticism in the study; therefore, sensitivity for this cutoff value was set to 100%, and the overall accuracy was artificially increased. For the control dogs, 18 of 57 had a basal cortisol concentration ≤ 2.0 µg/dL, which resulted in specificity of 68.4% and overall accuracy of 84.0%. When only dogs that were eventually included in the regression analysis were considered, 17 of 54 control dogs had a basal cortisol concentration ≤ 2.0 µg/dL, which resulted in specificity and accuracy that were virtually unchanged (68.5% and 83.5%, respectively).

Sensitivity and specificity of the logistic regression model for diagnosis of hypoadrenocorticism

Model 1 included the eosinophil count, Na-to-K ratio, SUN concentration, albumin concentration, creatine kinase activity, CiALP activity, and interaction between CiALP activity and creatine kinase activity **(Table 6)**. Cholesterol concentration modified the effect of the Na-to-K ratio, but was not itself significant. Because the primary purpose of the model was prediction of hypoadrenocorticism, and the addition of cholesterol concentration made no difference for the prediction, cholesterol concentration was excluded from the final model. Because of the interaction term and differing scales for the variables included in the

Table 4—Comparison of selected biochemical variables among dogs with hypoadrenocorticism, dogs with GDH, and control dogs on the basis of data collected retrospectively from review of medical records of dogs suspected of having hypoadrenocorticism that were examined at The Ohio State University Veterinary Medical Center between 2005 and 2013.

			All dogs with hypoadrenocorticism		Dogs with GDH		Control dogs	
Variable	Reference interval	Category	n	Median (range)	n	Median (range)	n	Median (range)
Creatinine (mg/dL)	0.6–1.4	NA	57	1.6 (0.6–5.3)	20	1.2 (0.6–2.8)	56	0.9 (0.2–5.5)*†
		< RI		0 (0)		0 (0)		0 (0)
		= RI		23 (40.4)		13 (65.0)		49 (87.5)
		> RI		34 (59.6)		7 (35.0)		7 (12.5)
SUN (mg/dL)	5–20	NA	57	39.5 (6.0–113.0)	20	25.0 (14.0–55.0)	56	14.0 (5.0–112)*†
		< RI		0 (0)		0 (0)		0 (0)
		= RI		13 (22.8)		6 (30.0)		42 (75.0)
		> RI		44 (77.2)		14 (70.0)		14 (25.0)
Na-to-K ratio	< 27	NA	57	23.5 (12.0–41.0)	20	32.9 (27.3–41.0)	57	33.2 (20.3–47.0)*
		< 27		38 (66.7)		0 (NA)		4 (07.0)
		> 27		19 (33.3)		20 (100)		53 (93.0)
ALT (U/L)	10–55	NA	57	66.5 (16–238)	20	76.0 (26–144)	57	40.0 (7–1,058)†
		< RI		0 (NA)		0 (NA)		1 (01.8)
		= RI		23 (40.4)		6 (30.0)		36 (63.1)
		> RI		34 (59.6)		14 (70.0)		20 (35.1)
AST (U/L)	12–40	NA	52	61.5 (19–873)	17	82.0 (24–873)	57	28.0 (13–184)*†
		< RI		0 (0)		0 (0)		0 (0)
		= RI		12 (23.1)		4 (23.5)		42 (73.7)
		> RI		40 (76.9)		13 (76.5)		15 (26.3)
ALP (U/L)	15–120	NA	57	40.0 (16–525)	20	45.0 (27–525)	57	57.0 (7–2,119)†
		< RI		0 (0)		0 (0)		2 (03.5)
		= RI		52 (91.2)		18 (90.0)		42 (73.7)
		> RI		5 (8.8)		2 (10.0)		13 (22.8)
CiALP (U/L)	0–6	NA	50	2.0 (0–144)	15	3.0 (0–35)	57	7.0 (0–2,021)*†
		< RI		NA		NA		NA
		= RI		40 (80.0)		11 (73.3)		27 (47.4)
		> RI		10 (20.0)		4 (26.7)		30 (52.6)
CK (U/L)	50–400	NA	50	367.5 (75–2,969)	16	755.5 (75–2,152)	56	158.0 (48–3,373)*†
		< RI		0 (0)		0 (0)		1 (01.8)
		= RI		26 (52.0)		5 (31.3)		50 (89.3)
		> RI		24 (48.0)		11 (68.7)		5 (08.9)
Cholesterol (mg/dL)	80–315	NA	55	121.0 (27–393)	18	116.0 (62–277)	56	217.5 (86–660)*†
		< RI		11 (20.0)		5 (27.8)		0 (0)
		= RI		43 (78.2)		13 (72.2)		44 (78.6)
		> RI		1 (01.8)		0 (0)		12 (21.4)
Albumin (g/dL)	2.9–4.2	NA	57	2.95 (1.4–4.5)	20	2.6 (1.4–3.5)	56	3.5 (0.9–4.4)*†
		< RI		26 (45.6)		15 (75.0)		12 (21.4)
		= RI		30 (52.6)		5 (25.0)		43 (76.8)
		> RI		1 (01.8)		0 (0)		1 (01.8)
Glucose (mg/dL)	77–126	NA	56	92.0 (28–684)	20	102.5 (34–318)	55	101.0 (64–227)
		< RI		19 (33.9)		5 (25.0)		3 (05.5)
		= RI		30 (53.6)		13 (65.0)		50 (90.9)
		> RI		7 (12.5)		2 (10.0)		2 (03.6)
Basal cortisol concentration (µg/dL)	1–11	NA	57	0.9 (0.9–1.8)	20	0.9 (0.9–0.9)	57	4.1 (0.9–16.5)
		≤ 2		5 (0.9)		20 (100)		18 (31.6)
		2–4		0 (0)		0 (0)		10 (17.5)
		> 4		0 (0)		0 (0)		29 (50.1)
Cortisol concentration after ACTH (µg/dL)	5–26	NA	57	0.93 (0.9–1.8)	20	0.9 (0.9–1.5)	29	13.2 (4.3–46.2)
		≤ 2		57 (100)		20 (100)		0 (0)
		2–4		0 (0)		0 (0)		0 (0)
		> 4		0 (0)		0 (0)		29 (100)

ALT = Alanine aminotransferase. CK = Creatine kinase.
See Tables 2 and 3 for remainder of key.

model, the coefficients could not be easily translated to useful ORs.

The likelihood of a positive diagnosis of hypoadrenocorticism was estimated for all study dogs for which predictor variable data were available (n = 103); estimations were determined by use of the aforementioned final logistic regression model (model 1). The resulting distribution of probabilities were plotted to illustrate the model's discriminatory power for differentiating dogs with hypoadrenocorticism or

Table 5—Hematologic and biochemical variables that could be used individually to discriminate between dogs with hypoadrenocorticism and control dogs and between dogs with GDH and control dogs on the basis of data collected retrospectively from review of medical records of dogs suspected of having hypoadrenocorticism that were examined at The Ohio State University Veterinary Medical Center between 2005 and 2013.

	Dogs with hypoadrenocorticism vs control dogs		Dogs with GDH vs control dogs	
Variable	***P* value***	**AUC (95% CI) for ROC curve**	***P* value***	**AUC (95% CI) for ROC curve**
Hematologic				
Lymphocyte count	< 0.001	0.754 (0.660–0.849)	0.010	0.727 (0.575–0.880)
Lymphocyte-to-neutrophil ratio	< 0.001	0.714 (0.615–0.812)	NS	ND
Eosinophil count	< 0.001	0.696 (0.597–0.795)	0.009	0.700 (0.546–0.855)
Hct	NS	ND	0.040	0.678 (0.519–0.837)
Biochemical				
SUN concentration	< 0.001	0.845 (0.771–0.919)	0.001	0.798 (0.697–0.899)
Creatinine concentration	< 0.001	0.820 (0.717–0.888)	0.025	0.698 (0.549–0.847)
Na-to-K ratio	< 0.001	0.834 (753–0.915)	NS	ND
AST activity	< 0.001	0.798 (0.713–0.883)	< 0.001	0.812 (0.686–0.938)
CK activity	< 0.001	0.782 (0.691–0.873)	< 0.001	0.795 (0.651–0.940)
Cholesterol concentration	< 0.001	0.811 (0.731–0.890)	< 0.001	0.856 (0.750–0.962)
CiALP activity	0.001	0.671 (0.569–0.774)	0.049	0.646 (0.494–0.798)
Albumin concentration	0.032	0.622 (0.518–0.727)	< 0.001	0.772 (0.640–0.903)
ALT activity	NS	ND	0.003	0.636 (0.478–0.793)

Data represent results for 57 dogs with hypoadrenocorticism, 20 dogs with GDH, and 57 control dogs.
*Values were considered significant at $P < 0.05$.
ND = Not determined. NS = Not significant.

Table 6—Results for multivariable logistic regression models for discrimination of dogs with hypoadrenocorticism and control dogs.

Model	Variable	Coefficient value	95% CI	*P* value
1	Intercept	19.8989	7.4763 to 43.3548	0.014
	Na-to-K ratio	–0.6532	–1.3076 to –0.3350	0.003
	CK activity	0.0081	0.0034 to 0.0178	0.010
	Eosinophil count	0.0027	0.0009 to 0.0061	0.021
	SUN concentration	0.1817	0.0429 to 0.4233	0.035
	Albumin concentration	–2.4255	–5.2966 to –0.4405	0.037
	CiALP activity	0.0167	–0.1222 to 0.0655	0.774
	CiALP activity X CK activity	–0.0003	–0.0006 to –0.0001	0.036
2	Intercept	16.3240	7.6594 to 28.3270	0.001
	Na-to-K ratio	–0.4859	–0.8259 to –0.2621	< 0.001
	CK activity	0.0017	0.0004 to 0.0033	0.010
	Eosinophil count	0.0021	0.0007 to 0.0042	0.016
	SUN concentration	0.1696	0.0571 to 0.3269	0.010
	Albumin concentration	–2.2911	4.1533 to –0.8721	0.004

Models were developed by use of results for 103 dogs (49 dogs with hypoadrenocorticism and 54 control dogs) examined at The Ohio State University Veterinary Medical Center between 2005 and 2013.

GDH from control dogs that did not have hypoadrenocorticism **(Figure 1)**. By use of a probability of ≥ 50% as a positive diagnosis and < 50% as a negative diagnosis, sensitivity and specificity of the model were calculated and an ROC curve was constructed **(Table 7)**. For the ROC curve, the AUC was 99.4 (95% CI, 98.2 to 100), with high sensitivity (97.96%; 95% CI, 93.90% to 100%) and high specificity (100%; 95% CI, 100% to 100%). Only 1 dog was misclassified (a dog with GDH that was classified as negative for hypoadrenocorticism). Thus, sensitivity of the model for the diagnosis of GDH was 93.3%.

Because CiALP activity is not routinely included in the biochemical panel of most reference laboratories, we evaluated the same variables as in model 1 but removed CiALP activity (model 2) to assess use for a more common clinical setting (Table 6). Exclusion of CiALP activity changed the model coefficients slightly, but model 2 was highly effective for use in discriminating dogs with hypoadrenocorticism from control dogs (AUC, 98.4; 95% CI, 96.2 to 100) but with a lower sensitivity than for model 1 (Table 7).

Exclusion of the Na-to-K ratio from the variables of model 1 resulted in model 3, which had a lower AUC, lower sensitivity and specificity, and overall lower fit (increased AIC) than for model 1 (Table 7). Use of model 3 resulted in misclassification of 7 control dogs and 2 dogs with hypoadrenocorticism. Of

these 2 dogs with hypoadrenocorticism, 1 had MGDH and the other had GDH and was the dog misclassified by the use of model 1; thus, removing the Na-to-K ratio from model 1 did not result in further misclassification of GDH dogs.

Discussion

The goal of the study reported here was to refine the decision-making process of clinicians who choose to measure cortisol concentrations of dogs suspected of having hypoadrenocorticism. We found that inclusion of a combination of variables from the CBC and biochemical analysis in a relatively simple logistic regression model (model 1) had greater diagnostic value than for any single variable alone. We also found that model 1 had greater discriminatory power than did basal cortisol concentration (albeit with a lower sensitivity) for the study population. Model 1 consisted of variables that differed significantly between dogs with hypoadrenocorticism and control dogs, but the absolute differences for each variable were small. Also, the magnitude of change of these variables was small, and values for the variables were often within the respective reference intervals. Therefore, it would be unlikely that clinicians would readily assimilate these variables into the diagnostic process for hypoadrenocorticism in clinical practice. However, test results of a suspected dog with hypoadrenocorticism can be entered into a computer software program.[e] For example, the following values can be entered into an equation based on model 1: Na-to-K ratio = 28, creatine kinase activity = 400 U/L, eosinophil count = 1,000 cells/dL, SUN concentration = 35 mg/dL, albumin concentration = 2 g/dL, and CiALP activity = 2 U/L. Those values would yield a probability of 0.9999 for the diagnosis of hypoadrenocorticism. If a value for CiALP activity were not entered, the program would automatically replace the missing value with the median value reported for the present study (4 U/L) and generate a slightly different probability of 0.9998. Importantly, the aforementioned calculations currently apply only to values generated by The Ohio State University Veterinary Medical Center Clinical Pathology Laboratory and might not apply to values measured by laboratories with different reference intervals.

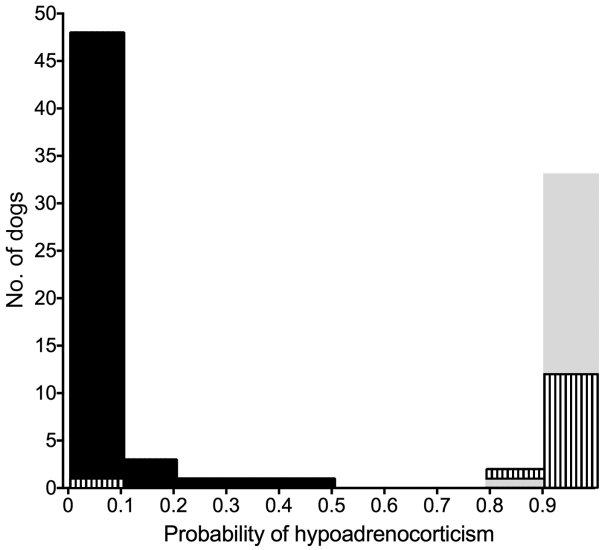

Figure 1—Probability of a diagnosis of hypoadrenocorticism for 103 dogs (54 control dogs [black bars], 15 dogs with GDH [striped bars], and 34 dogs with MGDH [light gray bars]) examined at The Ohio State University Veterinary Medical Center between 2005 and 2013. Probabilities were determined on the basis of a logistic regression model that included the CiALP activity, Na-to-K ratio, eosinophil count, creatine kinase activity, SUN concentration, and albumin concentration.

Only 1 dog (an 11-year-old Welsh Corgi examined because of vomiting and multifocal neurologic signs) was misclassified by use of model 1. That dog had an eosinophil count of 0 cells/μL, SUN concentration of 15 mg/dL, Na-to-K ratio of 33, creatine kinase activity of 83 U/L, and CiALP activity of 3 U/L, which made it unlikely the diagnosis would be classified as hypoadrenocorticism by use of model 1. However, that dog did have a lymphocyte count of 1,700 cells/μL. Interestingly, that was the only dog in the GDH group with a measurable cortisol concentration (1.5 μg/dL) after ACTH administration. Possibly, this could

Table 7—Diagnostic utility of logistic regression models for discrimination between dogs with hypoadrenocorticism and control dogs.

Model	Variables in model	AIC	Sensitivity (%)*	Specificity (%)*	AUC*	Cutoff
1	Na-to-K ratio, eosinophil count, CiALP activity, CK activity, SUN concentration, albumin concentration, and CiALP activity X CK activity	31.68	98.0 (93.9–100)	100 (100–100)	0.994 (0.982–1.000)	0.640
2	Na-to-K ratio, eosinophil count, CK activity, SUN concentration, and albumin concentration	41.82	93.9 (85.7–100)	100 (100–100)	0.984 (0.962–1.000)	0.635
3	Eosinophil count, CiALP activity, CK activity, SUN concentration, albumin concentration, and CiALP activity X CK activity	64.17	95.9 (89.8–100)	87.0 (77.8–96.3)	0.962 (0.928–0.996)	0.236

Models were developed by use of results for 103 dogs (49 dogs with hypoadrenocorticism and 54 control dogs) examined at The Ohio State University Veterinary Medical Center between 2005 and 2013. The AIC represents the goodness of fit of the model (smaller numbers indicate a better fit). The AUC represents the AUC for the ROC curve. The cutoff value is the optimized probability cutoff value used to calculate sensitivity and specificity (ie, a probability greater than or equal to the cutoff value was considered a positive test result for hypoadrenocorticism, and a probability less than the cutoff value was considered a negative result for hypoadrenocorticism); the cutoff value was chosen on the basis of results for the logistic regression equation.

*Values in parentheses are the 95% CI.

have represented a relatively early case of GDH, a misdiagnosis caused by inaccurate measurement of cortisol concentrations, or an actual diagnosis of hypoadrenocorticism with unrecorded exposure to glucocorticoids.

Traditionally, a lack of a stress leukogram in an ill dog has been considered a reason to suspect hypoadrenocorticism.[1] This likely was the reason that almost three-fourths of the dogs in the control group did not have lymphopenia. This finding is not indicative of a low frequency of lymphopenia in ill dogs. Rather, it is a reflection of clinicians' bias in choosing when to measure the cortisol concentration, a bias based on the traditional understanding of the pathophysiologic processes of hypoadrenocorticism. Because of this bias, it was difficult to assess the full utility of lack of a stress leukogram in the diagnosis of hypoadrenocorticism. One would need to test for hypoadrenocorticism in a population of dogs that are suspected of having hypoadrenocorticism regardless of their CBC results. This would pose ethical problems unless another test or combination of tests could be effectively used to make the decision to specifically test for hypoadrenocorticism. The model developed in the study reported here might be useful in that situation.

Despite the fact the selection process was biased against dogs with a stress leukogram, approximately 15% of dogs with hypoadrenocorticism in the study reported here were lymphopenic. Interestingly, the proportion of dogs with hypoadrenocorticism that were lymphopenic was much lower in earlier studies.[3,8] Similarly, the frequency of diagnosis of GDH has increased during the past 20 years, from 5% to 11% in retrospective studies[3,5] that included animals from 1979 through 1995 to 24% in a retrospective study[8] that included animals from 2005 through 2009 to 35% in the present study that included animals from 2005 through 2013. These changes over time likely were the result of 2 factors. First, greater awareness of hypoadrenocorticism has been developed over the years, and hypoadrenocorticism has been diagnosed in animals with more subtle disease. Second, validation of the measurement of basal cortisol concentration as a screening test in 2007[6] made it easier and less expensive to rule out hypoadrenocorticism, and a greater number of dogs with a wider variety of signalment and clinical signs are now being tested for hypoadrenocorticism. It is possible that this process of expanding recognition of hypoadrenocorticism will continue with the introduction of an even more accessible method, such as the one described in the present study. However, it is important to mention that model 1 was constructed on the basis of results for dogs with hypoadrenocorticism that were selected by use of traditional biases. This model will need to be further evaluated in a broader population with a wider range of clinical manifestations.

The study design and statistical analysis for the study reported here were similar to those in a previously reported study.[8] However, in contrast to the approach of the investigators for that study, we did not limit our model to variables that were hypothesized to predict hypoadrenocorticism. The final model in that previous study[8] was constructed by including the Na-to-K ratio and lymphocyte count and had an AUC for the ROC curve of 92.7 (95% CI, 87.5 to 96.2). That limited model appeared to be inferior to the model for the present study (AUC, 99.4; 95% CI, 98.2 to 100), especially when considering the fact that the test population for the present study had a higher proportion of dogs with GDH. Most importantly, the model for the present study could be used to correctly classify MGDH as well as GDH with unprecedented accuracy.

Some of the variables included in the model of the present study fit well with current knowledge about the pathophysiologic processes of hypoadrenocorticism and previous reports on the disease. For example, the number of eosinophils is expected to increase with cortisol deficiency, whereas CiALP activity is expected to remain unaffected. In contrast, creatine kinase activity and SUN concentration have not been previously reported as important variables in the diagnosis of hypoadrenocorticism. In the study reported here, we also found that AST activity differed significantly between dogs with hypoadrenocorticism and control dogs and that AST activity was correlated with creatine kinase activity. These variables have not previously been reported as important for the diagnosis of hypoadrenocorticism because they were not measured (eg, creatine kinase activity[3]) or not included in the assessment (SUN concentration and AST activity[8]) or because their importance was not appreciated, probably as a result of a lack of comparison with values for a control group (SUN concentration and AST activity[3]). It currently is unclear whether the subtle changes in these variables are a result of pathophysiologic processes that have previously been underappreciated in hypoadrenocorticism. For example, increased AST and creatine kinase activities could be the result of myopathy secondary to cortisol deficiency. Muscle cramps have been described in 2 dogs with hypoadrenocorticism.[21] Also, abdominal pain has been reported as a relatively common clinical sign in animals with hypoadrenocorticism (26% of cases[8]), and it is possible that the source of pain in these animals is the abdominal muscles. Abnormally high SUN concentrations in dogs with hypoadrenocorticism could be the result of dehydration, a decrease in the glomerular filtration rate, or gastrointestinal bleeding. Alternatively, high SUN concentrations might be caused directly by a cortisol deficiency. Interestingly, low SUN concentrations have been described in dogs with hyperadrenocorticism.[22]

Corticosteroid-induced ALP appears in canine serum after prolonged exposure to endogenous or exogenous glucocorticoids,[9] and stress-related elevations in CiALP activity have been reported in dogs with several diseases.[10,13,15,16] We hypothesized that a

lack of increase in CiALP activity would be a good discriminator between hypoadrenocorticism and the diseases it mimics. However, CiALP activity was often not increased in control dogs, and it had poor specificity when used as a single diagnostic test. On thte other hand, this was not surprising considering the fact that the basal cortisol concentration was also low in a large proportion of control dogs. Indeed, the specificity of the use of basal cortisol concentration as a single test for the diagnosis of hypoadrenocorticism was also low (68.4%). It was reported previously that the positive-predictive value of basal cortisol concentration for a diagnosis of hypoadrenocorticism is low, but its negative-predictive value is high.[6,7] Thus, both basal cortisol concentration and CiALP activity should be used only to rule out hypoadrenocorticism and are not extremely useful as single diagnostic tests. Also, it should be mentioned that the high sensitivity for basal cortisol concentration in those studies[6,7] was based on 13 and 14 affected dogs, respectively, and CIs for the sensitivity were not reported for either of those studies. Therefore, this reported high sensitivity could have been the result of random chance and is not necessarily higher than the sensitivity for the logistic regression model of the present study.

Dogs affected by hypoadrenocorticism lack the ability to secrete cortisol and should therefore be incapable of having high CiALP activity. Unexpectedly, 10 of 50 (20%) dogs with hypoadrenocorticism in the present study had CiALP activity above the reference interval. One possible explanation would have been previous exposure to exogenous corticosteroids that was not recorded by attending clinicians in the medical record (dogs with known exposure were excluded from the study). Another potential explanation would be analytic error. Activity of CiALP was measured with an automated levamisole-inhibition assay in which 4.2mM levamisole inhibited 98% of the liver isoenzyme activity and 42% of the corticosteroid-induced isoenzyme activity.[23] The analyzer was programmed to calculate the amount of corticosteroid-induced isoenzyme activity by use of a formula based on these inhibitions, as described elsewhere.[23] In an animal with a high liver isoenzyme activity, the remaining 2% of the activity could have contributed to the corticosteroid-induced activity. Finally, the half-life of CiALP is 72 hours,[23] which makes it theoretically possible for dogs with an acute injury to their adrenal glands to have some residual CiALP activity as a result of previous exposure to endogenous corticosteroids.

Of the 57 dogs with hypoadrenocorticism in the study reported here, 7 were Poodles. However, the control group included no Poodles. Generally, this difference in breed distribution would be a weakness of the present study. However, it would have been a weakness for the regression model only if Poodles were inherently different than other breeds with respect to the variables that were included in the model. To our knowledge, this was not the case. Some families of Poodles reportedly have a high mean cell volume, but because the Poodles of the present study did not have an unusually high mean cell volume (before or after treatment) and because mean cell volume was not included in the model, this difference in breed distribution between the groups was unlikely to have substantial effects. Regardless, clinical utility of the model reported here should be further assessed by testing a naïve population of dogs (with and without hypoadrenocorticism) that were not used for the generation of this model. This should be conducted with a variety of breeds and ideally a wide variety of clinical situations. Further validation should be performed with data from other laboratories. It is likely that such studies would result in further refinement and improvement of the model.

In the present study, dogs with hypoadrenocorticism and GDH had significantly lower CiALP activity, compared with CiALP activity for control dogs, but evaluation of CiALP activity alone could not be used as a reliable diagnostic test for hypoadrenocorticism. In addition, SUN concentration and AST and creatine kinase activities differed significantly between all dogs with hypoadrenocorticism and control dogs and between dogs with GDH and control dogs. Combined into 1 diagnostic test that calculated the probability of disease, the eosinophil count, Na-to-K ratio, CiALP activity, SUN concentration, and creatine kinase activity provided excellent power to discriminate between hypoadrenocorticism and diseases mimicking hypoadrenocorticism. The role of this model for use in screening to detect hypoadrenocorticism in dogs (most importantly, its negative predictive value) should be evaluated further.

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Footnotes

a. Advia 2120i, Siemens Healthcare Diagnostics, Tarrytown, NY.
b. Cobas 6000 c501, Roche Diagnostics, Indianapolis, Ind.
c. Immulite, Diagnostics Products Corp, Los Angeles, Calif.
d. R, version 3.2.3, The R Foundation for Statistical Computing, Vienna, Austria. Available at www.R-project.org. Accessed Dec 11, 2015.
e. Addison calculator. The Ohio State University. Available at: episim.osu.edu/AddisonCalc. Accessed Nov 16, 2016.

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