Prediction of serum ionized calcium concentration by use of serum total calcium concentration in dogs

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Objective—To determine whether total serum calcium (tCa) or adjusted tCa concentrations accurately predict ionized calcium (iCa) status in dogs.

Sample Population—1,633 canine serum samples.

Procedure—The tCa concentration was adjusted for total protein (TP) or albumin concentration by use of published equations. Correlations between iCa and tCa or adjusted tCa, tCa and TP, and tCa and albumin were calculated. Diagnostic discordance between tCa or adjusted tCa and iCa was determined. Diagnostic discordance in predicting iCa was also determined for 490 dogs with chronic renal failure (CRF). Sensitivity, specificity, positive and negative predictive values, and positive and negative diagnostic likelihood ratios were calculated for tCa, tCa adjusted for TP, and tCa adjusted for albumin.

Results—Diagnostic discordance was 27% when tCa concentration was used to predict iCa status. Use of adjusted tCa increased diagnostic discordance to approximately 37% for all dogs and 55% for dogs with CRF. Positive predictive value and positive diagnostic likelihood ratios were poor when tCa concentration was used to predict iCa status. The tCa concentration overestimated normocalcemia and underestimated hypocalcemia. Adjusted tCa overestimated hypercalcemia and underestimated hypocalcemia.

Conclusions and Clinical Relevance—Adjusted tCa or tCa concentrations are unacceptable for predicting iCa status in dogs. Use of adjustment equations is not recommended. Direct measurement of iCa concentration is necessary for accurate assessment of calcium status. Use of tCa or adjusted tCa concentrations to predict iCa status in dogs could cause serious mistakes in diagnosis and case management, especially in dogs with CRF. (*Am J Vet Res* 2005;66:1330–1336)

Serum calcium exists in 3 fractions: **ionized calcium (iCa)**, complexed calcium, and protein-bound calcium.[1,2] In clinically normal dogs, iCa, complexed calcium, and protein-bound calcium account for approximately 56%, 10%, and 34% of the serum **total calcium (tCa)** concentration, respectively.[3] Serum iCa is the most important biologically active fraction[4,5] and is a sensitive indicator of pathologic states.[6–10]

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Evaluation of calcium status continues to rely on the determination of serum tCa concentrations.[11] Before the development of ion-selective electrodes, researchers commonly used equations to adjust the tCa value on the basis of measured concentrations of **total protein (TP)** or albumin. Equations have been derived for use in humans[12–16] and dogs[17] in an effort to better predict and monitor the actual iCa status. These equations assume that serum tCa concentrations that are adjusted into the reference range are associated with a serum iCa concentration within the reference range and that samples with values that are not within the reference range after adjustment are associated with abnormal serum iCa concentrations. These equations have been accepted by veterinarians and are in widespread use. Unfortunately, adjustment equations were based solely on the correlation between serum concentrations of tCa and albumin or TP, with the assumption that measurement of TP and albumin concentrations correlates with the protein-bound calcium concentration. This assumption does not take into account the fraction of complexed calcium, which can vary for certain diseases, especially **chronic renal failure (CRF)**.[18] To our knowledge, the adjustment equations for serum tCa concentration have never been verified through comparison with actual iCa measurement. Thus, it is unknown whether serum tCa concentration or adjusted tCa concentration accurately predicts iCa concentration, especially in disease states.

The accuracy of diagnosis in dogs is unknown when tCa concentrations or correction equations are used to predict serum iCa concentration. Thus, the objectives of the study reported here were to determine the use of serum tCa measurement, serum concentration of **tCa adjusted on the basis of TP concentration (adjtCa[TP])**, and serum concentration of **tCa adjusted on the basis of albumin concentration (adjtCa[alb])** in predicting true iCa status in dogs with various diseases.

Materials and Methods

Sample population—Serum samples from 1,633 dogs were submitted to The Ohio State University clinical chemistry laboratory. Dogs were categorized on the basis of diagnosis of disease conditions into those with evidence of CRF (n = 490) and those with no evidence of CRF (1,143). Conditions diagnosed other than CRF included adenocarcinoma of the thyroid gland, hemangiosarcoma, lymphosarcoma, multiple myeloma, adenocarcinoma of the anal sacs, tumor at the base of the heart, leiomyoma, islet cell carcinoma, bronchogenic carcinoma, mammary masses, osteosarcoma, squamous cell carcinoma, chondrosarcoma, transitional cell carcinoma, histiocytoma, hepatic tumor, melanoma,

granulomatous dermatitis, seborrhea, pyoderma, toxoplasmosis, urinary tract infection, fever of unknown origin, primary hyperparathyroidism, erythema multiforme, hypoparathyroidism, ethylene glycol toxicosis, intervertebral disk disease, paraplegia, epilepsy, tracheal collapse, hyperadrenocorticism, portosystemic shunt, hepatic encephalopathy, pancreatitis, pyometra, retained fetus, eclampsia, dystocia, uterine rupture, mastitis, diabetes mellitus, infestation with whipworms, hypoadrenocorticism, myotonia, otitis, enteritis, gastric volvulus, distemper, disseminated intravascular coagulopathy, polyarthropathy, urolithiasis, incontinence, aspergillosis, heat prostration, septic shock, panosteitis, immune-mediated hemolytic anemia, pyothorax, trauma resulting from being hit by a vehicle, vestibular disease, heartworm disease, parvovirus, perineal hernia, trauma resulting from gunshot wounds, dilated cardiomyopathy, congestive heart failure, gastrointestinal foreign body, megaesophagus, pneumothorax, head trauma, and splenic torsion.

Analysis of serum calcium concentrations—Serum iCa, tCa, TP, and albumin concentrations were measured in all samples. Serum concentrations of iCa were measured anaerobically by use of an ion-selective electrode.[a] Serum concentrations of tCa, TP, and albumin were measured by use of a spectrophotometric method.[b]

Serum tCa concentration was adjusted on the basis of TP and albumin concentrations by use of the following adjustment equations[17]:

AdjtCa(TP) (in mg/dL) = Measured tCa concentration (in mg/dL) – (0.4 × serum TP concentration [in g/dL]) + 3.3, and

AdjtCa(alb) (in mg/dL) = Measured tCa concentration (in mg/dL) – albumin concentration (in g/dL) + 3.5.

Procedure—Dogs were classified as hypercalcemic, normocalcemic, or hypocalcemic on the basis of serum tCa, adjtCa(TP), and adjtCa(alb) concentrations as well as on the basis of measured serum iCa concentration. Normocalcemia was defined as serum tCa, adjtCa(TP), or adjtCa(alb) concentration within an established reference range[c] of 9.0 to 12.0 mg/dL and serum iCa concentration within an established reference range[c] of 5.0 to 6.0 mg/dL. Hypercalcemia was defined as serum tCa, adjtCa(TP), or adjtCa(alb) concentration > 12.0 mg/dL or a serum iCa concentration > 6.0 mg/dL. Hypocalcemia was defined as serum tCa, adjtCa(TP), or adjtCa(alb) concentration < 9.0 mg/dL or a serum iCa concentration < 5.0 mg/dL.

Classification of hypercalcemia, normocalcemia, or hypocalcemia on the basis of serum iCa measurement was compared with the classification on the basis of tCa, adjtCa(TP), and adjtCa(alb) concentrations in all dogs, the subpopulation of dogs with CRF, and the subpopulation of dogs with conditions other than CRF. Prevalence of hypercalcemia, normocalcemia, or hypocalcemia was calculated as the percentage of all dogs identified with hypercalcemia, normocalcemia, or hypocalcemia at the time of the study reported here.

Sensitivity, specificity, **positive predictive value (PPV)**, **negative predictive value (NPV)**, **positive diagnostic likelihood ratio (PDLR)**, and **negative diagnostic likelihood ratio (NDLR)** for serum concentrations of tCa, adjtCa(TP), and adjtCa(alb) were calculated for the diagnosis of hypercalcemia and hypocalcemia in all dogs, the subpopulation of dogs with CRF, and the subpopulation of dogs with conditions other than CRF. Sensitivity was the probability of identifying hypercalcemia or hypocalcemia by use of tCa, adjtCa(TP), or adjtCa(alb) concentrations in dogs with hypercalcemia or hypocalcemia identified by the use of iCa concentrations. Specificity was the proportion of dogs that were not hypercalcemic or hypocalcemic on the basis of iCa concentrations that were correctly identified by use of tCa, adjtCa(TP), or adjtCa(alb) concentrations. The PPV was the probability that a diagnosis of hypercalcemia or hypocalcemia was correct, and NPV was the probability that a diagnosis of normocalcemia was correct. The likelihood ratio of a test result is the probability of that test result in those animals that are truly affected divided by the probability of that test result in those animals that are not affected. The PDLR for hypercalcemia was the probability of identifying hypercalcemia by use of serum tCa, adjtCa(TP), or adjtCa(alb) concentrations in dogs that were truly hypercalcemic divided by the probability of identifying hypercalcemia in dogs that were not hypercalcemic. The NDLR for hypercalcemia was the probability of not identifying hypercalcemia in those dogs that were truly hypercalcemic divided by the probability of correctly identifying those dogs that were not hypercalcemic by use of tCa, adjtCa(TP), or adjtCa(alb) concentrations. The PDLR is a stronger indicator as the value increases, and NDLR is a more useful indicator as the value decreases.[19]

Diagnostic discordance was determined by use of serum concentrations of tCa, adjtCa(TP), and adjtCa(alb). Diagnostic discordance was the percentage of dogs with incorrect identification of calcium status on the basis of tCa, adjtCa(TP), or adjtCa(alb) concentration by use of the following equation: diagnostic discordance = (number of samples with diagnostic disagreement between measured iCa and tCa concentrations/total number of samples) × 100.

The κ coefficient assesses overall agreement by relating actual agreement with chance agreement.[20] Agreement between 2 methods of measurement is considered poor when κ is ≤ 0.20, fair when κ is > 0.20 but ≤ 0.40, moderate when κ is > 0.40 but ≤ 0.60, substantial when κ is > 0.60 but ≤ 0.80, and good when κ is > 0.80. The κ coefficients were compared by use of the Z test, and values were considered significantly different for values of $P < 0.05$. Correlations of serum TP and albumin concentrations with tCa concentration and serum concentrations of tCa, adjtCa(TP), and adjtCa(alb) with iCa concentration were assessed by use of least squares regression. Correlation coefficients, κ coefficients, and 95% confidence intervals were calculated by use of statistical software.[d]

Results

We detected a significant difference in mean serum iCa concentrations in dogs classified as hypercalcemic, normocalcemic, or hypocalcemic. Mean ± SD serum iCa concentrations for all hypercalcemic, normocalcemic, and hypocalcemic dogs were 7.22 ± 1.08 mg/dL (range, 6.01 to 12.50 mg/dL), 5.42 ± 0.26 mg/dL (range, 5.00 to 6.00 mg/dL), and 4.13 ± 0.75 mg/dL (range, 1.93 to 4.99 mg/dL), respectively. Correlation between serum tCa concentration and albumin or TP concentration was poor in all dogs (**Table 1**). Serum adjtCa(TP) or adjtCa(alb) concentration had a slightly better correlation with serum iCa concentration. Correlations were slightly better in dogs with conditions other than CRF, compared with correlations for dogs with CRF.

In our population of 1,633 dogs, 651 were identified as normocalcemic, 205 were hypercalcemic, and 341 were hypocalcemic on the basis of serum iCa and tCa concentrations (**Table 2**). In dogs identified as hypercalcemic by use of iCa concentration, 93 were identified as normocalcemic and 6 were identified as hypocalcemic on the basis of serum tCa concentration. In dogs identified as hypocalcemic by use of iCa concentration, 143 were identified as normocalcemic and

Table 1—Correlation coefficient (95% confidence interval) between serum concentrations of various analytes in dogs.

Comparison	All dogs (n = 1,633)	Dogs with CRF (490)	Dogs with conditions other than CRF (1,143)
tCa and albumin	0.30 (0.26–0.34)	0.40 (0.32–0.47)	0.31 (0.26–0.36)
tCa and TP	0.34 (0.29–0.38)	0.37 (0.29–0.44)	0.36 (0.30–0.41)
tCa and iCa	0.57 (0.53–0.60)	0.73 (0.69–0.77)	0.87 (0.86–0.89)
AdjtCa(alb) and iCa	0.73 (0.71–0.75)	0.66 (0.61–0.71)	0.83 (0.82–0.85)
AdjtCa(TP) and iCa	0.73 (0.71–0.75)	0.67 (0.62–0.72)	0.83 (0.81–0.85)

CRF = Chronic renal failure. tCa = Total calcium. TP = Total protein. iCa = Ionized calcium. AdjtCa(alb) = tCa adjusted on the basis of albumin concentration. AdjtCa(TP) = tCa adjusted on the basis of TP concentration.

Table 2—Comparison of the classification of calcium status in dogs determined by use of serum iCa concentration or tCa concentration with classifications determined on the basis of tCa, adjtCa(TP), or adjtCa(alb) concentration.

		NC*			HRC*			HOC*		
Group	Variable	NC†	HRC†	HOC†	NC†	HRC†	HOC†	NC†	HRC†	HOC†
All dogs (n = 1,633)	tCa (%)	40‡	5	6	6	13‡	< 1	9	1	21‡
	AdjtCa(TP) (%)	40‡	13	1	3	14‡	< 1	17	4	9‡
	AdjtCa(alb) (%)	42‡	12	1	3	13‡	< 1	19	3	8‡
Dogs with CRF (490)	tCa (%)	42‡	11	2	2	6‡	< 1	16	4	16‡
	AdjtCa(TP) (%)	29‡	25	0	1	8‡	< 1	18	9	10‡
	AdjtCa(alb) (%)	28‡	26	0	0	8‡	< 1	20	8	9‡
Dogs with conditions other than CRF (1,143)	tCa (%)	39‡	2	8	7	16‡	< 1	6	< 1	23‡
	AdjtCa(TP) (%)	46‡	7	1	4	17‡	< 1	16	< 1	9‡
	AdjtCa(alb) (%)	49‡	5	1	4	16‡	< 1	18	0	7‡

*Classification determined by use of serum iCa concentration. †Classification determined by use of serum tCa concentration. ‡Values represent those dogs with classification agreement between serum iCa concentration and tCa or adjusted tCa concentration.
NC = Normocalcemia. HRC = Hypercalcemia. HOC = Hypocalcemia.
See Table 1 for remainder of key.

Table 3—Prevalence of hypercalcemic, normocalcemic, or hypocalcemic dogs as determined on the basis of serum iCa, tCa, adjtCa(TP), or adjtCa(alb) concentration.

Group	Classification	iCa	tCa	AdjtCa(TP)	AdjtCa(alb)
All dogs (n = 1,633)	Hypercalcemic (%)	19	19	30	28
	Normocalcemic (%)	50	54	59	63
	Hypocalcemic (%)	31	27	10	9
Dogs with CRF (490)	Hypercalcemic (%)	9	22	42	43
	Normocalcemic (%)	55	60	47	48
	Hypocalcemic (%)	36	19	11	10
Dogs with conditions other than CRF (1,143)	Hypercalcemic (%)	23	17	24	20
	Normocalcemic (%)	48	52	66	72
	Hypocalcemic (%)	29	31	10	8

See Table 1 for key.

23 were identified as hypercalcemic on the basis of tCa concentration. In dogs identified as normocalcemic by use of iCa concentration, 75 were identified as hypercalcemic and 96 were identified as hypocalcemic on the basis of tCa concentration.

Of 490 dogs with CRF, 205 were normocalcemic, 29 were hypercalcemic, and 79 were hypocalcemic on the basis of serum iCa and tCa concentrations. In dogs with CRF identified as normocalcemic by use of iCa concentration, 56 were identified as hypercalcemic and 10 were identified as hypocalcemic on the basis of tCa concentration. In dogs with CRF identified as hypercalcemic by use of iCa concentration, 11 were identified as normocalcemic and 2 were identified as hypocalcemic on the basis of tCa concentration. In dogs with CRF identified as hypocalcemic, 76 were identified as normocalcemic and 22 were identified as hypercalcemic on the basis of tCa concentration. Classification of calcium status for the 1,143 dogs with conditions other than CRF was also determined (Table 2).

On the basis of serum iCa concentration for all 1,633 dogs, 304 were hypercalcemic, 822 were normocalcemic, and 507 were hypocalcemic. Thus, the prevalence of hypercalcemia, normocalcemia, and hypocalcemia in this population was 19%, 50%, and 31%, respectively (**Table 3**). Use of serum tCa concentration resulted in a similar prevalence in all dogs, with a slight overestimation for the prevalence of normocalcemia and underestimation for the prevalence of hypocalcemia. Use of an equation to adjust the tCa concentra-

tion on the basis of TP or albumin concentration resulted in an overestimation for the prevalence of hypercalcemia and underestimation for the prevalence of hypocalcemia.

Table 4—Diagnostic discordance in the prediction of serum iCa status in dogs.

	All dogs (n = 1,633)		Dogs with CRF (490)		Dogs with conditions other than CRF (1,143)	
Variable	**%**	**κ**	**%**	**κ**	**%**	**κ**
tCa	27	0.56	36	0.38	23	0.64
AdjtCa(TP)	37	0.35	53	0.19	28	0.48
AdjtCa(alb)	38	0.38	54	0.21	28	0.51

All κ values differ significantly ($P < 0.05$) from a perfect agreement (κ = 1.00) between the variable tested and iCa measurement.
See Table 1 for key.

Although analysis of serum tCa concentration revealed prevalences of hypercalcemia, normocalcemia, and hypocalcemia that were similar to the prevalences determined by use of iCa concentration for the entire population of dogs, this was not the case in the subpopulation of dogs with CRF. Use of serum tCa concentration greatly overestimated the prevalence of hypercalcemia and underestimated the prevalence of hypocalcemia in dogs with CRF. Use of an adjustment equation further overestimated the prevalence of hypercalcemia and underestimated the prevalence of normocalcemia and hypocalcemia in dogs with CRF. In dogs with conditions other than CRF, the use of an adjustment equation overestimated the prevalence of normocalcemia and underestimated the prevalence of hypocalcemia.

Serum tCa incorrectly predicted iCa concentration in 27% of all dogs and 36% of dogs with CRF (**Table 4**). The use of an adjustment equation increased

Table 5—Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive diagnostic likelihood ratio (PDLR), and negative diagnostic likelihood ratio (NDLR) for the prediction of hypercalcemic status as determined by the use of serum iCa concentrations in dogs on the basis of serum tCa, adjtCa(TP), or adjtCa(alb) concentrations.

Group	Variable	tCa	AdjtCa(TP)	AdjtCa(alb)
All dogs (n = 1,633)	Sensitivity (%)	67	83	82
	Specificity (%)	93	80	82
	PPV (%)	68	45	45
	NPV (%)	93	96	96
	PDLR	9	4	4
	NDLR	0.35	0.21	0.22
Dogs with CRF (490)	Sensitivity (%)	69	90	96
	Specificity (%)	83	63	63
	PPV (%)	27	20	20
	NPV (%)	97	98	99
	PDLR	4	2	3
	NDLR	0.38	0.16	0.06
Dogs with conditions other than CRF (1,143)	Sensitivity (%)	67	81	78
	Specificity (%)	98	91	94
	PPV (%)	90	71	76
	NPV (%)	91	95	95
	PDLR	30	9	13
	NDLR	0.34	0.21	0.23

See Table 1 for key.

Table 6—Sensitivity, specificity, PPV, NPV, PDLR, and NDLR for the prediction of hypocalcemic status in dogs by use of serum iCa concentration on the basis of tCa, adjtCa(TP), or adjtCa(alb) concentrations.

Group	Variable	tCa	AdjtCa(TP)	AdjtCa(alb)
All dogs (n = 1,633)	Sensitivity (%)	67	32	26
	Specificity (%)	91	99	99
	PPV (%)	77	89	90
	NPV (%)	86	78	76
	PDLR	8	20	20
	NDLR	0.36	0.70	0.75
Dogs with CRF (490)	Sensitivity (%)	45	28	25
	Specificity (%)	96	99	99
	PPV (%)	87	97	97
	NPV (%)	75	70	69
	PDLR	12	55	50
	NDLR	0.58	0.73	0.76
Dogs with conditions other than CRF (1,143)	Sensitivity (%)	79	35	27
	Specificity (%)	89	98	98
	PPV (%)	74	84	84
	NPV (%)	91	82	80
	PDLR	7	17	16
	NDLR	0.23	0.66	0.74

See Tables 1 and 5 for key.

the amount of misdiagnosis, with an incorrect diagnosis for approximately 37% of all dogs. In dogs with CRF, adjusted tCa incorrectly identified iCa status in approximately 53% of dogs. The κ coefficient indicated moderate agreement between classification based on serum tCa and iCa concentrations. Use of an adjustment equation decreased the κ value to only fair agreement. In dogs with CRF, the κ coefficient indicated only fair agreement between the classification of calcium status based on tCa and iCa concentrations, and use of an adjustment equation decreased the degree of agreement to poor. In dogs with conditions other than CRF, use of tCa concentration or an adjustment equation incorrectly identified iCa status in approximately 30% of dogs. The κ values were all < 1 (ie, less than perfect agreement), and z values differed significantly, which indicated that the lack of agreement among iCa, tCa, adjtCa(TP), and adjtCa(alb) values was significant and not attributable to chance alone (data not shown).

Use of serum tCa concentration to predict hypercalcemia for all dogs revealed sensitivity of 67%, specificity of 93%, PPV of 68%, NPV of 93%, PDLR of 9, and NDLR of 0.35 (**Table 5**). In dogs with CRF, use of serum tCa concentration to predict hypercalcemia had similar sensitivity but a decrease in specificity, PPV, and PDLR, compared with values for all dogs. Use of an adjustment equation to predict hypercalcemia increased sensitivity but decreased specificity, PPV, and PDLR. In dogs with conditions other than CRF, use of serum tCa concentration to predict hypercalcemia also had similar sensitivity but a decrease in NPV, compared with values for all dogs. Use of an adjustment equation to predict hypercalcemia increased sensitivity and NPV but decreased specificity, PPV, and PDLR.

Use of serum tCa concentration to predict hypocalcemia for all dogs revealed a sensitivity of 67%, specificity of 91%, PPV of 77%, NPV of 86%, PDLR of 8, and NDLR of 0.36 (**Table 6**). In dogs with CRF, use of serum tCa concentration to predict hypocalcemia had decreased sensitivity and NPV, higher NDLR, and increased specificity, PPV, and PDLR, compared with values for all dogs. Use of an adjustment equation to predict hypocalcemia decreased sensitivity and NPV, resulted in a higher NDLR, and increased specificity, PPV, and PDLR, compared with values for all dogs. In dogs with conditions other than CRF, use of serum tCa to predict hypocalcemia increased the sensitivity and NPV. Use of an adjustment equation to predict hypocalcemia decreased the sensitivity and NPV but increased the specificity, PPV, and PDLR.

Discussion

Utility of serum iCa measurement differs from that of other tests in that various disease states are possible, depending on whether an animal is hypercalcemic or hypocalcemic. Sensitivity, specificity, PPV, NPV, PDLR, and NDLR may differ depending on iCa classification; thus, these variables are calculated separately for the diagnosis of hypercalcemia and hypocalcemia.

Dogs in the study reported here had several conditions, and a high percentage (30%) had CRF. Because serum protein abnormalities (hypoalbuminemia or hypoproteinemia) are evident in many patients with CRF, which may impact the protein-bound fraction of serum tCa,[21] dogs with CRF were also analyzed separately, and their results were compared with those for dogs that were not affected with CRF.

Correlation of tCa concentration with albumin or TP concentration was poor in this study of 1,633 serum samples obtained from dogs. In 2 other studies,[17,22] correlation of tCa concentration with albumin concentration was slightly better than the correlation for the study reported here. In a study[17] of 209 dogs, the **correlation coefficient (*r*)** between tCa concentration and albumin concentration was 0.575, whereas the *r* was 0.411 for 9,041 dogs in another study.[22] Dogs with increased serum creatinine concentrations were excluded in the study of 209 dogs,[17] and the incidence of dogs with evidence of renal disease (9%) was much lower in the study of 9,041 dogs[22] than in the study reported here (30%). The *r* of tCa concentration with albumin concentration in the study reported here is similar to that for 2 studies in humans (*r*, 0.32 for 558 subjects[23];*r*, 0.25 for 63 subjects[24]).

The *r* (ie, 0.57) between tCa and iCa concentrations in the study reported here indicates a weak linear relationship and is lower than that seen in studies[9,23,25] in humans. Use of an adjustment equation improved the linear relationship between tCa and iCa concentrations (*r*, 0.73). However, the closeness of fit was still low, and only approximately half of the change in tCa concentration (r^2, 0.53) could be attributed to changes in iCa concentrations. The correlation between tCa and iCa concentrations differs when comparing patients with various diseases.[9] Correlation of tCa to iCa concentrations by use of an adjustment equation was poorer in dogs with evidence of CRF, and only approximately 45% of the change in tCa concentrations (r^2, 0.45) can be attributed to changes in iCa concentrations. Correlation between tCa and iCa concentrations was highest in dogs without evidence of CRF (*r*, 0.87), with 76% of the change in tCa attributed to a change in iCa (r^2, 0.76). There was no improvement in correlation between tCa and iCa concentrations with the use of either adjustment equation in dogs with conditions other than CRF.

A fairly high amount of diagnostic discordance (27%) was detected when tCa concentration was used to predict iCa concentration in all dogs. Diagnostic discordance when tCa concentration was used to predict iCa concentration was 31%[25] and 26%,[9] respectively, in 2 studies in humans, which is similar to the diagnostic discordance for the dogs in our study. Use of either adjustment equation increased the diagnostic discordance for all dogs, especially for dogs with evidence of CRF. In dogs with CRF, diagnostic discordance when tCa concentration was used to predict iCa concentration was 36%, which increased to approximately 54% when an adjustment equation was used. This indicates that iCa status would be incorrectly predicted in 54% of dogs on the basis of an adjusted tCa concentration. Concentration of tCa was a better predictor of iCa status, compared with adjusted tCa concentration, in dogs with CRF; however, the diagnostic discordance indicates that tCa concentration alone is an unacceptable predictor of iCa status.

In human medicine, a number of algorithms have been developed to adjust the measured tCa concentration on the basis of serum TP or albumin concentrations in an effort to improve the prediction of iCa concentration.[12–16] In a study[23] of 558 human patients, there was no improvement in the correlation between tCa and iCa concentrations when several adjustment equations were used.[23] Poor correlation between adjusted tCa and iCa concentrations was also observed for 3 adjustment equations in a study[26] that included patients with renal disease. The use of 6 adjustment equations revealed poor correlation between calculated and measured iCa concentrations in a patient with end-stage renal disease.[27] All algorithms overestimated iCa concentration and would have led to inappropriate treatment had the information been used by clinicians. Adjusting tCa concentration on the basis of albumin concentration did improve the diagnostic discordance in 1 study[25] of 1,213 human patients; however, the adjusted tCa value still incorrectly predicted iCa concentration in 18% of patients. Approximately 74% of patients in that study[25] in humans had iCa concentrations within the reference range, compared with only 50% of dogs in the study reported here, which may have accounted for a lesser degree of diagnostic discordance in the human study. Because of poor correlations between tCa and iCa concentrations and lack of improvement when an adjustment algorithm was used, use of adjustment equations is not recommended and direct measurement of iCa is the method of choice in human medicine.

Analytic variation in measurement may contribute slightly to diagnostic discordance.[13] Day-to-day precision of iCa measurement in the study reported here was 1.6%, and others[11] have calculated that approximately 7% of diagnostic discordance could be attributed to this degree of analytic variation. However, the observed diagnostic discordance in our study clearly exceeded the discordance that may result from day-to-day variation in measurements.

Diagnostic discordance observed when tCa concentrations were used to predict iCa concentrations was most likely a reflection of variation among dogs in the protein-bound or complexed calcium fractions. Correlations of serum TP and serum albumin concentrations with tCa concentrations were poor in this group of dogs, revealing considerable variation among dogs. Concentration of complexed calcium can also vary substantially, especially in dogs with CRF.[18]

Sensitivity of serum tCa concentration for use in the prediction of hypercalcemia was low as a result of the high number of hypercalcemic dogs (as determined by use of iCa concentrations) that were normocalcemic on tCa measurement (ie, false-negative results). Specificity was high, indicating a low number of truly normocalcemic dogs that were hypercalcemic on tCa measurement (ie, false-positive results). Similar values for sensitivity and specificity have been reported[23] for humans when tCa concentrations are used for the prediction of iCa concentrations. Published adjustment equations for use in dogs slightly improved sensitivity in the study reported here by decreasing the number of false-negative results and decreased specificity attributable to an increase in false-positive results.

The PPV and NPV are affected by prevalence of hypercalcemia in the population, whereas PDLR and NDLR have the advantage of not being affected by prevalence.[28] The PDLR and PPV for prediction of hypercalcemia (as determined on the basis of iCa concentration) were low in all dogs, especially dogs with CRF. The PDLR and PPV for tCa concentration were higher in dogs with conditions other than CRF but still not within an acceptable diagnostic range. Use of an adjustment equation actually decreased the PDLR and PPV for tCa concentration in prediction of iCa status. Thus, the adjustment equations offered no advantage over tCa measurement.

Sensitivity of tCa concentration for the prediction of hypocalcemia (as determined on the basis of iCa concentration) was fair in all dogs and higher in dogs with conditions other than CRF. Adjustment equations decreased the sensitivity in all dogs by increasing the number of dogs with false-negative results. Specificity increased slightly as a result of a decrease in the number of dogs with false-positive results. Sensitivity for tCa concentration or adjusted tCa concentration was poorest in dogs with CRF. Most dogs with CRF and hypocalcemia were identified as normocalcemic by use of serum tCa or adjusted tCa concentrations.

The PDLR and NDLR for prediction of hypocalcemia (as determined on the basis of iCa concentration) were low in all dogs. The low PDLR suggests that most hypocalcemic dogs were misidentified as normocalcemic when serum tCa concentration or adjusted tCa concentration was used to predict iCa status. Use of an adjustment equation provided a slight improvement in PDLR, but PDLR was still low, which indicated that adjusted tCa concentration is unacceptable for the prediction of iCa concentration.

Overall, serum tCa concentration or adjusted tCa concentration was a poor predictor of iCa concentration, with an observed high degree of diagnostic discordance. Adjustment equations for tCa concentrations offer little advantage over the use of tCa measurement, and their use should be discouraged. Measurement of tCa concentration or adjusted tCa concentration cannot be relied on for accurate assessment of calcium status in dogs, especially dogs with CRF in which abnormalities in TP, albumin, and complexed calcium fractions may be evident. When a calcium metabolic disturbance is suspected, the direct measurement of serum iCa concentration is the method of choice for accurate assessment of calcium status in dogs.

a. 634 Ca^{++}-pH analyzer, Ciba-Corning, Medfield, Mass.
b. Hitachi 911, Boehringer Mannheim, Indianapolis, Ind.
c. The Ohio State University Clinical Chemistry Laboratory, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio.
d. Minitab statistical software, Minitab Inc, State College, Pa.

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