Serum vitamin D metabolite and acute-phase protein concentrations are frequently abnormal in a cohort of hospitalized dogs and cats

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OBJECTIVE
To determine whether serum 25-hydroxyvitamin D (25(OH)D) and 1,25-dihydroxyvitamin D (1,25(OH)2D) concentrations are associated with survival and negatively correlate with acute-phase protein (APP) concentrations in ill dogs and cats admitted to nursing care units.

ANIMALS
Client-owned dogs (n = 79) and cats (16) admitted to 2 academic veterinary hospital nursing care units.

METHODS
A prospective cohort study was conducted between August 12, 2019, and October 26, 2021. A diagnostic laboratory measured 25(OH)D, 1,25(OH)2D, and haptoglobin (HPT) in dogs and cats; C-reactive protein (CRP) in dogs; and serum amyloid A (SAA) in cats. Serum was collected within 12 hours of admission. Illness severity (acute patient physiologic and laboratory evaluation [APPLEfast]) scores and survival data were recorded.

RESULTS
Serum 25(OH)D concentrations were in the deficient range for 22 of 79 dogs and 2 of 16 cats. There were no associations between serum analyte concentrations (25(OH)D, 1,25(OH)2D, and APP) or APPLEfast score and survival in dogs or cats. In dogs, HPT was negatively correlated with 25(OH)D (P = .002; r = −0.34) and 1,25(OH)2D (P = .012; r = −0.28), while CRP was positively correlated with HPT (P = .001; r = 0.32) and APPLEfast score (P = .014; r = 0.16). In cats, 1,25(OH)2D was negatively correlated with APPLEfast scores (P = .055; r = −0.49) and SAA was positively correlated with HPT (P = .002; r = 0.73).

CLINICAL RELEVANCE
Serum 25(OH)D or 1,25(OH)2D was not associated with survival in our hospitalized patient population. Relationships between APP and serum vitamin D metabolites with APPLEfast scores in cats warrant further investigation as illness severity biomarkers.

Keywords: vitamin D, acute-phase protein, calcitriol, canine, feline
with acute patient physiologic and laboratory evaluation fast (APPLE\textsubscript{fast}) scores.\textsuperscript{5} The veterinary literature is limited to a single study\textsuperscript{6} that found serum 25(OH)D concentration was an independent predictor of short-term risk of death in hospitalized cats. These studies suggest that vitamin D in critically ill dogs and cats could have similar overlapping roles with humans. However, to our knowledge the question of whether low vitamin D is a risk factor for illness, a consequence of illness or illness severity, or a combination thereof remains unresolved in veterinary species.

Vitamin D’s importance in critical illness may relate to its immunomodulatory properties as it augments the innate immune response in people by increasing phagocytosis and inducing antimicrobial peptide synthesis.\textsuperscript{10} Vitamin D reduces the production of proinflammatory cytokines, such as IL-6 and TNF-α,\textsuperscript{11} and has anti-inflammatory properties in people.\textsuperscript{12} Likewise, vitamin D has immunologic effects in dogs, with 1 study demonstrating that vitamin D caused an in vitro anti-inflammatory phenotype shift in critically ill dogs.\textsuperscript{5,13}

Changes in serum vitamin D metabolite concentrations over time in the acute phase of critical illness have been investigated in humans but not companion animals. A study\textsuperscript{4} of humans found that serum 25(OH)D concentrations decreased significantly in the first 2 to 3 days after hospital admission before gradually increasing. These decreases were associated with the severity of illness scores. Understanding the kinetics of serum vitamin D metabolites in companion animals during hospitalization may be clinically valuable.

We hypothesized that vitamin D metabolites and selected acute-phase proteins (APPs) would be complementary biomarkers for predicting survival in hospitalized cats and dogs. To test this hypothesis, we measured 25(OH)D, 1,25(OH)\textsubscript{2}D, and haptoglobin (HPT) in cats and dogs; CRP in dogs; and SAA in cats. Our objectives were to determine whether (1) concentrations of these biomarkers are frequently abnormal, (2) vitamin D metabolite and APP serum concentrations would be negatively correlated, (3) disease category would influence the severity of serum vitamin D metabolite and APP abnormalities, and (4) the selected biomarkers were predictive of survival in hospitalized cats and dogs.

**Methods**

**Animals**

We recruited hospitalized dogs and cats in a prospective cohort study with informed client consent. The Cornell University (2019-0091) and Midwestern University (protocol 2951) IACUCs approved this protocol. Cases were recruited and sampled between August 12, 2019, and October 26, 2021, with a COVID-19-related pause (March 13, 2020, to September 27, 2020).

Dogs and cats admitted to the Midwestern University College of Veterinary Medicine and Cornell University Hospital for Animals nursing care units were eligible for inclusion. Cases were enrolled by primary clinicians, authors, or both, and consent was obtained in person or via phone at both institutions. Additionally, notice about the study was maintained on the electronic emergency board at Cornell University. Exclusion criteria included pregnancy, lactation, hypercalcemia of malignancy, hyperparathyroidism, hypoparathyroidism, known history of chronic kidney disease, and vitamin D or calcium supplementation. Dogs and cats were also excluded if their admittance to the hospital was associated with postoperative care for elective surgical procedures without complications (eg, dental prophylaxis with or without tooth extractions, orthopedic procedures, orchectomy, or ovariohysterectomy). The APPLE\textsubscript{fast} scoring system, which ranges from 0 to 50, was used to assess illness severity.\textsuperscript{14,15} Score parameters obtained within 24 hours of hospital admission were obtained from medical records and used to generate each patient’s APPLE\textsubscript{fast} Score. Upon advice from the author of the APPLE\textsubscript{fast} score (personal communication, Galina Hayes, DVM, PhD, DACVS, DACVECC, Associate Professor, Cornell University, email), missing values were assigned a score of 0 under the assumption that in most cases the lack of a parameter reflected a lack of clinical concern.

**Recorded parameters**

Survival was assessed up to the time of manuscript preparation by medical record review and contacting owners or primary care veterinarians when lost to clinical follow-up. Sepsis was defined as a patient having a positive blood culture or cytological confirmation of bacterial infection (prior antimicrobial therapy was not an exclusion criterion) in addition to meeting 2 or more criteria of systemic inflammatory response syndrome as previously described for dogs\textsuperscript{16,17} (WBC count ≤ 4 X 10\textsuperscript{3} or ≥ 12 X 10\textsuperscript{9}/μL or ≥ 10% band neutrophils, heart rate ≥ 160 beats/min, respiratory rate ≥ 40 breaths/min, or temperature ≤ 37.8 °C or ≥ 39.7 °C) or cats\textsuperscript{18,19} (WBC count ≤ 5.0 X 10\textsuperscript{3} cells/μL or > 19.5 X 10\textsuperscript{9} cells/μL, or ≥ 5% band cells, heart rate ≤ 140 or ≥ 225 beats/min, respiratory rate ≥ 40 breaths/min, temperature ≤ 37.8 °C or ≥ 39.7 °C). Dogs and cats were categorized into 1 of 6 groups on the basis of primary clinical signs and diagnostic results. Groups were created to best represent common presentation in the cohort without influence from vitamin D metabolite or APP measurements. Groups included (1) neoplastic disease, (2) gastrointestinal foreign body, (3) infections/inflammatory/immune-mediated, (4) nonseptic, infections/inflammatory/immune-mediated and septic, (5) neurologic disease, and (6) miscellaneous (MISC). Individuals falling into defined groups with < 3 individuals were included in the MISC group.

**Sample collection**

Blood samples were acquired by venipuncture of jugular or peripheral veins or through sampling catheters based on clinical discretion. A total of 3 to 4 mL of whole blood was collected into serum separator or nonadditive tubes (BD Vacutainer; Becton, Dickinson and
Company). Patients hospitalized in the ICU for at least 48 hours had an additional 1 mL of blood obtained 48 hours after admission. All blood was centrifuged, and serum was harvested within 1 hour of sample collection. The serum was placed in airtight, freezer-resistant plastic tubes and stored at –80 °C for batch analysis of vitamin D metabolites and APP measurements.

**Vitamin D metabolites**

Concentrations of 25(OH)D were measured by a commercial direct competitive chemiluminescent immunoassay (LIAISON; DiaSorin Inc) as previously described by Veterinary Diagnostics Institute (VDI) Laboratory.20,21 The assay’s dynamic range is 4.0 to 150 ng/mL. Reference ranges for 25(OH)D in dogs were established from a prior study21 conducted by the same laboratory, VDI, which analyzed data from 282 healthy dogs. This study not only measured 25(OH)D levels but also assessed parathyroid hormone and phosphorus levels to determine reference categories.21 Similarly, reference ranges for cats were determined internally at VDI, employing a comparable methodology. Ranges are categorized as follows: vitamin D sufficiency, 100 to 150 ng/mL (all values > 100 ng/mL considered sufficient in this study); insufficiency, 40 to 100 ng/mL; and deficient, < 40 ng/mL. This assay has been validated for use in dogs with intra- and interassay precision (5 replicates) of 4.0% and 3.4%, respectively.21

Serum 1,25(OH)2D was measured in duplicate by chemiluminescent immunoassay (LIAISON; DiaSorin Inc) by VDI Laboratory using the manufacturer’s instructions. The 1,25(OH)2D assay is a modified 3-step sandwich assay that uses a recombinant fusion protein for capture of the 1,25(OH)2D molecule and a murine monoclonal antibody that specifically recognizes the complex formed by the recombinant fusion protein with the 1,25(OH)2D molecule. The solid phase contains a specific monoclonal antibody to bind with the complex. Using flash chemiluminescence, the light signal is measured by a photomultiplier as relative light units. It is proportional to the concentration of 1,25(OH)2D present in the calibrators, controls, and patient samples. Intra- and interassay precision is 0.2% to 2.9% and 4.1% to 6.6%, respectively. This assay’s measurement range is between 5.0 and 200 pg/mL. A laboratory reference interval was not established for 1,25(OH)2D. Routine submissions of serum to VDI by veterinarians reported as a healthy dog (n = 26) or cat (18) and that did not have elevated levels of inflammation (CRP in dogs, HPT in cats) were used to measure 1,25(OH)2D and provide a reference (REF) group of apparently healthy nonhospitalized dogs or cats. This group also included 6 research colony dogs fed a commercial diet enrolled in an observational aging study.

**Acute phase proteins**

Serum CRP and HPT concentrations were measured by VDI Laboratory as previously described.20,22,23 Briefly, CRP was measured with a canine-specific ELISA (TECO Canine cCRP assay; TECO Medical AG) and HPT by canine/feline-specific ELISA (TECO Canine/Feline HPT assay; TECOmedical AG). The VDI reference intervals for the CRP assay in dogs are as follows: normal (< 3.9 mg/L), mildly increased (4 to 9.9 mg/L), moderately increased (10 to 39.9 mg/L), and markedly increased (≥ 40 mg/L).20 The assay has intra- and interassay precision of 4.3% and 6.0%, respectively.21 The normal reference interval for the HPT assay in dogs is 30 to 250 mg/dL,20,22 and 34 to 112 mg/dL in cats on the basis of previously reported data.22 The assay has intra- and interassay precisions of 3.3% and 8.6%, respectively.22

Serum amyloid-A was measured in duplicate at VDI by a feline-specific fluorescent immunoassay (Vcheck Feline SAA 3.0; BioNote Inc). The test uses specific anti-feline SAA antibodies conjugated with fluorescence microparticles that bind to feline SAA. The solid phase is a nitrocellulose membrane coated with anti-feline SAA antibodies. Fluorescence line intensity compared against a known standard reflects the concentration. The method has a range of 5 to 200 µg/mL. There is no cross-reactivity to similar molecules (CRP, α-1-AG, haptoglobin). The assay’s normal reference interval for SAA in cats is < 5 µg/mL based on previous data cited by the manufacturer24 and the assay’s detection limit. The lower limits of quantification for serum CRP, HPT, and SAA are 0.5 mg/L, 10 mg/dL, and 5 µg/mL, respectively.

**Statistical analyses**

Values below detection limits were assigned a value of half the assay sensitivity (eg, 2.5 for an assay with a limit of detection of 5.0).26 Normality was assessed by the D’Agostino-Pearson omnibus normality test. As all parameters failed the test, except for feline 1,25(OH)2D, nonparametric statistics were applied on the basis of the non-Gaussian data distributions or small group sample sizes. Continuous data were presented as median and range. We also reported the IQR for analyte values. Serum 1,25(OH)2D concentrations were compared between hospitalized dogs and cats to corresponding species REF animals by the Mann-Whitney test. We compared serum analyte concentrations at admission to 48 hours postadmission by the Wilcoxon matched-pairs signed rank test. A correlation matrix with the Spearman test was conducted to evaluate relationships between individual dog vitamin D metabolites and APP concentrations and APPLEast scores. Spearman r values were interpreted as the following relationship strengths: 0.00 to 0.19 = very weak, 0.20 to 0.39 = weak, 0.40 to 0.59 = moderate, 0.60 to 0.79 = strong, and 0.80 to 1.0 = very strong.27 Serum analyte concentrations were compared among disease categories by the Kruskal-Wallis test with the Dunn multiple comparisons test (with adjusted P values reported). Associations between survival and serum analytes (serum concentration at admission and change after 48 hours), APPLEast scores, and study site, were tested by the Cox proportional hazard test. Kaplan-Meier curves were used to compare overall survival (ie, death at any time recorded during the study period), survival at 10 days postadmission, and survival at 1 year postadmission (ie, cases were censored if they were alive at 10 days or 1 year
postadmission, respectively) between or among groups categorized by analyte value cutoffs (median or laboratory reference ranges). Commercial software (Prism, version 9.0 or later; GraphPad Software) computed the statistical analyses and generated corresponding graphs. A P value < .05 established significance.

Results

Patient demographics

Client-owned dogs (n = 79) and cats (16) were included in this study (Figure 1). Forty-one male (4 intact) and 38 female (11 intact) dogs were included. A total of 1,800 dogs and 369 cats eligible for inclusion were not included in the final cohort. Most eligible cases were not enrolled due to a lack of owner consent or sufficient lab work/residual serum from admission (n = 1,962) and may not have been enrolled due to known exclusion criteria. Reasons for exclusion of initially enrolled cases included hospitalization for an elective procedure (n = 181), chronic renal disease (14), previously included or hospitalized (10), euthanized before admission (1), and parathyroid disease (1). The age range of dogs included was 6 weeks to 15 years, with a median of 6.7 years. The weights of included dogs ranged from 0.9 to 63 kg (median, 21.7 kg). Eight female (1 intact) and 8 male cats were included. The age range of cats included was 1 to 13 years, with a median of 6.4 years. The weights of included cats ranged from 2.7 to 7.3 kg (median, 4.9 kg). In dogs, disease categories included gastrointestinal foreign bodies (n = 9); infectious, inflammatory, immune-mediated, and nonseptic (24); infectious, inflammatory, and septic (7, all identified by positive blood culture); neoplasia (10); neurologic disease (9); and MISC (20). Diagnoses in the MISC category included increased gal tone (n = 1), persistent right aortic arch (1), jaw pain (1), cholelithiasis (1), urethral calculi (2), liver failure (2), phenobarbital-responsive sialadenitis, anemia from drug toxicity (1), hemoabdomen (1), pneumomediastinum (1), uroabdomen (1), hyperadrenocorticism (1), snake envenomation (1), diabetic ketoacidosis (1), urolithiasis with bacterial cystitis (1), peripheral vestibular disease (1), splenectomy for mass with extramedullary hematopoiesis (1), and mixed etiology (2). Disease categories for cats included the following: gastrointestinal foreign bodies (n = 3); infectious, inflammatory, immune-mediated, and nonseptic (8); and MISC (5). Diagnosis for cats in the MISC category included nasopharyngeal stenosis (n = 1), urethral obstruction (1), trauma (2), and anemia (1).

Canine APPLEfast scores ranged from 9 to 41 with a median of 20; the medians were 16 at Cornell University and 23 at Midwestern University. In cats, APPLEfast scores ranged from 7 to 39 with a median of 20; the medians were 19 at Cornell University and 25 at Midwestern University.

Serum analytes

At admission, 28% (22/79) of dogs hospitalized had 25(OH)D serum concentrations in the deficient range, with 50% (39/78) of dogs in the insufficient range and 23% (18/79) of dogs in the sufficient range (Figure 2).
Overall, the median serum 25(OH)D concentration for dogs was 66.5 ng/mL (range, 8.1 to 254 ng/mL; IQR, 38 to 95). A laboratory reference range was not established for 1,25(OH)\(_2\)D in dogs, and the median concentration was 9.2 pg/mL (range, 2.5 to 52.1 pg/mL; IQR, 5.1 to 14.3). The median 1,25(OH)\(_2\)D serum concentration in reportedly healthy (REF group) dogs obtained from samples submitted to VDI Laboratory for other testing purposes was 18.6 pg/mL (range, 5.3 to 50.2 pg/mL; IQR 15.0 to 23.3) and was significantly (P < .0001) higher than hospitalized dogs. C-reactive protein data were unavailable for 2 dogs. Eighty-nine percent (70/77) of dogs had increased serum CRP concentrations. The categorical distribution of serum CRP elevation in dogs was mildly increased (n = 9), moderately increased (15), and markedly increased (45). The median serum CRP concentration in dogs was 72.0 mg/L (range, 0.47 to 250.7 mg/L; IQR, 12.45 to 175.7). Sixty percent (47/79) of dogs had increased serum HPT concentrations (median, 444.5 mg/dL; range, 17.8 to 2,171.9 mg/dL; IQR, 138.4 to 919.7).

Samples collected 48 hours postadmission were obtained from 18 of 79 dogs. Lower 48-hour postadmission concentrations were measured for 25(OH)D in 10 dogs and 1,25(OH)\(_2\)D in 10 dogs. However, it is important to note that these changes were not consistent across all individual cases for both analytes. Median 25(OH)D and 1,25(OH)\(_2\)D in dogs for 48-hour samples were 45.1 ng/mL (range, 8.0 to 115 ng/mL; IQR, 35.0 to 88.4) and 6.2 pg/mL (range, 2.5 to 19.6 pg/mL; IQR, 2.5 to 11.3), respectively. Median CRP and HPT in dogs for 48-hour samples were 91.6 mg/L (range, 2.6 to 235 mg/L; IQR, 16.0 to 154.4) and 706.9 mg/dL (range, 45.9 to 1,897.6 mg/dL; IQR, 272.3 to 902.1), respectively. Overall, there was a median increase (median, 3.4 mg/L) in CRP, with decreased concentrations in 8 dogs. In contrast, there was an overall median decrease of 152.2 mg/dL in HPT 48 hours postadmission (negative change in 12/21). There were no significant changes between admission and postadmission samples for any analytes measured.

The median serum 25(OH)D and 1,25(OH)\(_2\)D concentrations in hospitalized cats on admission were 68.2 ng/mL (range, 23 to 111 ng/mL; IQR, 42.4 to 92.6) and 17.4 pg/mL (range, 2.5 to 54.25 pg/mL; IQR, 7.3 to 26.1), respectively (Figure 2). Serum concentrations of 25(OH)D in hospitalized cats were in the deficient

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**Figure 2**—Serum analyte values from a prospective cohort study in hospitalized dogs (A through D) and cats (E through H). Box-and-whisker plots depicting serum concentrations of the indicated metabolites measured in dogs (n = 79) and cats (16) hospitalized (HOSP) for various illnesses. For 25-hydroxyvitamin D (25(OH)D; A and E) the blue shaded area corresponds to the laboratory range for sufficient, the red shaded area indicates the deficiency range, and the intervening white area the insufficient range. For 1,25-dihydroxyvitamin D (1,25(OH)\(_2\)D; B and F), a laboratory reference range was not established and values measured in dog (n = 26) and cat (18) submissions to the laboratory reported as reportedly healthy are shown as a reference group. For C-reactive protein (CRP; C), haptoglobin (HPT; D and H), and serum amyloid-A (SAA; G), the blue-shaded areas correspond to the laboratory reference range. Boxes depict the IQR and horizontal lines within depict the median, whiskers depict the range, and dots depict individual values. Note that axis may not be the same for corresponding analytes between the 2 species (ie, data for each species are meant to stand alone). REF = Reference group. ****P < .0001.
range for 13% (2/16), insufficient for 69% (11/16), and in the sufficient range for 18% (3/16) of cats. The median 1,25(OH)₂D serum concentration in reportedly healthy (REF) cats was 15.9 pg/mL (range, 2.5 to 38.5 pg/mL; IQR, 9.0 to 25.8) and was not different (P = .96) than concentrations in hospitalized cats. On admission, median SAA and serum HPT in cats were 4.28 µg/mL (range, 2.5 to 200 µg/mL; IQR, 2.5 to 102.9) and 317.6 mg/dL (range, 37.2 to 1,503.4 mg/L; IQR, 76.9 to 605.3), respectively. Concentrations of SAA and HPT were abnormal in 50% (8/16) and 69% (11/16) of cats, respectively. Postadmission (48-hour) samples were only available for 3 cats, precluding meaningful analyses.

**Analyte relationships**

Significant correlations were identified between several analyte concentrations and clinical parameters in dogs (Figure 3). Serum 25(OH)D negatively correlated weakly with HPT (P = .002; r = −0.34). Serum 1,25(OH)₂D negatively correlated moderately with CRP (P < .0001; r = −0.43) and weakly with HPT (P = .012; r = −0.28). C-reactive protein positively correlated weakly with HPT and APPLE₉ score (P = .001; r = 0.32). A moderate, positive correlation existed between the study site and APPLE₉ score (P < .0001; r = 0.44).

In cats, there was a strong, positive correlation between serum SAA and HPT concentrations (P = .002; r = 0.73). Although it did not reach significance, a negative moderate relationship existed between 1,25(OH)₂D and APPLE₉ scores (P = .055; r = −0.49).

**Serum analytes in disease categories**

Dogs with sepsis had significantly lower serum 1,25(OH)₂D concentrations than dogs with gastrointestinal foreign bodies (P = .005) or those in the MISC (P = .007) disease category (Figure 4). C-reactive protein was significantly higher in dogs with sepsis than in dogs with neurologic (P = .007) or MISC (P = .02) diseases, and HPT was significantly higher in septic dogs than dogs in the MISC group (P = .02). In contrast, no significant differences in serum analytes among disease categories were detected in cats (Figure 5).

![Figure 3](image-url)

Figure 3—Correlation matrix comparing relationships between serum analytes from a prospective cohort study in hospitalized dogs and cats. Heat maps based on Spearman r values between parameters for (A) dogs (n = 78) and (B) cats (16) are provided in each cell. Scatterplots of parameters with significant and moderate or stronger relationships plotting 1,25(OH)₂D vs CRP (C) and SAA versus HPT (D). Study sites included the following: MWU (assigned 1), CU (assigned 0). Spearman r values were interpreted as the following relationship strengths: 0.00 to 0.19 = very weak, 0.20 to 0.39 = weak, 0.40 to 0.59 = moderate, 0.60 to 0.79 = strong, and 0.80 to 1.0 = very strong, as described in the methods. APPLE₉ = Acute patient physiologic and laboratory evaluation, abbreviated (fast) version. *P < .05. **P < .01. ***P < .001. ****P < .0001.
Figure 4—Serum analyte values in hospitalized dogs (n = 79) and cats (16) enrolled in a prospective cohort study segregated by disease category. Violin plots of serum concentrations in dogs (A through D) of 25(OH)D (A), 1,25(OH)_{2}D (B), CRP (C), and HPT (D) and in cats (E through H) of 25(OH)D (E), 1,25(OH)_{2}D (F), SAA (G), and HPT (H). Disease categories for dogs included the following: gastrointestinal foreign bodies (n = 9); infectious, inflammatory, immune-mediated, and nonseptic (24); infectious, inflammatory, and septic (7); neoplasia (10); neurologic disease (9); and MISC (20). Disease categories for cats included the following: gastrointestinal foreign bodies (n = 3); infectious, inflammatory, immune-mediated, and nonseptic (8); and MISC (5). Dots indicate individual values. Dashed lines indicate medians, and dotted lines indicate IQRs. Note that axis may not be the same for corresponding analytes between the 2 species (ie, data for each species are meant to stand alone). GI = Gastrointestinal. Inf/Infl/IMD = Infectious/inflammatory/immune-mediated disease. MISC = Miscellaneous. *P < .05. **P < .01.
Figure 5—Survival curves from a prospective cohort study in hospitalized dogs (n = 79) and cats (16). Survival data were compared between dogs (A through D), with 25(OH)D (A), 1,25(OH)2D (B), CRP (C), and HPT (D) concentrations above medians to at or below medians. Survival was also categorized by reference intervals for 25(OH)D (A inset) and HPT (D inset). Survival data were compared between cats (E through H) with 25(OH)D (E), 1,25(OH)2D (F), SAA (G), and HPT (H) concentrations above medians to at or below medians. Steps indicate death events. Tick marks indicate censored events. No significant differences were detected.
Serum vitamin D metabolites, APP, and survival

Individual survival data are summarized in Supplementary Table S1. In dogs, there were no associations between overall risk of death (HRs were approximately 1, with 95% CIs spanning 1) and admission concentrations of 25(OH)D, 1,25(OH)₂D, or APP (Table 1).

Table 1—Cox proportional hazard analysis of overall survival data obtained in a prospective cohort study conducted between August 12, 2019, and October 26, 2021, evaluating serum vitamin D metabolites and acute-phase protein concentrations in hospitalized dogs (n = 79) and cats (16).

<table>
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<th>Variable</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>P value</th>
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<td>.27</td>
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Hazard ratios < 1 indicate a reduced risk of death while ratios > 1 indicate an increased risk of death. The 95% CI reflects precision of the HR, with smaller ranges indicating greater precision. Study site comparison was Midwestern University (assigned 1) versus Cornell University (assigned 0).

1,25(OH)₂D = 1,25-dihydroxyvitamin D (calcitriol). 25(OH)D = 25-hydroxyvitamin D. APPLExn score = Acute patient physiologic and laboratory evaluation, abbreviated (fast) version.

or change in concentration at 48 hours. Similarly, when dogs were divided into 2 groups based on median analyte values, survival was not different between dogs with concentrations above or below median values (Figure 5). There was also no difference in survival in dogs categorized by reference intervals for 25(OH)D or HPT. Similarly, there was no survival difference between median-dichotomized groups at 10 days or 1 year (data not shown). Additionally, there were no significant differences in survival when patients were dichotomized by positive versus negative 48-hour changes in analyte concentrations.

In cats, serum analyte results, study site, and APPLExn score scores all lacked a reliable association with survival (Table 1). As in dogs, there was no significant difference in cats dichotomized by median serum analyte values.

Discussion

We aimed to measure the serum vitamin D metabolites 25(OH)D and 1,25(OH)₂D and APP, CRP, SAA, and HPT concentrations in dogs and cats hospitalized for illness and evaluate relationships with survival. Serum vitamin D metabolite and APP concentrations were frequently abnormal in our cohort of hospitalized dogs and cats. We also explored longitudinal changes over a 48-hour period postadmission. This aspect of our investigation is crucial as it begins to address the dynamic nature of these biomarkers during hospitalization. These data refuted our hypothesis and found that vitamin D metabolite and APP concentrations in dogs or cats in this cohort of hospitalized patients were not predictive of survival.

Lower 25(OH)D and higher APP serum concentrations have been described in various canine and feline conditions. Less commonly, 1,25(OH)₂D has been measured in the context of canine and feline illness beyond investigations linked to calcium homeostasis. Longitudinal evaluation of vitamin D metabolite status in cats and dogs in illness states is lacking. After 48 hours of hospitalization, the majority (10 out of 18) of cases exhibited lower concentrations of vitamin D metabolites. However, no statistically significant differences were observed in any analyte concentrations between the 2 time points. This lack of discernible variation could stem from several factors. First, the relatively small size of the cohort of dogs with longitudinal data may have rendered the study underpowered. Additionally, treatment variables were uncontrolled, potentially influencing vitamin D metabolite concentrations through medications, treatments, and individual patient responses.

Lastly, our investigation focused on a single time point soon after admission. To comprehensively assess longitudinal changes in serum vitamin D metabolites throughout hospitalization periods and evaluate the impacts of treatment, a larger cohort is needed. Therefore, additional studies are required to evaluate longitudinal changes in vitamin D metabolite status in hospitalized cats and dogs to address questions regarding any potential advantages for serial measurements or supplementation.

Although many studies have examined CRP concentrations in the context of critical illness, and some studies have looked at CRP in relationship to 25(OH)D, to the authors’ knowledge, no studies have investigated the relationship between 1,25(OH)₂D and CRP in cats or dogs. We hypothesized that 1,25(OH)₂D would negatively correlate with CRP. Our study confirmed that 1,25(OH)₂D was moderately negatively correlated with CRP, while 25(OH)D did not correlate with CRP. While our sample size was small, these data suggest a greater depletion of 1,25(OH)₂D than 25(OH)D levels during critical illness, particularly in disease processes that drive elevations in CRP. This phenomenon could be attributable to increased 1α-hydroxylase by immune cells in systemic inflammatory states.12,28,29 Another consideration is the relative half-lives of 25(OH)D (approx 2 weeks) and 1,25(OH)₂D (approx 6 hours)50 likely contribute to this finding. Future studies to further investigate this relationship are warranted, and an expanded panel of analytes may be informative, including other metabolites or enzymes involved in vitamin D metabolism, such as vitamin D–binding protein, 24,25(OH)D, and fibroblast growth factor-23 (which inhibits calcitriol production). Haptoglobin was weakly negatively correlated with both vitamin D metabolites.
However, it could act as a surrogate marker for vitamin D status in specific populations, and investigating how it may aid in future decision-making for supplementing vitamin D may still be warranted.

Several veterinary studies have linked hypovitaminosis D to outcomes in various disease categories in small animals. Jaffey et al found that critically ill dogs and dogs with sepsis had significantly lower serum 25(OH)D concentrations than healthy controls. These results contrast with our findings; however, our study included a cohort of hospitalized dogs that included critically ill patients and patients with more stable conditions. Our study’s median APPLE fast score in canine and feline patients was 20. In contrast, the previous research published by Jaffey et al had a median APPLE fast score of 23.2 (cats were not included in this study). Furthermore, the median canine 25(OH)D in our study was 66.5 ng/mL, while the median canine 25(OH)D in the previously mentioned study was 29.6 ng/dL. While 25(OH)D measurement in the Jaffey et al study was conducted using high-performance liquid chromatography, making the results not directly comparable, there is a stark contrast between our results. The contrasting results suggest that our cohort of hospitalized dogs did not have a wide enough distribution of illness severity to detect an association with 25(OH)D. Moreover, certain conditions within our cohort exhibited severity and chronicity patterns that may deviate from previous findings. For instance, our study included several cases involving gastrointestinal foreign bodies, whereas prior research on vitamin D metabolites in gastrointestinal conditions predominantly centered on chronic enteropathies.

While our study found that 25(OH)D did not predict survival overall, we did find that variation in concentration exists between specific disease categories (ie, sepsis vs gastrointestinal foreign bodies; Figure 4). It’s important to acknowledge that the grouping of cases was based on the distribution of conditions within our cohort, which may have introduced some bias into the data analysis. Therefore, while 25(OH)D was not a useful predictor of survival in our cohort of dogs, previous studies suggest it may be more beneficial when measured in the context of specific diseases or disease categories. In humans, the administration of vitamin D supplements has been linked to shorter durations of mechanical ventilation and hospital stays. While our cohort did not show a correlation between reduced concentrations of vitamin D metabolites and survival, further studies exploring alternative outcome measures and potential supplementation advantages are warranted.

Similarly, another study conducted in cats found that low serum 25(OH)D concentrations were predictive of survival. However, it is worth noting that there was considerable overlap in 25(OH)D concentrations measured in cats that died versus those that survived in this study. We also used slightly different approaches than the previous study in assessing relationships between 25(OH)D concentrations and survival, as they employed the Mann-Whitney test and logistic regression analysis. An intriguing discovery in our study was the observation of similar concentrations of 1,25(OH)₂D in hospitalized cats as apparently healthy controls. Should this finding hold true for broader populations of hospitalized versus healthy cats, it could imply species-specific distinctions in the regulation of 1α-hydroxylase activity in the face of reduced circulating levels of 25(OH)D.

In our study, there may have been an enrollment bias toward patients with lower disease severity, as median APPLE fast scores in our population were a quarter of the maximum score of 50 and ranged up to only about half the maximum score. In developing the canine APPLE fast score, most deaths occurred in dogs with scores > 20. Our maximum scores were approximately 40, where the CI ranged from about 50% to 100% risk of death. Therefore, our cohort’s range in illness severity may help explain the relative lack of death prediction by APPLE fast scores in this study.

Our goal to examine vitamin D status in dogs and cats hospitalized for nonelective reasons led to the recruitment of cases with various conditions, resulting in small numbers of patients in some disease categories. Although in this patient population vitamin D status was not a predictor of death, unfortunately, the patient populations in individual groups were limited. Further studies examining specific groups of patient populations are warranted to determine when vitamin D status serves as a predictor of death.

Various methodologies for measuring 25(OH)D exist, but discrepancies between assays hinder direct comparisons across previous studies. Mass spectrometry is regarded as the gold standard, yet its widespread adoption is limited by cost, sample size requirements, and specialized expertise. A prior investigation highlighted the misclassification of 25(OH)D sufficiency and insufficiency when comparing immunologic assays, like those used here, to mass spectrometry. Future efforts should prioritize refining the accuracy of more accessible immunologic assays to advance 25(OH)D research.

Benchmarks defining physiologic deficiency in serum levels of vitamin D metabolites remain elusive. Consequently, interpreting these values requires consideration of laboratory-specific reference ranges. Additionally, it is crucial to recognize that adequacy of vitamin D may vary depending on its roles in physiological functions, such as calcium regulation versus immune system balance.

Another limitation was the lack of a reference interval for this study’s 1,25(OH)₂D assay. We tackled this issue by assembling a cohort of dogs and cats reportedly in good health. Although these cases were deemed healthy by the veterinarians who submitted the serum samples, standardized diagnostics to confirm their health status were not mandated. While this lack of standardization introduces variability in our reference group, these cases serve as a valuable nonhospitalized control cohort with normal serum CRP or HPT concentrations. Additionally, it’s important to note that detailed diet histories for all recruited patients were not available, adding another layer of complexity to interpreting these results.

In a relatively small cohort of hospitalized cats and dogs, our results suggest measuring vitamin D metabolites was not a predictor of death. Future research should focus on expanding longitudinal
studies with larger sample sizes to further elucidate the dynamic changes in these biomarkers and their potential implications for patient management and outcomes. Additionally, interpretation of these biomarkers should consider the patient’s disease and clinical status, emphasizing the need for a personalized approach in clinical practice.

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Disclosures

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**Supplementary Materials**

Supplementary materials are posted online at the journal website: avmajournals.avma.org.