Evaluation of serum cortisol concentration as a prognostic indicator for nonsurvival to hospital discharge in critically ill dogs

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OBJECTIVE

To investigate whether serum cortisol (SC) concentration is a useful prognostic indicator for survival versus nonsurvival to hospital discharge in critically ill dogs.

ANIMALS

229 client-owned dogs.

PROCEDURES

Medical records were retrospectively reviewed to identify critically ill dogs that were hospitalized between January 2010 and May 2018 and that had SC concentrations measured ≤ 3 days after admission. Results for SC concentrations were compared for dogs grouped by survival versus nonsurvival to hospital discharge, with versus without sepsis, and other variables of interest. The predictive value of SC concentration for nonsurvival to hospital discharge was assessed (OR, sensitivity, and specificity) for cutoffs determined from a ROC curve or reference limit.

RESULTS

Median SC concentration was higher in dogs that did not survive to hospital discharge (8.5 μg/dL; interquartile [25th to 75th percentile] range, 4.8 to 11.8 μg/dL), compared with concentration in those that were discharged alive (4.5 μg/dL; interquartile range, 2.5 to 6.9 μg/dL). The area under the ROC curve was 0.72 (95% confidence interval [CI], 0.64 to 0.81) for SC concentration predicting nonsurvival. The calculated optimum cutoff of SC concentration was 7.6 μg/dL, at which the OR, sensitivity, and specificity for nonsurvival were 5.4 (95% CI, 2.7 to 10.9), 58%, and 80%, respectively. Alternatively, when the upper reference limit for SC concentration (5.8 μg/dL) was used as the cutoff, the OR, sensitivity, and specificity for nonsurvival were 3.6 (95% CI, 1.8 to 7.1), 67%, and 64%, respectively.

CONCLUSIONS AND CLINICAL RELEVANCE

Results indicated that SC concentration could be used as part of an overall assessment of prognosis in critically ill dogs. (*J Am Vet Med Assoc* 2020;256:1034–1040)

The role of the HPA in maintaining homeostasis in humans and animals is well established; however, its role in the presence of systemic disease is less defined.[1] In humans, studies[2,3] show that SC concentrations increase in the acute phase of systemic disease. Conflicting evidence exists regarding whether SC concentration changes in humans have prognostic value. For instance, findings disagree on whether adverse outcome is associated with SC concentrations that are high[2,4–11] versus low or at the lower reference limit,[1] and other studies[12,13] show no correlation between SC concentration and outcome.

Applicable translation of findings in human medicine to veterinary medicine is hampered by species differences, such as lower SC concentrations in dogs versus humans.[14] Furthermore, the potential association between SC concentration and outcome has not been investigated as much in veterinary patients as it has in human patients. However, studies show that high SC concentrations are associated with nonsurvival in dogs with parvovirus infection[15] or acute *Babesia canis* infection.[16] A laboratory-based study[17] in dogs with experimentally induced *Staphylococcus aureus* pneumonia similarly shows a correlation between SC concentration at 24 hours after onset of sepsis and nonsurvival to 96 hours.[17] Conversely, a small prospective study[18] in dogs admitted to an ICU shows no substantial correlation between any markers of HPA function and patient outcome.

The aim of the retrospective study presented here was to investigate whether SC concentration is a useful

ABBREVIATIONS

CI	Confidence interval
CLA	Chemiluminescent immunoassay
HPA	Hypothalamic pituitary axis
ICU	Intensive care unit
IQR	Interquartile (25th to 75th percentile) range
ROC	Receiver operating characteristic
SC	Serum cortisol
SOFA	Sequential organ failure assessment score

prognostic indicator for nonsurvival versus survival to hospital discharge in critically ill dogs. We hypothesized that SC concentrations would be higher in dogs that did not survive to hospital discharge, compared with those that were discharged alive, thereby conferring prognostic value for predicting outcome in terms of survival versus nonsurvival to hospital discharge.

Materials and Methods

Animals

In this retrospective study, the patient database of the University of Liverpool Small Animal Teaching Hospital was searched for records of all dogs that were admitted to the ICU for emergency or critical care and that had SC concentration measured with a CLA ≤ 3 days after admission between January 2010 and May 2018. Dogs were excluded if they did not initially require emergency or critical care, were referred for urgent care but not deemed critically ill (eg, dogs that had acute hemorrhagic diarrhea syndrome but were cardiovascularly stable) by the attending veterinarian, had received glucocorticoids or other medications known to affect the HPA (eg, ketoconazole, progestogens, or etomidate) by any route in the previous 6 weeks, had a concurrent condition (eg, hypercortisolism or hypoadrenocorticism) that could affect the HPA, or did not have SC concentration measured with a CLA within the first 3 days of hospitalization. If the suitability of including a dog was unclear, the authors reviewed the dog's medical history and decided by consensus for inclusion or exclusion. The study was approved by the University of Liverpool Veterinary Research Ethics Committee (VREC575).

Data collection

Data collected from the medical records included signalment, SC concentration (µg/dL), presence of sepsis (yes or no), and survival to hospital discharge (yes or no). Dogs were classified as having sepsis if they met the most recent human criteria for sepsis.[19] Sepsis was defined as "organ dysfunction caused by a dysregulated host response to infection."[19] Organ dysfunction was defined as an increase in SOFA score[20] ≥ 2 during hospitalization. An infection was determined present when the medical record evidenced a confirmed final diagnosis of an infectious process (whether primary [eg, pyelonephritis] or perceived to have substantially contributed to the dog's critical illness even if not the primary complaint [eg, aspiration pneumonia secondary to an esophageal foreign body]) or an attending veterinarian's strong suspicion of infection (eg, severe pneumonia) that could not be confirmed because the dog was too unstable to undergo confirmatory testing at the time. The final diagnosis recorded in the dog's clinical notes was that given by the attending veterinarian. While hospitalized, dogs were managed by interns, residents, or board-certified veterinarians, and for each dog managed by an intern or resident, a board-certified veterinarian supervised the care and confirmed the final diagnosis. On the basis of information in the medical records and with use of the modified Glasgow coma scale,[21] a SOFA score was calculated retrospectively for each dog. When medical records lacked sufficient information on individual parameters for scoring, those parameters were awarded a zero score. Dogs were grouped according to whether they died before hospital discharge (nonsurvivors) or were discharged alive (survivors), without attention to the duration of hospitalization. Dogs were then further considered on the basis of whether sepsis was or was not present and whether their CLA-measured SC concentration was or was not above the upper reference limit (5.8 µg/dL).

Cortisol CLA

Serum cortisol concentration was measured with a cortisol CLA.[a] The use of a cortisol CLA has been previously validated in dogs.[14,22,23] Intra-assay coefficients of variation range from 10% to 55% and are concentration dependent, with increased precision at higher concentrations.[22] The standard operating procedure at the facility during the study period was that blood samples intended for cortisol CLA were collected in serum tubes with or without a serum gel separator and then stored at room temperature until tested, typically ≤ 4 hours after collection.

Statistical analysis

An a priori sample size was calculated with statistical software.[b] To our knowledge, no veterinary study provided sufficient information to perform a sample size calculation for the investigation we intended; therefore, a comparative study[6] looking at SC concentrations and nonsurvival in human patients with septic shock was used as guidance. We determined that a sample size of 94 dogs, with 47 dogs in each group (survivors vs nonsurvivors), was required ($\alpha = 0.05$; $\beta = 0.8$; $d = 0.6$).

Data were retrieved from hospital records[c] and entered into a spreadsheet.[d] Statistical analysis was performed with available software.[e] Results were reported as mean ± SD or as median and IQR. Normality was assessed with the Kolmogorov-Smirnov test and visualization of Q-Q plots. To compare results for baseline group characteristics, the independent *t* test or Mann-Whitney *U* test was used for continuous variables and the χ^2 test was used for categorical variables. Comparisons of SC concentration results for survivors versus nonsurvivors as well as for dogs with versus without sepsis and for other variables of interest were performed with the Mann-Whitney *U* test. The effect size was calculated with the formula:

$$r = \frac{Z}{\sqrt{n}}$$

where r = effect size (small, $r = 0.10$ to < 0.30; medium, $r = 0.30$ to < 0.50; and large, $r = > 0.50$), Z = score derived from the Mann-Whitney *U* test, and n = the number of samples.

Univariate analysis to identify a potential relationship between SC concentration and nonsurvival to hospital discharge was performed with binary logistic regression, and ORs were calculated. A ROC curve was created by plotting the sensitivity (true-positive rate) versus 1 – specificity (false-positive rate) to assess the accuracy of SC concentration in predicting nonsurvival to hospital discharge, and the Youden index was used to find the optimum SC concentration cutoff. The predictive value of SC concentration for nonsurvival to hospital discharge was assessed (OR, sensitivity, and specificity) for cutoffs determined from the ROC curve (optimal cutoff determined with the Youden index) or upper reference limit (5.8 µg/dL [160 nmol/L]). Significance was set at $P < 0.05$.

Results

Animals

Our search of the medical records identified 2,507 dogs that had been admitted to the hospital and had SC concentrations measured between January 2010 and May 2018. Of these, 2,278 were excluded because they did not require emergency care (n = 2,002), had received glucocorticoids or other potentially interfering medication (79), had or were suspected of having hypercortisolism (65), had incomplete medical records (62), had hypoadrenocorticism (23), had intervertebral disk disease (22), or either did not have SC concentration measured ≤ 3 days after hospital admission (17) or had it measured by means other than CLA (8). Of the 229 dogs that met the inclusion criteria, all had initially been triaged and referred by other veterinarians, 184 survived to hospital discharge (survivors), and 45 did not survive to hospital discharge (nonsurvivors). Of the 45 nonsurvivors, 34 were euthanized and 11 died. Thus, the overall mortality rate during the study period was 20% (45/229), with 15% (34/229) from euthanasia and 5% (11/229) from death. The exact reason for euthanasia was not documented in any dog.

Dogs in the nonsurvivor group included 5 Labrador Retrievers; 3 each of Cocker Spaniel, English Springer Spaniel, and mixed-breed dog; 2 each of Boxer, Cavalier King Charles Spaniel, Flat-Coated Retriever, and Pug; and 1 each of 23 other breeds. Dogs in the survivor group included 33 mixed-breed dogs; 17 Labrador Retrievers; 8 each of Cavalier King Charles Spaniel and Cocker Spaniel; 7 each of Border Collie, Boxer, English Springer Spaniel, and German Shepherd Dog; 6 Yorkshire Terriers; 5 each of Border Terrier and Golden Retriever; 4 each of Beagle, Dogue de Bordeaux, Rottweiler, Shih Tzu, Siberian Husky, and Staffordshire Bull Terrier; 3 each of Chihuahua, Jack Russell Terrier, Lhasa Apso, Miniature Schnauzer, Pug, Standard Poodle, Weimaraner, and West Highland White Terrier; 2 each of Akita, Greyhound, Lurcher, Newfoundland, and Pomeranian; and 1 each of 6 other breeds.

The nonsurvivor group consisted of 28 males (11 castrated and 17 sexually intact) and 17 females (12 spayed and 5 sexually intact); the survivor group consisted of 108 males (66 castrated and 42 sexually intact) and 76 females (65 spayed and 11 sexually intact; **Table 1**). Although no difference in survival to hospital discharge was detected for male versus female dogs in general or for females that were sexually intact versus spayed, sexually intact males had greater odds (OR = 2.4; 95% CI, 1.2 to 4.6; $P = 0.038$) for nonsurvival to hospital discharge than did castrated males. Although median age did not differ significantly ($P = 0.46$) between survivors (6.0 years; IQR, 3.0 to 10.0 years) and nonsurvivors (7.0 years; IQR, 4.5 to 10.0 years), the median age was older in neutered dogs (spayed females and castrated males; 7.0 years; IQR, 4.5 to 10.0 years; $P < 0.001$) than in sexually intact dogs (4.0 years; IQR, 1.0 to 8.0 years). Median age also differed significantly ($P = 0.006$ and $P < 0.001$, respectively) for spayed females (7.0 years; IQR, 4.0 to 10.0 years) versus sexually intact females (4.0 years; IQR, 0.60 to 6.0 years) and for castrated males (8.0 years; IQR, 5.0 to 10.5 years) versus sexually intact males (4.0 years; IQR, 1.0 to 8.0 years).

Median body weight was not substantially different between survivors (19.2 kg [42.2 lb]; IQR, 9.0 to 31.6 kg [19.8 to 69.5 lb]) and nonsurvivors (20.0 [44.0 lb]; IQR,

Table 1—Results of analysis to identify potential factors associated with survival versus nonsurvival to hospital discharge in critically ill dogs that had SC concentrations measured with a CLA ≤ 3 days after admission between January 2010 and May 2018.

Variable	Nonsurvivors (n = 45)	Survivors (n = 184)	*P* value
Age (y)*	7.0 (4.5–10.0)	6.0 (3.0–10.0)	0.46
Weight (kg)*	20.0 (11.0–29.6)	19.2 (9.0–31.6)	0.82
Sex			0.67
Male	28	108	
Female	17	76	
Neuter status			0.01
Sexually intact	22	53	
Castrated or spayed	23	131	
No. (%) of dogs with sepsis	10 (22)	25 (14)	0.15
SC concentration (µg/dL)*	8.5 (4.8–11.8)	4.5 (2.5–6.9)	< 0.001
No. (%) of dogs with SC concentration > 5.8 µg/dL	30 (67)	66 (36)	< 0.001

*Reported as the median and IQR.

11.0 to 29.6 kg [24.2 to 65.1 lb]; Table 1). Similarly, the proportion of dogs that met the criteria for sepsis was not meaningfully different between survivors (25/184 [14%]) and nonsurvivors (10/45 [22%]).

SC concentration

Results for SC concentrations were nonnormally distributed, and the overall median SC concentration was 5.0 µg/dL (138 nmol/L; IQR, 2.7 to 8.2 µg/dL [74 to 226 nmol/L]; reference interval, 2.0 to 5.8 µg/dL [55 to 160 nmol/L]). Ninety-six of the 229 (42%) dogs had a SC concentration > 5.8 µg/dL, and the proportion of dogs with high SC concentration was significantly (P < 0.001) greater for nonsurvivors (30/45 [67%]), compared with survivors (66/184 [36%]; Table 1). In addition, the median SC concentration was significantly (P < 0.001) higher for nonsurvivors (8.5 µg/dL [234 nmol/L]; IQR, 4.8 to 11.8 µg/dL [132 to 326 nmol/L]) versus survivors (4.5 µg/dL [123 nmol/L]; IQR, 2.5 to 6.9 µg/dL [69 to 190 nmol/L]; **Figure 1**). The effect size was medium (r = 0.30). There was a 1.11 increase in the odds of nonsurvival (OR = 1.11; 95% CI, 1.05 to 1.17; P < 0.001) for every 1 µg/dL increase in SC concentration.

When SC concentration was evaluated for dogs grouped by those with versus without sepsis, the median SC concentration was significantly (P < 0.001) higher for dogs with sepsis (9.2 µg/dL [253 nmol/L]; IQR, 6.1 to 17.6 µg/dL [168 to 486 nmol/L]) versus those without sepsis (4.5 µg/dL [124 nmol/L]; IQR, 2.6 to 7.0 µg/dL [72 to 193 nmol/L]; **Figure 2**). The effect size was medium (r = 0.3).

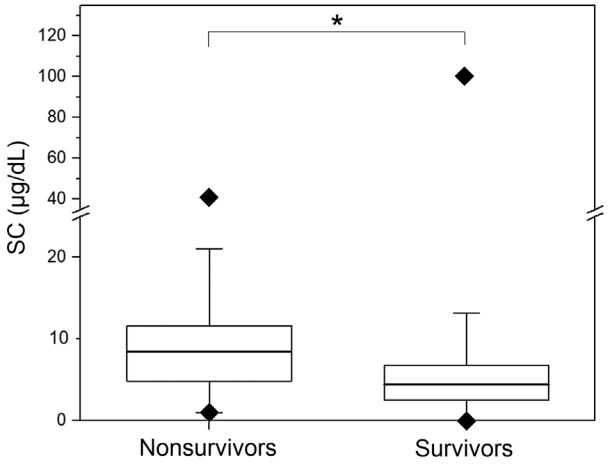

Figure 1—Box-and-whisker plots of SC concentration measured within 3 days of hospitalization in 229 critically ill dogs treated between January 2010 and May 2018 and grouped by whether dogs did not survive to hospital discharge (nonsurvivors; n = 45) or were discharged alive (survivors; 184). Each box represents the IQR, the central horizontal line in the box represents the median, whiskers represent the data points farthest from the median that are not outliers (ie, that are within 1.5 times the IQR of the first and third quartiles), and diamonds represent the maximum and minimum values. *Median SC concentration differed significantly (P < 0.001) for survivors versus nonsurvivors.

SC concentration as a predictive indicator for nonsurvival to hospital discharge

The area under the ROC curve was 0.72 (95% CI, 0.64 to 0.81; **Figure 3**), which indicated that SC concentration had good predictive value for nonsurvival to hospital discharge in critically ill dogs. With use of the Youden index, the SC concentration of 7.6 µg/dL (210 nmol/L) was identified as the optimum cutoff, at which the OR, sensitivity, and specificity for nonsurvival to hospital discharge were 5.4 (95% CI, 2.7 to 10.9), 58%, and 80%, respectively. Alternatively,

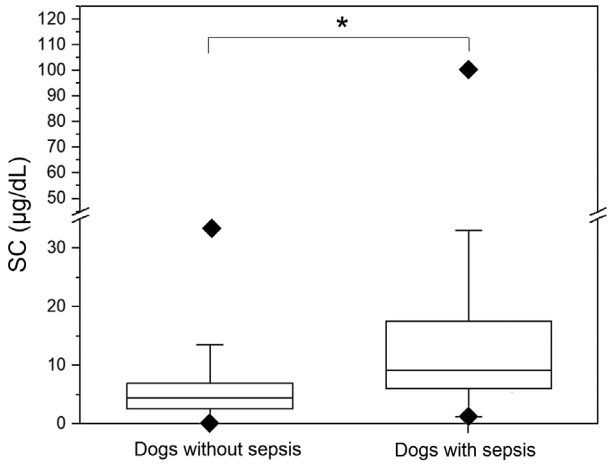

Figure 2—Box-and-whisker plots of SC concentration in the dogs in Figure 1 grouped by those with (n = 35) versus without (194) sepsis. *Median SC concentration differed significantly (P < 0.001) for dogs with versus without sepsis. ***See*** Figure 1 for key.

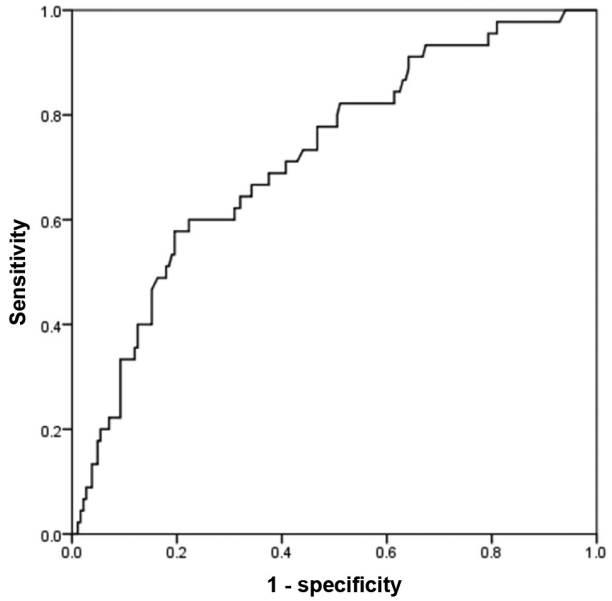

Figure 3—Results of ROC curve analysis to determine the predictive value of SC concentration for nonsurvival to hospital discharge in the critically ill dogs described in Figures 1 and 2. The area under the ROC curve is 0.72 (95% CI, 0.64 to 0.80)

when the upper reference limit (SC concentration, 5.8 µg/dL) was used as the cutoff, the OR, sensitivity, and specificity for nonsurvival were 3.6 (95% CI, 1.8 to 7.1), 67%, and 64%, respectively.

Discussion

Results of the present study indicated that the median SC concentration in critically ill dogs was higher in those that did not survive to hospital discharge, compared with those that were discharged alive. This finding supported our hypothesis and was consistent with studies in dogs with parvoviral diarrhea,[15] *B canis rossi* babesiosis,[16] or *S aureus* pneumonia[17] that show associations between high SC concentration and nonsurvival in affected dogs. However, the mortality rate in the present study (20% [45/229]) differed from that in the study[16] involving dogs with *B canis* infection (7% [7/95]), likely because of differences in underlying disease processes in dogs evaluated. However, the mortality rate of the present study did not have a comparative finding in the study[17] involving dogs with *S aureus* pneumonia because of the terminal design of that previous study. Another difference was that the SC concentration was > 5.8 µg/dL for all 7 dogs that died in the *B canis* study,[16] compared with 30 of the 45 (67%) nonsurvivors in the present study.

In contrast, our finding of an association between high SC concentration and nonsurvival to hospital discharge in critically ill dogs was inconsistent with a study[18] that shows no substantial difference in SC concentration for dogs that did versus did not survive to hospital discharge. The reason for the discrepancy was not clear but could have been because of a difference in inclusion criteria that resulted in differences between the groups of dogs evaluated. Although the proportion of critically ill dogs with SC concentrations > 5.8 µg/dL in the present study (42% [96/229]) and the previous study[18] (37% [19/52]) were similar, the mortality rate was lower in our study (20% [45/229]), compared with the previous study (35% [7/52]).

The area under the ROC curve was 0.72, which suggested a good overall predictive value of SC concentration for nonsurvival to hospital discharge in critically ill dogs of the present study. This finding was similar to the area under the curve (0.62) reported in 2 prospective human studies[5,10] involving patients with septic shock. The optimal cutoff calculated from the ROC curve was 7.6 µg/dL (210 nmol/L), at which the OR, sensitivity, and specificity were 5.4, 58%, and 80%, respectively. These findings mirrored those of studies[5,6,13] in human patients with septic shock that show high SC concentration is associated (OR, 1.8 to 7.89) with nonsurvival. When the SC concentration cutoff in the present study was lowered to the upper reference limit (5.8 µg/dL), the OR and specificity for nonsurvival also decreased to 3.6 and 64%, consistent with findings in the human studies,[5,6,13] and the sensitivity for nonsurvival in our study increased to 67%.

The cause of SC concentration increase during acute, severe illness is likely multifactorial. It is presumed to be in part the result of increased activity of the HPA itself, resulting from catecholamine release,[24] proinflammatory cytokines,[25] and ACTH-independent adrenal steroidogenesis, among other processes.[1,3,24,26] However, non-HPA mediated factors are also believed to be involved, such as those resulting from reduced cortisol binding proteins[1,27,28] and cortisol metabolism.[3] Thus, it could have been that the difference in SC concentration between survivors and nonsurvivors in our study was truly a pathophysiologic observation, reflecting differences in disease severity and physiologic stress, which our study was not designed to assess.

An unexpected finding in the present study was the greater odds of nonsurvival for sexually intact male dogs, compared with those that had been neutered. This finding could have reflected a greater proportion of certain diseases more commonly seen in young animals (eg, parvoviral enteritis) or a relatively lower tolerance to critical illness in younger dogs because sexually intact male dogs were notably younger than neutered male dogs in the present study. However, a similar association between neuter status and nonsurvival was not detected in female dogs of the present study, suggesting that factors other than age may have been present. Alternatively, this could have been a type I statistical error.

The main limitation of the present study was its retrospective nature. It is not standard practice to measure SC concentration in every critically ill patient at our hospital, and consequently our selection criteria resulted in selection bias toward dogs with suspected HPA dysfunction. This was made apparent during data collection when hypercortisolism and hypoadrenocorticism were identified frequently among differential diagnoses noted in the medical records of dogs with neurologic and gastrointestinal signs, respectively. Therefore, applying the results of the present study to all critically ill dogs must be done cautiously. Relatedly, avoidance of misapplication of results was the principal reason why we did not calculate positive and negative predictive values and believe that a prospective study would better determine the prognostic value of SC concentration in critical illness in dogs.

Aside from illnesses, many other factors can alter SC concentrations in dogs. Potential interpatient differences include, but are not limited to, breed,[29] weight,[30] sex,[31] stage of the estrous cycle,[32] activity levels,[33] stress,[34] recent surgery,[35] hypoglycemia,[36] and opioid or benzodiazepine administration.[37,38] In addition, SC concentration may also be impacted by potential differences in measurement methods (eg, sample timing[3,17,27] and handling[39]) and inter- and intra-assay variation.[14,22,23,40] The relatively large number of dogs in the present study may have mitigated differences in group characteristics; however, the influence of sample timing must be highlighted because studies[2,11,27] in humans show temporal changes in SC concentrations during the acute phase of illness, with an initial peak concentration followed by a return to

baseline in the subsequent 24 to 72 hours. In dogs, one study[17] shows a similar pattern, whereas another study[15] shows that SC concentrations only correlated with survival 48 and 72 hours after onset of illness but not at 24 hours after onset.[15] This temporal pattern may reflect the short half-life of cortisol in dogs[41,42] and humans[43,44] and has implications for the present study. Although SC concentration was measured ≤ 3 days after admission to the ICU in the present study, time between disease onset and hospitalization likely varied. The higher median SC concentration in the nonsurvivor group may have been the result of those dogs having been deemed less stable by the referring veterinarians, having been referred more quickly, and having had their SC concentration measured earlier in the disease course, compared with dogs in the survivor group. In a nonexperimental study, such as ours, it is difficult to compensate for differences in sample timing relative to onset of critical illness, especially in a referral population. However, the use of a hospital population, rather than an experimental population, allows for more appropriate application of the results of the present study to other hospital populations. An important caveat to highlight is that all dogs in the present study were referred, which may limit the application of results to primary care practices.

In addition, relative differences in cortisol metabolism between dogs that did and did not survive to hospital discharge in the present study could have influenced SC concentration results. Cortisol primarily undergoes hepatic metabolism, with a lesser degree of renal metabolism, and both processes may be reduced in the presence of severe systemic illness.[3] Thus, dogs in the present study with concurrent hepatic or renal dysfunction may have had an additive effect on the already reduced cortisol metabolism of systemic illness. However, studies[27,45–47] also show a resultant increase in total cortisol concentrations with hepatic dysfunction. The implication for our study was that potential greater organ dysfunction in nonsurvivors, compared with survivors, may have led to lower cortisol metabolism and resulted in higher SC concentration; however, the number of dogs with affected organs assessed with the SOFA score were too small to provide any evidence for this association. Further, the influence of euthanasia on evaluation of survival versus nonsurvival to hospital discharge must be considered, especially in a retrospective study such as ours in which the reason for euthanasia was unclear.

Results indicated that SC concentration could be used as part of an overall assessment for prognosis in critically ill dogs. We believe that the explanation for the difference in SC concentrations between dogs that did and did not survive to hospital discharge in the present study was most likely multifactorial and that further studies to investigate the relationship and explore interventions that may improve survival to hospital discharge in critically ill dogs are warranted.

Acknowledgments

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Extralabel use of antimicrobials may have occurred with dogs of the present study, but such use was not discussed in the manuscript.

Footnotes

a. Immulite 2000 Cortisol, Siemens Healthcare GmbH, Erlangen, Germany.
b. G*Power, version 3.1.9.2, G*Power Team, Düsseldorf, Germany.
c. Tristan, version 1.8.3.110, Orion Engineering Services, Aberdeen, Scotland.
d. Microsoft Excel, version 2016, Microsoft Corp, Redmond, Wash.
e. SPSS Statistics, version 22.0, IBM Corp, Armonk, NY.

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