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Nutritional and laboratory assessment as predictors of hospitalization time, severity of the underlying disease and mortality in dogs

Avaliação nutricional e laboratorial como preditores do tempo de hospitalização, gravidade da doença de base e mortalidade em cães

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Highlights

Malnutrition, anemia and elevated CRP/albumin are associated with disease severity.
Low TF and TIBC and high TS are associated with systemic diseases.
LOS indicators: low albumin, CRP/albumin ratio, BMI, HGB and hyporexia.
Hypoalbuminemia and elevated urea concentration are markers of mortality.

Abstract

In medicine, prognosis is defined as a prediction based on a patient's diagnosis and therapeutic possibilities, including the duration and severity of illness, and death rate. The prognostic indices used in human medicine, or even the few standardized indices for veterinary medicine, are not widespread or routinely applied in small animal clinics. Thus, this study aimed to identify commonly used variables in routine clinical practice that may be useful as prognostic markers, correlating specifically with length of stay (LOS), disease severity, and mortality. This study included 246 routine dogs from a teaching veterinary hospital in southern Brazil who underwent analyses for nutritional status (NS) (by anamnesis, physical, and laboratory examinations), hematological examinations, and several serum biochemical parameters. Frequencies, means and standard deviations of the variables, correlation analyses and comparative analyses (Kruskal-Wallis at α = 5%) were calculated. The following relevant markers of disease severity in dogs were considered: presence of malnutrition, including history of hyporexia, anorexia, body condition score (BCS) ≤3 and muscle mass score (MMS≤2); low values of transferrin

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(TF), total iron binding capacity (TIBC) and anemia; high values of transferrin saturation (TS), lactate and C-Reactive Protein (CRP)/albumin ratio. In animals without systemic diseases or with mild-to-moderate systemic diseases, low albumin values, CRP/albumin ratio, and hyporexia were indicators of a long LOS. In animals with critical systemic diseases, low body mass index (BMI) and hemoglobin (HGB) values were indicators of a short LOS (secondary to high mortality). Mortality indicators in sick dogs included hypoalbuminemia and high urea concentration. In conclusion, the observation of these alterations, especially when they occur simultaneously, helps in determining the prognosis in dogs from a hospital population in an objective, practical, and accessible manner.
Key words: Dogs. C-Reactive Protein/albumin. Systemic Diseases. Prognosis.

Resumo

Prognóstico é definido na medicina, como uma predição baseada no diagnóstico do paciente e nas possibilidades terapêuticas, acerca da duração da enfermidade, gravidade da doença e taxa de óbito. Os índices de prognóstico utilizados em medicina humana ou mesmo os poucos padronizados para medicina veterinária não são muito difundidos ou aplicados na clínica de pequenos animais. Dessa forma, esta pesquisa objetivou identificar variáveis de uso comum na rotina clínica que possam ser úteis como marcadoras de prognóstico, se correlacionando, especificamente como o tempo de internamento (TI), gravidade de doença e mortalidade. Para tal, foram avaliados 246 cães da rotina de um hospital veterinário escola do sul do Brasil, estudados em relação ao estado nutricional (EN) (por anamnese, exame físico e laboratorial), exames hematológicos e diversos parâmetros bioquímicos séricos. Foram calculadas as frequências, médias e desvios-padrões das variáveis, análises de correlação e análises comparativas (Kruskal-Wallis em $\alpha = 5\%$). Foram considerados marcadores relevantes de gravidade de doença em cães: presença de desnutrição, incluindo histórico de hiporexia, anorexia, escore de condição corporal (ECC) ≤3 e escore de massa muscular (EMM≤2); baixos valores de transferrina (TF), da capacidade total de ligação ao ferro (CTLF) e anemia; além de valores elevados de índice de saturação da transferrina (IST), lactato e da relação PCR/albumina. Nos animais sem doenças sistêmicas ou com doenças sistêmicas discretas a moderadas, os indicadores de elevado TI foram: baixos valores de albumina, relação Proteína-C-Reativa (PCR)/Albumina e hiporexia. Nos animais com doenças sistêmicas críticas, os indicadores de baixo TI (secundários a alta mortalidade) foram: baixos valores de índice de massa corporal (IMC) e hemoglobina (HB). Os indicadores de mortalidade em cães doentes foram: hipoalbuminemia e elevada concentração de ureia. Conclui-se que a constatação dessas alterações, especialmente quando ocorrem simultaneamente, embasa a determinação do prognóstico em cães de uma população hospitalar, de maneira objetiva, prática e acessível.
Palavras-chave: Cães. Proteína-C-Reativa/albumina. Doenças Sistêmicas. Prognóstico.

Introduction

Prognosis is defined in medicine as a prediction made by the doctor based on a patient's diagnosis and therapeutic possibilities, duration of the disease (including length of stay), severity of the underlying disease, and mortality (Niewiński et al., 2014;

da Silva et al., 2014). To determine this, it is necessary to investigate the presence of metabolic and potentially lethal disorders, which also guide the selection of patients who require intensive care (Rabelo et al., 2009; Fabretti et al., 2014).

Prognostic indices (PI) have been developed to make the prognostic estimates more objective. The Acute Physiology and Chronic Health Evaluation II (APACHE II), Simplified Acute Physiology Score II (SAPS II), Logistic Organ Dysfunction System (LODS), Multiple Organ Dysfunction Score (MODS), and Sequential Organ Failure Assessment (SOFA) are most commonly used in human medicine to analyze clinical and laboratory variables to predict mortality. There are several other PIs with similar objectives and methods and, recently, PIs based on genetic-immunological analyses have also been developed (Namendys-Silva et al., 2013; da Silva et al., 2014; Huang et al., 2021; Ma et al., 2022).

Few prognostic evaluation protocols have been developed in veterinary medicine. The Survival Prediction Index (SPI), Animal Trauma Triage Scoring (ATT), and Rapid Intensive Care Outcome (RICO) are inspired by human PIs, in which practically the same variables are monitored; however, these indices are not widely used or applied (King et al., 2001; Rabelo et al., 2009). During routine clinical examination of companion animals, measurements of albumin, C-reactive protein (CRP), and lactate are used as prognostic markers, without classification into indices (Rabelo et al., 2009; Portero et al., 2019; Ruggerone et al., 2021).

Therefore, this study aimed to identify variables commonly used in routine clinical practice that could be useful as prognostic markers, specifically correlating with length of stay (LOS), severity of illness, and mortality.

Material and Methods

All dogs treated over a period of three years and five months were considered for participation in the study, regardless of sex, age, or breed. The exclusion criteria were as follows: animals whose owners were not willing to participate in the study, animals with clinical dehydration >8%, animals that received blood transfusion within 21 days prior to evaluation for this study, aggressiveness, dyspnea, body weight <2 kg, nephropathy, proteinuria, and liver disease with liver cirrhosis. This study was approved by the Institutional Ethics Committee under registration number 1679 in 2013.

The following variables were evaluated: nutritional status (NS), using the body condition score (BCS), muscle mass score (MMS), body mass index (BMI), weight and food intake; as well as blood count, cholesterol, urea, albumin, globulin, albumin/globulin ratio (R:A/G), total protein (TP), total iron binding capacity (TIBC), iron, transferrin (TF), transferrin saturation (TS), lactate, CRP and CRP/albumin ratio. To our knowledge, studies on TF and TS as markers of LOS, disease severity, and mortality in dogs are unprecedented.

Patient data were collected through anamnesis, physical examinations, and laboratory tests within 48 h of hospitalization. The following data were recorded: breed, age, sex, occurrence, and duration of hyporexia or anorexia, diet type usually consumed, LOS (in days), reason for hospitalization

and/or diagnosis, and whether the outcome of hospitalization was discharge or death. Hospitalization was solely decided by the veterinarian in charge of the case, with no connection to the research. In these cases, the day of admission was recorded, but not the day of discharge or death. With regard to diet, situations other than the sole use of commercial feed, such as total or partial feeding of human food, use of snacks, or both, were considered inappropriate.

Weight was measured using an electronic scale. For the determination of CCS, the dogs were classified into grades 1–9 according to Laflamme's technique (Laflamme, 1997). MMS was recorded in four grades, as indicated by the World Small Animal Veterinary Association (World Small Animal Veterinary Association [WSAVA], 2013). BMI was classified according to the standard formula: body weight (kg)/[height (m)]2, and the height was measured using a measuring tape according to Muller's technique (Muller, 2008).

The severity of the patients' underlying diseases was classified according to Muir's (2007) disease score (DS) and used to classify them into three groups. The first group, G1, consisted of 96 dogs without systemic diseases (DS 1). The second group, G2, consisted of 80 dogs with non-disabling systemic diseases (DS 2 or 3), that is, the disease did not prevent them from walking, at least for short distances. The third group, G3, consisted of 70 dogs with disabling systemic diseases (DS 4 or 5), who remained in obligatory decubitus.

Blood analyses were performed in the laboratories of the same institution. Blood counts were performed using an automated device Mindray® BC-2800VET (Mindray do Brasil – Comércio e Distribuição de Equipamentos Médicos Ltda, São Paulo – SP), with platelet and leukocyte counts performed manually using optical microscopy. The serum was separated by centrifugation and stored in polypropylene tubes at -80°C until the biochemical examination was performed.

Albumin was measured by the bromocresol green method, analyzed by the semiautomatic device BIO-2000 (Bioplus® Produtos para Laboratórios Ltda, Baurueri - SP), using colorimetric tests with kits (Gold Analisa Diagnóstica Ltda, Belo Horizonte - MG). The other biochemical measurements were performed in an automated Siemens Dimensions device, model RXL, with enzymatic/colorimetric test kits (Siemens AG, São Paulo – SP), analyzed by the spectrophotometry method, according to the manufacturer's instructions. Additionally, CRP was analyzed using an ultrasensitive turbidimetric immunoassay.

From the TBIC values, TF was calculated using the following formula: TF (mg dL^(-1))=TBIC x 0.7, which was used because commercial reagents for determining human TF do not have good reproducibility in canine species. There are species-specific kits for evaluating dogs; however, they are imported, expensive, and inaccessible in our country (Pires et al., 2011). TF saturation was determined using the following formula:

$$Transferrin\ saturation\colon\ [TS\ (\%)] = 100\ x\ \frac{Iron}{TBIC}$$ (Pires et al., 2011; Veena et al., 2013).

The laboratory teams did not have access to the results of the physical examinations and experimental groups of the animals from whom blood samples were collected, and were blinded to the nutritional condition and prognosis of the studied dogs.

In each group, the correlation between hematological and serum biochemical variables, NS evaluation parameters (presence of hyporexia or anorexia, type of diet, BCS, MMS, and BMI), and the prognostic measures of LOS, DS, and mortality rate was tested.

Two statistical analyses were performed in this study. The statistical software ActionStat Pro® was used for both experiments. First, considering the possible equalities/differences between the means of the variables, the Kruskal-Wallis test was performed. Subsequently, the minimum significant difference was calculated using the H test, considering an error level (α) of 5% (Pimentel-Gomes, 2009). Second, correlation analysis (r) was applied between the quantitative variables to ascertain their strength (Spearman´s correlation) (Wissler, 1905). Next, a hypothesis test (p=0) was applied to the correlation coefficient, with a t-test attached (Bussab & Morettin, 2014).

Results and Discussion

Description of the groups

Based on the originally proposed inclusion and exclusion criteria, 246 dogs were recruited for further analyses. G1 included 96 animals; 41/96 (42.70%) were male, 55/96 (57.30%) were female, 35/96 (36.45%) were of mixed breeds, and 61/96 (63.55%) were of various breeds. The age of the dogs ranged from 3 months to 15 years, with a mean ± standard deviation of 70 ± 52.54 months. The weight of the animals ranged from 2.3 to 60 kg, with a mean of 15.14 ± 11.24 kg. In this group, 37/96 (38.54%) patients visited the Veterinary Hospital for elective orchiectomy or ovariohysterectomy, and physical evaluation and blood collection were performed before surgery. In addition, 22/96 (22.92%) underwent check-up examinations, 22/96 (22.92%) were evaluated for localized dermatopathies, and 15/96 (15.62%) were evaluated for reasons not associated with systemic diseases.

G2 consisted of 80 animals; 35/80 (43.75%) were male, 45/80 (56.25%) were female, 36/80 (45%) were of mixed breed, and 44/80 (55.00%) were of various breeds. The age of the dogs ranged from 2 months to 16 years, with a mean of 57.46 ± 56.50 months. The weight of the animals ranged from 2.6 to 54.6 kg, with a mean of 13.37 ± 11.59 kg. In this group, 27/80 (33.75%) underwent evaluation due to gastroenteritis, 6/80 (7.5%) due to pyometra, 3/80 (3.75%) due to pancreatitis and the remaining 44/80 (55.00%), due to the presence of various non-disabling systemic diseases.

G3 consisted of 70 animals; 42/70 (60.00%) were male, 28/70 (40.00%) were female, 42/70 (60.00%) were of mixed breed, and 28/70 (40,00%) were of various breeds. The age of the dogs ranged from 2 months to 20 years, with a mean of 92 ± 4.63 months. The weight of the animals ranged from 2.0 to 35.0 kg, with a mean of 12.0 ± 9 kg. In this group, 12/70 (17.14%) were evaluated due to neoplasms, 9/70 (12.85%) due to heart disease, 8/70 (11.43%) due to ehrlichiosis, and 41/70 (58.58%) due to other critical systemic diseases.

A comparative description of the variables studied in terms of frequency is presented in Table 1. A comparative description of the variables analyzed in terms of mean ± standard deviation is presented in Table 2. Statistically significant correlations ($p<0.05$) between the variables studied for G1, G2 and G3 are described in Table 3. The correlations presented in the table and which will be discussed subsequently have moderate strength ($[0.3 < r \leq 0.6]$, according to Callegari-Jacques, 2009), considering that weak correlations ($r \leq 0.3$) are of little significance.

Table 1
Comparative analysis of the occurrences and frequencies of the studied variables between the groups of dogs with disease score 1 (G1), 2 or 3 (G2) and 4 or 5 (G3)

Variable	Occurrence (n) Frequency (%)					
	G1 (n=96)		G2 (n=80)		G3 (n=70)	
	n	%	n	%	n	%
Hyporexia	4	4,16a	32	40,50b	27	38,57b
Anorexia	5	5,21a	28	35,44b	35	50,00c
Improper diet	39	40,62a	40	50,00b	39	55,71b
NM	79	82,30a	46	57,50b	12	17,14c
MMCM	15	15,62a	22	27,50b	24	34,28c
SCM	2	2,08a	12	15,00b	34	48,58c
OF	16	16,67a	12	15,00b	5	7,14c
Thinness	9	9,37a	18	22,55b	28	40,00b
Muscle loss	14	14,58a	30	37,50b	56	80,00c
Death	1	1,04a	6	7,5a	34	48,57 b

Different letters in the horizontal lines indicate significant differences shown by the Kruskal-Wallis test at 5%. BCS = body condition score; MMS = muscle mass score; NM = not malnourished (BCS = 4-5 and MMS=3); MMCM = mild to moderate clinical malnutrition (BCS=3 e/ou MMS=2); SCM = severe clinical malnutrition (BCS 1-2 e/ou MMS 0-1); OF = overweight/fat (BCS 6-9); thinness=BCS ≤3; muscle loss= MMS≤2.

Table 2
Comparative analysis of means (with standard deviation) of variables between groups of dogs with disease score 1 (G1), 2 or 3 (G2) and 4 or 5 (G3)

Variable	Means ± Standard Deviation (s)		
	G1 (n=96)	**G2** (n=80)	**G3** (n=70)
LOS (days)	0,72 ± 1,04a	3,18 ± 2,91b	6,50 ± 6,92c
BMI	15,04 ± 1,04a	13,39 ± 3,59b	14,00 ± 4,00c
Erythrocytes (x10^6 µL)	6,40 ± 1,26a	5,43 ± 0,18b	4,69 ± 2,10c
Hematocrit (%)	40,15 ± 7,73a	33,16 ± 9,78b	27,99 ± 11,65c
Hemoglobin (g/dL)	13,58 ± 2,94a	10,94 ± 3,52b	9,39 ± 4,17c
MCV (fl)	62,48 ± 3,47a	61,19 ± 4,00a	60,40 ± 4,51a
MCH (pg)	21,20 ± 1,84a	20,03 ± 1,98a	20,08 ± 3,31a
MCHC (%)	33,89 ± 2,36a	32,83 ± 2,73a	33,56 ± 3,60a
Platelets (x10^6 µL)	490 ± 232a	409 ± 223b	341 ± 255c
Lymphocytes (/µL)	2.398 ± 1.464a	2.167 ± 1.964b	1.336 ± 1.113c
Transferrin (mg/dL)	254,50 ± 76,10a	211,92 ± 76,22b	223,18 ± 99,46b
Iron (µg/dL)	129,08 ± 64,99a	124,99 ± 79,25a	141,07 ± 88,06a
TIBC (µg/dL)	363,57 ± 108,72a	302,75 ± 108,89b	318,83 ± 142,08b
Transferrin saturation (%)	35,79 ± 14,88a	42,97 ± 23,89ab	47,73 ± 23,94b
Albumin (g/dL)	3,13 ± 1,24a	2,54 ± 0,90b	2,20 ± 1,03b
Globulin (g/dL)	3,29 ± 1,57a	3,75 ± 1,93ab	3,97 ± 1,62b
R:A/G (g/dL)	1,77 ± 3,22a	0,92 ± 0,73b	0,80 ± 0,93c
Total Protein (g/dL)	6,42 ± 1,48a	6,36 ± 1,73a	6,26 ± 1,66^a
Urea (mg/dL)	37,70 ± 15,64a	40,84 ± 37,85a	151,96 ± 183,17b
Cholesterol (mg/dL)	217,17 ± 69,67a	220,86 ± 84,74a	238,76 ± 140,90a
CRP (µg/mL)	2,13 ± 1,36a	3,61 ± 1,73b	4,97 ± 7,01c
CRP/albumin	0,90 ± 1,09a	1,81 ± 1,72b	2,62 ± 2,80c
Lactate (mmol/L)	3,89 ± 2,86a	3,88 ± 2,77a	4,65 ± 2,86b

Same letters indicate that the compared variables do not differ statistically, whereas different letters indicate a significant difference shown by the Kruskal-Wallis test at 5%. LOS = length of stay, BMI = body mass index, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, TBIC = Total iron-binding capacity, CRP = c-reactive protein. **Reference values:** BMI: 11,8 a 15; erythrocytes = 5,5-8,5x106 µL; hematocrit = 37-55%; hemoglobin = 12-18 g/dL; MCV = 60-77 fl; MCH = 19-23 pg; MCHC = 32-36%; lymphocytes = 1.000 a 4.800/µL; platelets = 200.000 a 500.000/µL; cholesterol = 135 a 270 mg/dL; albumin = 2,6 a 3,3 g/dL; R:A/G = 0,59 -1,11 g/dL; total protein = 5,5 a 8,0 g/dL; urea = 21,4 a 59,92 mg/dL; globulin = 2,7 a 4,4 g/dL (Kaneko, 2008); TIBC = 326,4 à 415,8 µg/dL; iron = 120,2-174,6 µg/dL; transferrin = 228,5 à 291,1 mg/dL; transferrin saturation = 34,8% à 46,1% (Pires et al., 2011); CRP = <5,05-8,70 µg/mL; lactate = <2,5 mmol/L (Cortellini, et al., 2015).

Table 3
Significant correlations (p<0.05) between the parameters: length of stay (LOS), mortality rate (death) and disease score (DS) with food intake data, nutritional physical examination and laboratory variables in dogs from a hospital population

Correlation	p-value	Pearson correlation coefficient (r)
	G1	
LOS x albumin	<0,01	-0,33
LOS x CRP/albumin	<0,01	0,38
	G2	
LOS x Death	0,01	-0,38
LOS e CRP/albumin	<0,01	0,35
LOS e Hyporexia	<0,01	0,38
Death x Urea	0,01	0,34
Disease Score x Lymphocytes	<0,01	-0,36
	G3	
LOS x BMI	<0,01	0,52
LOS x Hemoglobin	<0,01	0,33
LOS x Death	0,01	- 0,40
Death x Albumin	0,04	-0,34
Disease Score x Hyporexia	<0,01	0,35

G1 = dogs with disease score 1, G2 = dogs with disease score 2 or 3, G3 = dogs with disease score 4 or 5, LOS = Length of stay, CRP = c-reactive protein, BMI = body mass index.

The main prognostic markers observed in this study were NS, TF, TBIC, erythrogram, TS, lactate, albumin, the CRP/albumin ratio, and urea.

Disease severity markers

Regarding eating disorders, the higher prevalence of food intake abnormalities (hyporexia and anorexia) and the frequency of inadequate diet consumption in animals with systemic diseases (G2 and G3) are notorious, resulting in a high risk of malnutrition (Table 1). A significant direct correlation was observed between DS and hyporexia in G3 (Table 3). This may have facilitated the onset and/or worsening of health disorders and contributed to the higher mortality rates in these groups.

In addition, it was evident that the presence of clinical malnutrition, including thinness and muscle loss, were more frequent in animals with systemic diseases, especially in those with critical illnesses (G3) (Table 1). In summary, subclinical and clinical malnutrition were more frequent with greater severity of the underlying disease.

The prevalence of malnutrition in patients is high, and this direct correlation has been well established in the literature (Fabretti et al., 2015; Niseteo et al., 2020; Arnau-Barrés et al., 2021). Malnourished organisms are immunosuppressed because of the lack of nutrients for the synthesis of cytokines, complement, and gamma globulins, and for the promotion of mitosis and phagocytosis by leukocytes (Stelmasiak et al., 2021). This results in atrophy of the organs related to the immune system, an increase in the frequency and severity of infections, and worsening of prognosis (Lombardi, 2019; Stelmasiak et al., 2021), as noted in G2 and G3.

Additionally, in G2, a negative correlation was noted between DS and MMS, which indicates that the greater the severity of the disease, the lower the muscle mass (Table 3). It is known that, in patients, acute phase proteins (APP) are synthesized in priority over the usual proteins, and myofibrils are catabolized to make aminoacids available for immune system and collagen synthesis, to enhance defense and tissue regeneration or healing (Cohen et al., 2015). Therefore, protein catabolism is higher in sick malnourished individuals than in healthy malnourished individuals (Cohen et al., 2015; Sharma et al., 2019).

There was a statistical difference between the mean BMI in the three studied groups; however, these were within the reference range (Table 2). This demonstrates that BMI does not correlate with disease severity. The results described in the literature regarding this association are conflicting, depending on the disease studied (Islam et al., 2011; Bove et al., 2013).

Analysis of TF and TBIC between the groups revealed a statistically significant difference between animals with DS1 (G1) and those with systemic illness (G2 and G3), but not between G2 and G3 (Table 2). For these parameters, the means were within the normal range in G1 and below the normal range in G2 and G3. These results indicate that below-normal TF and TBIC values are associated with the presence of systemic diseases.

Other studies have described low TF and TBIC values as indicators of disease or poor prognosis (Nakajima et al., 2012, 2014; Fabretti et al., 2021). This reduction in serum concentrations of TF and TBIC is a result of the action of cytokines (such as IL-1, IL-6 and TNF-α), secreted in inflammatory processes, which alter the profile of hepatic synthesis of proteins (TF is a negative APP) (Pires et al., 2011; Zalles Cueto et al., 2012).

In addition to some previous studies, the present study showed that the mean TS increased as the DS increased (Table 2) (Mainous et al., 2013; Stack et al., 2014). In G3, the mean of this variable exceeded the normal range, whereas in G2, the data variation evidenced by the standard deviation showed that there were several cases of measures above the reference value, which did not occur in G1 (Table 2).

It is possible that the increase in TS is justified by a reduction in the number of TF molecules in cases of protein malnutrition or even inflammatory processes (since TF is a negative APP), situations that are progressively more frequent as DS increases. In other words, TS elevation above the reference range suggests malnutrition, inflammation, and worse prognosis (Pires et al.,

2011; Ghadiri-Anari et al., 2014; Kwiatkowska et al., 2019). Interestingly, TS will be reduced in hypoferremia, which is closely associated with iron deficiency anemia, which is also associated with a poor prognosis (Mainous et al., 2013; Campodonico et al., 2021).

A statistically significant difference was noted in the erythrocyte count, HCT, HGB, and platelets between the groups (Table 2). G1 was the only group with normal means for these parameters, and systemically sick animals (G2 and G3) had values below the normal range, especially G3. The exception was the platelet count, whose mean values were normal in all three groups. Thus, anemia was considered indicative of disease severity, with thrombocytopenia having little sensitivity for this purpose, which is supported by other studies (Warner et al., 2020; Campodonico et al., 2021).

There were no statistical differences between the MCV, MCH, and MCHC means of the three groups. In addition, the mean values were within the reference range (Table 2), indicating a predominance of normochromic normocytic anemia. This type of anemia is most common in malnutrition and inflammatory processes (Zaninetti et al., 2018; Weiss et al., 2019; Mrimi et al., 2022).

The average lactate levels in the three groups were above the reference values. In G1, it is possible that this occurred (at least in part) due to the agitation of these dogs at the time of evaluation, with many showing signs of fear (including vasoconstriction of the mucous membranes) or hyperactivity during the consultation. Comparative analysis showed statistically significant differences between G1 and G3, and G2 and G3, but not between G1 and G2 (Table 2). This indicates that animals with critical illness have higher serum concentrations than those with mild-to-moderate illness. Hyperlactatemia is associated with several diseases, particularly the critical ones. This is a consequence of hypermetabolism and tissue hypoperfusion that occur in pathological situations and are common in critically ill patients (Botteon, 2012; Fabretti et al., 2016). Hyperlactatemia during critical illness indicates cellular stress even when lactate levels are not correlated with total oxygen debt (Fabretti et al., 2016).

A statistically significant difference was observed between the albumin concentrations in animals with DS1 (G1), whose mean value was within the reference range, and dogs with systemic diseases (G2 and G3), whose mean values were below normal (Table 2). Hypoalbuminemia was indicative of the disease, but not of its severity. An association between hypoalbuminemia and disease has been previously reported (Bohl et al., 2016; Eckart et al., 2020; Arnau-Barrés et al., 2021).

Furthermore, a negative correlation ($p<0.01$) was observed between DS and lymphocytes in G2. Therefore, the more severe the illness of the dog, the lower the circulating lymphocyte count (CLC). In agreement with this finding, several publications have stated that CLC is a marker of disease severity (Oliveira et al., 2008; Ong et al., 2014; J. Liu et al., 2020). However, as the mean number of lymphocytes was within the reference values in the three groups, although there was a statistical difference among them, CLC was not considered a sensitive prognostic predictor in dogs in this study.

Comparing the means of the albumin/globulin ratio between the groups, the results showed that they decreased as DS increased, with a statistically significant difference between the three groups (Table 2). This is due to the reduction in albumin, which is related to the severity of diseases (APP), and the increase in globulins in response to these diseases (Azab et al., 2013; B. Liu et al., 2021). Therefore, low values of this ratio indicate a disease.

Several reports in medicine indicate that subnormal values of the albumin/globulin ratio are associated with lower survival. It is possible that this variable is a better prognostic marker in specific and chronic diseases when there is time and stimulus for a significant increase in globulins and a reduction in albumin; cancer and chronic infections are examples cited in the human and veterinary medical literature (Azab et al., 2013; B. Liu et al., 2021; Zhang et al., 2020). This ratio may not be as useful as a prognostic marker in general populations, especially in cases of acute illness, which may explain the marked variations of the ratio observed in this study (B. Liu et al., 2021).

Although most clinically malnourished dogs in G1 had the lowest cholesterol values, the mean of this parameter among the three groups was within the reference range, with no statistical difference between the groups (Table 2). Therefore, the cholesterol level was not considered a marker of disease severity. On the other hand, some authors have indicated that hypocholesterolemia is a marker of high mortality and LOS (Corkins et al., 2014; Ong et al., 2014). However, other studies along with the present study did not support these findings (Oliveira et al., 2008; Fabretti et al., 2014).

CRP is associated with the level of inflammation in underlying diseases and is a widely used prognostic marker in human and veterinary medicine. The reduction in albumin due to the inflammatory process intensified the increase in the CRP/albumin ratio in these cases (Gibson et al., 2018; Eckart et al., 2020).

In the present study, dogs with more severe disease had the highest CRP levels (Table 2). However, the fact that the CRP means were normal in all groups indicates the low sensitivity of this variable as an indicator of disease severity. Similar results have been reported in humans and animals (Squassoni et al., 2011; Qin et al., 2016).

The CRP/albumin ratio increased markedly in proportion to the severity of the underlying disease. Thus, CRP/albumin was a better marker of disease severity than albumin or CRP levels interpreted separately. Similar results have been reported previously (Gibson et al., 2018; Park et al., 2018).

Length of stay (LOS)

Notably, a negative correlation was observed between LOS and mortality rate in G2 and G3 (Table 3). In other words, in populations with a high mortality rate, such as G3 (death rate = 48.57%), poor prognosis coincides with a shorter LOS, suggesting that the reason for these short hospitalizations is the early occurrence of death. Conversely, in groups with a low number of deaths (G1 and G2), poor prognosis was indicated by a longer LOS, which demonstrates a delay in recovery and, consequently, in patient discharge. Similar conclusions regarding this correlation have been reported in veterinary medicine (Brunetto, 2006; Fabretti et al., 2014).

In G1 animals, there was a negative correlation ($p<0.01$) between LOS, albumin, and TP (Table 3). Therefore, a low concentration of these parameters is a marker of a long hospital stay. Hypoproteinemia, an indicator of poor prognosis, has been extensively reported in medical literature (Nakajima et al., 2014; Bohl et al., 2016; Arnau-Barrés et al., 2021).

In G3, animals with hypohemoglobinemia remained hospitalized for a shorter time (Table 3). However, human studies have demonstrated the opposite results (Zaninetti et al., 2018; Shah, et al., 2019). In the present study, the reduction in the LOS of animals with low hemoglobin values occurred due to early death and not due to clinical discharge; therefore, cellular hypochromia was interpreted as a marker of poor prognosis.

In G3, a direct correlation was observed between BMI and LOS ($p<0.01$) (Table 3). Thus, animals with low BMI had a short LOS. In contrast, most reports on the association between BMI and LOS in humans are negative, with reduced BMI implying a prolonged LOS (Silva et al., 2012; Hecht et al., 2015). However, the mortality rate in the G3 of this study had an influence on the LOS, as previously discussed.

There was also a positive correlation between the CRP/albumin ratio and LOS in G1 and G2 ($p<0.01$) (Tables 3 and 4). This relationship is considered superior to that of CRP alone as a marker of LOS (Gibson et al., 2018; Park et al., 2018). These correlations were not observed in animals with critical illnesses (G3), probably because the mortality rate was significant and interfered with the LOS of the animals in these groups.

Finally, a positive correlation was observed between hyporexia and LOS in G2, indicating that animals with reduced appetite had slower clinical recovery, indicating poor prognosis. Several studies in humans have correlated poor diet with changes in LOS (Cohen, et al., 2015; Peres et al., 2020; Lengfelder et al., 2022).

Mortality markers

There was no statistically significant difference among the three groups regarding iron concentration, which was within the normal range, or strong or moderate correlations with LOS and mortality. Therefore, ferremia was not considered a prognostic marker in this study. In contrast, in humans, hypoferremia is recognized as an important marker of mortality, particularly in patients with heart failure (Rössler et al., 2020; Graham et al., 2022).

In G3, there was a negative correlation between mortality and albumin levels (Table 3). Thus, hypoalbuminemia is associated with higher mortality rates in critically ill dogs. There are extensive reports in the literature on the correlation between hypoproteinemia, especially hypoalbuminemia, and greater severity of underlying diseases, hospital stay, and mortality (Cooper et al., 2004; Luis et al., 2006; Gupta et al., 2011; Bohl et al., 2016; Arnau-Barrés et al., 2021). Some authors have suggested that this may be associated with a more severe inflammatory state than with malnutrition (Cooper et al., 2004; Gupta et al., 2011). In addition to these correlations, studies have found that patients with hypoproteinemia are more susceptible to infection, pulmonary complications, severe

anemia, and a greater chance of readmission (Luis et al., 2006; Bohl et al., 2016).

Additionally, in sick animals, there was a positive correlation between urea and the mortality rate; that is, dogs with higher serum urea concentrations were at greater risk of death (Table 3). It should be noted that animals with > 8% dehydration were excluded from this study. Elevation of urea in these patients may indicate metabolic stress, increased protein catabolism, intestinal bleeding (with absorption of blood proteins), and a lower glomerular filtration rate, with the association of high serum concentrations and high mortality documented in several medical situations (Ugajin et al., 2012; Arihan, et al., 2018; Cheng et al., 2020).

Conclusions

Veterinary medicine requires PIs that can be routinely used in clinics and hospitals. Further studies to develop such protocols or discover accessible prognostic markers are required. Based on the results of this study, the following variables are associated with disease severity in dogs: presence of malnutrition (due to the occurrence of hyporexia, anorexia, BCC ≤3 and MMS≤2); low TF and TBIC values and anemia; and high values of TS, lactate and CRP/albumin ratio.

In animals without systemic diseases or with mild-to-moderate systemic diseases, low albumin values, CRP/Albumin ratio, and hyporexia indicated longer LOS. In animals with critical systemic diseases, low BMI and HB values indicated shorter LOS (secondary to high mortality).

In human medicine, ICUs provide specialized services; consequently, the mortality rate, even in the face of serious illnesses, is relatively low. In these cases, a long LOS indicates a poor prognosis, as it demonstrates a delay in recovery of patients, and a short LOS indicates a good prognosis, suggesting rapid discharge. In many cases, intensive care veterinary services do not have the same resources and have comparatively high mortality rates. In this context, the interpretation of LOS can eventually be inverted, with longer LOS indicating a good prognosis, suggesting that patients survive serious illnesses, and shorter LOS indicating early deaths.

Hypoalbuminemia and high urea concentration were the mortality indicators in sick dogs. The findings of the aforementioned alterations, especially when they occur simultaneously, support the determination of prognosis in dogs from hospital populations in an objective, practical, and accessible manner.

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