Determination of the reference interval of the C-reactive protein/albumin ratio and its efficiency, CRP and albumin as prognostic markers in dogs

Andrei K. Fabretti2*, Raquel C.S. Siqueira2, Higo F. Amaral3, Daniella A.G. Kemper4, Rafael O. Chaves5 and Patricia M. Pereira2

ABSTRACT.- Fabretti A.K., Siqueira R.C.S., Amaral H.F., Kemper D.A.G., Chaves R.O. & Pereira P.M. 2020. Determination of the reference interval of the C-reactive protein/albumin ratio and its efficiency, CRP and albumin as prognostic markers in dogs. Pesquisa Veterinária Brasileira 40(3)202-209. Universidade Estadual de Londrina, Rod. Celso Garcia Cid PR-445 Km 380, Londrina, PR 86057-970, Brazil. E-mail: akfabretti@gmail.com

The objective of this research was to create a reference interval for C-reactive protein (CRP)/albumin ratio (CAR) in the canine species and to analyze the potential of CRP, albumin and the relationship between both, to serve as indicators of disease severity, length of hospital stay (LoS) and mortality in this species. For this, an outcome study was conducted in a Veterinary Teaching Hospital in southern Brazil. One hundred ninety dogs were included randomly, without distinction of gender, age, or breed, from June 2013 to November 2016. Plasma was collected from them and analyzed for assessment of CRP and albumin. The reference range stipulated for CAR in dogs was 0.36-0.60, as determined by the confidence interval of mean resamplings (in percentiles). The frequencies mean, and standard deviations of the variables, correlation analysis, and comparative analysis (Kruskal-Wallis in α = 5%) were calculated. Elevation (above reference) of CAR was determined to be proportional to the severity of the underlying disease, and CRP means were reasonable. Besides, hypoalbuminemia was indicative of systemic disease, but not of severity. Thus, CAR was a better marker of disease severity than were CRP and albumin, analyzed separately. Concerning LoS, there was a positive correlation with CAR (p<0.01) in patients, and the same was not observed with CRP and albumin. Concerning mortality, hypoalbuminemia was the only marker valid in animals with a critical illness (p=0.04). In conclusion, CAR is a better marker of disease severity and LoS in dogs than are CRP and albumin analyzed separately.

INDEX TERMS: C-reactive protein, albumin, prognosis, mortality, length of stay, disease severity, dogs.
Rubio & Schmidt 2014, Reimann et al. 2016). At this time, in dogs and their peak is reached in 24-48 hours. (Davis et al. 2012, Eckersall & Schmidt 2014). In dogs and humans, their elevation proportional to the inflammatory level. (Dabrowski 2014, Wong et al. 2016).

For the prognosis to be as precise as possible, it is necessary to use effective markers to predict the severity of the underlying disease, clinical recovery time and chance of death in each case. Among the markers most commonly used in medicine, both human and veterinary, are serum levels of C-reactive protein (CRP) and albumin (Yeun et al. 2000, Cerón et al. 2008, Eckersall & Schmidt 2014, Fabretti et al. 2014, Wong et al. 2016).

C-reactive protein is the main positive acute phase protein in dogs and an excellent modulator and marker of initial inflammation, being measured as the magnitude of its elevation proportional to the inflammatory level. (Dabrowski et al. 2013, Kjelgaard-Hansen et al. 2013, Christensen et al. 2014, Eckersall & Schmidt 2014). In dogs and humans, their serum concentration increases four to six hours after injury, and their peak is reached in 24-48 hours. (Davis et al. 2012, Rubio & Schmidt 2014, Reimann et al. 2016). At this time, in dogs, C-reactive protein can reach serum concentrations above 100 to 1,000 times the reference range, and normalization occurs approximately one to two days after the end of the inflammatory stimulus (Jitpean et al. 2014, Tizard 2014, Venco et al. 2014). Healthy dogs have less than 5mg/L of this protein in plasma (Kuribayashi et al. 2003, Jitpean et al. 2014, Rubio & Schmidt 2014, Reimann et al. 2016).

As a prognosticmarker in companion animals, C-reactive protein is especially useful because its increase precedes any leukocyte alteration, enabling the recognition of subclinical disorders (Anziliero et al. 2013, Karlsson et al. 2013, Viitanen et al. 2014). High serum concentrations have been associated with a higher death rate in dogs, with normalizing being associated with clinical recovery (Michelsen et al. 2012, Anziliero et al. 2013, McClure et al. 2013).

In addition, albumin is the most abundant plasma protein, with its main function being to transport nutrients, hormones, metabolites and pigments in blood and tissues (Cerón et al. 2008, Eckersall 2008). It is a negative acute phase protein; therefore, its serum level decays in inflammatory processes (Cerón et al. 2008, Eckersall 2008). Albumin is considered, in human and veterinary medicine, a sensitive marker of morbidity, mortality and hospitalization time; in this way, the more serious the disease becomes, the lower the albumin concentration will be, and the worse the prognosis will be (Corkins et al. 2010, Fabretti et al. 2014, Ong et al. 2014, Qin et al. 2016).

One advantage is that use is simple and has a high accuracy with a reduced cost compared with other prognostic indicators (Kinoshita et al. 2015, Park et al. 2016). Although this marker is used in human medicine, there are no known studies, to the best of the authors’ knowledge, of this relationship in veterinary medicine of companion animals. Thus, the present work creates a reference interval for CAR in dogs and analyzes the potential of CRP, albumin and the relationship between both as prognostic markers in dogs, studying their correlations with disease severity, LoS and mortality in this species. This study is, therefore, intended to innovate in the prognostic evaluation of dogs.

**MATERIALS AND METHODS**

One hundred ninety dogs admitted in a veterinary teaching school hospital, from June 2013 to November 2016, were randomly included in this study, regardless of gender, age or race and with client approval by the informed consent document. The project was approved by the institutional ethical committee, under registration 1679/2013. Exclusion criteria were: animals whose guardians did not agree to the study; animals with clinical dehydration above 8%; blood transfusion

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**INTRODUCTION**

The strong bond of affection between humans and companion animals, coupled with easy access to information (especially online) has generated a high level of questioning of animal owners to veterinarians, with the clinical course of their sick animals (Rabelo et al. 2009, Fabretti et al. 2014). A similar scenario occurs in human medicine (Niewinski et al. 2014, Silva et al. 2014).

In this way, correctly determining a patient’s prognosis is an increasingly valued medical action. Prognosis is defined as an early forecast of the patient’s progression and chances of recovery based on clinical and diagnostic information from the patient. (Niewinski et al. 2014, Silva et al. 2014). This prediction is useful in several ways in human and veterinary medicine: it supports the type of treatment indicated (at home or in hospital, including intensive care); goal decisions regarding monitoring protocols; estimation of length of hospital stay (LoS); and when applicable, medical costs and chances of survival, as well as providing helpful information for making euthanasia decisions in animal patients (Cockcroft 2007, Rabelo et al. 2009, Davis et al. 2012, Fabretti et al. 2014).

As a tool that directs the patient’s clinical conduct and serves as an argument for the veterinarian to receive authorization for expensive procedures, an erroneous estimate can have disastrous consequences, as a wrong choice of medical protocols can result in worse clinical outcomes (possibly death) in addition to the possibility of unnecessary expenses. As a result, conflicts may occur between veterinarians and their clientele (Cockcroft 2007, Rabelo et al. 2009, Fabretti et al. 2014).

For the prognosis to be as precise as possible, it is necessary to use effective markers to predict the severity of the underlying disease, clinical recovery time and chance of death in each case. Among the markers most commonly used in medicine, both human and veterinary, are serum levels of C-reactive protein (CRP) and albumin (Yeun et al. 2000, Cerón et al. 2008, Eckersall & Schmidt 2014, Fabretti et al. 2014, Wong et al. 2016).

To innovate in the prognostic evaluation of dogs.
within 21 days prior to project evaluation; aggressiveness, dyspnea or less than 2kg of body weight; nephropathies and proteinuria or liver disease with cirrhosis.

The following patient data were recorded: race, age, gender; hospitalization period (in days), reason for hospitalization and the outcome (discharge or death). The weight was measured in the electronic balance. The severity of the underlying disease was classified according to Disease Score (DS) as described by Muir (2007) and was a parameter for the distribution of dogs in three groups. The first group was the Control Group (CG), which was composed of 40 healthy and asymptomatic dogs. The second group was composed of 80 dogs classified in DS 2 or 3 and was called the moderate diseases group (MDG). These were patients with nondisabling systemic diseases who were able to move, at least at short distances. The third group was called the severe diseases group (SDG), consisting of 70 animals classified in DS 4 and 5, that is, with disabling and critical systemic diseases. These dogs remained in decubitus position.

Blood was collected from the jugular vein and stored in serum tubes (2mL). Samples were centrifuged within 15 minutes at 1500 x g for 10 minutes, and serum was immediately separated and stored at -80°C until analysis. All analyses were performed simultaneously on each sample.

C-reactive protein and serum albumin analyses were performed in veterinary laboratories. The albumin was measured by the bromocresol green method, analyzed by the semiautomatic apparatus BIO-2000 (Bioplus Products for Laboratories Ltda, Bauru/SP, Brazil), using colorimetric tests with kits of Analisa brand (Gold Analisa Diagnostica Ltda, Belo Horizonte/MG, Brazil). Additionally, the CRP was analyzed by the ultrasensitive turbidimetric immunoassay technique in the Siemens Dimensions (automated clinical chemistry analyzer), model RXL, with test kits from the same company. The measurements were made according to the manufacturer’s instructions and the kits used were validated for use on dogs (Christensen et al. 2014, Vitannen et al. 2014). The laboratory teams did not have access to other data of the studied patients and were blinded to the prognosis of these patients.

For the determination of the reference range of clinical laboratory variables in veterinary medicine, a sample between 40 and 120 is indicated (Friedrichs et al. 2012) This research had 40 samples (CG) for determination of the reference range of CAR; therefore, the data are significant. For that, the confidence interval (α = 95%) of the mean of the resamplings, based on percentiles, was calculated using the software BioEstat 5.0 (BioEstat - Institute of Sustainable Development Mamirauá/AM, Brazil).

In all groups, the correlation between CRP, albumin and CAR was tested with the prognostic measures of LoS and mortality. Comparative analysis of these variables between groups was allowed to investigate whether they were correlated to the severity of underlying disease.

The means and standard deviations of the variables for CG, MDG and SDG were also investigated. The Pearson correlation between the variables within each group was then analyzed. Finally, the Kruskal-Wallis nonparametric test at α = 5% (significant) and α = 1% (highly significant) was used to compare the groups (which did not have the same number of animals). Statistical software Action Stat Pro (Estatcamp - Statistical Consulting and Quality - São Carlos/SP, Brazil) was used for these analyses.

## Results

One hundred ninety dogs were evaluated in this study. The characteristics of each group are described in Table 1.

In relation to GC, 25/40 (62.5%) were admitted for checkup, 14/40 (35.0%) for elective castration and 1/40 (2.5%) for blood donation. The animal assessment and blood collection were performed before the surgeries. Regarding the reasons for attendance in MDG, 27/80 (33.75%) were due to gastroenteritis, 6/80 (7.5%) for pyometra, 3/80 (3.75%) due to pancreatitis and the remainder, 44/80 (55.00%), due to the presence of several other nondisabling systemic diseases. In SDG, these animals were taken to hospital care due to neoplasms 12/70 (17.14%); heart diseases 9/70 (12.85%), ehrlichiosis 8/70 (11.43%) and 41/70 (58.58%) due to other critical systemic diseases.

The description of CG relative to the values of CRP, albumin and CAR is shown in Table 2. The calculated reference range

| Group | Sex | Races | Age | Weight |
|-------|-----|-------|-----|--------|
| CG (n=40) | Males: 22/40 (55.00%) | Non-breed: 13/40 (32.50%) | 5 months - 10 years | 2.3 to 60kg |
| | Females: 18/40 (45.00%) | Different races: 27/40 (67.50%) | (44 ± 32) | (15.84 ± 12.49) |
| MDG (n=80) | Males: 35/80 (43.75%) | Non-breed: 36/80 (45.00%) | 2 months - 16 years | 2.6 to 54.6kg |
| | Females: 45/80 (56.25%) | Different races: 44/80 (55.00%) | (57.46 ± 55.50) | (13.37 ± 11.59) |
| SDG (n=80) | Males: 42/70 (60.00%) | Non-breed: 42/70 (60.00%) | 2 months - 20 years | 2.0 to 35.0kg |
| | Females: 28/70 (40.00%) | Different races: 28/70 (40.00%) | (92 ± 64.63) | (12.0 ± 9.00) |

| Variable data | CRP (µg/mL) | Albumin (g/dL) | CAR |
|---------------|-------------|----------------|------|
| Minimum       | 0.10        | 2.40           | 0.02 |
| 1st quartile  | 0.70        | 2.90           | 0.19 |
| Mean          | 1.64        | 3.30           | 0.48 |
| Median        | 1.40        | 3.34           | 0.41 |
| 3rd quartile  | 2.30        | 3.60           | 0.71 |
| Maximum       | 5.20        | 5.10           | 1.57 |
| Standard deviation | 1.26 | 1.27           | 0.08 |
| Coefficient of variation | 0.73 | 0.33           | 0.97 |

Notes: CRP = C-reactive protein, Albumin = C-reactive protein/albumin ratio.
for CAR (using the GC of 40 dogs) was 0.36 to 0.60, with a mean of 0.48 (α = 5%) (Fig. 1A).

Descriptive analyses of the CRP, albumin and CAR variables for sick animals (MDG and SDG) are shown in Tables 3 and 4, respectively. Likewise, the analysis of CAR values in these groups is shown in Figure 1B and 1C.

The comparative analysis of the means (and standard deviation) of the variables between groups are listed in Table 5 and allowed to evaluate their correlations with the severity of the underlying diseases. The correlations between the variables studied with LoS and mortality, for sick animals (MDG and SDG), are described in Table 6.

### DISCUSSION

#### Severity of underlying disease

In this research, the dogs had a progressive increase in the CAR values as the severity of the underlying disease increased (Table 5), with the mean values in MDG (1.81) and SDG (2.62) being much higher than the reference interval suggested in this study (0.36-0.60). In fact, compared to the upper reference limit, the MDG mean was increased more than three times, and in SDG, this increase was greater than 4.3 times. As expected, the difference in means between groups was statistically significant, proving that CAR is a good marker of disease severity. There are several studies in human
Table 3. Descriptive analysis of the group of 80 dogs with moderate systemic diseases in relation to the C-reactive protein, albumin and C-reactive protein/albumin ratio

| Variable              | CRP[^a^] (µg/mL) | Albumin (g/dL) | CAR[^b^] |
|-----------------------|-------------------|----------------|----------|
| Minimum               | 0.40              | 0.30           | 0.12     |
| 1st quartile          | 3.00              | 1.90           | 0.93     |
| Mean                  | 3.61              | 2.54           | 1.81     |
| Median                | 3.60              | 2.60           | 1.54     |
| 3rd quartile          | 4.10              | 3.20           | 2.33     |
| Maximum               | 13.30             | 5.10           | 12.0     |
| Standard deviation    | 1.73              | 0.90           | 1.72     |
| Coefficient of variation | 0.48             | 0.35           | 0.95     |

[^a^] CRP = C-reactive protein,[^b^] CAR = C-reactive protein/albumin ratio.

Table 4. Descriptive analysis of the group of 70 dogs with severe systemic diseases in relation to the C-reactive protein, albumin and C-reactive protein/albumin ratio

| Variable              | CRP[^a^] (µg/mL) | Albumin (g/dL) | CAR[^b^] |
|-----------------------|-------------------|----------------|----------|
| Minimum               | 0.10              | 0.57           | 0.03     |
| 1st quartile          | 3.10              | 1.58           | 1.31     |
| Mean                  | 4.97              | 2.29           | 2.62     |
| Median                | 3.90              | 2.20           | 1.88     |
| 3rd quartile          | 4.80              | 3.00           | 3.14     |
| Maximum               | 59.40             | 6.30           | 19.80    |
| Standard deviation    | 7.01              | 1.03           | 2.80     |
| Coefficient of variation | 1.41             | 0.45           | 1.07     |

[^a^] CRP = C-reactive protein,[^b^] CAR = C-reactive protein/albumin ratio.

Table 5. Comparative analysis of death rates and means (with standard deviation) of the variables: length of hospital stay, albumin, C-reactive protein and C-reactive protein/albumin ratio between the Control Group and the diseased dog groups

| Variable | Frequency or means ± Standard deviation (s) |
|----------|---------------------------------------------|
| Death    | Control Group (n=40) Moderate diseases group (n=80) Severe diseases group (n=70) |
| LoS (days) | 0 (0%)[^a^] 6 (7.5%)[^b^] 34 (48.57%)[^c^] |
| CRP (µg/dL) | 0.34 ± 1.04a 3.18 ± 2.91b 6.50 ± 6.92c |
| Albumin (g/dL) | 1.64 ± 1.26a 3.61 ± 1.73b 4.97 ± 7.01c |
| CAR       | 0.48 ± 0.08a 1.81 ± 1.72b 2.62 ± 2.80c |

[^a^] CRP = C-reactive protein,[^b^] CAR = C-reactive protein/albumin ratio; a,b,c = equal letters indicate that the variables compared do not differ statistically, while distinct letters indicate a significant difference by the Kruskal-Wallis test at 5%. Reference values: albumin = 2.6 to 3.3 g/dL (Thrall et al. 2015), CRP = <5.0-8.70 µg/mL (Kuribayashi et al. 2003, Kaya et al. 2012, Anziliero et al. 2013).

Table 6. Analysis of correlations between C-reactive protein, albumin, and C-reactive protein/albumin ratio with length of hospital stay and death rate in sick dog groups

| Correlation | Moderate diseases group | Severe diseases group |
|-------------|-------------------------|-----------------------|
| p-value     | r                       | p-value               | r                       |
| CRP[^a^] x LoS[^b^] | 0.08 0.19 | 0.73 -0.04 |
| CRP x Death | 0.99 0.01 | 0.21 0.15 |
| Albumin x LoS | 0.12 -0.17 | 0.26 -0.13 |
| Albumin x Death | 0.42 -0.09 | 0.04 -0.24 |
| CAR[^a^] x LoS | <0.01 0.45 | 0.86 0.02 |
| CAR x Death | 0.73 0.03 | 0.71 -0.01 |

[^a^] CRP = C-reactive protein,[^b^] LoS = length of hospital stay,[^c^] CAR = C-reactive protein/albumin ratio.
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Mortality

There was no correlation between the CAR and the death rate in this study (Table 6). However, articles in human medicine, especially in the area of oncology and in patients with sepsis show the opposite. A survey, aiming to analyze the residual effects of inflammation on survival after discharge from an intensive care unit, analyzed the mortality rate six months after a severe sepsis or septic shock event (Kim et al. 2015). Another group of researchers elaborated a similar study, analyzing mortality 90 days after discharge from the ICU with the same profile of patients (Ranzani et al. 2013, Kim et al. 2015). Both groups concluded that CAR was an excellent marker of mortality during follow-up periods (Ranzani et al. 2013, Kim et al. 2015). Numerous studies in oncology emphasize that CAR predicts the mortality of long-term human patients after cancer treatment (Kim et al. 2015). Zhou et al. 2015, Tao et al. 2016, Toiyama et al. 2016, Zhang et al. 2017). Therefore, it is possible to infer that CAR is a better indicator of long-term mortality compared to observations only at the time of hospitalization.

Regarding CRP, there was also no significant correlation with the mortality rate. There are researches in dogs with immune-mediated hemolytic anemia and ehrlichiosis stating the same (Mylonakis et al. 2011, Griebsch et al. 2019). In humans, most published works demonstrate the inverse (Kunitoshi et al. 1999, Yeun et al. 2000, Kristine et al. 2009, Boulware et al. 2011).

However, there was a negative correlation between death and albumin levels in SDG (Table 6); therefore, in dogs with critical diseases, hypoalbuminemia indicates a higher chance of death. In the literature, reports of correlations between low concentrations of albumin and high mortality are extensive (Nakajima et al. 2014, Wang et al. 2014, Garwe et al. 2016, Slee 2016).

CONCLUSIONS

The reference range of CAR for normality in dogs in this study was 0.36 to 0.60. This relationship is a better marker of disease severity and LoS than the CRP or albumin analyzed separately.

However, CAR is not a sensitive marker of mortality during the hospitalization period, and serum albumin is better for dogs with critical diseases, for this purpose. Surveys are needed to assess the accuracy of CAR as a predictor of mortality in dogs over the long term (including postadmission).

Acknowledgments. - To Danielle Venturini, Mara Regina Stipp Balarin and the entire team involved in the project.

Conflict of interest statement. - The authors declare no conflicts of interest.

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