Chemotherapy has been associated with long survival times in dogs with multicentric lymphoma. However, treatment is generally only palliative, rather than curative, and relapse is common. In dogs in which the disease relapses, changes in the chemotherapeutic regimen are typically required. Currently, however, relapse can only be detected through observation of clinical signs, such as enlargement of the peripheral lymph nodes, with the result that detection of a relapse and alterations in the drug regimen that could possibly increase survival time may be delayed. For this reason, it would be helpful to identify factors that could be used as markers for disease relapse, allowing relapse to be identified earlier.

C-reactive protein is an acute-phase protein, and serum CRP concentration in dogs typically increases in response to inflammation or infection. In human medicine, serum CRP concentration has been used as a prognostic factor for patients with lymphoid neoplasia and as a marker for cancer remission and relapse. However, it is not known whether serum CRP concentration is altered in dogs with lymphoma or whether the concentration will change in dogs undergoing chemotherapy. The purposes of the study reported here, therefore, were to determine whether serum CRP concentration is high in dogs with multicentric lymphoma, whether serum CRP concentration changes in response to chemotherapeutic protocols that do or do not include prednisone, and whether CRP concentration can be used as a marker for relapse in dogs with multicentric lymphoma.

**Materials and Methods**

**Dogs**—Twenty-eight healthy dogs and 20 dogs with multicentric lymphoma were included in the study. The

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The experimental protocol was approved by the Bioethics Committee of the University of São Paulo School of Veterinary Medicine and Animal Science in São Paulo, Brazil.

For the 28 healthy dogs, results of a complete physical examination, CBC, serum biochemical profile (measurement of urea nitrogen, total protein, albumin, calcium, and phosphorus concentrations and alanine transaminase and alkaline phosphatase activities), and urinalysis were unremarkable, and none of the dogs had any signs of disease. Twenty of the 28 healthy dogs were privately owned and were used to determine serum CRP concentrations in healthy dogs. The remaining 8 healthy dogs were owned by the University of São Paulo and were used to determine the effects of chemotherapy on serum CRP concentrations in healthy dogs.

Four of these dogs were randomly assigned to undergo chemotherapy with a combination of CVP. The other 4 were assigned to undergo chemotherapy with a combination of VCMA.

The 20 dogs with multicentric lymphoma were all privately owned. In all 20 dogs, the diagnosis of multicentric lymphoma had been made on the basis of results of cytologic examination of a fine-needle aspirate from an enlarged, peripheral lymph node. A CBC, serum biochemical profile, urinalysis, thoracic radiography, and abdominal ultrasonography were performed in all dogs, and all dogs were classified as having stage IV or V multicentric lymphoma. Dogs with signs of concurrent disease or localized infection and dogs that had previously undergone chemotherapy or had been treated with prednisone were excluded from the study. In addition, dogs that developed complications before or during treatment (eg, immune-mediated hemolytic anemia, pancreatitis, pyelonephritis, or sepsis) were excluded.

The 20 dogs with multicentric lymphoma were treated at the University of São Paulo Veterinary Hospital. Ten were randomly assigned to undergo chemotherapy with a combination of CVP. The other 10 were assigned to undergo chemotherapy with a combination of VCMA. Response to chemotherapy was assessed weekly by measuring solid tumors, and overall response was determined for both groups at week 4. Response was classified as complete remission (ie, disappearance of all solid tumors and clinical signs), partial remission (ie, ≥ 50% decrease in the size of all solid tumors), or no response (ie, < 50% reduction in size of all solid tumors). Only dogs that had a complete or partial remission were included in the study.

**Chemotherapy protocol**—The CVP chemotherapeutic protocol consisted of administration of cyclophosphamide (250 mg/m², PO), vincristine (0.75 mg/m², IV), and prednisone (40 mg/m², q 24 h, PO) during week 1, followed by administration of vincristine once a week for 3 weeks and then at 3-week intervals, cyclophosphamide at 3-week intervals, and prednisone (20 mg/m²) on alternate days until relapse was documented. The VCMA chemotherapeutic protocol consisted of administration of vincristine (0.75 mg/m², IV) during weeks 1 and 3, administration of cyclophosphamide (250 mg/m², PO) during week 2, and administration of methotrexate (0.8 mg/kg [0.36 mg/lb], IM) during week 4. A single dose of L-asparaginase (10,000 U/m², SC) was given on day 1. This monthly schedule was maintained until relapse was detected. A CBC was performed before every treatment was administered, and chemotherapy was delayed if the neutrophil count was < 2,500 cells/µL.

**Blood sample collection**—A single blood sample was collected from the 20 privately owned healthy dogs. In the 8 healthy dogs undergoing chemotherapy, blood samples were collected during weeks 1 (prior to treatment), 2, 3, 4, 7, 10, 13, and 16. In the 20 dogs with multicentric lymphoma, blood samples were collected during weeks 1 (prior to treatment), 2, 3, and 4. After the induction period of chemotherapy (ie, the first month), blood samples were collected every 21 days. When enlargement of the lymph nodes was detected (ie, relapse), an additional blood sample was collected. Blood samples collected during weeks 1, 2, 3, and 4 and at the time relapse was detected were submitted for determination of serum CRP concentration. In addition, the last blood sample collected prior to detection of relapse (ie, when the dog's condition was stable, clinical signs were not evident, and lymph node enlargement could not be detected) was submitted for analysis (stable-disease blood sample). For all blood samples, serum was harvested and frozen at −70°C until assayed for CRP concentration.

**CRP assay**—Serum CRP concentration was determined by the use of a solid-phase sandwich immunoassay. Serum samples at room temperature were diluted 1:500 in buffer solution and applied to coated microwells. Wells were washed, and anti-canine CRP antibody labeled with horseradish peroxidase was added to each well. Tetramethylbenzidine substrate solution was added, and absorbance was measured at 450 nm in a spectrophotometer. All samples were processed in duplicate, and a standard calibration curve was constructed for each plate. Samples with absorbance higher than that for the highest standard on that plate were diluted and reanalyzed.

**Data analysis**—Data for serum CRP concentration were analyzed by use of repeated-measures ANOVA incorporating factors for study group and week of treatment and assuming a symmetric matrix of correlation between observations. When results of the ANOVA were significant, the Tukey multiple comparison test was used to identify differences in serum CRP concentration among time points and among groups. Standard software was used for all analyses. Values of P < 0.05 were considered significant.

**Results**

The 20 healthy dogs ranged from 2 to 6 years old (mean, 3.7 years) and weighed between 5 and 26 kg (11 and 57.2 lb; mean, 7 kg [15.4 lb]). There were 9 males (3 neutered) and 11 females (2 neutered). Fifteen were purebreds and 5 were of mixed breeding. Mean ± SEM serum CRP concentration in these dogs was 2.77 ± 0.45 µg/mL. The 4 healthy dogs assigned to the CVP protocol ranged from 2 to 4 years old and weighed between 12 and 23 kg (26.4 and 50.6 lb; mean, 16 kg [35.2 lb]).
There were 3 males (all sexually intact) and 1 female (neutered). The 4 healthy dogs assigned to the VCMA protocol ranged from 2 to 4 years old and weighed between 12 and 19 kg (26.4 and 41.8 lb; mean, 16 kg). There were 3 males (all sexually intact) and 1 female (neutered). Dogs with lymphoma assigned to the CVP protocol ranged from 3 to 10 years old (mean, 6.2 years) and weighed between 14 and 42 kg (30.8 and 92.4 lb; mean, 21.9 kg [48.2 lb]). There were 4 males (all sexually intact) and 6 females (2 neutered). Seven were purebreds and 3 were of mixed breeding. Dogs with lymphoma assigned to the VCMA protocol ranged from 4 to 14 years old (mean, 7.5 years) and weighed between 11 and 33 kg (24.2 and 72.6 lb; mean, 21.9 kg [48.2 lb]). There were 3 males (1 neutered) and 7 females (3 neutered). Nine were purebreds and 1 was of mixed breeding.

Of the 10 dogs with lymphoma assigned to the CVP protocol, 7 had a complete remission and 3 had a partial remission. Mean time to collection of the stable-disease blood sample was 78 days (SD, 43 days; range, 49 to 161 days), mean duration of first remission was 108 days (SD, 58 days; range, 50 to 222 days), and mean survival time was 242 days (SD, 122 days; range, 75 to 426 days). Of the 10 dogs with lymphoma assigned to the VCMA protocol, 6 had a complete remission and 4 had a partial remission. Mean time to collection of the stable-disease blood sample was 121 days (SD, 79 days; range, 49 to 245 days), mean duration of first remission was 153 days (SD, 82 days; range, 56 to 268 days), and mean survival time was 294 days (SD, 157 days; range, 56 to 518 days). Data for time to collection of the stable-disease blood sample, duration of the first remission, and survival time were considered to be normally distributed (Kolmogorov-Smirnov test). Mean time to collection of the stable-disease blood sample, mean duration of first remission, and mean survival time were not significantly different between dogs with lymphoma assigned to the CVP protocol and dogs with lymphoma assigned to the VCMA protocol.

For both groups of dogs with lymphoma, mean serum CRP concentration during week 1 (prior to treatment) was significantly higher than mean concentration at the time the stable-disease blood sample was collected ($P = 0.01$) and mean concentration at the time of relapse ($P = 0.012$). For all other comparisons within each group, mean serum CRP concentrations during weeks 1, 2, 3, and 4; at the time the stable-disease blood sample was collected; and at the time of relapse were not significantly different (Figure 1), and mean concentrations were not significantly different between groups at any time point ($P = 0.999$). Mean serum CRP concentrations for dogs with lymphoma during chemotherapy were significantly higher than mean concentration for the 20 healthy control dogs at all points ($P = 0.047$).

For both groups of healthy dogs that underwent chemotherapy, mean serum CRP concentrations at all time points were not significantly different from mean concentration for the 20 healthy control dogs. In addition, mean concentrations were not significantly different ($P = 0.485$) between the 2 groups of healthy dogs that underwent chemotherapy at any time point.

**Discussion**

Theoretically, serum CRP concentration might increase in dogs undergoing chemotherapy as a result of induction by the tumor itself, induction caused by chemotherapy, or induction caused by any concurrent inflammatory or infection process. Groups included in the study reported here were structured to allow evaluation of these possibilities and more accurate interpretation of serum CRP concentrations. Thus, dogs with concurrent diseases and dogs that developed complications before or during chemotherapy were excluded from the study; and 2 groups of healthy dogs treated with CVP or VCMA were enrolled so that the effects of chemotherapy on serum CRP serum concentrations could be assessed.

The finding that mean serum CRP concentration was significantly higher prior to the initiation of treatment in dogs with lymphoma than the concentration during treatment suggests that multicentric lymphoma plays a role in induction of the acute phase response. A similar role for cancer in the acute phase response has been reported for humans and animals. With the stimuli that induce the acute phase response are diverse, all include, as a common denominator, production of cellular or tissue injury or death. With cancer, there is constant death of neoplastic cells because of high cell turnover or as a result of ischemia in fast-growing tumors. Also, adjacent tissues can be damaged by volumetric expansion of tumors, which may also cause cellular death. The acute phase response is nonspecifically triggered by most forms of tissue damage and generally reflects quantitatively the extent of

![Figure 1](image-url)
such damage.\textsuperscript{30} In our study, induction of a complete or partial remission led to a decrease in serum CRP concentrations, probably as a result of a decrease in the tissue damage caused by the neoplasm.

In another study,\textsuperscript{29} serum $\alpha_1$-acid glycoprotein concentrations were measured in 12 dogs with lymphoma treated with doxorubicin. Serum $\alpha_1$-acid glycoprotein concentrations were higher in the dogs with lymphoma prior to chemotherapy than in a group of healthy dogs, which was similar to results of the study reported here. However, in that study, serum $\alpha_1$-acid glycoprotein concentration was significantly decreased 3 weeks after the first dose of doxorubicin was administered, and a subsequent increase in concentration was detected 3 weeks before relapse and at the time of relapse, which is different from findings of our study. Of the 20 dogs with lymphoma in our study, only 4 had an increase in serum CRP concentration at the time the stable-disease blood sample was collected or at the time of relapse.

It may be speculated that the acute phase response mediated by CRP is different from that mediated by $\alpha_1$-acid glycoprotein, which could explain the delay in the decrease in serum CRP concentration following initiation of chemotherapy and the failure for CRP concentration to increase at the time of relapse. It appears that serum CRP concentrations are high only under conditions during which the tumor load is large enough to cause substantial tissue damage.\textsuperscript{31} Thus, serum CRP concentrations could be expected to be higher at the time of diagnosis, when tumor burden would be the highest, than immediately prior to relapse, when tumor burden would be expected to be low. In some dogs in the study reported here in which rescue chemotherapy was not administered, an increase in serum CRP concentration was observed after relapse, when enlargement of the lymph nodes was already clinically evident. Therefore, serum CRP concentration appeared to be a late marker for relapse, rendering it useless in monitoring progression of the disease. In humans with lymphoid neoplasias such as lymphoma, multiple myeloma, and Hodgkin's disease, a high serum CRP concentration prior to administration of chemotherapy is correlated with a poor prognosis.\textsuperscript{16-19} Further study is needed to establish the prognostic value of serum CRP concentration in dogs.

Chemotherapy alone did not alter serum CRP concentration in healthy dogs in the study reported here. Similarly, another study\textsuperscript{28} indicated that $\alpha_1$-acid glycoprotein concentrations measured in 8 healthy dogs before and 3 weeks after a single dose of doxorubicin was administered were not significantly different.

Corticosteroids have various anti-inflammatory, metabolic, and immunosuppressive effects,\textsuperscript{32} and studies\textsuperscript{33,34} in rats have revealed that the mechanism through which corticosteroids could stimulate synthesis of acute phase proteins is not yet fully understood. Thus, additional studies are needed on how corticosteroids affect the acute phase response during chemotherapy. In our study, serum CRP concentrations in dogs given a chemotherapeutic protocol that incorporated prednisone were not significantly different from concentrations in dogs given a chemotherapeutic protocol that did not incorporate prednisone. Similarly, in another study,\textsuperscript{24} administration of a single dose of methylprednisolone acetate and prednisone for a maximum of 21 days did not change concentrations of the acute phase proteins ceruloplasmin, serum amyloid A, and CRP in dogs. However, the effects of long-term use of prednisone in a combined chemotherapeutic protocol, such as that described in the study reported here, were not evaluated.

In conclusion, results of our study suggest that serum CRP concentrations are high in dogs with multicentric lymphoma but that serum CRP concentration is not a useful marker for relapse and that chemotherapeutic protocols do not affect serum CRP concentrations, regardless of whether they do or do not include prednisone.

References


