Serum C-Reactive Protein Concentration as an Indicator of Remission Status in Dogs with Multicentric Lymphoma

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Background: The acute-phase protein C-reactive protein (CRP) is used as a diagnostic and prognostic marker in humans with various neoplasias, including non-Hodgkin’s lymphoma.

Objective: To evaluate if CRP could be used to detect different remission states in dogs with lymphoma.

Animals: Twenty-two dogs with untreated multicentric lymphoma.

Methods: Prospective observational study. Blood samples were collected at the time of diagnosis, before each chemotherapy session, and at follow-up visits, resulting in 287 serum samples.

Results: Before therapy, a statistically significant majority of the dogs (P = .0019) had CRP concentrations above the reference range (68%, 15/22). After achieving complete remission 90% (18/20) of the dogs had CRP concentrations within the reference range, and the difference in values before and after treatment was statistically significant (P < .001). CRP concentrations of dogs in complete remission (median, 1.91; range, 0.2–103) were significantly different (P = .031) from those of dogs with partial remission (median, 2.48; range, 0–89), stable disease (median, 1.77; range, 1.03–42.65), or progressive disease (median, 8.7; range, 0–82.5). There was profound variation of CRP measurements within each dog.

Conclusions: CRP is useful in determining complete remission status after treatment with cytotoxic drugs. However, the individual variation between dogs means CRP concentration is not sufficiently different in other remission states to permit its use in monitoring progression of the disease. Greater reliability in determining remission status might be achieved by combining CRP concentration with other serum markers.

Key words: Acute phase protein; Complete remission; C-reactive protein; Serum markers.

Malignant lymphoma is one of the most common canine neoplasms: prevalence is 13–24 cases/100,000 dogs.1 Combination chemotherapy achieves significant periods of remission and a median survival time of 250–300 days.2 Prognostic markers for remission and survival time include age, clinical stage, immunophenotype, and malignancy grade.3,4 Currently, remission status is assessed by clinical examination alone or in combination with other diagnostic modalities, such as imaging. However, clinical examination along with measurement of lymph node size recently proved insufficient to determine remission status.5 It is also well recognized from the human literature that predicting life expectancy of individual patients is problematic during treatment and once treatment has finished because there are few objective markers.6,8

C-reactive protein (CRP) is an acute-phase protein produced in the liver that is released in response to microbial invasion, acute inflammation, and tissue injury. Serum CRP concentration in humans is a nonspecific but very sensitive systemic marker of inflammation and tissue damage, and it has shown potential as a diagnostic and prognostic marker in patients with various types of neoplasia, including epithelial ovarian cancer,9 thymic cell carcinoma,10 pancreatic cancer,11 colorectal cancer,12 renal cell carcinoma,13 multiple myeloma,14 and Hodgkin’s and non-Hodgkin’s lymphoma.15–17 CRP concentrations in dogs are elevated in infectious disease (leishmaniasis, leptospirosis, parvovirus, ehrlichiosis), specific inflammatory conditions (inflammatory bowel disease, uremia, allergies, and immune-mediated disease), endocrine and metabolic disease, and nonspecified neoplastic conditions.18–22

CRP is the most sensitive acute-phase protein in dogs, and it does not appear to be altered by corticosteroid administration in contrast to haptoglobin, another acute-phase protein.23,24 Therefore, CRP is a good candidate for assaying dogs with lymphoma that are receiving treatment. Furthermore, the concentration of this protein appears to be higher in untreated dogs with lymphoproliferative and myeloproliferative disorders.25,26

The main objective of the current study was to investigate whether CRP concentration could be used to monitor the remission status of lymphoma during therapy. This was evaluated by examining whether CRP concentration was increased in dogs with untreated high-grade multicentric lymphoma, whether this variable changed when the disease was in remission, and whether it was possible to differentiate between the different remission states by the use of the CRP concentration.

Material and Methods

This study was performed with the approval of the Glasgow University Faculty of ethics and welfare committee.
**Table 1.** World Health Organization clinical staging system for lymphosarcoma in domestic animals.  

<table>
<thead>
<tr>
<th>Stage and Substage</th>
<th>Description</th>
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<tbody>
<tr>
<td>I</td>
<td>Involvement limited to a single node or lymphoid tissue in a single organ (excluding bone marrow)</td>
</tr>
<tr>
<td>II</td>
<td>Involvement of many lymph nodes in a regional area (with or without the tonsils)</td>
</tr>
<tr>
<td>III</td>
<td>Generalized lymph node involvement</td>
</tr>
<tr>
<td>IV</td>
<td>Liver and/or spleen involvement (with or without stage III disease)</td>
</tr>
<tr>
<td>V</td>
<td>Manifestation in the blood and involvement of bone marrow and/or other organ systems (with or without stages I-IV disease)</td>
</tr>
<tr>
<td>Substage a</td>
<td>Without systemic signs</td>
</tr>
<tr>
<td>Substage b</td>
<td>With systemic signs</td>
</tr>
</tbody>
</table>

**Dogs**

Untreated dogs with malignant multicentric lymphoma presented to University of Glasgow Veterinary Hospital between January 2004 and July 2006 were eligible for entry into the study.

All dogs were evaluated by physical examination and measurements of peripheral lymph nodes were made whenever possible to ensure that future assessments of remission by different clinicians would be as objective as possible. A diagnosis of malignant lymphoma was confirmed by lymph node cytology or histopathology, and immunophenotyping was carried out by immunocytochemistry using antibodies against CD79a and CD3. All dogs were staged and substaged according to the World Health Organization staging system (Table 1) evaluating CBC, blood smear examination, serum biochemistry, urinalysis, thoracic radiography, and abdominal ultrasonography with splenic and hepatic cytology. Bone marrow evaluation was performed in dogs with haematologic bicytopenia or pancytopenia. Dogs were treated with a standard multidrug chemotherapy protocols for malignant lymphoma: 25 weeks of cyclophosphamide, Adriamycin, vincristine, prednisolone (CHOP) in 20 dogs and cyclophosphamide, vincristine, prednisolone (COP) in 2 dogs. Both protocols consisted of an induction and maintenance part. At relapse dogs were re-staged and reinduced with CHOP (1 dog) or treated with an alternative protocol; dexamethasone, melphalan, actinomycin D, cytosine arabinoside (DMAC) (6 dogs).

**Exclusion Criteria**

Dogs were excluded from the study if they had known concurrent inflammatory and infectious disease, if they had other anatomic subclassification of malignant lymphoma, or if they had been treated previously for malignant lymphoma.

**Clinical Assessment**

Response to chemotherapy was assessed at each visit as complete response (CR, no visible disease) partial response (PR, reduction in size by >50%), stable disease (SD, reduction in size by < 50% or < 25% increase in visible tumor), or progressive disease (PD, > 25% increase in visible tumour). The dimensions of the peripheral lymph nodes were measured with callipers, and the internal mediastinal and subiliac lymph nodes were measured by metric rulers as part of the ultrasonographic equipment.

**C-reactive Protein**

Serum samples were obtained from the dogs at the point of diagnosis; then weekly before each chemotherapy session; and then subsequently at 1, 3, and 6 months after treatment. At relapse, blood samples were taken as part of the restaging procedure and subsequently at routine visits before each chemotherapy dose of the relapse protocol. Using jugular venipuncture, 1–2 mL whole blood was collected from the tumor-bearing dogs into serum tubes. Blood was allowed to clot at room temperature then centrifuged at 538 g for 3 minutes with a fixed rotor centrifuge. At least 200 μL serum was collected and frozen at −20°C for batch analysis at a later date. No grossly hemolysed or lipemic samples were used.

CRP concentration was measured in duplicate by a solid-phase sandwich immunoassay. The test has previously been validated in this laboratory and by others to have a low within-run imprecision with intraassay coefficients of variance (n=8) of 1.0% and 2.8% at mean control values of 18 mg/L and 74 mg/L, respectively. It also had an acceptable between-run imprecision with interassay coefficients of variance (n=11) of 11.1% and 12.6% at mean control values of 19 and 75 mg/L, respectively. The population-based reference for normal CRP concentration, based on recent studies of 29 normal dogs (determined by clinical examination, CBC, and serum biochemistry) was 0.46–9.6 mg/L.

**Statistical Analysis**

Statistical analyses were carried out using SAS. For all analyses, a P value ≤0.05 was considered significant.

The initial CRP concentration before therapy was compared to the reference range for normal dogs using the Wilcoxon one-sided test. The effect of stage and substage on the initial CRP concentration was analyzed to adjust for potential influence these classifications might have on the CRP concentrations by the use of the Wilcoxon test. CRP serum concentration before therapy was compared to the CRP concentration at first CR using the Wilcoxon signed rank test.

Differences between remission states (CR, PR, SD, and PD) and CRP concentration were analyzed by use of a general linear mixed model, with a random effect of dog nested within the remission status. For this analysis, CRP concentration was log-transformed to improve the assumption of Gaussian distribution and variance homogeneity.

Finally, the ability of CRP concentration to detect CR versus other remission states was evaluated using alternating logistic regression which estimates the within-dog association as a constant odds ratio across dogs. In this analysis, CRP concentration was log-transformed to improve the assumption of linearity on the logit-scale. Data are presented as median and range of CRP concentration in the different clinical stages (CR, PR, SD, and PD).

**Results**

**Animals**

Within the study period, 64 dogs were examined for malignant lymphoma; 48 of these had the multicentric form of the disease. Of these 48 dogs, 26 were excluded from the study because they were euthanized at the point of diagnosis (4 dogs), the owners elected not to treat the dog (2 dogs), dogs had already been treated with chemotherapeutics by their local veterinarian (6 dogs),...
or the owners elected at the point of diagnosis to treat the dog at their local veterinarian (10 dogs). Finally, 4 dogs were excluded because of concurrent diseases, such as cardiomyopathy (1 dog), kidney disease (1 dog), or bacterial cystitis (2 dogs).

Twenty-two dogs with malignant multicentric lymphoma met the inclusion criteria and entered the study. All dogs had high-grade lymphoblastic lymphoma diagnosed on morphologic examination (large immature lymphocytes, high nuclear:cytoplasm ratio, anisokaryosis, high mitotic index, stippled chromatin patterns, multiple nucleoli), 9 of which were B-cell (CD79a positive) and 3 of which were T-cell (CD3 positive); the remaining dogs were not immunophenotyped on immunocytochemistry. Fourteen dogs were males (9 entire and 5 castrated), and the remaining 8 dogs were female (2 entire and 6 neutered). Thirteen breeds were represented: most prevalent were Labrador Retrievers (3 dogs), Bullmastiffs (3 dogs), German Shepherds (3 dogs), Weimaraners (3 dogs) and Boxers (2 dogs). Ages ranged from 3 to 11 years (median, 7.5 years). CRP before treatment ranged from 0.5 to 198 (median, 22.20). All stages were represented, but the majority of the dogs were in stages 3 and 4. The majority of the dogs (14/22) presented with substage a disease.

Serum samples (n=287) were obtained from the 22 dogs. The total number of samples per dog varied from 2 to 35 (median, 11.5 samples per dog). Of the 22 dogs, 20 achieved complete remission after treatment. In addition, 14 dogs achieved complete remission by the second visit to the hospital (1 week), 3 dogs achieved complete remission at the 3rd visit (2 weeks), and 3 dogs achieved complete remission at the 5th visit (4 weeks). By the end of the study (July 2006) 8 dogs were still alive, and the remaining dogs had died. All the dead dogs had been euthanized because of the malignant lymphoma.

**CRP Concentration**

Mean serum CRP concentrations for substages a (39.1, standard error = 14.6) and b (34.7, standard error = 15.7) at the first presentation were very similar and no statistically significant difference could be found between dogs with substage a and substage b (P = .45). Evaluation of the serum CRP concentration in relation to the different stages of disease was not assessed statistically because of low numbers in several categories.

At the first presentation, before treatment commenced, the majority of the dogs (68%, 15/22) had CRP concentrations above the reference range (9.6 mg/L), and this increase in CRP concentration was statistically significant (P = .0019). After achieving complete remission 90% (18/20) of the dogs had CRP concentrations within the reference range. The difference between CRP concentration before treatment and the first point of complete remission was statistically significant (P < .001).

Median and range of CRP concentration for complete remission (median, 1.91; range, 0.2–103), partial remission (median, 2.48; range, 0–89), stable disease (median, 1.77; range, 1.03–42.65), and progressive disease (median, 8.7; range, 0–82.5) showed substantial similarities. There was no significant difference (P = .087) in the mean log (CRP) between the 4 different states of remission (CR, PR, SD, PD). Variance components were estimated at σ²_Dog = 1.22 and σ² = 1.32, for the effect of dog and residual variance, respectively, which implies that 48% (1.22/1.22+1.32) of the variation in log(CRP) was attributable to differences in CRP concentration between dogs rather than differences between remission status. Alternating logistic regression analysis was used to evaluate CRP as an indicator of CR compared to all other remission states (PR, SD, PD). There was a statistically significant effect of log(CRP) on the probability of CR (P = .031). The effect of log(CRP) was estimated at −0.31, with an associated odds ratio of 1.36, whereas the estimated within-dog association of odds ratio was 4.47.

**Discussion**

This longitudinal study of dogs with lymphoma has shown that CRP concentration is able to determine complete remission status after therapy with cytotoxic drugs. However, there is substantial individual variation between patients, and CRP concentration is not sufficiently different in other remission states to permit its use in monitoring these different remission states or relapse of the disease. Greater reliability in determining remission status may be achieved by combining CRP concentration with other serum markers or by monitoring CRP using a baseline for each patient. Further longitudinal studies involving larger numbers of dogs are needed to evaluate conclusively the usefulness of this acute-phase protein in dogs with cancer.

Several prognostic markers at the point of diagnosis have proved useful for determining survival times in dogs with lymphoma, but few markers have been evaluated to monitor the progression of the disease. Evaluation of remission status at each visit is subjective and often inaccurate, especially as relapse may occur at a site different from that of the initial presentation. Identification of a serum marker to aid assessment of remission status would therefore be of value. The potential benefit of the acute-phase protein CRP was evaluated in this small study to provide a more objective measurement of remission status in dogs with multicentric lymphoma.

The CRP concentration increased in most dogs with untreated multicentric lymphoma and decreased to within the normal range at the first point where the animal went into complete remission. Similar findings have recently been reported in dogs as well as in people with non-Hodgkin’s lymphoma. Although the decrease in CRP concentration was statistically significant overall, it is important to note that 7/22 (32%) of the individual dogs had CRP concentrations inside the reference range before therapy; therefore, there was minimal change in CRP concentration in CR in these dogs. This finding is in contrast to previous studies examining dogs with untreated hematopoietic and nonhematopoietic neoplasia along with inflammatory and autoimmune disease conditions where the CRP concentrations were above the
It is difficult to explain why some dogs in our study, despite presenting with major systemic disease, did not mount a profound inflammatory response reflected by a raised CRP concentration. None of the dogs in our study had presented with hepatic failure secondary to neoplastic infiltration, which in theory could result in a reduced acute-phase protein metabolism.

Although the CRP concentration decreased from before treatment to the first CR in most cases, and there was a significant difference between CR and other remission states, this study failed to show a significant difference overall between each individual remission state. In general, the CRP concentration was considerably lower when the dogs were in CR compared with when they were in PR, SD, or PD. However, a large overlap of CRP concentrations was seen in all other remission states (PR, SD, and PD), and no consistent trend was noted toward higher CRP concentrations at relapse. The failure to detect a significant difference in CRP concentration between remission states may have been for a number of reasons. There was a wide range in the number of samples per dog, and in particular, some dogs had only 2 CRP measurements, making comparisons of different remission states difficult. The small total sample size of the study (22 dogs) also contributed to the lack of statistical significance, and the results need to be confirmed with a larger study population.

Investigation of the variation of log(CRP) detected a much stronger within-dog association than between-dog association. The observation that the CRP concentration fluctuated more within 1 dog than between dogs in this study is worth noting, but it may be difficult to explain. In contrast, a previous study examining 10 healthy Beagles during a 28-day period, observed that CRP did not vary significantly in individual dogs, but there were significant differences between dogs. Because the dog itself contributed as much variation to the CRP concentration as remission status, this suggests there may be a need for a CRP-baseline to be established for each individual dog to make monitoring valid.

In humans, CRP has been used in lymphoid neoplasia as an objective measure of response to therapy and, more importantly, to monitor or predict progression of disease. It is not used in isolation, however, but it forms part of a larger panel of objective markers such as interleukins, lactate dehydrogenase, and tumor necrosis factor.

Measuring a similar panel of objective markers along with serum CRP concentration in dogs may have greater value in estimating remission status of lymphoma. Other serum markers have been investigated in dogs with lymphoid neoplasia. The acute-phase proteins alpha-1 glycoprotein and haptoglobin have both been shown to be increased in dogs with untreated lymphoid neoplasia. Both proteins are considered moderate acute-phase proteins in dogs, which indicates that they have a slower increase and a slower decrease in concentration compared with CRP. As haptoglobin concentrations alter with corticosteroid therapy, and this medication is often included in the lymphoma protocols, it precludes haptoglobin as a protein for monitoring disease progression during therapy. In contrast, alpha-1 glycoprotein has shown some promise as an indicator of relapse. Alpha-1 glycoprotein is present in lymphocytes in the dog, and a combination of CRP and this protein might be more accurate in determining remission status in canine lymphoma patients undergoing treatment. Thymidine kinase (TK), an enzyme involved in DNA synthesis and cellular proliferation, has been investigated in dogs with lymphoma and leukemia and shows great potential as a marker of relapse and a prognostic indicator for survival. This marker, however, is not necessarily specific for dogs with neoplasia as serum activity of TK is sometimes elevated in cases of viral infections; it has also been reported raised in a case of pyometra and tongue necrosis.

As CRP is a nonspecific acute-phase protein, it is essential to assess lymphoma patients for concurrent inflammation or infections, which could falsely elevate CRP concentrations, rather than neoplastic disease, itself. Other factors influencing CRP concentration could include chemotherapeutics, which might create an inflammatory state, for example, by free radical release in the tissues or gastrointestinal irritation. The effect of chemotherapeutics on CRP concentrations was not examined in this study because of the nonuniformity of protocols used and the lack of a constant time interval between the use of drugs and CRP measurements. Raised CRP concentration secondary to chemotherapy has not been documented in the human literature, and earlier studies in dogs have not observed that chemotherapy had an effect on this acute-phase protein either.

Increasing evidence from human medicine suggests that a systemic inflammatory response, defined by a raised CRP concentration, predicts survival time in gastrointestinal and lung cancer, independent of stage. A recent study investigating the serum CRP concentration in people with non-Hodgkin’s lymphoma in relation to survival found high pretreatment concentrations to be a strong independent predictor of death, despite lack of information on clinicopathologic stage and treatment along with variable time of sampling. Too many variables were involved to apply survival statistics to our canine study. In particular, the different chemotherapeutic protocols would have affected the survival of these dogs.

Footnotes

a Tridelta Development, Ltd, Ireland
b Version 9.1, SAS Institute, Cary, NC

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References


