Serum α1-acid glycoprotein and serum amyloid A concentrations in cats receiving antineoplastic treatment for lymphoma

Valter M. Winkel MSc
Tatiana L. R. Pavan DVM
Vera A. B. F. Wirthl DVM
Ana L. N. Alves DVM
Silvia R. R. Lucas PhD

OBJECTIVE
To characterize serum α1-acid glycoprotein (AGP) and serum amyloid A (SAA) concentrations at diagnosis and during treatment in cats with lymphoma.

ANIMALS
16 cats with various anatomic forms of lymphoma and 25 healthy cats.

PROCEDURES
Blood samples were collected from healthy cats once and from cats with lymphoma at diagnosis and 2-week intervals until the 12th week of antineoplastic treatment. Serum harvested from blood samples was assessed for AGP and SAA concentrations. Differences in serum AGP and SAA values were investigated between healthy cats and cats with lymphoma (at diagnosis) and, for cats with lymphoma, between diagnosis and various points during treatment.

RESULTS
Serum AGP and SAA concentrations were higher in cats with lymphoma at diagnosis (median, 832.60 and 1.03 µg/mL, respectively), compared with those in healthy cats (median, 269.85 and 0.10 µg/mL). Treatment resulted in a gradual decrease in serum AGP concentration after 4 weeks and in SAA concentration after 8 weeks of treatment, and these concentrations returned to values comparable with those of healthy cats by 12 weeks of treatment, by which point all cats had achieved complete remission of the disease.

CONCLUSIONS AND CLINICAL RELEVANCE
Serum AGP and SAA concentrations in cats with lymphoma were higher at diagnosis than after antineoplastic treatment. Decreases to values established for healthy cats corresponded with achievement of complete disease remission. Serum AGP and SAA may be useful protein markers for monitoring of antineoplastic treatment in cats with lymphoma. (Am J Vet Res 2015;76:983–988)

In infectious, traumatic, neoplastic, immune-mediated, and inflammatory processes, organisms develop a general, nonspecific, acute reaction to local or systemic injuries that affects homeostasis. This reaction is known as the acute-phase response and is part of the innate defense system of the organism.1,2 Proinflammatory cytokines are released, and the vascular system and inflammatory cells are activated, resulting in the production of cytokines and other inflammatory mediators, including interleukin-1, interleukin-6, and tumor necrosis factor.3 These mediators target the liver, which reacts to the stimulus by producing APPs.4,5

Acute-phase proteins have been used as markers of infection, inflammation, and trauma in humans for several decades, but they are not commonly used in clinical veterinary practice.6 The proteins are classified in accordance with the magnitude of their response to stimuli as major (10- to 100-fold change), moderate (2- to 10-fold change), or negative (serum concentration decreases in the presence of the stimuli).2

The most widely known APPs are proteins with antibacterial or immunomodulatory activity, such as AGP and C-reactive protein; transport proteins, such as haptoglobin and ceruloplasmin; and proteins that protect tissues against reactive oxygen species (free radicals), such as SAA.7 Haptoglobin, AGP, and SAA are useful indicators of the acute-phase status of cats.8,10 However, the same is not true of C-reactive protein.8

A highly glycosylated protein, AGP is the main protein component of seromucoid, the fraction of plasma that is most resistant to acid precipitation.2 In cats affected by infectious peritonitis and lymphoma, serum concentrations of AGP increases.8,11 In humans, serum AGP concentration is an important variable in the monitoring and staging of malignancies such as lymphoma and colorectal cancer; and it increases when the disease is active.12,13

In humans, SAA concentration can increase up to 1,000-fold within 24 to 48 hours after an inflammatory stimulus.10 Similarly, in cats, SAA concentration can increase markedly when inflammation occurs and is rec-
recognized as a useful inflammatory marker.\textsuperscript{10} High SAA concentrations have been identified in cats with experimentally induced inflammation, infectious diseases (e.g., infectious peritonitis), and other diseases.\textsuperscript{8,10,14}

Lymphomas are the most common neoplasia in cats, representing 30\% of all diagnosed neoplasias.\textsuperscript{15,16} The treatment for lymphoma involves antineoplastic chemotherapy, and because this treatment is usually palliative rather than curative, clinical relapse frequently occurs. The behavior of APPs during the acute-phase response in cats with lymphoma has not yet been investigated. Therefore, the objective of the study reported here was to measure serum AGP and SAA concentrations in cats with lymphoma and compare these concentrations at diagnosis and during chemotherapy.

**Materials and Methods**

**Animals**

Two groups of cats were enrolled in the study. The first consisted of 25 healthy adult (≥1 year old) privately owned cats and was used as a control group. To qualify for inclusion in the control group, cats were required to be in good clinical condition as confirmed through results of physical examination and laboratory tests (CBC; measurement of serum BUN, creatinine, total protein, and albumin concentrations and alanine aminotransferase and alkaline phosphatase activities; urinalysis; and serologic tests\textsuperscript{a} for FIV and FeLV infection). Cats were also required to be up-to-date on vaccinations against panleukopenia virus, feline calicivirus, feline herpes virus type I, and rabies virus infections and to be housed with no access to the outdoors.

The second group consisted of 16 cats with lymphoma undergoing antineoplastic treatment. Diagnosis of lymphoma and eligibility for inclusion were established by consideration of data pertaining to clinical history, physical examination, laboratory tests (same as performed for the healthy cats), thoracic radiography, abdominal ultrasonography, and cytologic analysis of material collected through aspiration biopsy of affected organs. When results of cytologic assessment were not conclusive or cytologic assessment could not be performed, cats underwent biopsy and histologic assessment of collected tissue samples was performed.

No restrictions were placed on sex, age, or breed for inclusion of cats in either study group; however, prior administration of any antineoplastic or corticosteroid drug and the presence of concomitant diseases were considered exclusion criteria. Cats with lymphoma that developed complications during treatment, did not respond to the treatment, or could not be followed up for at least 3 months were also excluded from the study. After diagnosis of lymphoma and initiation of treatment, cats were monitored for remission of the disease by abdominal ultrasonography (those with renal and alimentary lymphoma) or thoracic radiography (those with mediastinal lymphoma), every 4 weeks until the 12th week of treatment, when complete remission status was achieved for all cats.

Antineoplastic treatment included prednisolone, chlorambucil, L-asparaginase, doxorubicin hydrochloride, or vincristine sulfate, as described previously.\textsuperscript{17} The chemotherapy protocol was selected in accordance with the anatomic form of the disease. Cats with small cell lymphoma were treated with L-asparaginase (10,000 U/m\textsuperscript{2}, SC, twice with an interval of 15 days), chlorambucil (2 mg/cat, PO, 3 times/wk), and prednisolone (40 mg/m\textsuperscript{2}, PO, q 24 h for 7 days, then q 48 h). Animals with large cell, renal, or mediastinal lymphomas were also treated with vincristine (0.5 mg/m\textsuperscript{2}, IV, q 15 days for 4 times, then every 21 days) or doxorubicin (1.0 mg/kg, IV, q 21 days). During treatment, the protocol was adjusted by reducing the dosages on the basis of the response to chemotherapy and development of adverse effects.

**Blood sample collection and processing**

Blood samples were collected from the cats by jugular or cephalic venipuncture by use of 1-inch, 21-gauge needles. For healthy cats, blood samples were collected only once at the time of screening for study inclusion. For cats with lymphoma, blood samples were collected at the time of diagnosis; at 2, 4, 6, 8, 10, and 12 weeks after the onset of chemotherapy; and when the cats were in remission and clinically stable (provided that remission was achieved during the 12-week follow-up period).

Two milliliters of each collected blood sample was placed in a vial containing 10\% EDTA for performance of a CBC.\textsuperscript{3} The remainder of each sample was placed in a siliconized vial without anticoagulants and centrifuged to separate the serum, which was subsequently harvested and divided into aliquots for laboratory tests.\textsuperscript{6} Serum samples were stored at −70°C until measurements were performed. This decision was made because APPs are stable in frozen samples, and serum or plasma samples should be stored at −70°C if the storage is to exceed a few weeks. In addition, it is recommended that serially collected samples be analyzed in batches to minimize assay imprecision.\textsuperscript{18}

**Serum AGP and SAA measurement**

Serum AGP concentration was measured in batches with a commercial single radial immunodiffusion kit\textsuperscript{7} as described previously.\textsuperscript{8,17,19} Serum amyloid A concentration was measured with a multispecies solid-phase sandwich immunoassay kit\textsuperscript{8} as described previously.\textsuperscript{18,19} All samples were processed in duplicate.

**Statistical analysis**

Data analysis was performed by use of statistical software.\textsuperscript{1} Data were assessed for Gaussian distribution, and normally distributed data are reported as mean ± SD. Nonnormally distributed data are reported as median (range). Because serum AGP and SAA data were not normally distributed, the nonparametric
Mann-Whitney test was performed to compare serologic results between healthy control cats and cats with lymphoma at the time of diagnosis. Nonparametric ANOVA for paired samples (Friedman test with posttest) was performed to compare serologic results at various points in cats with lymphoma. Correlations between serum AGP and SAA concentrations were evaluated by calculation of Spearman correlation coefficients. Values of $P < 0.05$ were considered significant for all analyses.

**Results**

**Control cats**

Mean ± SD age of the 25 healthy control cats was 3.0 ± 1.1 years (median, 3.0 years; range, 1 to 4 years). Eight (32%) cats were male, and 17 (68%) were female. Most (72%) control cats did not have a defined breed, and all control cats had negative results of FIV and FeLV testing. Results of CBCs and serum biochemical analyses for the control group were summarized (Table 1). Median serum AGP and SAA concentrations were 269.85 μg/mL (range, very low or undetectable to 536.20 μg/mL) and 0.10 μg/mL (range, very low or undetectable to 0.96 μg/mL), respectively. No differences between males and females were identified with respect to serum AGP and SAA concentrations in the control group.

**Cats with lymphoma**

Mean age of the 16 cats with lymphoma was 9.3 ± 4.0 years (median, 9.0 years; range, 3 to 16 years). The majority (10) were male, and none had a defined breed. Two had positive results of FeLV testing, and 1 had positive results of FIV testing.

Histologic analysis of tumor samples was performed for 12 of 16 cats with lymphoma, and cytologic analysis was performed for the remaining 4 cats. Ten cats with lymphoma had small cell lymphoma, and 6 had large cell lymphoma. Immunophenotyping was not performed. All cats had small cell lymphoma, and 6 had large cell lymphoma. Medullary lymphoma was the most common anatomic form of lymphoma (9 with small cell and 2 with large cell lymphoma), followed by renal (1 with small cell lymphoma and 1 with intermediate cell lymphoma), mediastinal (1 with large cell lymphoma), multicentric (1 with large cell lymphoma), and extranodal (1 with large cell lymphoma) forms.

By 4 weeks of antineoplastic treatment, complete remission was achieved and persisted for the 12-week follow-up period for 4 cats with small cell alimentary lymphoma, 1 cat with large cell alimentary lymphoma, and the sole cat with mediastinal, multicentric, or extranodal lymphoma. By 8 weeks and up to 12 weeks of treatment, those cats were joined in remission by 2 cats with small cell alimentary lymphoma, the other cat with large cell alimentary lymphoma, and the cat with small cell renal lymphoma. By 12 weeks of treatment, all remaining cats had also achieved complete remission (3 with small cell alimentary lymphoma and 1 with intermediate cell renal lymphoma).

**Serum AGP and SAA concentrations**

Hematologic and biochemical data for the cats with lymphoma were summarized (Table 1). Median serum AGP and SAA concentrations were 832.60 μg/mL (range, 50.00 to 2,825.40 μg/mL) and 1.03 μg/mL (range, undetectable to 18.80 μg/mL), respectively. No significant differences in serum AGP and SAA concentration were identified between the cats with small and large cell alimentary lymphoma.

Median serum AGP and SAA concentrations were significantly ($P < 0.001$ and $P = 0.003$, respectively) higher in cats with lymphoma at the time of diagnosis than in the control group. During the course of antineoplastic treatment, serum AGP concentration in cats with lymphoma was variable (Figure 1). However, SAA concentration was high at diagnosis, decreased gradually during the treatment period ($P = 0.005$), and was significantly lower than at diagnosis from weeks 8 (median, 0.22 μg/mL; $P < 0.05$ for all comparisons) through 12 (median, 0.00 μg/mL; $P < 0.01$ for all comparisons), by which point remission was achieved for all cats with lymphoma and all were in good clinical condition. Serum amyloid A concentration returned to a value comparable with that for the control group by 12 weeks of treatment ($P = 0.70$).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Lymphoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC count (X 10⁶ RBCs/µL)</td>
<td>7.02 ± 1.07 (5.30–9.00)</td>
<td>6.69 ± 1.21 (3.40–8.20)</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>39.3 ± 7.3 (30.0–48.0)</td>
<td>35.0 ± 5.0 (18.0–42.0)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.2 ± 2.3 (9.8–15.7)</td>
<td>11.9 ± 1.6 (6.4–14.0)</td>
</tr>
<tr>
<td>WBC count (X 10³ WBCs/µL)</td>
<td>10.45 ± 4.00 (5.00–16.40)</td>
<td>13.84 ± 7.21 (7.00–18.30)</td>
</tr>
<tr>
<td>Neutrophil count (X 10³ neutrophils/µL)</td>
<td>6.04 ± 3.51 (3.50–13.50)</td>
<td>9.57 ± 2.80 (4.90–14.36)</td>
</tr>
<tr>
<td>Lymphocyte count (X 10³ lymphocytes/µL)</td>
<td>3.34 ± 1.41 (1.65–5.88)</td>
<td>1.80 ± 1.56 (0.50–6.04)</td>
</tr>
<tr>
<td>Platelet count (X 10³ platelets/µL)</td>
<td>492.65 ± 143.66 (287.00–660.00)</td>
<td>453.94 ± 161.21 (221.00–610.00)</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>52.3 ± 7.3 (38.6–55.8)</td>
<td>107 ± 84.8 (42.6–480.0)</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.33 ± 0.21 (0.93–1.60)</td>
<td>1.56 ± 1.24 (0.79–6.10)</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>7.3 ± 0.8 (6.3–8.0)</td>
<td>7.5 ± 0.9 (6.4–9.1)</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.2 ± 0.4 (2.8–3.8)</td>
<td>3.1 ± 0.5 (1.9–3.8)</td>
</tr>
<tr>
<td>Alanine aminotransferase (U/L)</td>
<td>42.8 ± 21.1 (13.5–52)</td>
<td>31.0 ± 19.0 (9.5–62.1)</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>20.9 ± 9.8 (9.8–42.2)</td>
<td>14.9 ± 7.3 (6.9–29.5)</td>
</tr>
</tbody>
</table>
Serum AGP concentration in cats with lymphoma behaved similarly to SAA concentration (Figure 2). Specifically, values decreased gradually during the treatment period ($P < 0.001$) and were significantly lower than at diagnosis from weeks 4 (median, 544.10 µg/mL; $P < 0.01$ for all comparisons) through 12 (median, 322.15 µg/mL; $P < 0.001$ for all comparisons). Serum AGP concentration returned to a value comparable with that of the control group by 12 weeks of treatment ($P = 0.06$).

Because most (11/16) cats with lymphoma had an alimentary form of the disease, an analysis was...
performed comparing only those cats with the control group. In that analysis, a significant difference was observed between the groups with respect to serum AGP (median, 840.6 µg/mL; \( P < 0.001 \)) and SAA (median, 1.03 µg/mL; \( P = 0.005 \)) concentrations, but no significant difference in values was identified between cats with alimentary lymphoma and those with other anatomic forms of the disease. Values pertaining solely to the subset of cats with alimentary lymphoma decreased significantly for serum AGP concentration from weeks 6 (\( P < 0.05 \)) through 12 (\( P < 0.001 \)) and for SAA concentration from weeks 8 (\( P < 0.05 \)) through 12 (\( P = 0.002 \)). When cats with retrovirus infections were compared with those without infections, no significant difference in the results previously described for serum AGP and SAA concentration at diagnosis and during the treatment were identified. Similarly, no significant differences were identified in serum AGP or SAA concentration when comparing cats with small cell lymphoma with those with large cell lymphoma or cats that had a complete treatment response with those that had a partial response. Despite similar behavior, there was no correlation between serum AGP and SAA concentrations.

Discussion

Serum concentrations of APPs can increase in cats with lymphoma because of the tumor itself or any inflammatory process or concomitant disease, similar to findings for other animal species. To avoid such confounding influences in the present study in which serum AGP and SAA concentrations were evaluated in cats with lymphoma, cats with concomitant diseases or those that developed complications during treatment were excluded. However, cats with FIV or FeLV infections were included because these viruses are related to lymphoma. For similar reasons, only healthy indoor immunized adult cats were considered for inclusion in the control group. Age and sex were not matched between the 2 groups; however, we did not believe this was necessary because, as has been reported for dogs, serum AGP concentrations in cats are not associated with age. Moreover, serum AGP and SAA concentrations in the control group in the present study were similar to those reported by other investigators.

The significantly higher serum APP concentrations in cats with lymphoma prior to treatment, compared with values for control cats, suggested that lymphoma induced an acute-phase response in cats, as has been reported for other species. In the present study, baseline AGP and SAA concentrations increased 10- to 20-fold, compared with those of the control group. Induction of a full response to antineoplastic treatment and stabilization of the clinical status of the cats resulted in a gradual reduction of AGP and SAA concentrations, and this decrease was significant after 4 and 8 weeks of treatment, respectively. Both concentrations returned to values comparable with those of the control group after 12 weeks of treatment.

In a study involving cats with lymphoma receiving chemotherapy, despite identification of an increase in serum AGP concentration at the time of diagnosis, no differences were observed between values measured before and after treatment, in contrast to our findings. However, that study involved only 8 cats, and there was no serial collection of serum samples. In humans, serial measurement of serum AGP concentration can be useful for monitoring various types of cancer, such as lung cancer and lymphomas, insofar as the concentration of this APP increases at diagnosis and decreases gradually during treatment until returning to healthy concentrations when remission is detected, similar to the findings in this study.

Increases in serum AGP and SAA concentrations identified in cats with lymphoma in the present study agreed with the results of another study involving the acute-phase response in cats hospitalized for various diseases. In that study, significant increases were identified for these proteins as well. The present findings confirmed that serum AGP and SAA concentrations increased in the presence of inflammatory stimuli and decreased after antineoplastic treatment in cats, as reported by other investigators.

To the authors’ knowledge, no studies have been published that assess the influence of glucocorticoids on the amount of circulating APPs in cats. We presumed that glucocorticoids would have no influence on serum concentrations of any APP except haptoglobin, as has been reported for dogs.

A study involving 118 cats (signalment unknown) revealed a positive correlation (\( r = 0.644 \)) between serum AGP and SAA concentrations. When a strong correlation (ie, \( r > 0.9 \)) exists, the 2 proteins can be interpreted as behaving similarly. In the present study, no correlation was evident between serum AGP and SAA concentrations, perhaps because of the smaller number of cats than in the other study or because AGP and SAA did not behave similarly.

Results of the present study indicated that serum AGP and SAA concentrations were higher in cats with lymphoma than in healthy cats and that, during the treatment, concentrations decrease significantly when the disease was in remission. We consequently believe that the APPs SAA and, in particular, AGP could be clinically useful protein markers for monitoring the effect of antineoplastic treatment in cats with lymphoma.

Acknowledgments

This manuscript represents a portion of a thesis submitted by Dr. Winkel to the University of São Paulo, Department of Internal Medicine, in partial fulfillment of the requirements for a Master of Science degree. Supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo.


Footnotes

a. IDEXX Laboratories, Westbrook, Me.
b. Vet abc, Horiba Ltd, Kyoto, Japan.
c. Labmax, Labtest Diagnóstica SA, Lagoa Santa, MG, Brazil.
d. PHASE feline α-1 acid glycoprotein SRID assay kit, Tridelta Development Ltd, Maynooth, County Kildare, Ireland.
e. PHASE serum amyloid A-multispecies, Tridelta Development Ltd, Kildare, Ireland.
f. Instat, version 3.10, GraphPad Software Inc, La Jolla, Calif.

References