The APR is part of the innate immune system triggered by inflammatory stimuli such as trauma, infection, and neoplasia. Activation of the APR results in increased or decreased synthesis of APPs by the liver. Positive APPs are categorized on the basis of their response to inflammation as major (>10-fold increase), moderate (2- to 10-fold increase), or minor (< 2-fold increase). Negative APPs decrease in concentration in response to inflammation. When the liver responds to inflammation by producing positive APPs, the production of negative APPs is downregulated. Albumin is the most widely measured negative APP. The most widely measured positive APP in equine practice is fibrinogen, which is a moderate APP in horses. The wide reference interval for fibrinogen, moderate increase, and slow response time of 24 to 72 hours after an inflammatory insult make it a relatively insensitive APP, particularly as a predictive marker of inflammation. In contrast, SAA is a major APP in horses characterized by low or undetectable concentrations in clinically normal horses. After inflammatory stimuli, there is a rapid and robust SAA response, with increases of 10- to > 100-fold within 6 to 12 hours after the inflammatory insult and a short biological plasma half-life of approximately 20 to 35 hours in several species.

Evaluation of serum amyloid A and haptoglobin concentrations as prognostic indicators for horses with inflammatory disease examined at a tertiary care hospital

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Crystal M. Foster BS
Keith P. Poulsen DVM, PhD

OBJECTIVE
To evaluate use of serum amyloid A (SAA) and haptoglobin concentrations as prognostic indicators for horses with inflammatory disease in regard to euthanasia, complications, and hospitalization duration and cost.

ANIMALS
20 clinically normal horses and 53 horses with inflammatory disease.

PROCEDURES
Total WBC count, neutrophil count, and fibrinogen, SAA, and haptoglobin concentrations were determined for clinically normal horses and horses with suspected inflammatory disease. Clinicopathologic values at admission were compared to test the use of SAA and haptoglobin concentrations in predicting euthanasia, complications, and hospitalization duration and cost. Haptoglobin and SAA concentrations of 22 horses were monitored during hospitalization to test the use of serial measurements in predicting survival and complications.

RESULTS
Neutrophil count and SAA and haptoglobin concentrations were significantly different at admission for horses with inflammatory disease, compared with those for clinically normal horses. Horses with colitis and peritonitis had significantly higher SAA and haptoglobin concentrations than clinically normal horses. A moderate positive correlation (r = 0.355) between hospitalization duration and haptoglobin concentration was identified. Horses with an increase in SAA concentration between 24 and 72 hours after admission, compared with admission SAA concentration, were significantly more likely (OR, 7.0; 95% confidence interval, 1.1 to 45.9) to be euthanized or develop complications.

CONCLUSIONS AND CLINICAL RELEVANCE
Concentrations of SAA and haptoglobin at admission were not significantly correlated with outcome in horses with inflammatory conditions. Acute-phase proteins likely have more utility in serial analysis rather than testing at a single time point for horses with inflammatory conditions. (Am J Vet Res 2015;76:882–888)
This response makes SAA concentrations a sensitive marker during the early inflammatory response and useful for monitoring treatment efficacy. Haptoglobin is a moderate APP in horses that may be useful as a marker of chronic inflammation. Haptoglobin is typically found in detectable concentrations in plasma, increases 12 to 24 hours after an inflammatory stimulus, and has a half-life of approximately 3.5 days.

Measurement of APP concentrations is becoming routine in human health care for both diagnostic and prognostic purposes in numerous disease processes. Measurement of APP concentrations has not gained widespread use in equine practice because of limited test availability and turnaround time, which has limited its clinical usefulness. Veterinary practitioners who measure APP concentrations often consider them to be of high clinical value as one of the most important biomarkers of inflammation. Validation of assays that use automated serum biochemistry analyzers has facilitated APP evaluation during case management of horses; however, test availability remains limited.

In addition to limited availability, the demand for these assays in equine practice currently remains low because of the small number of studies conducted to evaluate the ability of APP measurement to improve diagnostic and prognostic abilities of clinicians. This is likely to change because of studies in which investigators found increased APP serum concentrations in horses with inflammatory diseases such as enterocolitis, pneumonia, and placentitis and in foals with septicemia.

The objectives of the study reported here were to compare serum SAA and haptoglobin concentrations in horses referred to a tertiary care facility for evaluation of disease with a suspected inflammatory component, to evaluate the association of serum SAA and haptoglobin concentrations with duration and cost of hospitalization and the development of complications, and to assess the use of serial analysis of SAA and haptoglobin concentrations in predicting survival outcome and complication development. The null hypothesis was that serum SAA and haptoglobin concentrations would not differ significantly between clinically normal horses and horses with inflammatory disease and, within hospitalized horses with inflammatory disease, would not differ significantly between surviving versus nonsurviving (euthanized) horses with regard to mortality rate, development of complications, and hospitalization cost and duration.

Materials and Methods

Animals

Twenty clinically normal horses that were part of the Oregon State University Teaching Herd or were privately owned animals were used in the study. The study also included a convenience sample of 53 horses admitted to a tertiary care facility (Oregon State University Veterinary Teaching Hospital) for evaluation of disease with a suspected inflammatory component. Horses euthanized for financial reasons with no treatment attempted were excluded. Horses admitted because of acute colic were included only if an underlying inflammatory disease (eg, peritonitis or colitis) was diagnosed; horses admitted for surgical or medical treatment of colic (eg, intestinal obstruction, strangulation, gas colic, and colic of unknown cause) were excluded. The Oregon State University Institutional Animal Care and Use Committee approved procedures and sample collection protocols used in the study. Informed consent was obtained for use of client-owned horses.

Experimental design and sample collection

A blood sample was collected from each of the 20 clinically normal horses. Blood (approx 15 mL) was collected from a jugular vein and placed into tubes that contained EDTA or sodium heparin or that contained no anticoagulant (serum tube). Similarly, a blood sample was obtained at time of admission from each of the 53 hospitalized horses. Additional blood samples for SAA and haptoglobin measurement were obtained from 22 horses up to once daily during hospitalization. Heparinized blood was centrifuged (2,200 × g for 10 minutes) and plasma was harvested. A CBC and serum biochemical analysis were performed on EDTA-anticoagulated and heparinized plasma, respectively, by use of automatic analyzers and standard methods by personnel at the Oregon State University Veterinary Diagnostic Laboratory during business hours or at the Oregon State University Veterinary Teaching Hospital during emergency hours. Plasma fibrinogen concentration was determined by a heat precipitation method. Blood collected in the serum tube was allowed to clot and centrifuged at 3,000 × g for 10 minutes; serum was removed and stored at -80°C for later batch analysis of SAA and haptoglobin concentrations. Serum biochemical analyses, which included measurement of albumin concentrations, were performed for 48 hospitalized horses. Albumin concentration was not measured in clinically normal horses because of financial limitations.

Additional data collected for hospitalized horses included diagnosis, development of complications, cost and duration of hospitalization, and survival outcome. Complications were defined as new problems that developed during hospitalization that were not present at admission.

Measurement of SAA and haptoglobin concentrations

Serum amyloid A and haptoglobin concentrations were measured with an automated chemistry analyzer by use of commercially available assays validated for use on samples obtained from horses. The assays were validated in accordance with manufacturer instructions and standard operating procedures for new tests in the Oregon State University Veterinary Diagnostic Laboratory.
Data analysis

Hospitalsed horses were grouped on the basis of survival outcome. Horses euthanized because of a poor prognosis following initial examination and diagnostic testing or during treatment comprised the nonsurvivor group, and horses discharged from the hospital after treatment comprised the survivor group. For analysis of complications and hospitalization cost, horses (n = 51) were included if treatment was elected at time of admission following initial examination and diagnostic testing. Analysis of hospitalization duration was performed only on surviving horses.

Total WBC and neutrophil counts and fibrinogen, albumin, SAA, and haptoglobin concentrations were tested for normality by use of the Shapiro-Wilk test. The WBC and neutrophil counts and albumin and haptoglobin concentrations had normal distributions, but fibrinogen and SAA concentrations had nonnormal distributions. Mean and 95% confidence interval were determined for normally distributed data; median and interquartile range were determined for all variables for continuity of data reporting. A 1-way ANOVA was performed to identify significant differences between the clinically normal, survivor, and nonsurvivor groups for normally distributed data (WBC count, neutrophil count, and haptoglobin concentration). Albumin concentration was not evaluated in clinically normal horses; therefore, a t test was performed to identify significant differences between survivors and nonsurvivors. A Kruskal-Wallis test and Dunn multiple comparison posttest were performed on data with nonnormal distributions (fibrinogen and SAA concentrations) to identify significant differences between the clinically normal, survivor, and nonsurvivor groups. Pearson correlation was used to assess the association between cost and hospitalization duration in relation to WBC count, neutrophil count, albumin concentration, and haptoglobin concentration. Nonparametric Spearman correlation was used to assess the association between cost and hospitalization duration in relation to SAA concentration and fibrinogen concentration. The Mann-Whitney U test was used to identify significant differences in clinicopathologic variables between horses with and without complications.

Survival data and hospitalization cost and duration for peritonitis and colitis subgroups were analyzed. Fibrinogen concentration was not analyzed for subgroups because of the lack of test availability during emergency hours, which resulted in a small dataset for the subgroups. All variables, except for SAA concentration, were normally distributed for the peritonitis subgroup. The WBC count and albumin and haptoglobin concentrations were normally distributed for the colitis subgroup, but neutrophil count and SAA concentration were not normally distributed. Categorical data were compared by use of a Fisher exact test with OR and 95% confidence interval reported when significant.

Significance was set at P < 0.05 for all statistical analyses. Statistical analysis was performed with commercially available statistical software.

Results

Fifty-three horses with suspected inflammatory disease were admitted. Diagnosis included peritonitis (n = 16), colitis (15), trauma (4), renal insufficiency (3 [2 with concomitant cystitis]), pneumonia (2), cellulitis (2), fever of unknown origin (2), lymphangitis (1), chronic laminitis (1), septic osteomyelitis (1), hyperammonemic encephalopathy (1), urethral obstruction with bladder rupture (1), tooth root fracture and abscess (1), middle uterine artery rupture (1), pyometra (1), and placentitis (1). Thirty-six (68%) horses survived, and 17 (32%) horses were euthanized because of a poor prognosis. Eleven of 16 horses with peritonitis survived, and 5 were euthanized because of a poor prognosis. Causes of peritonitis included idiopathic, septic, and neoplastic conditions. Eight of 15 horses with colitis survived, and 7 were euthanized because of a poor prognosis. Potomac horse fever, right dorsal colitis, coronavirus infection, and colitis of unknown etiology comprised the causes of colitis.

The distribution of abnormal clinicopathologic findings was summarized (Table 1). Forty-four of 53

<table>
<thead>
<tr>
<th>Variable</th>
<th>Clinically normal (n = 20)</th>
<th>Survivors (n = 36)</th>
<th>Nonsurvivors (n = 17)</th>
<th>Peritonitis subgroup (n = 11)</th>
<th>Survivors</th>
<th>Nonsurvivors (n = 5)</th>
<th>Colitis subgroup (n = 8)</th>
<th>Survivors</th>
<th>Nonsurvivors (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukopenia</td>
<td>5 (25)</td>
<td>9 (25)</td>
<td>3 (18)</td>
<td>2 (18)</td>
<td>1 (20)</td>
<td></td>
<td></td>
<td>6 (75)</td>
<td>2 (29)</td>
</tr>
<tr>
<td>Leukocytosis</td>
<td>0 (0)</td>
<td>8 (22)</td>
<td>2 (12)</td>
<td>2 (18)</td>
<td>0 (0)</td>
<td></td>
<td></td>
<td>0 (0)</td>
<td>1 (14)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>2 (10)</td>
<td>7 (19)</td>
<td>4 (24)</td>
<td>2 (18)</td>
<td>1 (20)</td>
<td></td>
<td></td>
<td>5 (63)</td>
<td>3 (43)</td>
</tr>
<tr>
<td>Neutrophilia</td>
<td>0 (0)</td>
<td>18 (50)</td>
<td>8 (47)</td>
<td>7 (64)</td>
<td>1 (20)</td>
<td></td>
<td></td>
<td>0 (0)</td>
<td>2 (29)</td>
</tr>
<tr>
<td>Hyperalbuminemia</td>
<td>ND</td>
<td>7/32 (22)</td>
<td>7/16 (44)</td>
<td>4/36</td>
<td>2/40</td>
<td></td>
<td></td>
<td>2 (25)</td>
<td>5 (71)</td>
</tr>
<tr>
<td>Hyperalbuminemia</td>
<td>ND</td>
<td>3/32 (9)</td>
<td>1/16 (6)</td>
<td>0/3 (0)</td>
<td>0 (0)</td>
<td></td>
<td></td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Hyperfibrinogenemia</td>
<td>0 (0)</td>
<td>7/19 (37)</td>
<td>4/10 (40)</td>
<td>2/3 (67)</td>
<td>1/3 (33)</td>
<td></td>
<td></td>
<td>0/2 (0)</td>
<td>1/2 (50)</td>
</tr>
<tr>
<td>High SAA</td>
<td>0 (0)</td>
<td>29 (81)</td>
<td>15 (88)</td>
<td>10 (91)</td>
<td>4 (80)</td>
<td></td>
<td></td>
<td>8 (88)</td>
<td>6 (86)</td>
</tr>
<tr>
<td>Low haptoglobin</td>
<td>1 (5)</td>
<td>2 (6)</td>
<td>3 (18)</td>
<td>1 (9)</td>
<td>1 (20)</td>
<td></td>
<td></td>
<td>0 (0)</td>
<td>2 (29)</td>
</tr>
<tr>
<td>High haptoglobin</td>
<td>0 (0)</td>
<td>23 (64)</td>
<td>8 (47)</td>
<td>7 (64)</td>
<td>3 (60)</td>
<td></td>
<td></td>
<td>4 (44)</td>
<td>2 (29)</td>
</tr>
</tbody>
</table>

1 Values represent the number (percentage) of horses with abnormal findings. Abnormalities were defined as follows: leukopenia, < 6,000 cells/µL; leukocytosis, > 12,000 cells/µL; neutropenia, < 3,000 cells/µL; neutrophilia, > 6,000 cells/µL; hypoalbuminemia, < 2.8 mg/dL; hyperalbuminemia, > 3.9 mg/dL; hyperfibrinogenemia, > 400 mg/dL; high SAA, > 20 µg/mL; low haptoglobin, < 10 mg/dL; and high haptoglobin, > 70 mg/dL.

ND = Not determined.
horses had a high SAA concentration (> 20 μg/mL) at time of admission. Of the 9 horses with an SAA concentration within the reference interval at time of admission, 4 had an elevated haptoglobin concentration (> 70 mg/dL), and those horses had clinical signs for ≥ 5 days associated with laminitis, renal insufficiency, pyometra, and placentalitis. The other 5 horses had both SAA and haptoglobin concentrations within reference intervals at time of admission, and all 5 had clinical signs for ≤ 2 days. A low haptoglobin concentration (< 10 mg/dL) at time of admission was detected in 5 horses (2 survivors with rhabdomyolysis, 2 nonsurvivors with disseminated intravascular coagulation, and 1 nonsurvivor with hemorrhagic colitis).

The WBC and neutrophil counts and albumin, fibrinogen, SAA, and haptoglobin concentrations of clinically normal and hospitalized horses were summarized (Table 2). Neutrophil count, SAA concentration, and haptoglobin concentration of the clinically normal group differed significantly from those of both the survivor and nonsurvivor groups. Nonsurvivors had a significantly lower albumin concentration, compared with the albumin concentration of surviving horses. Survival outcome was not associated with WBC count, neutrophil count, fibrinogen concentration, SAA concentration, or haptoglobin concentration.

Clinicopathologic variables for the peritonitis (n = 16) and colitis (15) subgroups were summarized (Table 3). The WBC and neutrophil counts were significantly higher for survivors in the peritonitis subgroup, compared with counts for the clinically normal group. Haptoglobin and SAA concentrations were significantly higher in both survivors and nonsurvivors for the peritonitis subgroup, compared with concentrations for the clinically normal group. For the colitis subgroup, SAA concentration was significantly higher in both survivors and nonsurvivors, compared with the SAA concentration for the clinically normal group. Haptoglobin concentration was significantly higher for survivors in the peritonitis subgroup, compared with the haptoglobin concentration for the clinically normal group. Survival outcome in horses with peritonitis and colitis was not significantly associated with any of the analyzed clinicopathologic variables.

Cost analysis of hospitalized horses (n = 51) revealed a moderate positive correlation (r = 0.342) between hospitalization cost and fibrinogen concentration and a weak negative correlation (r = -0.279) between hospitalization cost and albumin concentration. No significant correlation was detected between hospitalization cost and SAA and haptoglobin concentrations for treated horses. A strong positive correlation (r = 0.474) was identified between hospitalization cost and haptoglobin concentration in horses with colitis. Analysis of hospitalization duration for surviving horses revealed a moderate positive correlation (r = 0.355) between duration of hospitalization and haptoglobin concentration. A strong positive correlation (r = 0.587) was identified between duration of hospitalization and haptoglobin concentration in horses with peritonitis that survived.

Complications were detected in 12 hospitalized horses and included the development of phlebitis or thrombophlebitis (n = 3), laminitis (2), colitis of the right dorsal colon (1), polysynovitis (1), retained fetal membranes following dystocia (1), abortion (1), impaction of the large colon (1), septic peritonitis (1), and bladder rupture (1). No significant correlation was found between the clinicopathologic variables evaluated at time of admission and development of complications.

Serial analysis of SAA and haptoglobin concentrations performed 24 to 72 hours after start of hospitalization for 22 horses was categorized as an increased or decreased concentration, compared with the concentration at time of admission, to assess the correlation with euthanasia or development of complications. Horses with an SAA concentration > 2,500 μg/mL at time of admission and for successive samples were placed in the increased category, which included 1 survivor with a complication and 1 survivor with

Table 2—Values for clinicopathologic variables of clinically normal horses and survivor and nonsurvivor horses hospitalized because of suspected inflammatory conditions.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Clinically normal (n = 20)</th>
<th>Hospitalized survivors (n = 36)</th>
<th>Hospitalized nonsurvivors (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Minimum Maximum</td>
<td>n (Median (IQR))</td>
</tr>
<tr>
<td>WBCs (cells/µL)</td>
<td>6.690 (5.933–7.178)</td>
<td>4.820 9.800</td>
<td>36 (8,525 (5,598–11,450)</td>
</tr>
<tr>
<td>Neutrophils (cells/µL)</td>
<td>3.365 (3.120–3.830)</td>
<td>2.653 4.711</td>
<td>36 (6,228* (3.294–6,575)</td>
</tr>
<tr>
<td>Albumin (mg/dL)</td>
<td>ND</td>
<td>ND ND</td>
<td>32 (3.1)</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>200 (125–300)</td>
<td>100 400</td>
<td>19 (300)</td>
</tr>
<tr>
<td>SAA (µg/mL)</td>
<td>&lt; 5 (&lt; 5)</td>
<td>&lt; 5 &lt; 5</td>
<td>36 (40* (120–1,437)</td>
</tr>
<tr>
<td>Haptoglobin (mg/dL)</td>
<td>42.8 (27.6–49.8)</td>
<td>6.0 60.5</td>
<td>36 (87.0* (58.0–127.0)</td>
</tr>
</tbody>
</table>

*Value differs significantly (P < 0.05) from the value for the clinically normal group. †Value differs significantly (P < 0.05) from the value for the nonsurvivor group.

IQR = Interquartile range.

See Table 1 for remainder of key.
no complications. Ten of 22 horses with serial analysis had complications (5) or were euthanized (5). Of these 10 horses, 7 had increasing SAA concentrations in serial analysis, whereas only 3 of 12 surviving horses with no complications had an increasing SAA concentration during serial analysis. Horses with an increase in SAA concentration between 24 and 72 hours of hospitalization, compared with the SAA concentration at time of admission, were significantly more likely to be euthanized or develop complications during hospitalization than horses with decreasing SAA concentrations (OR, 7.0; 95% confidence interval, 1.1 to 45.9). No correlation was detected between serial haptoglobin concentrations and survival outcome or the development of complications.

### Discussion
Assessment of APP concentrations is routinely used by physicians for diagnostic purposes and to monitor response to treatment in hospitalized patients. The use of APP concentrations is becoming more widely recognized in veterinary medicine with the increase in number of published studies and availability of automated assays. The purpose of the study reported here was to evaluate the clinical importance of SAA and haptoglobin measurement for horses admitted to a tertiary care facility because of inflammatory disease.5,13 Previous studies5–7,15 have found the SAA concentration to be a reliable marker of inflammation in diverse processes, including diseases caused by bacteria and viruses and aseptic processes such as surgical trauma. Serum amyloid A concentration more consistently identified and characterized severity of the inflammatory response at the time of initial evaluation, compared with measurement of the fibrinogen concentration alone. Measurement of fibrinogen concentrations allows assessment of the magnitude of the inflammatory response days after the initial insult as a result of a slow response time of 24 to 72 hours with peak concentrations not being reached until 72 to 144 hours after the initial insult.4 Although typically high in hospitalized horses in this study, SAA concentrations may not have been high in some horses early in the APR prior to upregulated synthesis of SAA, which occurs at 6 to 12 hours after the initial insult, with a peak response up to 48 hours after the initial insult.4,10 Another potential explanation for an SAA concentration within the reference range at time of admission would be resolving inflammation and the short plasma half-life of SAA, which would have resulted in an initial measurement obtained too late to identify a high SAA concentration in a particular horse. Although the half-life of SAA has not been determined in horses, it has been found that SAA serum concentrations can return to reference range values within days after an inflammatory insult.15,17 Studies18,19 conducted to evaluate clearance of SAA in mice revealed a half-life of 30 to 75 minutes. Species differences and ongoing inflammation likely affect biological half-life in disease states, as has been reported for patients with community-acquired pneumonia in which the SAA half-life is 55 hours.6 Similarly, SAA half-life is approximately 20 hours in sheep receiving treatment for *Psoroptes ovis* infestations.5

Moderate APPs such as fibrinogen and haptoglobin with a longer APR are valuable in APP measurement because of the information they provide regarding chronicity. Divergences between major and moderate APPs for dogs and cattle can provide useful information in regard to differentiating between acute and chronic inflammation and when monitoring chronic disease.6,20 In the present study, 4 horses had SAA concentrations within the reference range and concurrent high haptoglobin concentrations. Each

Table 3—Values for clinicopathologic variables of clinically normal horses and survivor and nonsurvivor hospitalized horses in peritonitis and colitis subgroups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Clinically normal (n = 20)</th>
<th>Peritonitis (n = 16)</th>
<th>Colitis (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Survivors (n = 13)</td>
<td>Nonsurvivors (n = 3)</td>
<td>Survivors (n = 8)</td>
</tr>
<tr>
<td>WBCs (cells/µL)</td>
<td>6,690 (5,933–7,178)</td>
<td>9,270* (6,400–12,300)</td>
<td>7,150 (5,186–9,890)</td>
</tr>
<tr>
<td>Neutrophils (cells/µL)</td>
<td>3,365 (3,120–3,830)</td>
<td>7,100* (5,100–10,400)</td>
<td>5,186 (2,967–7,000)</td>
</tr>
<tr>
<td>Albumin (mg/dL)</td>
<td>ND</td>
<td>2.9 (2.7–3.3)</td>
<td>2.9 (2.7–3.4)</td>
</tr>
<tr>
<td>SAA (µg/mL)</td>
<td>&lt; 5</td>
<td>489* (423–2390)</td>
<td>511* (186–842)</td>
</tr>
<tr>
<td>Haptoglobin (mg/dL)</td>
<td>43 (28–50)</td>
<td>84* (54–108)</td>
<td>113 (29–195)</td>
</tr>
</tbody>
</table>

*Values reported are mean (IQR).

**Value differs significantly (P < 0.05) from the value for the clinically normal group.
of these horses had chronic disease, and although it was a small subset of the tested population, assessment of haptoglobin concentration may be a valuable diagnostic tool for monitoring inflammation and treatment efficacy in diseases such as chronic laminitis or placentitis. Haptoglobin concentration, although not significantly correlated with survival, had a moderate positive correlation with duration of hospitalization for all horses and a strong positive correlation for the subgroup of horses with peritonitis. These correlations likely reflected the fact that horses with chronic ongoing inflammation may have required a longer period of hospitalization for appropriate treatment. Haptoglobin concentrations may also be useful in identifying hemolytic processes. The main function of haptoglobin is to bind free hemoglobin; however, haptoglobin can also bind free myoglobin.21 Haptoglobin concentrations can be overwhelmed by large amounts of free hemoglobin or myoglobin, and haptoglobin concentrations will be abnormally low in animals with intravascular hemolysis or rhabdomyolysis.22,23 Only a few hospitalized horses (n = 5) in the present study had an abnormally low haptoglobin concentration; this included 2 surviving horses with rhabdomyolysis attributable to trauma and 3 nonsurviving horses (2 with disseminated intravascular coagulation and 1 with hemorrhagic colitis). If SAA and haptoglobin concentrations are used to monitor horses with inflammatory diseases, assessment of haptoglobin concentrations may have an additional benefit as an indicator of hemostatic diseases such as disseminated intravascular coagulation in patients already at increased risk. Future studies will be necessary to determine whether haptoglobin concentration will be a reliable biomarker in horses with hemostatic disease.

No association between SAA concentration at time of admission and survival outcome was identified in the present study. Investigators of another study24 found an association between survival outcome and increased SAA concentration in horses examined because of all types of colic; however, this may have reflected typical survival outcome associated with inflammatory disorders such as colitis or peritonitis, compared with survival outcome for noninflammatory disorders such as intestinal obstruction or gas colic. In another study25 conducted by our research group, we found a significant association for horses with noninflammatory causes of colic between SAA concentration and the likelihood of developing complications or of being euthanized because of a poor prognosis. On the basis of differences identified in that study and the study reported here, it is important to analyze APPs in the context of the primary disease process. In the present study, SAA concentration at time of admission was typically high, which would be expected in inflammatory conditions. However, SAA concentration at time of admission was not associated with survival outcome. These findings are reflected in the human literature on APP, whereby APP concentrations at time of admission do not correlate well with survival, whereas serial analysis of APP concentration is a much better predictor of death.25 For example, increasing concentrations of procalcitonin and C-reactive protein, both of which are APPs used in human medicine, are significantly associated with an increased mortality rate.20,26 Although sequential analysis was performed on only a subset of 22 horses, an increasing SAA concentration was significantly associated with the development of complications during hospitalization or a recommendation for euthanasia. Serial analysis of SAA concentrations, rather than analysis of a single concentration at a specified time point, likely will have the most benefit for clinical management of horses with an inflammatory condition. The cost-benefit relationship of APP analysis in equine medicine remains unknown. However, the study reported here highlighted the potential benefits of serial monitoring of SAA concentrations for clinical management and prognosis.

The inflammatory response is a complex cascade of molecular and cellular signals triggered by distinct disease processes such as trauma, neoplasia, and infection.1 The population of horses with various conditions in the present study reflected the diverse causes of inflammation. The purpose for use of a broad population of horses with suspected inflammation was to investigate the clinical usefulness of SAA and haptoglobin concentrations as a general prognostic indicator. One of the drawbacks for use of a population with various disease processes included the small number of horses with each specific condition. The peritonitis and colitis subgroups were large enough to allow us to analyze them separately, which revealed that both SAA and haptoglobin concentrations may be useful in the diagnosis and clinical management of these conditions. Further studies to investigate the prognostic use of APPs for specific disease processes may need to be designed as multicenter studies to improve the power of the data. Another weakness of the present study was the lack of information on fibrinogen concentrations for emergency cases and albumin concentrations in clinically normal horses because of a lack of availability and cost. Future studies should include assessment of negative APPs, such as albumin.

In the present study, SAA and haptoglobin concentrations at time of admission for horses hospitalized because of inflammatory conditions were not significantly associated with survival outcome. However, we rejected the null hypothesis that SAA and haptoglobin concentrations were the same between hospitalized horses and clinically normal horses because SAA and haptoglobin concentrations were significantly higher in horses with inflammatory conditions, including peritonitis and colitis, compared with concentrations in clinically normal horses. Assessment of haptoglobin concentrations will likely have the most use for monitoring cases of chronic inflammation, with a need for further investigations regarding specific disease processes. Single-point analysis of SAA concentrations is likely to identify the presence of inflammation; however, serial analysis during treatment is likely to
be a more effective diagnostic and prognostic tool for horses with an inflammatory condition.

Acknowledgments

This manuscript represents a portion of a thesis submitted by Dr. Westerman to the Oregon State University Department of Clinical Sciences as partial fulfillment of the requirements for a Master of Science degree.

Supported by the Oregon State University College of Veterinary Medicine Department of Clinical Sciences Resident Training Grants (Westerman) and Oregon State University New Faculty Funding (Poulsten).

Footnotes

a. Advia 120 Hematology System, Siemens Health Care Diagnostics SL, Tarryton, NY.

b. Beckman Coulter AU-480 Chemistry Analyzer, Beckman Coulter Inc, Brea, Calif.

c. HemaTrue Hematology Analyzer, Heska Corp, Loveland, Colo.

d. Element DC Chemistry Analyzer, Heska Corp, Loveland, Colo.

e. LZ Test Eiken SAA, EIKEN Chemical Co Ltd, Tokyo, Japan.

f. Haptoglobin assay OSR6165, Beckman Coulter Inc, Brea, Calif.

g. GraphPad Prism, GraphPad Software Inc, La Jolla, Calif.

h. Westerman TL. Evaluation of serum amyloid A and haptoglobin as prognostic indicators for horses in a referral population. MS thesis, Department of Clinical Sciences, Oregon State University, Corvallis, Ore., 2014.

References


