Assessment of serum amyloid A testing of horses and its clinical application in a specialized equine practice

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Objective—To compare serum amyloid A (SAA) concentration, plasma fibrinogen concentration, total WBC count, and serum albumin-to-globulin concentration ratio (A:G ratio) in clinically normal (CN) and clinically abnormal (CA) horses.

Design—Prospective cohort study.

Animals—111 CN horses and 101 CA horses hospitalized at a specialty clinical practice.

Procedures—Shortly after admission, a blood sample (20 mL) was collected from each horse for a CBC, serum protein electrophoresis, and determination of plasma fibrinogen concentration; SAA concentration was assessed with a previously validated immunoturbidometric assay. Similar testing of a subset of CA horses was conducted at various points during treatment.

Results—Total WBC count, A:G ratio, and SAA concentration were determined for all 212 horses; data regarding plasma fibrinogen concentration were available for 127 horses (of which 47 were CN and 80 were CA). Median SAA concentration, total WBC count, and plasma fibrinogen concentration and mean A:G ratio differed significantly between CN horses and CA horses. Correlations between these variables were poor to weak. For discrimination of CN horses from CA horses, the SAA assay had sensitivity of 53% and specificity of 94% (diagnostic accuracy, 75%); for the other assessments, accuracy ranged from 59% to 62%. Repeated assessment of SAA concentration in some CA horses revealed a gradual return to normal concentrations.

Conclusions and Clinical Relevance—Results indicated that assessment of SAA concentration can provide valuable information regarding the clinical state of horses and may be more useful for patient monitoring and as a prognostic indicator than are traditional markers of inflammation. (*J Am Vet Med Assoc* 2013;243:113–119)

The APR is a well-documented nonspecific phenomenon that rapidly occurs in the body and is incited by a variety of stimuli that may be related to infection, trauma, neoplasia, inflammation, or stress.[1–4] The response is mediated by a plethora of reactants produced primarily by the liver; those reactants respond in either a negative or positive manner to the inflammatory stimulus. In response to inflammation, the plasma concentrations of some APPs decrease (negative APPs), whereas the plasma concentrations of other APPs increase (positive APPs). Negative APPs include albumin and transferrin. Positive APPs include haptoglobin, C-reactive protein, ceruloplasmin, fibrinogen, and SAA. The positive APPs are further classified as major, moderate, and minor APPs. Major APPs maintain very low plasma concentrations in healthy mammals; however, there may be as much as a 1,000-fold increase in those concentrations in response to inflammation. Moderate and minor APPs are typically present in the plasma of healthy individuals; for these APPs, there may be only a 1- to 10-fold increase in concentration during an APR.

Abbreviations

A:G ratio	Serum albumin-to-globulin concentration ratio
APP	Acute-phase protein
APR	Acute-phase response
CA	Clinically abnormal
CN	Clinically normal
IQR	Interquartile range (25th to 75th percentile)
SAA	Serum amyloid A

To date, SAA has been the major APP identified in horses.[5,6] Serum amyloid A has garnered attention as a reliable indicator of inflammation or infection across species.[1,4,7] The fact that SAA concentration undergoes up to a 1,000-fold increase in response to inflammatory stimuli, in addition to SAA's early response and increase in concentration when stimulated by the inflammatory cascade of cytokines, has substantiated the assessment of SAA concentration as a means of monitoring patients with infectious and inflammatory diseases. In addition, given the short half-life of SAA, a more rapid decrease in concentration is observed in response to treatment and resolution of the disease process, relative to treatment-associated changes in circulating concentrations of other markers of inflammation.[7]

Previous investigations[8–12] of SAA in horses have been limited in sample size or type of natural or ex-

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perimental stimuli. The primary objective of the study reported here was to compare SAA concentration, plasma fibrinogen concentration, total WBC count, and A:G ratio in CN and CA horses. By using patients admitted to a specialty equine hospital, we intended to include horses with diverse clinical signs, times of disease onset, breeds, and ages. We hypothesized that SAA concentration would be a more reliable indicator of the inflammatory process, compared with other clinicopathologic variables (eg, plasma fibrinogen concentration, total WBC count, and A:G ratio) that are considered more traditional markers of inflammation. Furthermore, in addition to its use as a primary tool in the initial evaluation of equine patients, we hypothesized that SAA concentration would also function as a strong prognostic indicator for CA horses.

Materials and Methods

Horses—The study included 212 horses that were admitted to a specialty veterinary practice. Among the horses, there were > 23 breeds; the predominant breeds were Standardbred (n = 64), Thoroughbred (39), warmblood (21), Quarter Horse (21), and Paso Fino (19). There were 93 male horses and 119 female horses. The horses' ages ranged from 4 months to 26 years.

The horses were classified as either CN (n = 111) or CA (101). The CA group included severely ill patients that were admitted to the hospital because of a variety of infectious or inflammatory conditions; many of these horses had a history of fever or nasal discharge. Among the CA horses, clinical signs or diagnoses included bacterial and viral pneumonia, bacterial cholangiohepatitis, *Streptococcus equi* subsp *equi* infection, meningitis, enterocolitis, various forms of colic and neoplasia, abscesses (pulmonary and musculoskeletal), septic tenosynovitis, and retropharyngeal lymphadenopathy. The CN group included horses that were not considered ill on the basis of history and results of physical examination. These horses were healthy mares admitted to the hospital with sick foals or patients admitted for treatment of noninfectious and noninflammatory processes such as osteochondrotic lesions.

Sample acquisition—A blood sample (20 mL) was collected from each horse within a 3-hour period after admission to the hospital. Ten milliliters of blood was placed into a tube containing EDTA as well as into a tube without anticoagulant.[a] The latter was allowed to clot for 20 minutes and was centrifuged at 3,400 × *g* for 10 minutes. Serum was separated to an inert transport tube. The whole blood and serum samples were stored under refrigeration and then shipped on cold packs to the University of Miami Acute Phase Protein Laboratory. A CBC, serum protein electrophoresis, and assessment of SAA concentration were performed for samples from all 212 horses. Determination of plasma fibrinogen concentration was performed at the discretion of the clinician at time of each horse's hospital admission; thus, samples from only 127 horses were analyzed for plasma fibrinogen concentration at the specialty veterinary practice. All assays were conducted within 24 hours after blood sample collection. For a small subset of CA horses (n = 23), additional blood samples were collected and similarly analyzed at various times during treatment to illustrate the pattern of change in SAA concentration as a result of successful treatment or resolution of disease processes. The duration of the hospitalization ranged from 1 to 20 days and was dictated by duration of illness and time at the hospital.

All CN horses were monitored (including measurement of rectal temperature) for a minimum of 7 days after last sample collection (either at the hospital or at their barns via follow-up calls) to ensure that they remained CN. The CA horses were similarly monitored for a minimum of 7 days after discharge from the hospital.

CBC—For each horse, the sample of EDTA-anticoagulated blood underwent a CBC via an automated analyzer.[b] A manual review of the blood smear was also conducted. On the basis of in-laboratory–derived reference intervals, total WBC counts ≥ 12,500 WBCs/µL were considered abnormally high.

Determination of plasma fibrinogen concentration—For 127 of the 212 horses, the sample of EDTA-anticoagulated blood was also used for determination of plasma fibrinogen concentration via the heat precipitation method[13] at the specialty veterinary practice. On the basis of in-clinic–derived reference intervals, plasma fibrinogen concentrations ≥ 400 mg/dL were considered abnormally high.

Serum protein electrophoresis—Serum samples were analyzed with split beta gels according to the manufacturer's specifications.[c] The resultant gel was scanned and the electrophoretogram was produced following previously published fraction delimitation conventions as well as those developed within the laboratory.[14] The total protein concentration was determined by refractometry. The absolute value for each protein fraction was determined by multiplication of the total protein concentration by the percentage of the fraction. The A:G ratio was calculated as serum concentration of albumin divided by the sum of the serum concentrations of globulins. On the basis of in-laboratory–derived reference intervals, A:G ratios ≤ 0.84 were considered abnormally low.

Assessment of SAA concentration—Serum amyloid A concentration was quantitated with a kit[d] on an analyzer[e] as previously described.[15] The interassay coefficient of variation was calculated as 2.8%, and the intraassay coefficient of variation was calculated as 5.4%. The analyzer was subject to routine quality control measurements throughout the study. This assay has previously been described as having acceptable linearity within clinically relevant ranges of SAA concentration in horses.[15] On the basis of in-laboratory–derived reference intervals, which were consistent with previously published data,[15] SAA concentrations ≥ 20 mg/L were considered abnormally high.

Statistical analysis—The distribution of data for SAA concentration, plasma fibrinogen concentration, total WBC count, and A:G ratio were tested for normality with a Shapiro-Wilk test. Serum amyloid A concentration, plasma fibrinogen concentration, and total WBC count data all had nonnormal distributions ($P < 0.001$).

Therefore, these data are reported as the median and IQR; the Mann-Whitney *U* test was used to identify significant differences between the CN and CA horses. The A:G ratio data were normally distributed ($P = 0.685$). Therefore, these data are reported as the mean and 95% confidence interval; a *t* test was performed to identify significant differences between the CN and CA horses. For continuity of data presentation, both median (and IQR) and mean (and 95% confidence interval) were summarized for SAA concentration, plasma fibrinogen concentration, total WBC count, and A:G ratio in CN and CA horses. Spearman rank correlation was used to assess the relationship between SAA concentration and plasma fibrinogen concentrations, total WBC count, or A:G ratio. Reference intervals had previously been determined at the laboratory (total WBC count, A:G ratio, and SAA concentration) and clinic (plasma fibrinogen concentration). Data obtained at various times during treatment for a small subset of CA horses were not formally analyzed but served, in a descriptive manner only, to illustrate the pattern of change in SAA concentration as a result of successful treatment or resolution of disease processes. All analyses were conducted with statistical software.[f] Values of $P \leq 0.05$ were considered significant.

Results

Comparison of SAA concentration, plasma fibrinogen concentration, total WBC count, and A:G ratio—The median SAA concentration, plasma fibrinogen concentration, and total WBC count and mean A:G ratio for the CN and CA horses were compared (**Table 1**). Significant differences between the 2 groups were observed for all variables. The magnitude of the difference in SAA concentration between the CN and CA horses was marked, whereas the magnitudes of the between-group differences for total WBC count, plasma fibrinogen concentration, and A:G ratio were much less profound. Among the CA horses, there was a 20.5-fold increase in median SAA concentration yet only a 1.2-fold increase in median mean total WBC count (and the value was within reference limits for this analyte), compared with the value in CN horses. There was a 1.7-fold and a 1.2-fold between-group difference for plasma fibrinogen concentration and for A:G ratio, respectively.

The Spearman correlation analysis revealed a poor correlation between total WBC count and SAA concentration ($r = 0.11$; $P = 0.127$) and also between plasma fibrinogen and SAA concentrations ($r = 0.16$; $P = 0.079$). A weak negative correlation was observed between A:G ratio and SAA concentration ($r = –0.42$; $P < 0.001$). Results for the diagnostic properties of individual assays were summarized (**Table 2**). For discrimination of CN horses from CA horses, assay sensitivity ranged from 17% for total WBC count to 59% for plasma fibrinogen concentration; specificity was excellent for both the SAA concentration assay (94%) and total WBC count (97%). Positive predictive value was good for SAA concentration and WBC count (89% and 85%, respectively). Negative predictive value was lower for SAA concentration (69%) and A:G ratio (61%). The overall accuracy for SAA concentration assay was 75%, with the accuracy for the other analyte assays ranging from 59% to 62%.

Table 1—Median (IQR) and mean (95% confidence interval [CI]) values of SAA concentration, plasma fibrinogen concentration, and total WBC count and of A:G ratio in CN and CA adult horses.

	CN horses					CA horses						
Variable	No. of horses	Median (IQR)	Mean (95% CI)	Minimum	Maximum	No. of horses	Median (IQR)	Mean (95% CI)	Minimum	Maximum	*P* value	Value considered abnormal
SAA (mg/L)	111	3.5 (2.1–11.9)	6.8 (5.6–8.1)	0.1	26.6	101	71.7 (3.0–800.8)	513.0 (347.5–678.6)	0.1	3,800.0	< 0.001	≥ 20
Plasma fibrinogen (mg/dL)	47	300 (200–400)	349 (296–402)	100	800	80	500 (300–800)	514 (456–573)	100	1,200	0.001	≥ 400
Total WBC count (× 10^3 WBCs/μL)	111	7.1 (6.3–8.1)	7.4 (7.1–7.8)	3.9	12.9	101	8.4 (6.1–10.7)	9.3 (8.4–10.2)	1.8	27.0	0.006	≥ 12.5
A:G ratio	111	0.91 (0.82–1.01)	0.92 (0.89–0.94)	0.67	1.21	101	0.83 (0.67–0.97)	0.82 (0.78–0.87)	0.32	1.35	< 0.001	≤ 0.84

Horses were admitted to a specialty equine clinic and classified as CN (horses with noninfectious and noninflammatory processes [eg, osteochondrotic lesions] and healthy mares admitted with sick foals) or CA (horses with infectious or inflammatory conditions). A blood sample was collected for analysis within a 3-hour period after admission. The *P* value for differences in median or mean values between CN and CA horses was determined by use of a Mann-Whitney *U* test (SAA concentration, plasma fibrinogen concentration, and total WBC count) or a *t* test (A:G ratio).

Table 2—Diagnostic performance characteristics associated with testing for SAA concentration, plasma fibrinogen concentration, total WBC count, and A:G ratio as a means of discriminating CA horses from CN horses.

	Clinical status						
Variable and test result	CA (No. of horses)	CN (No. of horses)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy (%)
SAA concentration			53	94	89	69	75
Abnormal (≥ 20 mg/L)	54	7					
Not abnormal (< 20 mg/L)	47	104					
Plasma fibrinogen concentration			59	51	71	49	62
Abnormal (≥ 400 mg/dL)	55	23					
Not abnormal (< 400 mg/dL)	25	24					
Total WBC count			17	97	85	56	59
Abnormal (≥ 12.5 × 10^3 WBCs/μL)	17	3					
Not abnormal (< 12.5 × 10^3 WBCs/μL)	84	108					
A:G ratio			52	67	59	61	60
Abnormal (≤ 0.84)	53	37					
Not abnormal (> 0.84)	48	74					

Clinical cases—Three horses that were originally considered CN were moved to the CA group after the onset of clinical signs within 24 hours after collection of the initial blood sample. This included a horse with metritis and 2 horses with colic, in which SAA concentrations ranged from 91 to 155 mg/L.

Data for a selection of CA horses were summarized (**Table 3**) to illustrate the patterns of SAA response to some of the major APR stimuli, such as trauma, inflammation, infection, and neoplasia. In cases involving a bacterial component, SAA concentrations were abnormally high, with the exception of 2 horses with bacterial sinus infections. Horses with neoplasia (n = 2) had SAA concentrations within the normal reference interval, as did a horse with a fracture of a second metatarsal bone. Two horses with equine herpesvirus-5–associated pneumonia had contrasting SAA concentrations.

Repeated collections of blood samples were obtained for a subset of 23 CA horses at various times during the course of treatment for their illnesses. Data for a selection of those horses were summarized (**Table 4**) to illustrate the patterns of change in the variables of interest. In all but 1 case, the SAA concentration gradually returned either to or toward normality during treatment. One day after admission, the SAA concentration in 1 horse had increased, compared with the value at admission; at day 6, SAA concentration was less than the value at admission (albeit still abnormally high). The changes in plasma fibrinogen concentration were more erratic; in 1 horse, the SAA concentration was considered normal at day 9 after admission, but the plasma fibrinogen concentration remained markedly high (as it had been at admission), despite a return to health of the patient. For the other evaluated horses, plasma fi-

Table 3—Data obtained for 12 CA horses in which SAA concentration was considered abnormal (n = 6) or not abnormal (6) at the time of hospital admission to illustrate the variation in that variable and other markers of inflammation (plasma fibrinogen concentration, total WBC count, and A:G ratio) depending on signalment, diagnosis, and duration of clinical signs.

Horse	Clinical diagnosis (duration of clinical signs prior to assessment)	SAA (mg/L)	Plasma fibrinogen (mg/dL)	WBC count (X 10^3 WBCs/μL)	A:G ratio
1	Fracture of second metatarsal bone (1 wk)	4.0	400	8.1	1.06
2	Granulosa cell tumor (several wk)	< 0.1	400	12.9	0.85
3	Ethmoid hematoma and bacterial sinusitis (several wk)	3.4	600	8.8	0.88
4	Chronic active cholangiohepatitis (1 wk)	412.5	200	14.5	0.67
5	Equine herpesvirus-5–associated pneumonia and bacterial pneumonia (2 wk)	993.0	400	4.0	0.70
6	Equine herpesvirus-5–associated pneumonia (several mo)	4.4	1,000	6.0	1.12
7	Bacterial meningitis (48 h)	1,292.8	600	16.6	0.42
8	Lymphadenopathy (*Streptococcus equi* subsp *equi* infection [4 d])	3,347.0	200	19.0	0.72
9	Enterocolitis (24 h)	1,426.8	900	8.1	0.91
10	Bacterial pneumonia (7 d)	1,220.0	600	10.2	0.57
11	T-cell lymphoma of the liver (several wk)	11.5	600	5.4	0.61
12	Ethmoid hematoma and bacterial sinusitis (30 d)	2.9	400	6.1	1.05

Values considered abnormal were as follows: SAA concentration, ≥ 20 mg/L; plasma fibrinogen concentration, ≥ 400 mg/dL; total WBC count, ≥ 12.5 × 10^3 WBCs/μL; and A:G ratio, < 0.84.

Table 4—Serial evaluations of SAA concentration, plasma fibrinogen concentration, total WBC count, and A:G ratio in 6 of the 101 CA study horses during hospitalization to illustrate the variation in those variables in response to treatment and improving clinical condition.

Horse	Clinical diagnosis	Time of blood sample collection	SAA (mg/L)	Plasma fibrinogen (mg/dL)	WBC count (X 10^3 cells/μL)	A:G ratio
1	Pleuropneumonia	Admission	1,220.0	600	10.2	0.57
		Day 2	1,084.4	600	7.4	0.62
		Day 5	299.5	400	8.6	0.67
		Day 20	1.7	200	8.8	0.98
2	Enterocolitis	Admission	1,426.8	900	8.1	0.91
		Day 3	1,214.8	800	9.0	0.70
		Day 9	1.0	900	18.8	0.81
3	Portal hepatitis and bile duct hyperplasia	Admission	3,628.0	800	6.4	0.54
		Day 2	3,049.0	1,200	6.5	0.54
		Day 6	341.0	500	11.0	0.63
4	Rectal mass	Admission	494.6	100	8.8	1.01
		Day 2	341.6	600	5.8	0.95
		Day 6	1.03	300	6.8	0.89
5	*S equi* subsp *equi* infection	Admission	1,346.9	800	23.4	0.47
		Day 2	1,921.0	800	23.2	0.62
		Day 6	176.6	200	20.5	0.59
		Day 16	4.2	800	14.1	0.69
6	Enterocolitis	Admission	800.8	800	6.4	0.70
		Day 1	1,269.0	600	5.8	0.70
		Day 6	345.3	400	20.1	0.78

See Table 3 for key.

brinogen concentrations changed during treatment but with no consistent pattern. With respect to total WBC counts, most horses had counts that were considered normal, despite clear clinical evidence of underlying inflammation or infection. In 2 horses with enterocolitis, the WBC count was abnormally high at the time of disease resolution, when SAA concentration had returned to or was near normal value. For the former of those horses, the WBC differential count findings were within reference intervals; for the latter, lymphocytosis was present.

Discussion

It is known that a variety of inflammatory stimuli are capable of inducing abnormally high SAA concentrations in horses. In equine experimental models of inflammation or infection, IM injection of turpentine oil as well as induction of synovitis, arthritis, and bacterial pneumonia results in 5- to 1,000-fold increases in SAA concentrations.[9–12,16,17] Castration and surgery also increase the concentration of this APP in horses.[8,12,18] Concentrations of SAA were found to be high in horses with colic, infectious joint disease, *Rhodococcus*-associated pneumonia, and equine influenza virus infection.[19–22] Although these reports and several excellent reviews form the basis for the use of SAA concentration as a marker for inflammation in equine medicine, many cited studies[5–7] have low sample size and limited focus. A primary goal of the present study was to determine the practical application of assessment of SAA concentration as a means of determining the clinical status of horses; to achieve this goal, the study was conducted at a specialized equine practice with patients that had a broad spectrum of clinical signs of inflammation and infection.

In the present study, there was a 20.5-fold increase in median SAA concentration (and 75-fold mean increase) in CA horses, compared with findings in CN horses. Many CA horses had an SAA concentration > 1,000 mg/L at the time of admission to the hospital. This contrasted the comparatively small-scale (< 2-fold) increases in median plasma fibrinogen concentration and total WBC count in CA horses, compared with findings in CN horses. Approximately half of the CA horses had abnormally high SAA concentrations, including horses with bacterial infections such as pneumonia, *S equi* subsp *equi* infection, bacterial cholangiohepatitis, enterocolitis, and meningitis. These data are consistent with previous reports[5,7,21] that infections of bacterial origin provoke an especially strong response in terms of increased SAA concentration, and support the proposal that SAA concentration can be used as a differentiator of infectious versus noninfectious diseases. However, not all conditions were associated with abnormally high SAA concentration in horses in the present study. Two horses with ethmoid hematomas and secondary bacterial sinusitis had SAA concentrations that were not considered abnormal, as did 2 horses with neoplasia (hepatic or ovarian). The lack of high SAA concentrations in horses with bacterial sinusitis may reflect an inability of the infection to incite a systemic inflammatory response because of sequestration of infection within the sinuses. Concentrations of APPs have traditionally been thought to be increased in neoplastic conditions, and they have been used as a biomarker in cases of lymphoma in dogs.[23] The reasons for the lack of induction of SAA production in the 2 horses with neoplastic processes in the present study are unknown but may be related to the duration of the disease. Also, SAA concentration is known to increase rapidly in association with acute inflammation. With chronic inflammation, other markers, such as plasma fibrinogen and haptoglobin concentrations, are often high.[7] Assessments performed at single time points, such as those performed throughout most of the present study, may not detect the period of SAA concentration elevation. This possibility was proposed in the report of a study[24] of foals with *Rhodococcus*-associated pneumonia; in that study, SAA concentration did not appear to be a sensitive marker for the disease, but a sampling interval of only 2 weeks was used.

To our knowledge, there have been limited studies of horses in which the use of SAA concentration assessment as a clinical monitoring tool has been investigated. Previous studies[8,20–22,25] have included horses with surgical disorders, septic arthritis, *Rhodococcus equi* infection, and colic. In the present study, SAA concentrations were monitored in 23 horses with various medical disorders during their respective courses of treatment to evaluate the pattern of change in SAA concentration in parallel with clinical response to treatment. Plasma fibrinogen concentrations were also evaluated over time in those patients. The SAA and plasma fibrinogen concentrations increased and decreased in parallel during treatment of a horse with pleuropneumonia; for most of the other CA horses that were repeatedly evaluated, the plasma fibrinogen concentrations changed more erratically during treatment. Interestingly, for 1 horse, the plasma fibrinogen concentration remained markedly high despite resolution of the disease and normalization of the SAA concentration (Table 4). This was likely a reflection of the contrasting kinetics of circulating SAA and fibrinogen, which are considered major and minor APPs, respectively. Concentrations of major APPs, such as SAA, typically increase very quickly in response to inflammation (6 to 12 hours, with a peak of 48 hours) and decrease quite rapidly upon resolution of the disease process.[6,7] In contrast, plasma fibrinogen concentration may begin to increase 24 to 72 hours after the inflammatory insult and may remain elevated for weeks.[7] Other factors (eg, consumptive coagulopathies or increased vascular permeability) that are commonly observed in critically ill equine patients may falsely lower plasma fibrinogen concentrations, thereby making determination of plasma fibrinogen concentration a somewhat inconsistent and unpredictable monitoring tool. Additionally, it should be noted that a manual technique for determination of plasma fibrinogen concentration was used at the specialty equine clinic during this study. As with other manual methods, this technique has been demonstrated to have a higher coefficient of variation, compared with automated methods.[26]

In the horses of the present study, the pattern of change in total WBC count also differed from that which occurred for SAA concentration. During the

acute stages of infection, a decrease in the WBC count may be observed initially as a result of margination, followed by a cytokine-mediated increase in WBC numbers over the next 36 hours. In the present study, several of the horses that were repeatedly evaluated during treatment had a high WBC count at later time points, often concomitant with normalization of SAA concentration and resolution of disease. In addition, most of the CA horses in this study had an apparently normal total WBC count and an absence of band neutrophils. A poor correlation was demonstrated between SAA concentration and either plasma fibrinogen concentration or total WBC count. This is consistent with findings of previous studies[16,22] in which total WBC counts were evaluated in horses following castration or total WBC count and fibrinogen concentration were evaluated in foals with infectious diseases.

The weak negative correlation of SAA concentration with A:G ratio identified in the present study was expected, given that SAA is one of many APPs that migrate in the globulin fractions resolved by serum protein electrophoresis.[27] With an ongoing APR, the concentrations of SAA and the globulins increase and the concentration of albumin (a negative APP) can decrease, resulting in a lower A:G ratio (ie, negative correlation). A stronger correlation might be detected with data from horses that have more severe inflammatory processes; however, the weak correlation observed in this study likely reflected the relative sensitivity of serum protein electrophoresis (g/dL) versus that of the SAA concentration assay (mg/L).

The SAA concentration assay provided the highest diagnostic accuracy despite the variety of clinical disease processes in the horses included in the present study. Compared with findings for the total WBC count, the SAA concentration assay had a similar specificity, but sensitivity was greater (approx 4-fold difference). Overall, these data from horses have indicated that SAA concentration is not directly correlated with plasma fibrinogen concentration, A:G ratio, or total WBC count, which is reflected in their differing kinetics and sensitivity to inflammatory stimuli. On the basis of data from companion animals, it is known that SAA is present in negligible concentrations and fibrinogen is present in detectable concentrations in the circulation of healthy individuals.[7] Thus, in CA animals with an appropriate APR, there is a possibility of a marked increase in magnitude of SAA concentration. Because of the short half-life of SAA, SAA concentration appears to be a reliable indicator of inflammation and infection, has prognostic value, and can be used as a consistently reliable clinical index for the progression of healing of patients.

In addition to its use as an aid in prognostic assessment, the wide spectrum of inflammatory conditions and infectious diseases associated with high concentrations of SAA in the present study and other investigations[5,6] had indicated that measurement of SAA concentration should be considered as a primary diagnostic tool. During the present study, 3 horses that were considered healthy at the time of initial blood sample collection were removed from the CN group after abnormally high SAA concentrations developed in concert with illnesses that became clinically apparent within 24 hours after the time of sample collection. This finding underscores the value of the use of SAA concentration measurements for the early detection of certain diseases in adult horses.

It is important to note that the present study was not conducted in a blinded fashion because it was not practical to use this type of experimental design given that many of the study horses had clinical signs that were severe enough to warrant admission to the hospital for treatment and observation. Thus, the overall positive impression of the usefulness of SAA concentration data may be linked to the severity of those cases. Furthermore, the present study involved blood samples collected at a single time point from horses with various diseases and conditions that had been ongoing for variable periods (days to months). It should also be recognized that horses were allocated to the CN group at the time of admission on the basis of clinicians' assessments of physical examination findings and clinical history and were followed for only a 7-day period. Although these facets of the experimental design are important, they represent the situation often seen in the clinical population at a specialty equine practice. To understand the clinical impact of the application of SAA concentration assessment as a diagnostic, prognostic, or monitoring aid, future studies should better focus on specific diseases, horse breeds and ages, the timing of onset of disease, and the effects of any prior history or treatment.

Serum amyloid A analysis has been validated and automated for use in horses and is presently available at some reference laboratories.[7,15] The results of the present study have demonstrated the accuracy with which SAA concentration reflects the presence of most forms of inflammation in horses. Compared with plasma fibrinogen concentration or total WBC count, SAA concentration was found to be a more reliable indicator of inflammation or infection and a more reliable index of a patient's return to health. Monitoring of SAA concentration in ill horses may aid in determining the response to treatment and be of prognostic value. Serum amyloid A concentration assessment should be routinely used in any diagnostic workup and during treatment in clinically ill horses, in addition to measurement of other, more traditional markers of inflammation, such as the total WBC count and plasma fibrinogen concentration.

a. BD Vacutainer, Butler Schein Animal Health, Dublin, Ohio.
b. Hemavet 9600, Drew Scientific, Waterbury, Conn.
c. SPIFE 3000 system, Helena, Beaumont, Tex.
d. Eiken, Tokyo, Japan.
e. Daytona Analyzer, Randox, Kearneysville, Va.
f. SAS, version 9.3, SAS Institute Inc, Cary, NC.

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