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Sequential measurement of serum amyloid A concentrations in ill hospitalized neonatal foals

Astrid J. van den Brom-Spierenburg[1,*], Esther W. Siegers[1], Cornélie M. Westermann[1], Johannes C. M. Vernooij[2], Marianne M. Sloet van Oldruitenborgh-Oosterbaan[1], Mathijs J. P. Theelen[1]

[1]Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands
[2]Department Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands
*Corresponding author: Astrid J. van den Brom-Spierenburg, Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 114, Utrecht 3584 CM, the Netherlands (a.j.spierenburg@uu.nl).

Abstract

Background: **In hospitalized foals, limited data are available on the utility of sequential measurement of SAA concentrations and the value of these data in a clinical setting.**

Hypothesis/Objectives: **To determine serum amyloid A (SAA) concentrations in ill neonatal foals at multiple timepoints during hospitalization, and to evaluate a potential association with systemic inflammatory response syndrome (SIRS) status, blood culture (BC) result, and survival.**

Animals: **Hospitalized ill foals ($n = 90$, ≤ 14 days).**

Methods: **In this retrospective study, foals were classified based on SIRS criteria: "SIRS" or "NonSIRS," BC results, and survival. Serum amyloid A concentrations on admission (ADM), day 1 (D1) and day 2 (D2) were compared within and between groups, using nonparametric tests. Results are presented as median (IQR) (effect size; 95% CI).**

Results: **Serum amyloid A concentrations were higher in SIRS than in NonSIRS foals both on ADM (401 mg/L (99; 855) vs 67 mg/L (23; 685), [ES 129, 95% CI, 15-385]), and on D1 (836 mg/L (616; 1240) vs 212 mg/L (69; 890), [ES 501, 95% CI, 121-724]). On D2, but not on ADM, foals with a positive BC had higher SAA (1244 mg/L (757; 2004)) than BC negative foals (153 mg/L (57; 695), [ES 1002; 95% CI, 282-1418]). On D1, but not on ADM, SAA was higher in non-survivors (729 mg/L (469; 1347) than in survivors (323 mg/L (75; 889), [ES 373; 95% CI, 28-651]).**

Conclusions and clinical importance: **Serum amyloid A measurements on D1 and D2 of hospitalization likely reflect both the severity of disease and response to initial treatment. Repeated SAA measurements during hospitalization can aid clinicians in determining severity of disease and can be useful as a prognosticator in ill neonatal foals.**

Keywords acute phase protein, blood culture, blood stream infection, neonatology, prognosticator, SAA, sepsis, SIRS

Abbreviations BC, blood culture; ES, effect size estimate; SAA, serum amyloid A; SIRS, systemic inflammatory response syndrome; "SIRS" foals, foals with > 2 signs of SIRS; "NonSIRS" foals, foals with < 2 signs of SIRS

Introduction

Serum amyloid A (SAA) is a major acute phase protein the concentration of which increases rapidly after the start of an acute infection, inflammation, or trauma[1–4] and peaks after 36-48 h. It can be easily measured in peripheral blood samples.

SAA concentrations are higher in critically ill neonatal foals with sepsis compared to those without sepsis.[5,6] However, SAA cannot reliably discriminate septic from non-septic foals,[5,6] because of much overlap in SAA concentrations in these groups. Serum amyloid A does seem to be useful to distinguish healthy from ill foals.[7] Serum amyloid A can be a valuable component of the routine newborn examination, with SAA < 100 mg/L having a high negative predictive value for infectious disease.[7] However, these recommendations should be used with caution in hospitalized ill foals in which the discriminatory value of SAA might be less.

Sepsis is a leading cause of death in neonatal foals[8] and early treatment of neonatal sepsis is crucial to increase chances of survival.[9] However, neither the definition nor diagnosis of equine neonatal sepsis is straightforward and under constant revision.[10] In addition, scoring systems developed for early detection of sepsis in foals[11–13] lack sensitivity and are difficult to validate in the absence of a clear definition of the disease.

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In human medicine, sepsis is currently defined as "life-threatening organ dysfunction caused by a dysregulated host response to infection".[14] This host response is demonstrable by measuring clinicopathological data and clinical signs compatible with the systemic inflammatory response syndrome (SIRS). In most studies, foal sepsis is defined as a combination of clinical signs of SIRS and a positive blood culture or a positive culture in samples collected from more than one site of infection (during life or postmortem).[5,6,10] Blood cultures can be used to diagnose blood stream infections (BSIs), however, both false negatives (due to a low sensitivity)[15] and false positives occur. The latter can be caused by transient bacteriemia, as was reported in 13% of healthy foal samples,[16] or by contamination of the sample. Furthermore, bacterial culture and sensitivity testing typically require at least 48-72 h before results become available.

In adult horses and foals with pneumonia, sequential SAA measurements can be used to assess disease progression and response to treatment.[17,18] However, no data have been published on follow-up SAA samples during the first days of hospitalization of ill neonatal foals. Consequently, it is currently unknown whether sequential SAA concentrations have diagnostic value, prognostic value, or both, in ill neonatal foals.

Therefore, this study aimed to compare SAA concentrations in neonatal foals at hospital admission, on day 1 (after 12-36 h of hospitalization) and day 2 (after 37-60 h hospitalization), in relation to SIRS status, blood culture results, and survival, to evaluate their usefulness as diagnostic and prognostic variables.

Materials and methods

Study design

Retrospective study.

Animals

All foals $\leq$ 14 days of age that were admitted for veterinary care to "masked for review" Equine University Hospital between January 2019 and December 2022 of which SAA was measured at admission and between 12 and 36 h of hospitalization, were included in the study. Foals considered healthy (defined by having no clinical complaints and showing no abnormalities in a clinical examination) and foals that were euthanized primarily for financial considerations were excluded.

In a subgroup of these foals, SAA concentrations after 37-60 h of hospitalization were also available and included in the study. Repeated hospitalizations were excluded. For each case, reason for presentation, clinical data (respiratory rate and breathing pattern, heart rate and body temperature, laboratory data (white blood cell count [WBCC]) and blood culture results were extracted from the patient file.

Classification

Foals were classified based on criteria for SIRS, blood culture result and survival. Foals were classified as "SIRS" when they met at least 2 of the following criteria (collected on admission): rectal temperature $<$ 37.2°C or $>$ 39.2°C, tachycardia ($>$115 beats/min when 0-3 days old and $>$ 120 beats/min when 4-14 days old), tachypnoea ($>$56 breaths/min), or abnormal WBCC ($<$6.9 cells/μL or $>$ 14.4 cells/μL when 0-3 days old and $<$ 4.0 cells/μL or $>$ 12.5 cells/μL when 4-14 days old).[19] Foals that met $<$ 2 of the SIRS criteria were classified as "NonSIRS."

Foals that had not enough variables recorded to determine their SIRS status, were considered "SIRS-undefined" and were only included in analyses regarding blood culture results and survival.

Foals that had a blood culture taken were classified as either "Blood Culture Positive" or "Blood Culture Negative" based on results from bacteriological culturing. Foals that had no blood culture taken were only included in analyses regarding SIRS status (if known) and survival.

Foals were classified as "survivor" when discharged from the hospital and as "non-survivor" when euthanized or died during hospitalization.

Sample collection and serum amyloid A measurement

Serum amyloid A concentration was measured routinely in peripheral blood of neonatal foals admitted to Utrecht University Equine University Hospital during the study period. Admission samples were taken before initiation of therapy. Repeated SAA measurements were performed every 24 h during the initial days of hospitalization, but only when blood was collected for other analyses (convenience samples). Serum amyloid A was measured in full heparinized blood using a point of care analyzer (Zoetis Stablelab EQ [Zoetis NL, Rivium Westlaan 74, 2909 LD Capelle aan den IJssel, the Netherlands]).[20] This membrane based immuno-assay measures SAA concentrations up to 3000 mg/L. Results exceeding the maximum value of 3000 mg/L were included as 3000 mg/L.

Serum amyloid A concentrations measured at 0-6 h after hospital admission were designated as admission (ADM) samples. SAA measurements collected after 12-36 h of hospitalization, but a minimum of 12 h apart from the admission sample, were included as day 1 (D1) samples. Serum amyloid A measurements collected 37-60 h after hospitalization, and a minimum of 12 h apart from the D1 sample, were included as day 2 (D2) samples. When more than one measurement was available within the interval, only the first sample was included in the study.

Samples for blood culture were collected based on clinical necessity as determined by the treating clinician. Two syringes of 20 mL of whole blood were taken aseptically from the jugular or cephalic vein (depending on the preference of the clinician), before administration of antimicrobials and before or during placement of a long-stay catheter. Each syringe was then added to a bottle of 70 mL of broth (BHI Broth + SPS by Biotrading [BioTRADING, Communicatieweg 7, 3641 SG Mijdrecht, the Netherlands]) and incubated at 37°C before being sent to the Veterinary Microbiologic Diagnostic Centre (VMDC, faculty of Veterinary Medicine, Department I&I, VMDC, Postbus 80165, 3508 TD Utrecht, the Netherlands) for aerobic and anaerobic culture.

Statistics

Serum amyloid A concentrations are presented as median and IQR. Descriptive statistics were performed. Serum amyloid A concentrations were compared between groups of foals using the Mann–Whitney *U* test (2 groups), while for the longitudinal

Table 1 Sampling moments, survival, and age on admission in groups of foals based on SIRS status, blood culture result, and survival.

	Sample time points (*n*)			Age on admission		
	Admission and day 1	**Admission, day 1 and day 2**	**Survival *n* (%)**	**Median age (h)**	**IQR (h)**	**Range (h)**
ALL	*90*	*40*	**71 (79)**	**31**	13; 72	0-264
SIRS	*32*	*15*	**23 (72)**	**26**	18; 70	0-216
NonSIRS	*51*	*23*	**42 (82)**	**34**	12; 96	0-264
SIRS-undefined	*7*	*2*	**6 (86)**	**36**	21; 60	0-240
BC positive	*26*	*14*	**19 (73)**	**24**	10; 48	0-168
SIRS	*11*					
NonSIRS	*15*					
BC negative	*23*	*11*	**17 (74)**	**42**	16; 72	0-264
SIRS	*7*					
NonSIRS	*13*					
No BC taken	*41*	*15*	**35 (85)**	**26**	18; 96	0-240
Survivors	*71*	*35*		**36**	15; 79	0-264
Non-survivors	*19*	*5*		**27**	11; 70	0-216

Number of samples in *italics*, data in **bold**. Abbreviations: BC = blood culture; NonSIRS = foals with <2 signs of SIRS; SIRS = foals with >2 signs of SIRS (systemic inflammatory response syndrome). There were no significant differences between groups.

comparison of the admission samples to the follow-up samples, the Wilcoxon signed rank test (2 timepoints) and the Friedman's 2-way ANOVA by ranks with pairwise comparisons with Bonferroni correction for multiple tests (3 timepoints) were used. Effect size estimates (ES) and 95% CI were acquired by the Hodges–Lehmann estimator. Comparison of proportion change in SAA level <100 mg/L to >100 mg/L between timepoints was tested by McNemar tests and between groups by Fisher's exact tests. Significance was set at $P < .05$.

Ethical approval

Ethical review and approval were waived for this study by the Institutional Review Board, as only convenience blood samples were used, and no additional procedures were performed on the foals included in this study. Informed consent was obtained from all owners.

Results

Foals: age, time of sampling, and reasons for presentation

Ninety foals met the inclusion criteria of the study. The median age of the foals on admission was 31 (IQR 13; 72) h. The age did not differ between groups regarding SIRS status, blood culture result, survival, or number of samples present (Table 1). Median time of sampling after admission was 0 h (IQR 0; 0). In all 90 foals, samples for SAA measurement were also collected at D1 between 12 and 36 h (median 21 h [IQR 18; 25 h]) after hospital admission. The interval between the ADM and D1 sample was 20 (IQR 17; 24) h. In a subset of 40 foals SAA measurements were additionally performed on D2, between 37 and 60 h after admission, with a median of 44 (IQR 42; 53) h after admission. The median interval between the D1 and D2 follow-up samples was 24 h (IQR 24; 25).

Table 2 Reasons for presentation.

	Number of foals (total *n* = 90)
Weakness/lethargy/poor nursing	35
Colic/straining	22
Diarrhea	17
Gait abnormality/lameness/limb deformity	9
Dyspnea	4
Fever	5
Suspected uroperitoneum	4
Suspected neonatal isoerythrolysis	3
Born at clinic from dystocia/high-risk pregnancy	3
Omphalophlebitis	2
Orphan foal	1
Miscellaneous	12

Foals can be included in multiple categories as many were referred for more than 1 presenting complaint.

Foals were presented for various reasons (Table 2), with weakness, lethargy, or insufficient nursing (or a combination of these) most often recorded, followed by colic or straining (or both), and diarrhea. There was some overlap in these categories, with 5 foals presenting with a combination of aforementioned problems.

SAA concentrations

Median SAA for all 90 foals increased from ADM to D1 ($P < .001$; effect size (ES) 170 mg/L; 95% CI, 75-273; see Table 3a). Median SAA concentrations for all 90 included ill foals and for the groups based on SIRS status, BC result and survival are presented in Table 3a and for the subgroup of 40 foals for which a D2 sample was also available, in Table 3b.

Table 3a Serum amyloid A concentrations in ill hospitalized foals on admission and day 1 (n = 90), and proportions of foals with serum amyloid A concentrations < 100 mg/L, in groups based on systemic inflammatory response syndrome status, blood culture result, and survival.

Group		Number of foals (n)	SAA ADM (median (IQR))	SAA ADM < 100 (n (%))	SAA D1 (median (IQR))	SAA D1 < 100 (n (%))	ΔSAA ADM to D1 (median (IQR))	SAA < 100 on ADM to > 100 at D1 (n (%))	SAA > 100 on ADM to < 100 at D1 (n (%))
ALL		*90*	**132**[#] (28; 708)	*41*[ω] (46)	**572**[#] (98; 1008)	*24*[ω] (27)	**+99** (−23; +453)	*21* (23)	*4* (4)
	NonSIRS	*51*	**67**[a,^] (23; 685)	*30*[e,ϑ] (59)	**212**[b,^] (69; 890)	*19*[f,ϑ] (37)	**+61** (−23; +366)	*13* (25)	*2* (4)
	SIRS	*32*	**401**[a,⁎] (99; 855)	*8*[e] (25)	**836**[b,⁎] (616; 1240)	*3*[f] (9)	**+200** (+16; +541)	*6* (19)	*1* (3)
	BC−	*23*	**310** (36; 870)	*9* (40)	**530** (148; 868)	*5* (22)	**+58**[c] (−50; +316)	*4* (17)	*0* (0)
	BC+	*26*	**398**[&] (20; 927)	*12*[ℋ] (46)	**896**[&] (663; 1550)	*4*[ℋ] (15)	**+393**[c] (+46; +761)	*8* (31)	*0* (0)
	Survivors	*71*	**146**[Ⓒ] (34; 696)	*31* (44)	**323**[d,Ⓒ] (75; 889)	*23*[g] (32)	**+98** (−23; +372)	*12* (17)	*4* (6)
	Non-survivors	*19*	**94** (23; 1126)	*10*[ת] (53)	**729**[d] (469; 1347)	*1*[g,ת] (5)	**+155** (−20; +608)	*9* (47)	*0* (0)

Abbreviations: ADM = on admission; BC = blood culture; D1 = day 1; NonSIRS = foals with < 2 signs of SIRS; SIRS = foals with > 2 signs of SIRS (systemic inflammatory response syndrome). Median SAA and median ΔSAA in **bold,** SAA and IQR in mg/L, and numbers of foals per group in *italics*. Group values with the same letters (group-wise comparisons) or symbols (longitudinal comparisons) in superscript are significantly different from each other (P < .05).

Table 3b Serum amyloid A concentrations in a subgroup of the ill hospitalized foals presented in Table 3a of which a day 2 sample was available. Concentrations on admission, day 1, and day 2 (n = 40), and proportions of foals with serum amyloid A concentrations < 100 mg/L, in groups based on systemic inflammatory response syndrome status, blood culture result, and survival.

Group		Number of foals (n)	SAA ADM (median (IQR))	SAA ADM < 100 (n (%))	SAA D1 (median (IQR))	SAA D1 < 100 (n (%))	SAA D2 (median (IQR))	SAA D2 < 100 (n (%))	ΔSAA ADM to D1 (median (IQR))	ΔSAA ADM to D2 (median (IQR))	ΔSAA D1 to D2 (median (IQR))	SAA < 100 on ADM to > 100 at D1/D2 (n (%))	SAA > 100 on ADM to < 100 at D1/D2 (n (%))
ALL		*40*	**133**[∞] (37; 471)	*18*[Θ] (45)	**683**[∞] (104; 874)	*9*[Θ] (23)	**773** (64; 1178)	*12* (30)	**+280** (−9; +651)	**+101** (−33; +780)	**−35** (−172; +240)	*11* (28)/*10* (25)	*2* (5)/*4* (10)
	NonSIRS	*23*	**56** (17; 521)	*14*[Γ,⍁] (61)	**725** (87; 1233)	*6*[Γ] (26)	**591** (73; 1180)	*7*[⍁] (30)	**+191** (+9; +724)	**+82** (−12; +648)	**−35** (−266; +95)	*8* (35)/*8* (35)	*0* (0)/*1* (4)
	SIRS	*15*	**226**[¥] (0; 471)	*4* (27)	**729**[¥] (399; 835)	*2* (13)	**817** (373; 1178)	*3* (20)	**+342** (89; 541)	**+633** (+29; +875)	**−29** (−125; +389)	*3* (20)/*2* (13)	*1* (7)/*1* (7)
	BC−	*11*	**189** (36; 569)	*5* (45)	**323** (148; 822)	*2* (18)	**153**[h] (57; 695)	*5* (45)	**+61** (−26; +316)	**+7**[i] (−132; +79)	**−71** (−**198**; −35)	*2* (18)/*2* (18)	*0* (0)/*2* (18)
	BC+	*14*	**141**[¤] (23; 842)	*7* (50)	**842** (132; 1678)	*3* (21)	**1244**[h,¤] (757; 2004)	*3* (21)	**+651** (+46; +761)	**+645**[i] (0; +1415)	**+34** (−165; +625)	*4* (29)/*4* (29)	*0* (0)/*0* (0)
	Survivors	*35*	**118**[§] (37; 370)	*16* (46)	**590**[§] (100; 853)	*9* (26)	**591** (45; 1154)	*12* (34)	**+191** (−17; +558)	**+38** (−33; +653)	**−35** (−168; +74)	*9* (26)/*8* (23)	*2* (6)/*4* (11)
	Non-survivors	*5*	**301** (78; 674)	*1* (20)	**729** (641; 2191)	*0* (0)	**1037** (867; 1185)	*0* (0)	**+563** (+428; +1270)	**+746** (+549; +884)	**+396** (−968; +456)	*2* (40)/*2* (40)	*0* (0)/*0* (0)

Abbreviations: ADM = on admission; BC = blood culture; D1 = day 1; D2 = day 2; NonSIRS = foals with < 2 signs of SIRS; SIRS = foals with > 2 signs of SIRS (systemic inflammatory response syndrome). Median SAA and median ΔSAA in **bold,** SAA and IQR in mg/L, and numbers of foals per group in *italics*. Group values with the same letters (group-wise comparisons) or symbols (longitudinal comparisons) in superscript are significantly different from each other (P < .05).

SAA concentrations < 100 mg/L

At ADM, more foals had SAA concentrations < 100 mg/L than on D1 ($P < .001$; Table 3a). NonSIRS foals more frequently had SAA < 100 mg/L compared to SIRS foals, both on ADM ($P = .003$) and on D1 ($P = .005$; Table 3a). In foals with a positive blood culture and in non-surviving foals the proportion of foals with SAA < 100 mg/L was significantly lower on D1 compared to ADM ($P = .008$ and $P = .004$, respectively; Table 3a).

In the subset of 40 foals for which also a D2 sample was available, SAA did not significantly change from D1 to D2, nor did the proportion of foals with SAA > 100 mg/L. For more detail, see Table 3b.

SAA concentrations in SIRS and NonSIRS foals

The number of foals classified as "NonSIRS," "SIRS," and "SIRS-undefined" (because of missing data) are presented in Table 1. Group-wise comparisons showed that SAA concentrations were significantly higher in SIRS foals compared to NonSIRS foals both in the ADM samples (ES 129 mg/L; 95% CI, 15-385; $P = .019$) and in the D1 samples (ES 501 mg/L; 95% CI, 121-724; $P = .003$) (Figure 1a). Longitudinal comparison of repeated samples showed a significant rise in SAA between ADM and D1 in both SIRS (ES 260 mg/L (95% CI, 100-459 mg/L; $P = .002$) and NonSIRS foals (ES 117 mg/L; 95% CI, 26-284 mg/L; $P = .01$) (Figure 1a). No difference was found between SAA concentrations of SIRS and NonSIRS foals on D2 (Figure 1b).

SAA concentrations and blood culture results

The BC status of foals in different subgroups are presented in Table 1.

Longitudinal comparison of repeated samples showed a significant rise in SAA between ADM and D1 in foals with a positive blood culture (ES 413 mg/L; 95% CI, 128-722 mg/L; $P = .002$) (Figure 2a). Serum amyloid A did not significantly change between ADM and D1 in foals with a negative culture.

In the subgroup of foals with D2 samples additionally available, longitudinal comparison of repeated samples showed that SAA on D2 increased significantly from ADM in the BC positive foals (ES 701 mg/L; 95% CI, 275-1301 mg/L; $P = .042$), but not in the blood culture negative foals (Figure 2b).

Furthermore, group-wise comparison showed that SAA concentrations on D2 (but not on D1 or ADM), were higher in foals with a positive BC than in foals with a negative BC (ES 1002 mg/L; 95% CI, 282-1418 mg/L; $P = .025$) (Figure 2b).

SAA concentrations and survival

Overall survival was 79% and did not differ based on SIRS status or blood culture result (Table 1). When foals were euthanized, the decision was primarily based on poor prognosis and animal welfare. In some cases, financial considerations contributed, but no foals were euthanized solely for financial restraints.

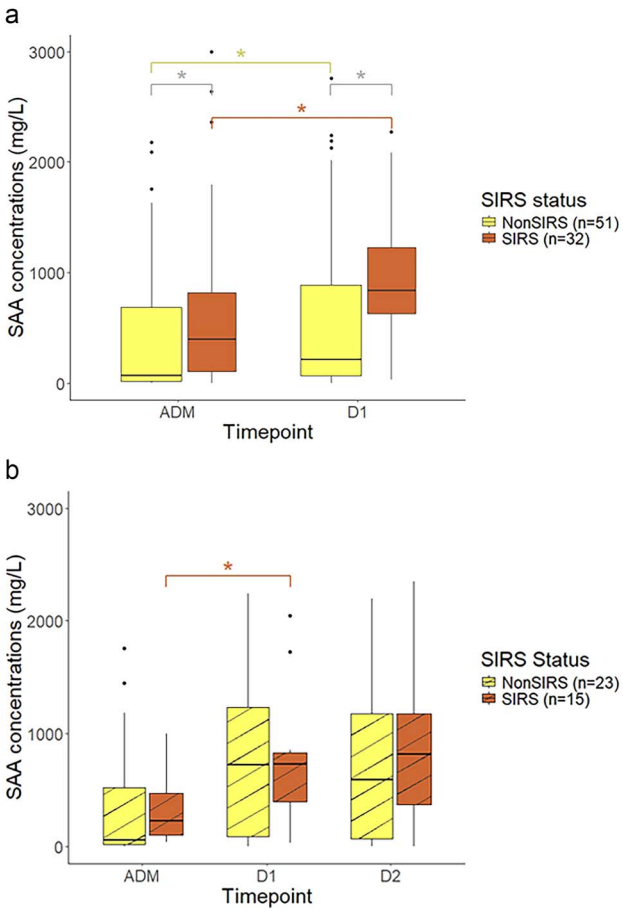

Figure 1 (a) Sequential SAA concentrations in foals both at ADM and D1, related to SIRS status ($n = 83$). NonSIRS = foals with < 2 signs of SIRS, SIRS = foals with > 2 signs of SIRS. *$P < .05$. Abbreviations: ADM = admission; D1 = day 1; SAA = serum amyloid A; SIRS = systemic inflammatory response syndrome (b) sequential SAA concentrations in a subset of foals at ADM, D1, and D2, related to SIRS status ($n = 38$). NonSIRS = foals with < 2 signs of SIRS, SIRS = foals with > 2 signs of SIRS. *$P < .05$. Abbreviations: ADM = admission; D1 = day 1; D2 = day 2; SAA = serum amyloid A; SIRS = systemic inflammatory response syndrome.

Group-wise comparisons showed that SAA concentrations at hospital admission were not different between surviving and non-surviving foals (Figure 3a). However, in the follow-up samples (D1), non-survivors had a higher median SAA concentration (729 mg/L; IQR 469; 1347) compared to surviving foals (323 mg/L, IQR 75; 889) with an ES of 373 mg/L (95% CI, 28-651 mg/L; $P = .037$) (Figure 3b).

Of the 40 foals for which a D2 sample was also available, only 5 did not survive. Due to the small numbers of foals, no additional analyses were performed on the D2 samples.

Discussion

In this study SAA concentrations in hospitalized foals were evaluated at 3 timepoints, related to SIRS status, blood culture results and survival. Associations between SAA concentrations and SIRS status, blood culture results and survival were observed, showing more pronounced and persistent rises of SAA in foals with SIRS, positive BC results and in non-survivors.

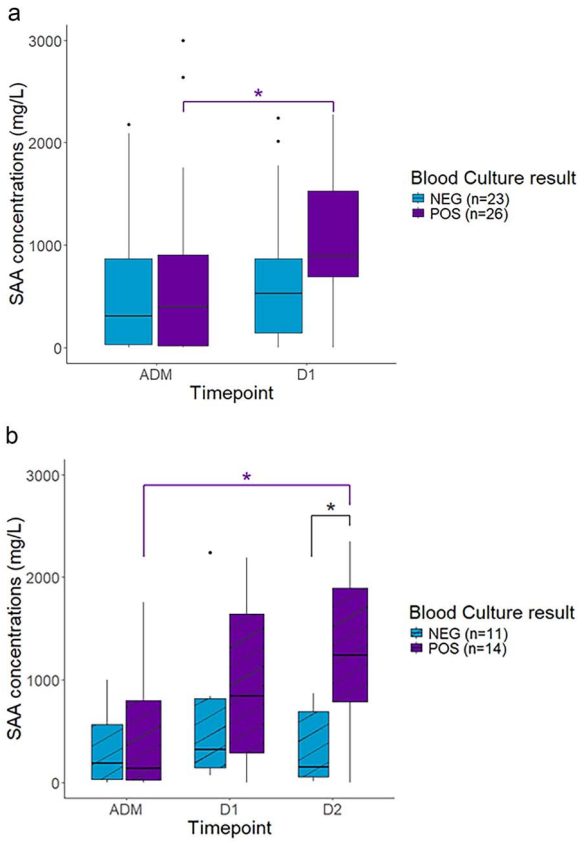

Figure 2 (a) Sequential SAA concentrations in foals both at ADM and D1, related to blood culture result ($n = 49$). *$P < .05$. Abbreviations: ADM = admission; D1 = day 1; NEG = blood culture negative, POS = blood culture positive; SAA = serum amyloid A; SIRS = systemic inflammatory response syndrome (b) sequential SAA concentrations in a subset of foals at ADM, D1, and D2, related to blood culture result ($n = 25$). *$P < .05$. Abbreviations: ADM = admission; D1 = day 1; D2 = day 2; NEG = blood culture negative, POS = blood culture positive; SAA = serum amyloid A; SIRS = systemic inflammatory response syndrome.

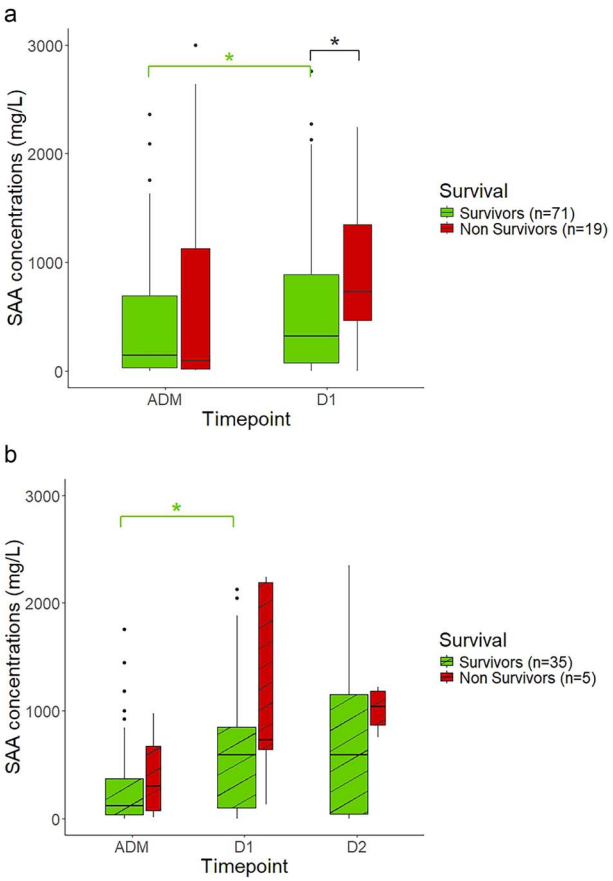

Figure 3 (a) Sequential SAA concentrations in foals both at ADM and D1, related to survival ($n = 90$). *$P < .05$. Abbreviations: ADM = admission; D1 = day 1; SAA = serum amyloid A (b) sequential SAA concentrations in a subset of foals both at ADM, D1, and D2, related to survival ($n = 40$). *$P < .05$. Abbreviations: ADM = admission; D1 = day 1; D2 = day 2; SAA = serum amyloid A.

In several studies, researchers demonstrated that SAA can be used to classify neonatal foals as healthy, ill without sepsis or ill with sepsis.[5–7] Serum amyloid A concentrations < 100 mg/L were reported to have a good predictive value to define a foal as healthy in a farm-based study.[7] However, in this hospital-based study, including only ill foals, 46% of all foals, and 59% of the foals in the NonSIRS group, had SAA concentrations on admission < 100 mg/L. Similar results are reported in hospitalized foals, with overlap in SAA results between ill and healthy neonatal foals.[4–7] This illustrates the limitation of (single) SAA concentration as a single variable to assess health status in a hospitalized foals.

Inflammation, but especially infection, initiates an acute phase response leading to the clinical signs of SIRS and an increase in SAA.[2] The physiologic responses that are part of the SIRS criteria (tachycardia, tachypnoea, and increased body temperature), are fast processes, whereas the rise of SAA takes 6 h to develop and 48 h to reach the peak value.[1,3] In other studies, a relation between positive blood culture/sepsis and higher SAA concentrations was also observed, but no follow-up samples have been studied.[5,6,21]

A significant and clinically relevant rise in SAA concentration was observed in foals with a positive BC, from ADM to D1 ($n = 26$, ES 413 mg/L) or D2 ($n = 14$, ES 701 mg/L) samples. This was not the case in foals with a negative BC. Also, at D2 of hospitalization, foals with a positive BC had much higher SAA concentrations than foals with a negative BC, with an effect size > 1000 mg/L, irrespective of SAA concentrations on admission. This is likely the result of the type and magnitude of the infectious stimulus at time of the blood collection for culture and the time the foal's immune system needs to respond.[1,3] Although SIRS criteria are easy and quickly obtained, BC results are generally not available until 48-72 h after the sample has been collected. Follow-up blood SAA results are available immediately after collection of the sample and can, also in absence of SAA measurements on admission, provide information on the infectious/inflammatory component of the disease and could, therefore, be helpful in making treatment decisions, while awaiting culture results.

The data in this study also demonstrate the additional value of collecting samples for follow-up SAA measurement to assess likelihood of survival. Follow-up SAA concentrations on D1 of hospitalization, but not SAA concentrations on admission, were significantly higher in non-survivors than in surviving foals with a clinically relevant ES of 373 mg/L. This difference being present on D1 likely reflects the (time to respond to the) severity of disease combined with the response to initial treatment. In critically ill neonatal foals, broad-spectrum antimicrobial treatment is often

started immediately after hospital admission while awaiting culture results. If the chosen antimicrobials are effective against the causative pathogen(s), this will likely contribute to eliminating the infection, thereby tempering the acute phase response and the increase in SAA. In contrast, in foals in which the clinical disease deteriorates, either because of ineffective antimicrobial treatment or as a result of exacerbation of disease, a larger increase in SAA is expected. We found no difference between survivors and non-survivors in the SAA concentrations on admission, in contrast to another study.[5] This might be related to the time between the start of illness and the moment the foal is admitted to the hospital; this might differ between different hospitals. In another study in hospitalized foals, the median age of the foals was 2 days and the mean SAA in the septic group was > 1000 mg/L on admission already,[5] suggesting that some time had passed between the start of disease and admission. Foals in the present study were younger when admitted (median 31 h) and transport to the hospital is usually short in the Netherlands, so most foals likely were admitted in the earlier stages of the disease. This is supported by the lower admission SAA (median of all foals 132 mg/L) in the foals studied.

As in other studies, overlap between different groups in this study is substantial. Therefore, SAA might be more suited for use as an additional variable to determine type and severity of disease and prognosis and not as a single variable on which to base clinical decisions. Based on the findings in this study, SAA concentrations at 1-2 days after admission are superior to admission SAA concentrations for this purpose.

The authors acknowledge that several aspects of the design of this study might have influenced the results obtained. First, the relatively small number of foals in some of the subgroups, limits the statistical power. Also, with multiple testing and a significance level of 0.05, results need to be interpreted with caution. However, the Bonferroni correction was used and observed differences between groups were (mostly) large and therefore considered clinically relevant. Furthermore, blood cultures were not collected routinely from all foals admitted to the hospital. This was a result of the decision to only collect convenience samples to not cause any additional harm to the foals included in this study by performing additional venipuncture. Another limitation is the unknown and probably variable interval between the start of disease and hospital admission, which is likely to influences SAA concentrations. Unfortunately, it is impossible to determine the exact starting point of disease in a hospitalized foals. The fact that the median age of the foals in this study was 31 h and that there was no difference in age between groups indicates a limited effect of the variation in time between start of disease and admission.

Since SAA also declines relatively quickly after cessation of the inflammatory response and the follow-up was only 1 or 2 days, this gives no complete picture of the kinetics of SAA in these foals. Also, foals that improved quickly were less likely to have follow-up samples collected and no samples were available of foals that died or were euthanized within 12 h of admission.

All foals were admitted to a single center. Care should be taken when extrapolating these results to other (hospitalized) foals.

Not all factors known to influence SAA concentrations were taken into account in this study. The higher SAA concentrations in most follow-up samples, also in the surviving and NonSIRS foals, might partly reflect a small physiological rise in SAA (1 mg/L [IQR 0; 4] on D2 vs 4 mg/L [IQR 0; 12] on D1) 48 h after birth,[4] since the median age of the foals was 31 h on admission. However, this will not explain differences between groups since age on admission was not different between groups (Table 1) and the observed concentrations in the present study were much higher. Mammary SAA is present in milk and colostrum but the effect on blood SAA is not clear.[22] Hyperimmune plasma increases SAA in one study,[23] but no effect was reported in another.[24] Therefore, these factors were not considered in the present study.

Regarding blood culture results; some foals might have received antimicrobials before referral, decreasing the chance of a positive culture. Unfortunately, this information was not consistently available and could therefore not be taken into account.

Finally, the point-of-care membrane based immuno-assay used in this study was validated in horses, but SAA measurements in the high range can be less accurate.[20] Only one sample measured > 3000 mg/L and was included as 3000 mg/L. This had no statistical effect because the data were not normally distributed and therefore nonparametric tests were used.

More research into timing of SAA sampling in different groups of ill foals, including longer follow-up would be valuable to gain more insight in the value of SAA in foals at different ages and with different types of disease.

Conclusions

Based on the results of this study, SAA measurement at hospital admission cannot be used reliably as prognosticator or to discriminate between blood culture negative and blood culture positive foals. However, a significant rise in SAA was observed between admission and D1 in blood culture positive foals only. On D2, SAA is significantly higher in foals with a positive blood culture compared to those with a negative blood culture. Furthermore, a higher SAA on D1 after hospital admission is associated with non-survival. This likely reflects the combined effect of severity of disease and the response to initial treatment. Therefore, routine collection of follow-up samples for SAA measurement could be helpful in ill hospitalized neonatal foals to aid in clinical assessment and prognosis.

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Author contributions

Astrid van den Brom-Spierenburg (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing—original draft, Writing—review & editing), Esther Willemijn Siegers (Conceptualization, Data curation, Investigation, Methodology, Project administration, Writing—review & editing), Cornélie Martine Westermann (Conceptualization, Data curation, Methodology, Supervision, Writing—review & editing), Hans Vernooij (Formal analysis, Investigation, Methodology, Software, Supervision, Writing—review & editing), Marianne Sloet-van Oldruitenborgh-Oosterbaan (Conceptualization, Methodology, Supervision, Writing—review & editing), and Mathijs Theelen (Conceptualization, Data curation, Methodology, Project administration, Supervision, Writing—review & editing)

Conflicts of interest

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Off-label antimicrobial declaration

The horses that were part of this study were treated with antimicrobials as deemed necessary by the treating clinician. Off-label antimicrobial use in our clinic is always accompanied by bacteriological culture to guide antimicrobial choice.

Institutional animal care and use committee or other approval declaration

Ethical review and approval were waived for this study, as only convenience blood samples were used and no additional procedures had to be performed on the foals included in this study. Informed consent was obtained from all owners.

Human ethics approval declaration

The authors declare human ethics approval was not needed.

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