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Assessment of platelet biology in equine patients with systemic inflammatory response syndrome

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Abstract. In addition to maintaining hemostasis, platelets have an important role in modulating innate and adaptive immune responses. A low platelet count has been found to be a negative prognostic factor for survival in humans and horses with critical illnesses, such as sepsis or systemic inflammatory response syndrome (SIRS). Decreased platelet aggregation, caused by in vivo activation, has been found in human patients with severe sepsis. In our prospective controlled study, we assessed platelet biology in blood samples from 20 equine SIRS cases and 120 healthy control horses. Platelet variables such as platelet count, large platelet count, clumps, plateletcrit, mean platelet volume, and mean platelet component concentration were analyzed by laser flow cytometry (Advia 2120) from K_3EDTA blood and from citrate blood. Hirudin blood samples were analyzed by impedance aggregometry (Multiplate analyzer; Roche) for platelet aggregation, including spontaneous aggregation and aggregation by 4 different agonists: adenosine diphosphate (ADPtest), ADP + prostaglandin E1 (ADPtestHS), arachidonic acid (ASPItest), and collagen (COLtest). SIRS cases had significantly lower platelet counts in K_3EDTA blood ($p < 0.0001$) compared to control horses. There were no significant differences in aggregation values between SIRS cases and controls. Non-surviving SIRS horses did not have statistically significant lower platelet counts or lower aggregation values for COLtest, ADPtest, or ADPtestHS compared to surviving SIRS horses, although 5 non-survivors were thrombocytopenic.

Key words: horses; impedance aggregometry; multiplate analyzer; platelet function.

Introduction

It is becoming increasingly clear that, in addition to being the main mediator of hemostasis, platelets play a substantial role in inflammatory processes and immunity.[46] Platelets are activated by direct contact with pathogens such as bacteria,[4] viruses,[24] or parasites,[30] by lipopolysaccharide (LPS)[13] and by the complement system.[36] Platelets have the ability to bind bacteria by functional immune receptors and secrete immunomodulatory chemokines.[4] It has been shown that platelets bind to malarial-infected red blood cells and kill the parasite within.[30] During sepsis, the interaction of platelets with adherent neutrophils leads to the formation of neutrophil extracellular traps (mesh-like DNA structures), which capture circulating bacteria and prevent bacterial dissemination.[13,29]

Systemic inflammatory response syndrome (SIRS) is a multifactorial event that was introduced in human medicine in 1991 to define a systemic hyperinflammatory reaction to nonspecific insults of either infectious or noninfectious origin.[9] Similarly in equine medicine, the term SIRS is used to describe cases of systemic inflammatory disease in which 2 or more of the following criteria are fulfilled (although reference intervals [RIs] vary by study): hyperthermia or hypothermia, tachycardia, tachypnea, and/or leukocytosis or leukopenia.[10,14,18,22,26,41,43]

In humans, a low platelet count was found to be a negative prognostic indicator for survival in critically ill patients.[15,25,42] Additionally, progressively declining platelet counts throughout stays in an intensive care unit were associated with higher mortality.[3,32,49] A retrospective analysis of platelet counts in patients in an equine clinic showed that thrombocytopenia was present especially in patients with systemic inflammatory disease, and that thrombocytopenia was also a negative prognostic factor for survival.[23]

Whole blood impedance aggregometry (Multiplate analyzer; Roche) is used routinely to determine platelet function in humans.[20,35,48] In human patients with severe sepsis, decreased platelet aggregation as a result of in vivo activation was found,[1,15] and impedance aggregometry was even better for predicting diagnosis and survival than platelet count.[1] RIs for platelet aggregometry on the Multiplate analyzer have been established for healthy horses[21] and ponies.[17]

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However, platelet function has not yet been investigated on the Multiplate analyzer in equine SIRS patients, to our knowledge.[10,14,18,22,26,41,43]

Evaluating platelet biology in horses with systemic inflammation could become a meaningful test to assess prognosis in equine patients. Our hypothesis was that equine SIRS patients have lower platelet counts and lower platelet aggregation values compared to healthy equids. We performed a prospective controlled study to evaluate the effects of SIRS on equine platelet variables and platelet function.

Materials and methods

Animals

SIRS patients had been presented to the Equine Clinic, Internal Medicine, Department of Veterinary Clinical Science, Justus Liebig University (Giessen, Germany), and blood sampling was performed as part of the routine diagnostic workup of the cases prior to any treatment in the equine clinic. Warmblood horses and ponies (height ≤148 cm) >3 y old were included. SIRS cases fulfilled at least 2 of the following criteria: heart rate >52 beats per min; respiratory rate >24 breaths per min; body temperature ≥39.0°C or ≤36.0°C; platelet count (Advia 2120; Siemens Healthcare) in K_3EDTA blood <90 × 10^9/L or >370 × 10^9/L, and in citrate blood <82 × 10^9/L or >345 × 10^9/L; leukocyte count (Advia 2120) <3.0 × 10^9/L or >15.0 × 10^9/L; bicarbonate concentration (Cobas b 221; Roche) ≤20.0 mmol/L; lactate concentration (Cobas b 221) ≥5 mmol/L.

Equids pretreated with acetylsalicylic acid (ASA) or clopidogrel, and pregnant mares as well as miniature horses, were excluded. In all cases, body condition score (BCS) was evaluated.[12] Blood was collected from a jugular vein with a sterile cannula (18 ga; B. Braun) or a sterile Teflon catheter (80 mm, 14 ga; Walter). A vacuum system was used to fill the following blood sample tubes (S-Monovette; Sarstedt): K_3EDTA (1.6 mg/mL), trisodium citrate (0.106 mol/L, mix ratio 1:10), hirudin (20 µg/mL), and one tube without anticoagulant for serum. If blood was taken from a catheter, the first 2 mL were discarded.

The control group included 60 healthy Warmbloods and 60 healthy ponies (height ≤148 cm) >3 y old. Blood sampling for health monitoring with the owner's consent was performed as outlined above. Exclusion criteria for control equids were: miniature horses and animals with a BCS <2 of 5,[12] an angiopathy (e.g., phlebitis) in the last 6 mo, or abnormalities of 1 or both jugular veins in the course of the clinical examination. We also excluded equids that had received medication in the past 14 d or had blood analysis results outside the RIs of 2 or more inflammation variables (leukocyte count RI: 4.4–9.0 × 10^9/L [Advia 2120], globulin concentration RI: 23–42 g/L [Pentra 400; Horiba], fibrinogen concentration RI: 1.25–3.29 g/L [STA Compact; Stago]).

Laser flow cytometry (Advia 2120)

The following platelet variables were analyzed by laser flow cytometry within 60 min after sampling of blood in SIRS cases and within 4 h in controls, from K_3EDTA blood and from citrate blood: platelet count, large platelet count, clumps, plateletcrit (PCT = [platelet count × MPV] ÷ 10,000), mean platelet volume (MPV), and mean platelet component concentration (MPC). Data for platelet volume distribution width were not considered because of a suspected software malfunction in the evaluation of results.[39]

Whole blood impedance aggregometry (Multiplate analyzer)

Hirudin blood samples were analyzed by the use of impedance aggregometry as described previously.[40] Platelet aggregation was measured using 4 different agonists to activate platelets according to the previous manufacturer's (Dynabyte) information: adenosine diphosphate (6.5 µM final concentration, ADPtest; Roche), ADP + prostaglandin E1 (6.5 µM/9.4 nM final concentration, ADPtestHS; Roche), arachidonic acid (0.75 mM final concentration, ASPItest; Roche), and collagen (1.6 µg/mL final concentration, COLtest; Roche). Spontaneous platelet aggregation (SPA) was also measured by adding isotonic saline instead of a platelet agonist. In 42 control equids and in all SIRS patients, aggregation was measured both with and without stirring for 3 min with a magnetic bar.

Statistical analysis

Data were analyzed (Prism v.6; GraphPad). The level of statistical significance was set at $p \leq 0.05$. All variables were assessed for normality using a Shapiro–Wilk test, and logarithmic transformation was performed if necessary.

Variables measured with the Advia 2120 were compared between SIRS cases and healthy controls by a 2-way ANOVA with repeated measurements concerning the type of anticoagulant, followed by a Bonferroni-adjusted post-hoc test with paired multiple comparison. Variables determined by the Multiplate analyzer were compared between SIRS patients and controls using a 2-way ANOVA with repeated measurements concerning the method (with or without stirring), and again a Bonferroni-adjusted post-hoc analysis.

Within the SIRS group, measurement results of survivors were compared with those of non-survivors by use of a 2-way ANOVA and a Bonferroni-adjusted post-hoc test. For platelet counts, positive likelihood ratios for non-survivors were calculated by use of receiver operating characteristic curve (ROC) analysis (Prism v.6) within all SIRS cases.

Results

SIRS group

We included 20 patients that met the SIRS criteria in our study: 11 Warmblood horses and 9 ponies. All subjects were presented to the equine clinic between September 2014 and March 2016. There were 12 geldings and 8 mares 4–25 y old (median: 17 y old). Six patients survived and were discharged. Fourteen patients were euthanized, 8 of them because of poor prognosis, 4 of them for economic reasons, and 2 were classified as inoperable during surgery.

Laser flow cytometry (Advia 2120)

SIRS patients had lower platelet counts than control equids in K_3EDTA blood samples ($p < 0.0001$). However, there was no statistical significance in platelet counts in citrate samples between SIRS patients and control animals. In SIRS patients, significantly more platelets were measured in blood samples anticoagulated with citrate compared to samples anticoagulated with K_3EDTA ($p = 0.003$; Fig. 1). Individual platelet counts in the SIRS group were almost identical or greater in citrated blood samples compared to the samples with K_3EDTA. EDTA-dependent pseudothrombocytopenia (EDTA-PTCP), as defined by thrombocytopenia in EDTA-anticoagulated blood with a platelet count within the RI in the citrated sample, was detected in 2 individuals of the SIRS group (10%) and in 5 individuals of the control group (4.2%). Except for PCT in citrate blood samples, no other variables had significant differences between SIRS cases and controls (Table 1). The differences found for PCT are not relevant clinically, and all measurements in citrated blood were within RIs.

There were significant differences in multiple platelet parameters between K_3EDTA-anticoagulated blood and citrated blood samples from SIRS patients, including: platelet count ($p = 0.003$), MPV ($p = 0.0001$), MPC ($p < 0.0001$), and PCT ($p < 0.0001$). For large platelet count and clumps, no significant differences were found.

Although there were no significant differences in platelet counts between survivors and non-survivors, none of the survivors were thrombocytopenic, whereas 5 of the non-survivors were thrombocytopenic. There were no significant differences in platelet variables for either anticoagulant between the 14 non-survivors and the 6 survivors (Fig. 2). SIRS patients with a lower platelet count ($<117 \times 10^9$/L in K_3EDTA blood, and $<119 \times 10^9$/L in citrate blood) had a positive likelihood ratio for non-survival of 3.9 (K_3EDTA) and 1.8 (citrate).

Whole blood impedance aggregometry (Multiplate analyzer)

Patients with SIRS had significantly lower platelet aggregation values for ASPItest with stirring only ($p = 0.031$); SIRS patients had significantly higher platelet aggregation values for ASPItest without stirring ($p = 0.001$; Table 2). In the control group, there was an obvious effect of the method with significantly lower values without stirring. In SIRS patients, this methodical impact was only measurable for COLtest ($p = 0.022$). No significant differences related to the method were found for ADPtest, ADPtestHS, or SPA.

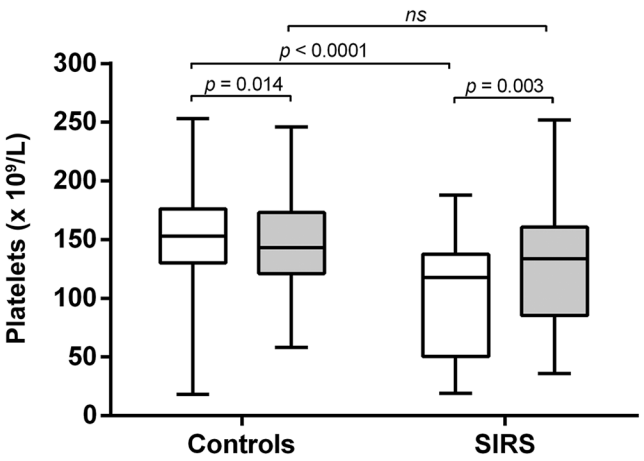

Figure 1. Platelet number (Advia 2120) in K_3EDTA blood (white boxes) and citrate blood (gray boxes, adjusted by dilution factor 1.1) in controls ($n = 120$) and systemic inflammatory response syndrome (SIRS) cases ($n = 20$). *ns* = not significantly different.

Within SIRS patients, no significant differences in aggregation values were found between survivors and non-survivors. Regarding the method in non-survivors, only COLtest showed significantly lower aggregation values without stirring ($p = 0.044$; Fig. 3). Non-survivors of the SIRS group had an overall trend towards lower aggregation values compared to survivors. Within the survivors, significant differences related to the method were found for COLtest ($p = 0.0001$), ADPtest ($p = 0.049$), and ADPtestHS ($p = 0.05$; Table 3).

Discussion

Equine SIRS patients had significantly lower platelet counts in K_3EDTA blood compared to the control group. In a retrospective study, no significant differences in platelet count between SIRS patients and controls were found in K_3EDTA blood analyzed by the Advia 2120.[45] However, given the unclear inclusion criteria for the SIRS cases in that study, a direct comparison with our results is not possible. A study in dogs also showed no differences in platelet count between healthy controls and animals with inflammatory disease; however, there were no specific SIRS criteria used, and inclusion was based on fever, neutrophilia, and band neutrophil counts $>1.0 \times 10^9$/L.[34] In humans, no comparable studies based on platelet count are available, to our knowledge.

In SIRS patients, platelet counts were higher in citrated blood than in K_3EDTA blood. This finding is in contrast to

Table 1. Platelet variables (Advia 2120) in K_3EDTA blood and in citrate blood in equid control group and systemic inflammatory response syndrome (SIRS) cases.

	Platelets ($\times 10^9$/L)		MPV (fL)		MPC (g/dL)		LargePLT ($\times 10^9$/L)		Clumps (*n*)		PCT (%)	
	E	C*	E	C	E	C	E	C*	E	C*	E	C
Controls												
n	120	119	120	120	120	120	120	120	116	120	116	120
Min.	18.0	58.0	5.1	6.3	15.9	17.7	0.0	0.0	0.0	41.0	0.02	0.04
25% P	130	121	6.9	8.1	22.9	20.3	1.0	2.0	90.0	80.2	0.10	0.10
Median	153	143	7.7	8.8	24.7	21.4	3.0	5.0	112	102	0.12	0.12
75% P	176	173	8.5	9.7	26.3	23.0	7.0	9.0	159	169	0.14	0.14
Max.	253	246	10.9	12.3	30.2	26.6	25.0	20.0	2,774	1,578	0.21	0.19
SIRS												
n	20	20	20	20	19	20	20	20	20	20	20	19
Min.	19.0	36.0	6.0	6.0	21.0	17.6	0.0	0.0	26.0	41.0	0.03	0.03
25% P	50.2	85.2	6.6	8.4	23.5	19.9	2.2	3.2	61.0	69.5	0.07	0.12
Median	118	134	7.5	8.9	25.8	21.1	5.5	7.5	74.5	99.0	0.10	0.17
75% P	138	161	7.9	10.3	27.3	22.7	8.0	12.7	109	151	0.13	0.21
Max.	188	252	11.1	11.2	30.0	30.5	18.0	26.0	1,299	736	0.19	0.25
p	<0.0001	0.102	0.777	0.826	0.131	>0.9	0.057	>0.9	0.181	0.833	0.107	<0.0001

C = citrated blood; E = K_3EDTA blood; LargePLT = large platelets; MPC = mean platelet component concentration; MPV = mean platelet volume; *n* = number; P = percentile; PCT = plateletcrit.
* Platelet number adjusted by dilution factor 1.1.

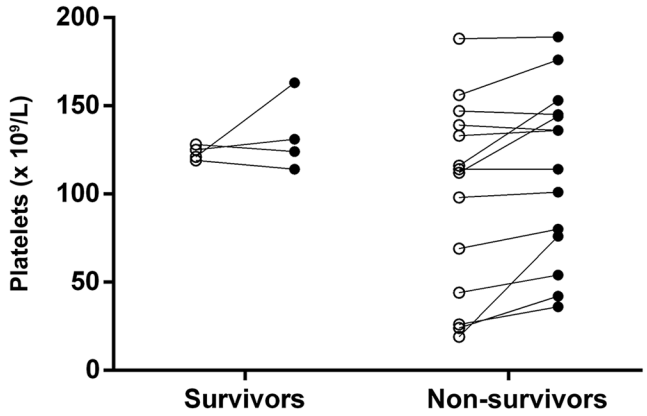

Figure 2. Platelet counts (Advia 2120) in K_3EDTA blood (open circles) and citrate blood (filled circles) within systemic inflammatory response syndrome (SIRS) cases, depending on survival (*n* = 6) or non-survival (*n* = 14).

the control group, which had higher platelet counts in K_3EDTA blood than in citrated blood. This difference is likely attributable to increased in vitro aggregation in SIRS patients as a result of EDTA-PTCP. In humans, the prevalence of EDTA-PTCP is shown to be higher in seriously ill patients and has the potential to develop as a result of disease.[8] Two patients with SIRS (10%) in our study had evidence of EDTA-PTCP; this proportion is twice as many as those seen in the control animals (5 of 120; 4.2%). EDTA-PTCP was documented upon presentation in both SIRS patients, and both equids had been ill for several days prior. Therefore, it may be that EDTA-PTCP developed before these patients were presented to the equine clinic.

Platelet counts were lower in non-survivors than in survivors, although statistically not significant. Furthermore, none of the horses with thrombocytopenia survived. One survivor had a platelet count of 37 × 10^9/L in K_3EDTA blood and 156 × 10^9/L in citrate blood, consistent with EDTA-PTCP. Four of the non-survivors were euthanized for economic reasons. Excluding these 4 cases from statistical analysis, a significant difference for platelet count was found in citrate blood between survivors and non-survivors (p = 0.038), with lower platelet counts in non-survivors. In K_3EDTA blood, the difference was not statistically significant. Positive likelihood ratios for non-survival without those 4 patients were 4.2 in K_3EDTA blood (cutoff 117 × 10^9/L) and 2.0 in citrate blood (cutoff 119 × 10^9/L). These results are consistent with those of a retrospective study in which thrombocytopenia was a negative prognostic factor for survival, with an odds ratio of 3.7.[23] Low platelet count was found to be a negative prognostic factor for survival in humans with critical illness as well.[3,15,25,32,42,49] To our knowledge, low platelet counts in equine SIRS patients have not been associated previously with increased mortality. Therefore, the platelet count could become a diagnostically conclusive variable for algorithms to evaluate the prognosis of equine SIRS cases.

Inflammatory processes are accompanied by platelet activation.[4,16,24,30,51] In humans, it has been shown in vitro that degranulation, especially of α-granules, leads to a decrease in refraction index and a subsequent decline in MPC.[2,28,53]

Table 2. Aggregation values (Multiplate analyzer) in equid control group and systemic inflammatory response syndrome (SIRS) cases with and without stirring during incubation.

	COLtest (U)		ADPtest (U)		ADPtestHS (U)		ASPItest (U)		SPA (U)	
	Stirring	No stirring	Stirring	No stirring	Stirring	No stirring	Stirring	No stirring	Stirring	No stirring
Controls										
n	42	42	42	41	42	42	41	42	41	42
Min.	44.0	16.0	16.0	9.0	13.0	5.0	2.0	0.0	0.0	0.0
25% P	209	96.3	74.5	48.0	54.5	38.8	12.0	9.8	0.0	0.0
Median	239	136	113	72.0	77.0	64.5	78.0	31.5	7.0	0.0
75% P	303	156	179	102	168	98.3	174	100	18.5	7.0
Max.	345	259	319	244	331	263	325	239	157	24.0
SIRS										
n	20	20	20	19	20	20	20	20	19	18
Min.	10.0	39.0	10.0	16.0	9.0	24.0	0.0	0.0	0.0	0.0
25% P	120	97.0	35.5	59.0	30.5	45.0	7.5	13.5	0.0	0.0
Median	339	170	121	85.0	124	90.0	21.5	66.0	4.0	1.0
75% P	404	259	280	203	256	239	56.8	182	54.0	18.3
Max.	526	323	414	340	376	360	171	282	214	126
p	>0.9	0.103	>0.9	0.597	0.9	0.061	0.031	0.305	>0.9	0.539

n = number; P = percentile; SPA = spontaneous platelet aggregation; U = units.

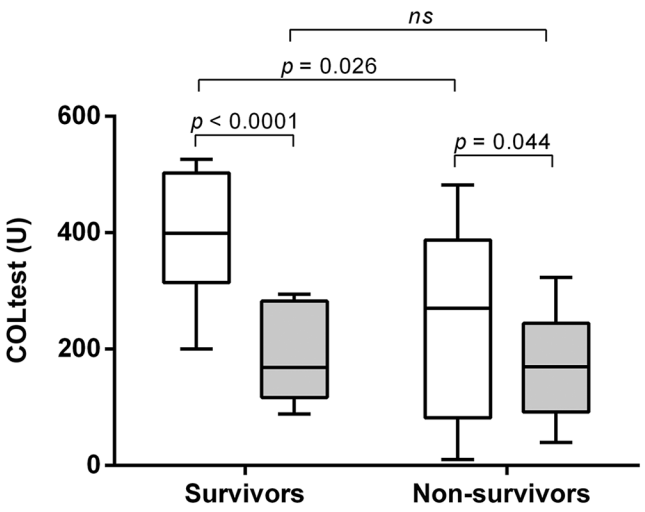

Figure 3. Aggregation values (Multiplate analyzer) for COLtest with (white boxes) and without (gray boxes) stirring during incubation within systemic inflammatory response syndrome (SIRS) cases, depending on survival and non-survival. *ns* = not significantly different.

Degranulation of α-granules in vitro after activation with thrombin has also been demonstrated in horses.[19,44] We found no significant differences for MPC between SIRS cases and controls. By contrast, in another equine study, SIRS patients had significantly lower MPC in K_3EDTA blood (235 ± 47 g/L) compared to the control group (262 ± 35 g/L).[45] Direct comparison of our results with that study is difficult, however, because different inclusion criteria were used. Furthermore, in the previous study, MPC measurements were performed in the SIRS group up to 24 h after blood collection; it has been shown that storage of blood samples for 24 h can cause a 15% decrease in MPC.[37] Therefore, the difference in MPC could be attributed to storage and could explain why we did not identify similar findings. In SIRS cases, we carried out measurements within 60 min after sampling of blood. The majority of MPC measurements in the control group were performed 2 h after blood collection; however, the maximum time interval was 4 h. The calculated MPC for the controls in our study (247 ± 26 g/L) was lower than in previous studies (275 g/L after 2 h,[37] 262 ± 35 g/L[45]) but higher than the mean value (202 g/L[33]) evaluated for the Advia 120.

Contrary to our hypothesis, there were no significant differences in platelet function between SIRS cases and controls. In humans, higher aggregation values were demonstrated in SIRS patients, whereas the values were decreased in patients with sepsis and septic shock.[1,15] Lower aggregation values are explained by existing activation of platelets in vivo linked to the primary disease. In vivo activation results in predominantly hyporesponsive platelets in the blood, whereas adding an agonist leads to no or considerably reduced aggregation.[1,15,27] We did not subdivide cases into SIRS, sepsis, and septic shock. For this reason, both increased and decreased aggregation values compared to the controls can be expected. This assumption is confirmed by the fact that, in SIRS cases in all aggregation tests, minimal achieved aggregation values were lower than in the control group and maximal achieved aggregation values were higher than in the control group. It can be assumed that the oppositely occurring aggregation values in cases with different severity of SIRS cancel each other out in sum. Furthermore, the relatively

Table 3. Aggregation values (Multiplate analyzer) with and without stirring during incubation within systemic inflammatory response syndrome (SIRS) cases depending on survival or non-survival.

	COLtest (U)		ADPtest (U)		ADPtestHS (U)		ASPItest (U)		SPA (U)	
	Stirring	No stirring	Stirring	No stirring	Stirring	No stirring	Stirring	No stirring	Stirring	No stirring
Non-survivors										
n	14	14	14	14	14	14	14	14	13	12
Min.	10.0	39.0	10.0	19.0	9.0	28.0	0.1	0.1	0.1	0.1
25% P	81.8	91.5	27.8	54.5	24.3	47.3	5.3	9.8	0.1	0.1
Median	270	170	104	74.0	123	117	28.5	66.0	3.0	1.0
75% P	387	244	261	232	247	264	56.3	237	26.5	14.5
Max.	482	323	386	340	376	360	171	282	214	57.0
Survivors										
n	6	6	6	5	6	6	6	6	6	6
Min.	200.0	88.0	80.0	16.0	49.0	24.0	6.0	13.0	0.1	0.0
25% P	314	117	95.8	37.5	87.3	36.0	10.5	41.5	2.3	0.1
Median	399	168	133	107	124	57.5	17.5	67.5	70.0	6.0
75% P	503	282	348	172	313	202	79.5	109	126	45.0
Max.	526	294	414	203	333	249	90.0	140	140	126
p	0.026	>0.9	>0.9	>0.9	>0.9	0.826	0.809	>0.9	0.365	0.788

n = number; P = percentile; SPA = spontaneous platelet aggregation; U = units.

small sample size of 20 SIRS cases is a significant limitation of our study.

In vitro tests have shown that platelet aggregation induced by ADP, collagen, and arachidonic acid in human blood was increased by adding LPS, whereas adding LPS alone without an agonist did not induce aggregation. This observation was explained as a priming effect of LPS to human platelets.[31,52] This effect of LPS on ADP-induced and arachidonic acid–induced aggregation was found in quite low concentrations of 0.1–10 ng/mL. In higher concentrations of 100 ng/mL, there was no increase in aggregation values.[31] In collagen-induced aggregation, this priming effect was also detected in higher LPS concentrations of 200–300 ng/mL.[52] Such concentration of LPS can be seen in human patients with shock[50] and in horses with acute gastrointestinal disease.[47]

Adding the magnetic stir bar 3 min after incubation resulted in significantly lower aggregation values in healthy horses, a finding consistent with a study in humans.[6] Within the SIRS group, this method effect was also present, but mostly not statistically significant. In assessing the individual aggregation values, it became obvious that the additive effect of stirring while incubating was present predominantly in SIRS cases with high aggregation values, whereas in cases with low values this effect was almost completely missing. The higher aggregation values of stirred samples could be explained by an in vivo priming of platelets in SIRS cases and the additive effect of increased ADP concentrations owing to stirring. Obviously, the additive effect of stirring was lost in most of the SIRS cases with lower aggregation values. In vitro examination of human platelets showed that incubation for one hour with an ADP analog causes selective desensitization of certain ADP receptors. Although the $P2Y_1$ receptor was not activatable additionally by adding ADP, functionality of the $P2Y_{12}$ receptor was unaffected.[7] The missing additive effect of stirring in some of the cases in our study could be the result of in vivo activation of platelets in severely ill patients with desensitization of $P2Y_1$ receptors. In this case, ADP-induced aggregation could only be mediated by $P2Y_{12}$ receptors leading to reduced aggregation.

The lower aggregation values for the ASPItest in SIRS cases in our study could have resulted from prior treatment with nonsteroidal anti-inflammatories; we defined only premedication with ASA or clopidogrel as exclusion criteria. Administration of 2 non-selective COX inhibitors (flunixin meglumine and phenylbutazone) can cause a significant decrease of thromboxane B_2 serum concentration, presumably as a result of inhibition of COX in platelets.[11] Comparable to ASA, this inhibition could explain the lower aggregation values for the ASPItest. Medication with flunixin meglumine and phenylbutazone had no effect on collagen-induced aggregation.[11] In our study, aggregation values for the COLtest in SIRS cases were also not decreased.

In human patients with SIRS, sepsis, and septic shock, decreased aggregation values were associated with increased mortality.[1,15] Our study provides indications that lower aggregation values measured with the Multiplate analyzer in equine SIRS patients might be associated with increased mortality. Our results suggest that aggregometry could also be a meaningful variable in diagnostic algorithms to assess prognosis in equine SIRS patients. This outcome needs to be confirmed in a larger sample.

SIRS criteria used in our study were adjusted to the human S2k guideline,[38] according to the physiologic differences in horses, and complemented with further variables.

The added variables of platelet count, [lactate], and [bicarbonate] were selected based on criteria formulated in the S2k guideline for "organ complication": encephalopathy, hypotension and shock, relative or absolute thrombocytopenia, arterial hypoxemia, renal dysfunction, and metabolic acidosis. Nevertheless, with restriction to the criteria listed in the human SIRS guideline and in other equine studies (heart rate, respiratory rate, temperature, and WBC),[18,26,41] 18 of 20 equids fulfilled the inclusion criteria. Therefore, the adjusted SIRS criteria of platelet count, [lactate], and [bicarbonate] probably do not cause a bias for patient inclusion compared with other human or equine studies. It has been shown that SIRS criteria overall have low specificity and are inappropriate for diagnosis of specific diseases,[5] although in human medicine SIRS criteria are used for a multitude of prospective controlled studies and allow for good comparability of data. Furthermore, biomarkers can be examined for their suitability in the diagnosis of SIRS and could possibly be integrated in diagnostic algorithms.[5] It can be assumed that the adjusted SIRS criteria in our study were suitable to provide preliminary information that platelet variables and platelet function may serve as biomarkers for systemic inflammation in horses.

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Declaration of conflicting interests

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