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

Carolin Ehrmann, Julia Engel, Andreas Moritz, Katja Roscher

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$_3$EDTA 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$_3$EDTA 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. Platelets are activated by direct contact with pathogens such as bacteria, viruses, or parasites, by lipopolysaccharide (LPS) and by the complement system. Platelets have the ability to bind bacteria by functional immune receptors and secrete immunomodulatory chemokines. It has been shown that platelets bind to malarial-infected red blood cells and kill the parasite within. 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.

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. 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.

In humans, a low platelet count was found to be a negative prognostic indicator for survival in critically ill patients. Additionally, progressively declining platelet counts throughout stays in an intensive care unit were associated with higher mortality. 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.

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

Equine Clinic, Internal Medicine (Ehrmann, Engel, Roscher) and Clinical Pathophysiology and Veterinary Clinical Pathology (Moritz), Department of Veterinary Clinical Science, Justus Liebig University, Giessen, Germany.

Corresponding author: Carolin Ehrmann, Equine Clinic, Internal Medicine, Department of Veterinary Clinical Science, Justus Liebig University, Giessen, Germany. carolin.ehrmann@vetmed.uni-giessen.de
However, platelet function has not yet been investigated on the Multiplate analyzer in equine SIRS patients, to our knowledge. 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. Warm-blood 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$_3$EDTA 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. 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$_3$EDTA (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, 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$_3$EDTA 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.

Whole blood impedance aggregometry (Multiplate analyzer)

Hirudin blood samples were analyzed by the use of impedance aggregometry as described previously. 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–Wilks 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$_3$EDTA 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$_3$EDTA ($p = 0.003$; Fig. 1). Individual platelet counts in the SIRS group were almost identical or greater in citrated blood samples compared to the samples with K$_3$EDTA. 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$_3$EDTA-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 × 10$^9$/L in K$_3$EDTA blood, and <119 × 10$^9$/L in citrate blood) had a positive likelihood ratio for non-survival of 3.9 (K$_3$EDTA) 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.

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$_3$EDTA 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$_3$EDTA 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 × 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$_3$EDTA blood. This finding is in contrast to
the control group, which had higher platelet counts in K₃EDTA 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 37×10⁹/L in K₃EDTA blood and 156×10⁹/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 K₃EDTA blood, the difference was not statistically significant. Positive likelihood ratios for non-survival without those 4 patients were 4.2 in K₃EDTA blood (cutoff 117×10⁹/L) and 2.0 in citrate blood (cutoff 119×10⁹/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 refractive 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 |
| Controls       |             |             |               |              |         |             |         |             |
| n              | 42          | 42          | 42            | 41           | 42      | 42          | 41      | 42           | 42 |
| Min.           | 44.0        | 16.0        | 16.0          | 9.0          | 13.0    | 5.0         | 2.0     | 0.0          | 0.0 |
| 25% P          | 209         | 96.3        | 74.5          | 48.0         | 54.5    | 38.8        | 12.0    | 9.8          | 0.0 |
| Median         | 239         | 136         | 113           | 72.0         | 77.0    | 64.5        | 78.0    | 31.5         | 7.0 |
| 75% P          | 303         | 156         | 179           | 102          | 168     | 98.3        | 174     | 100          | 18.5 |
| Max.           | 345         | 259         | 319           | 244          | 331     | 263         | 325     | 239          | 157 |
| SIRS           |             |             |               |              |         |             |         |              |
| n              | 20          | 20          | 20            | 19           | 20      | 20          | 20      | 19           | 18 |
| Min.           | 10.0        | 39.0        | 10.0          | 16.0         | 9.0     | 24.0        | 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 |
| Median         | 339         | 170         | 121           | 85.0         | 124     | 90.0        | 21.5    | 66.0         | 4.0 |
| 75% P          | 404         | 259         | 280           | 203          | 256     | 239         | 56.8    | 182          | 54.0 |
| Max.           | 526         | 323         | 414           | 340          | 376     | 360         | 171     | 282          | 214 |
| p              | >0.9        | 0.103       | >0.9          | 0.597        | 0.9     | 0.061       | 0.031   | 0.305        | >0.9 |

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

Degranulation of α-granules in vitro after activation with thrombin has also been demonstrated in horses. 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$_3$EDTA blood (235 ± 47 g/L) compared to the control group (262 ± 35 g/L). 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. 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) but higher than the mean value (202 g/L) 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. Lower aggregation values are explained by existing activation of platelets in vivo linked to the primary disease. In vivo activation results in predominantly hypo-responsive platelets in the blood, whereas adding an agonist leads to no or considerably reduced aggregation. 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
Platelet biology in equine SIRS patients

A 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. 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. In collagen-induced aggregation, this priming effect was also detected in higher LPS concentrations of 200–300 ng/mL. Such concentration of LPS can be seen in human patients with shock and in horses with acute gastrointestinal disease.

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. 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 P2Y1 receptor was not activatable additionally by adding ADP, functionality of the P2Y12 receptor was unaffected. 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 P2Y1 receptors. In this case, ADP-induced aggregation could only be mediated by P2Y12 receptors leading to reduced aggregation.

The lower aggregation values for the ASPTest 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 B2 serum concentration, presumably as a result of inhibition of COX in platelets. Comparable to ASA, this inhibition could explain the lower aggregation values for the ASPTest. Medication with flunixin meglumine and phenylbutazone had no effect on collagen-induced aggregation. 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. Our study provides indications that lower aggregation values measured with the Multiplate analyzer in equine SIRS patients might be associated with increased mortality.

| 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) | | | | | |
| | | | | | | |
| | ASPTest (U) | | | | | |
| | | | | | | |
| | SPA (U) | | | | | |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Non-survivors   | Stirring | No stirring | Stirring | No stirring | Stirring | No stirring | Stirring | No stirring |
| | | | | | | | | |
| n     | 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 |
| 25% P | 81.8 | 91.5 | 27.8 | 54.5 | 24.3 | 47.3 | 5.3 | 9.8 | 0.1 |
| Median | 270 | 170 | 104 | 74.0 | 123 | 117 | 28.5 | 66.0 | 3.0 |
| 75% P | 387 | 244 | 261 | 232 | 247 | 264 | 56.3 | 237 | 26.5 |
| Max.  | 482 | 323 | 386 | 340 | 376 | 360 | 171 | 282 | 214 |
| Survivors | Stirring | No stirring | Stirring | No stirring | Stirring | No stirring | Stirring | No stirring |
| | | | | | | | | |
| n     | 6 | 6 | 6 | 6 | 5 | 6 | 6 | 6 | 6 |
| Min.  | 200.0 | 88.0 | 80.0 | 16.0 | 49.0 | 24.0 | 6.0 | 13.0 | 0.1 |
| 25% P | 314 | 117 | 95.8 | 37.5 | 87.3 | 36.0 | 10.5 | 41.5 | 2.3 |
| Median | 399 | 168 | 133 | 107 | 124 | 57.5 | 17.5 | 67.5 | 70.0 |
| 75% P | 503 | 282 | 348 | 172 | 313 | 202 | 79.5 | 109 | 126 |
| Max.  | 526 | 294 | 414 | 213 | 333 | 249 | 90.0 | 140 | 140 |
| p     | 0.026 | >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.
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.

Acknowledgments

We thank Kim Theuerkauf for collecting SIRS cases and Lisa Held for collecting control horses and establishing RIs for the Multiplate analyzer (Roche) for Warmbloods. We also thank the laboratory technicians for their assistance in analyzing blood samples.

Declaration of conflicting interests

Material for the testing of platelet function with the Multiplate analyzer (Roche) was partly provided free of charge from the former manufacturer Dynabyte. This company had no influence on the design of the study; the collection, management, analysis or interpretation of the data; or the preparation of the manuscript. None of the authors of this manuscript has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the manuscript.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Carolin Ehrmann https://orcid.org/0000-0003-4526-2779

References

1. Adamzik M, et al. Whole blood impedance aggregometry as a biomarker for the diagnosis and prognosis of severe sepsis. Crit Care 2012;16:R204.
2. Ahmadi CE, et al. Assessment of platelet activation in several different anticoagulants by the Advia 120 hematology system, fluorescence flow cytometry, and electron microscopy. Thromb Haemost 2003;90:940–948.
3. Akca S, et al. Time course of platelet counts in critically ill patients. Crit Care Med 2002;30:753–756.
4. Arman M, et al. Amplification of bacteria-induced platelet activation is triggered by FcγRIIA, integrin αIIbβ3, and platelet factor 4. Blood 2014;123:3166–174.
5. Balk RA. Systemic inflammatory response syndrome (SIRS): where did it come from and is it still relevant today? Virulence 2014;5:20–26.
6. Bampalis VG, et al. Why and how to eliminate spontaneous platelet aggregation in blood measured by multiple electrode aggregometry. J Thromb Haemost 2012;10:1710–1714.
7. Baurand A, et al. Desensitization of the platelet aggregation response to ADP, differential down-regulation of the P2Y1 and P2cycy receptors. Thromb Haemost 2000;84:484–491.
8. Berkman N, et al. EDTA-dependent pseudothrombocytopenia, a clinical study of 18 patients and a review of the literature. Am J Hematol 1991;36:195–201.
9. Bone RC, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest 1992;101:1644–1655.
10. Bonelli F, et al. Plasma procalcitonin concentration in healthy horses and horses affected by systemic inflammatory response syndrome. J Vet Intern Med 2015;29:1689–1691.
11. Burkett BN, et al. Effects of firocoxib, flunixin meglumine, and phenylbutazone on platelet function and thromboxane synthesis in healthy horses. Vet Surg 2016;45:1087–1094.
12. Carroll CL, Huntington PJ. Body condition scoring and weight estimation of horses. Equine Vet J 1988;20:41–45.
13. Clark SR, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. Nat Med 2007;13:463–469.
14. Daniel AJ, et al. Concentrations of serum amyloid A and plasma fibrinogen in horses undergoing emergency abdominal surgery. J Vet Emerg Crit Care (San Antonio) 2016;26:344–351.
15. Davies GR, et al. The role of whole blood impedance aggregometry and its utilisation in the diagnosis and prognosis of patients with systemic inflammatory response syndrome and sepsis in acute critical illness. PLoS One 2014;9:e108589.
16. Elzey BD, et al. Cooperation between platelet-derived CD154 and CD4+ T cells for enhanced germinal center formation. J Leukoc Biol 2005;78:80–84.
17. Engel J, et al. Impedanzaggregometrisch bestimmte Thrombozytenfunktion bei klinisch unauffälligen Ponys [Determination of platelet function by impedance aggregometry in healthy ponies]. Abstract presented at: 25. Jahrestagung der FG “Innere Medizin und klinische Labordiagnostik” der DVG (InnLab), 03./04; February 2017; Göttingen, Germany.
18. Epstein KL, et al. Thrombelastography in horses with acute gastrointestinal disease. J Vet Intern Med 2011;25:307–314.
19. Gader AGMA, et al. The ultrastructure of camel blood platelets: a comparative study with human, bovine, and equine cells. Platelets 2008;19:51–58.
20. Grove EL, et al. Effect of platelet turnover on whole blood platelet aggregation in patients with coronary artery disease. J Thromb Haemost 2011;9:185–191.
21. Held LV. Ermittlung von Referenzintervallen der Thrombozytenfunktion mittels Multiplate® Analyzer beim klinisch unauffälligen Warmblutpferd [Determination of reference intervals for platelet function on the Multiplate Analyzer in healthy Warmblood horses]. Dissertation. Justus Liebig University, 2018. German.

22. Hooijberg EH, et al. Diagnostic and predictive capability of routine laboratory tests for the diagnosis and staging of equine inflammatory disease. J Vet Intern Med 2014;28:1587–1593.

23. Hübers E, et al. Thrombopenie beim Pferd [Thrombocytopenia in horses]. Tierarztl Prax Ausg G Grosstiere Nutztiere 2018;46:73–79. German.

24. Jenne CN, et al. Neutrophils recruited to sites of infection protect from virus challenge by releasing neutrophil extracellular traps. Cell Host Microbe 2013;13:169–180.

25. Kim HK, et al. Prognostic value of platelet indices as determined by Advia 120 in patients suspected of having disseminated intravascular coagulation. Int J Lab Hematol 2008;30:117–123.

26. Lambert JL, et al. Association of presence of band cells and toxic neutrophils with systemic inflammatory response syndrome and outcome in horses with acute disease. J Vet Intern Med 2016;30:1284–1292.

27. Levi M. Platelets at a crossroad of pathogenic pathways in sepsis. J Thromb Haemost 2004;2:2094–2095.

28. Macey MG, et al. Use of mean platelet component to measure platelet activation on the Advia 120 haematology system. Cytometry 1999;38:250–255.

29. McDonald B, et al. Intravascular neutrophil extracellular traps capture bacteria from the bloodstream during sepsis. Cell Host Microbe 2012;12:324–333.

30. McMorran BJ, et al. Platelets kill intraerythrocytic malarial parasites and mediate survival to infection. Science 2009;323:797–800.

31. Montrucchio G, et al. Mechanisms of the priming effect of low doses of lipopolysaccharides on leukocyte-dependent platelet aggregation in whole blood. Thromb Haemost 2003;90:872–881.

32. Moreau D, et al. Platelet count decline: an early prognostic marker in critically ill patients with prolonged ICU stays. Chest 2007;131:1735–1741.

33. Moritz A. Der Einsatz lasergestützter Multiparameter-Hämatologysysteme in der Veterinärmedizin [The use of automated laser-based multiparameter hematology systems in veterinary medicine]. Habilitation. Justus Liebig University Giessen, 2000. German.

34. Moritz A, et al. Evaluation of flow cytometric and automated methods for detection of activated platelets in dogs with inflammatory disease. Am J Vet Res 2005;66:325–329.

35. Pedersen SB, et al. Evaluation of aspirin response by Multiplate whole blood aggregometry and light transmission aggregometry. Platelets 2009;20:415–420.

36. Peerschke EI, et al. Platelet activation by C1q results in the induction of alpha IIb/beta 3 integrins (GPIIb-IIIa) and the expression of P-selectin and procoagulant activity. J Exp Med 1993;178:579–587.

37. Prins M, et al. Stability and reproducibility of Advia 120-measured red blood cell and platelet parameters in dogs, cats, and horses, and the use of reticulocyte haemoglobin content (CH) in the diagnosis of iron deficiency. Tijdschr Diergeneesk 2009;134:272–278.

38. Reinhart K, et al. Prävention, Diagnose, Therapie und Nachsorge der Sepsis. Erste Revision der S2k-Leitlinien der Deutschen Sepsis-Gesellschaft e.V. (DSG) und der Deutschen Interdisziplinären Vereinigung für Intensiv- und Notfallmedizin (DIVI) [Prevention, diagnosis, treatment, and follow-up care of sepsis. First revision of the S2k Guidelines of the German Sepsis Society (DSG) and the German Interdisciplinary Association for Intensive and Emergency Care Medicine (DIVI)]. Anaesthetist 2010;59:347–370. German.

39. Roscher K. Thrombozyten der Equiden – ein unterschätzter Biomarker? [Platelets in equids—an underrated biomarker?]. Habilitation. Justus Liebig University Giessen, 2018. German. [cited 2020 Dec 2]. http://geb.uni-giessen.de/geb/volltexte/2019/14904/pdf/RoscherKatja_2019_07_01.pdf

40. Roscher KA, et al. Inhibition of platelet function with clopidogrel, as measured with a novel whole blood impedance aggregometer in horses. Vet J 2015;203:332–336.

41. Roy M-F, et al. Prognostic value and development of a scoring system in horses with systemic inflammatory response syndrome. J Vet Intern Med 2017;31:582–592.

42. Russwurm S, et al. Platelet and leukocyte activation correlate with the severity of septic organ dysfunction. Shock 2002;17:263–268.

43. Schwarz BC, et al. Diagnostic value of the neutrophil myeloperoxidase index in horses with systemic inflammation. Vet J 2012;191:72–78.

44. Segura D, et al. Association between trends in clinical variables and outcome in horses with systemic inflammatory response syndrome. J Vet Intern Med 2016;30:581–588.

45. Segura D, et al. Mean platelet component as an indicator of platelet activation in foals and adult horses. J Vet Intern Med 2007;21:1076–1082.

46. Smyth SS, et al. Platelet functions beyond hemostasis. J Thromb Haemost 2009;7:1759–1766.

47. Steverink PJGM, et al. Laboratory and clinical evaluation of a chromogenic endotoxin assay for horses with acute intestinal disorders. Vet Q 1994;16:117–121.

48. Toth O, et al. Multiple electrode aggregometry: a new device to measure platelet aggregation in whole blood. Thromb Haemost 2006;96:781–788.

49. Tridente A, et al. Association between trends in clinical variables and outcome in intensive care patients with faecal peritonitis: analysis of the GenOSept cohort. Crit Care 2015;19:210.

50. van Deventer SJ, et al. Endotoxaemia: an early predictor of septicemia in febrile patients. Lancet 1988;1:605–609.

51. Verschoor A, et al. A platelet-mediated system for shuttling blood-borne bacteria to CD8α+ dendritic cells depends on glycoprotein GPIb and complement C3. Nat Immunol 2011;12:1194–1201.

52. Whitworth NH, et al. An investigation into the effects of bacterial lipopolysaccharide on human platelets. Eur J Haematol 1989;43:112–119.

53. Zelmanovic D, et al., inventors. Automated method and device for identifying and quantifying platelets and for determining platelet activation state using whole blood samples. United States patent US 5817519 A. 1998 Oct 06.