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Association of Presence of Band Cells and Toxic Neutrophils with Systemic Inflammatory Response Syndrome and Outcome in Horses with Acute Disease

J.L. Lambert, N.J. Fernandez, and M.-F. Roy

Background: Band cells and toxic neutrophils are thought to indicate acute and severe systemic illness but no studies have investigated their importance in adult equine emergency admissions.

Objectives: The objective of this study was to evaluate the association of band cells and toxic neutrophils with systemic inflammatory response syndrome (SIRS) and outcome in horses.

Animals: One hundred and five adult horses admitted on emergency basis to a private primary referral practice.

Methods: Prospective observational study with sample collection. All horses admitted on emergency basis over the study period were included in the study if they had a CBC and a blood smear collected on admission.

Results: The detection of band neutrophils on admission was associated with the SIRS status of the horse (RR = 2.80; 95% CI = 1.57–4.99; $P < .001$) and with poor outcome (RR = 1.88; 95% CI: 1.05–3.37; $P = .038$). Similarly, the presence of a neutrophil toxic grade higher than 3 was associated with SIRS (RR = 1.71; 95% CI: 1.03–2.82; $P = .034$) and death (RR = 2.34; 95% CI: 1.22–4.50, $P = .007$). The information gained from the blood smear review could not be captured by looking at only the WBC or neutrophil count.

Conclusions and Clinical Importance: The microscopic review of blood smears from critically ill horses to detect band cells or neutrophil toxic change is valuable and could help in assessing disease severity and prognosis in adult equine emergency admissions.

Key words: Blood smear; Prognosis; Survival.

The storage pool of neutrophils can be quickly depleted if tissue demand for neutrophils is high.[1] In such cases, particularly in conditions associated with an acute systemic inflammatory response syndrome (SIRS), band neutrophils, immature in morphology and potentially function, can be released from the marrow.[2,3] While little is known about the functional capacity of immature neutrophils in vivo, ex vivo studies have shown that these cells might have reduced capability to manage infection compared to their more mature counterparts because of reduced migration in response to chemoattractants, decreased production of reactive oxygen species, and decreased phagocytic and bactericidal activity.[2,4,5] The clinical significance of such altered innate immune function of immature neutrophils remains unclear, however, both hospitalized dogs[6] and cats[1] are more likely to die if they presented with a degenerative left shift (DLS), suggesting a link between an increased proportion of circulating band neutrophils and poor outcome.

Abbreviations:

95% CI	95% confidence interval
DLS	degenerative left shift
GI	gastrointestinal
RR	relative risk
SIRS	systemic inflammatory response syndrome
WBC	white blood cell

Acute systemic inflammatory or infectious processes, while associated with the release of band cells from the bone marrow, can also induce toxic change in neutrophils. These are defined as a collection of cytoplasmic alterations resulting from accelerated production in the bone marrow, including cytoplasmic basophilia, cytoplasmic vacuolation, Döhle bodies, and toxic granulation.[3,7,8] Neutrophil toxic change might be an early indicator of acute systemic illness and has been associated with disease severity, case fatality, hospitalization duration, and cost of treatment in dogs.[7] Similarly, in cats, the detection of toxic neutrophils is associated with disease severity, duration of hospitalization, and treatment cost.[8]

To our knowledge, no studies have been conducted that have specifically evaluated band neutrophils and toxic change in adult equine emergency admissions and therefore, the value of the microscopic review of peripheral blood smear in adult horses is unknown. Additionally, the microscopic evaluation of the blood smear is

From the Department of Veterinary Clinical and Diagnostic Sciences, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB (Lambert, Fernandez, Roy).

The cases and samples used in this study were collected from Moore Equine Veterinary Center, North Clinic, 260048A Writing Creek Crescent, Rocky View County, AB T4A 0M9. The blood smears review, data analysis and manuscript preparation were done at the Department of Veterinary Clinical and Diagnostic Sciences, Faculty of Veterinary Medicine, University of Calgary, 3330 Hospital Drive, NW, Calgary, AB, T2N 4N1.

Some of these results were presented at the XVII International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD) in Saskatoon, June 2015.

Corresponding author: M.-F. Roy, Department of Veterinary Clinical and Diagnostic Sciences, Faculty of Veterinary Medicine, University of Calgary, HRIC GA12, 3330 Hospital Drive, NW, Calgary, AB T2N 4N1; e-mail: mfroy@ucalgary.ca.

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frequently underutilized in private veterinary clinics that have in-house hematology analyzers, potentially leading to loss of clinically valuable information.[9] The objective of this study was therefore to determine the clinical value of microscopic review of the peripheral blood smear in adult equine emergency admissions. More specifically, we aimed to determine if the presence of band neutrophils and toxic change detected on admission to a private primary referral center was associated with disease severity and outcome.

Methods

Case Selection, Data and Sample Collection

From June 2012 to May 2014, as part of a prospective study on adult (>1 year old) equine emergency admissions to a private primary referral practice, clinicians and clinic staff were encouraged to prepare a blood smear using blood collected at the time of admission for a CBC. Blood samples were collected by jugular venipuncture directly into potassium EDTA tubes[a] and processed within 30 minutes. CBCs were performed on an in-house automated hematology analyzer.[b] Simultaneously, a blood smear was prepared and air dried. Case-related information was collected from the horse record and included signalment, history, physical examination findings on admission, diagnostic test results, treatment, final diagnosis, and outcome. For analysis, horses that were euthanized because of poor prognosis and horses that died were treated as the same nonsurvivor group. The study protocol was approved by the Veterinary Science Animal Care Committee of our institution. Signed owner or agent consent was obtained at the time of admission.

Preparation and Microscopic Review of Blood Smears

Blood smears were stained with a modified Wright-Giemsa stain using an automated slide stainer.[c] A board-certified clinical pathologist (N.J. Fernandez), who was blinded to the clinical status and outcome of the horses, performed the microscopic blood smear evaluation. Smear evaluation focused on the detection of band neutrophils and toxic change (Fig 1). When band neutrophils were present, a 100-cell leukocyte differential count was performed.

Grading of toxic change was adapted from a previous study in cats,[8] on a modified scale of 0–9 (Table 1). The toxic grade was determined based on both the severity of morphologic change and approximate percentage of cells affected. The presence of Döhle bodies, cytoplasmic basophilia, and cytoplasmic vacuolation was each graded as mild, moderate, or marked, and then adjusted for the percentage of cells affected (<10, 10–30, or >30%). This was done as previously published,[8] with the following exceptions: mild vacuolation was defined as observation of 1–2 small cytoplasmic vacuoles and moderate cytoplasmic vacuolation was the observation of 3 or more vacuoles. Toxic granulation and giant neutrophils were not seen in our patient population and thus were not included in the grading scheme. The overall toxic grade was the sum of the 3 scores for each morphologic feature. A toxic grade of 0–3 was considered to be mild and clinically irrelevant, whereas a toxic grade of 4 or more was considered to reflect clinically relevant toxic change.

SIRS Criteria

Based on available admission data, horses were categorized as SIRS or non-SIRS cases. Horses were categorized as SIRS cases if they met 2 or more of the following criteria, adapted for horses from the human literature:[10] heart rate >50 bpm, temperature above or below 37.0–38.5°C, respiratory rate >20 bpm and WBC count above or below 5.0–12.5 $\times$ 10^9 cells/L. If a horse met only one or none of these criteria, it was considered a non-SIRS case.

Statistical Analysis

Case fatality rates and proportion of cases with band neutrophils or significant toxic change are presented as percentage. Individual values for the band neutrophil count and toxic grade on admission are presented as scatter plots with median and interquartile range. Differences between SIRS and non-SIRS or survivors and nonsurvivors for the admission band neutrophil count or toxic grade were assessed using the Mann-Whitney test. Band neutrophils, toxic grade, WBC, and neutrophil count were also treated as dichotomous variables and horses were grouped within the following categories: bands or no bands; clinically relevant (4–9) or clinically irrelevant toxic grade (0–3); normal (within the reference interval) or abnormal (below or above the reference interval) WBC or neutrophil count. The relationship between band neutrophils, toxic grade, and WBC or neutrophil count as well as differences in SIRS or survival proportions for grouping variables (bands, toxic grade, WBC, and neutrophil count) were compared using the Fisher's exact test and are reported as relative risk (RR) with 95% confidence intervals (CIs) and associated P value. The survival proportions during hospitalization according to the presence of band neutrophils or a clinically relevant toxic grade on admission was plotted on Kaplan-Meier curves and compared using the log-rank test. The chi-square test for trends was used to test for an association between increasing mortality rates and increasingly more severe findings on the blood smear as well as on the WBC and neutrophil count. For all statistical analyses, a 2-sided P value of less than .05 was taken to indicate statistical significance. All statistical analyses were performed using Prism version 6.[d]

Results

Study Population

From June 2012 to May 2014, 479 adult horses were admitted as emergencies. Of these, 109 cases had both a CBC and a blood smear of sufficient quality collected on admission. Four cases (all surgical colic cases) were euthanized after refusal of surgical treatment because of financial constraints despite what appeared to be a fair prognosis and were removed from further analysis, leaving 105 cases for the present study. The 105 blood smears evaluated were collected from 104 individual horses, with one horse being admitted twice, 6 months apart. The study population consisted of 60 geldings, 41 mares, and 3 stallions. The horses' age ranged from 1 to 25 years with a median age of 10 years. The age was not recorded for one horse. Breeds included Quarter horses (n = 43), Thoroughbreds (n = 17), Warmbloods (n = 16), Paints (n = 5), ponies and miniatures (n = 5) and one each of the following breeds: Andalusian, Arabian, Belgian, Caspian, Clydesdale, Fjord, Friesian, and Tennessee Walker. Nine horses were of mixed breeds and 1 horse did not have its breed recorded. The overall survival rates for the 105 emergency admissions studied was 67% (n = 70). Among the 90 cases for which enough information was recorded on admission to allow classification as SIRS or non-SIRS, 47% (n = 42) were SIRS cases. By comparison, the overall survival rate for all adult emergencies admitted over the same

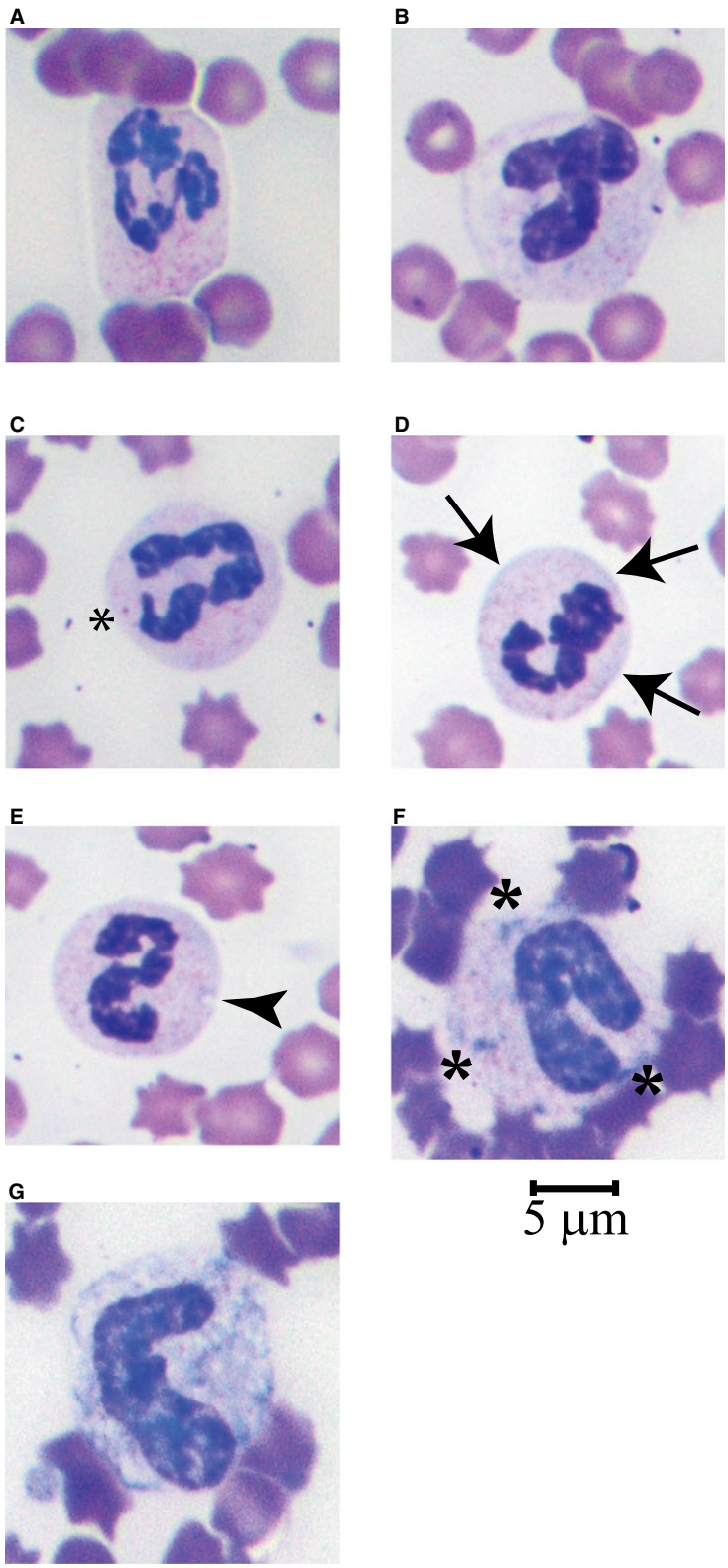

Fig 1. Mature, band, and toxic neutrophils in equine peripheral blood. (**A**) Mature segmented neutrophil with tight nuclear constrictions and darkly staining, clumped chromatin. Pink granules were typical with our stain protocol. (**B**) Band neutrophil. Note lack of nuclear constrictions and smooth parallel nuclear walls. (**C**–**E**) Low grade toxic change. (C) Neutrophil with single small Döhle body (asterisk). (D) Neutrophil with focal peripheral cytoplasmic basophilia (arrows). (E) Neutrophil with a single clear vacuole (arrowhead). (**F**–**G**) High grade toxic change. (F) Neutrophil band. Note several large irregular Dohle bodies (asterisks). (G) Neutrophil band. Note diffuse foamy cytoplasm and cytoplasmic basophilia. Modified Wright-Giemsa stain; bars = 5 μm (original magnification 1000×).

Table 1. Criteria for grading toxic change. The final toxic grade is the sum of the grades for each criterion, for a maximum grade of 9. A grade of 0–3 was considered mild and clinically irrelevant while a grade of 4–9 was considered clinically relevant.

	% Cells Affected		
Severity of Morphologic Change	<10	10–30	>30
Döhle bodies			
Mild	1	1	1
Moderate	1	1	2
Marked	2	2	3
Cytoplasmic basophilia			
Mild	1	1	2
Moderate	2	2	3
Marked	2	3	3
Cytoplasmic vacuolation			
Mild	1	1	2
Moderate	2	2	3
Marked	2	3	3

period was 79%, with a SIRS rate of 34%, indicating that the collection of CBC and blood smears on admissions was biased toward horses with more severe disease compared to the whole emergency population.

Band Cells, Toxic Change, WBC, and Neutrophil Count

The final diagnostic categories, outcome, and rates of cases presenting with band neutrophils or significant toxic change are shown in Table 2. Fifty percent (n = 53) of the cases had band neutrophils detected on their blood smear whereas 55% (n = 58) had an admission toxic grade higher than 3. The highest percentages of cases with bands or clinically relevant toxic change were found in surgical colics and colitis whereas the lowest percentages were found in medical colics. Seventy-seven percent (n = 41) of the cases that presented with bands also had a toxic grade higher than 3 and there was an association between these 2 variables such that horses presenting with bands were 2.4 times more likely to also have a toxic grade of 4–9 compared to horses with no bands (Table 3).

Among the horses with abnormal (increased [n = 44] or decreased [n = 22]) total WBC or neutrophil count on admission, 33% (n = 22) did not have bands or clinically important toxic change on their blood smear. Moreover, 68% (n = 26) of the horses with total WBC and neutrophil counts within reference intervals had bands or a toxic grade higher than 3 detected on examination of their blood smear. The results of the total WBC and neutrophil counts obtained from the in-house hematology analyzer were, therefore, not associated with the presence of bands or clinically relevant toxic change (Table 3).

Association between Blood Smear Findings and SIRS

Of the 105 cases, 90 had sufficient admission information recorded to allow classification as SIRS (47%; n = 42) or non-SIRS (53%; n = 48) cases. The SIRS cases had significantly higher numbers of band neutrophils and higher toxic grade compared to the non-SIRS cases (Fig 2). Additionally, there was a significant association between the presence, on admission, of any band neutrophils or a toxic grade of 4–9 and the SIRS status of the horse (Table 4).

Table 2. Diagnostic category, outcome, and rates of band cells and neutrophil toxic change for the study population.

	Total (n)	Case Fatality Rate (%)	Band Cells > 0 (%)	Toxic Grade 4–9 (%)
All cases	105	33.3	50.5	55.2
All GI cases	67	33	55	60
Medical colics	23	0	26	39
Surgical colics	22	64	64	77
Colitis	14	50	79	64
Musculoskeletal-skin	17	18	35	47
Other systems	21	48	48	48

GI, gastrointestinal.

Other systems: Cardiovascular (n = 1), Liver (n = 3), Multisystemic (n = 1), Neurologic (n = 1), Ophthalmologic (n = 1), Reproductive (n = 2), Respiratory (n = 7), Urogenital (n = 3) and Unknown (n = 2).

Table 3. Relationship between band cells, neutrophil toxic change, WBC, and neutrophil count.

Admission Variable	Total (n)	Toxic Grade 4–9 (%)	RR	95% CI	*P* Value
Band cells > 0	53	77	2.37	1.56–3.59	<.001
No Band cells = 0	52	33			
		Toxic grade 4–9 or bands (%)			
Abnormal count	66	67	0.97	0.74–1.28	1.000
Normal count	38	68			

Abnormal count: abnormal WBC or neutrophil count. Normal count: normal WBC and neutrophil count.

Association between Blood Smear Findings and Outcome

Horses that died had higher numbers of band neutrophils and higher toxic grade on admission compared to horses that survived (Fig 3). The RR of death for a horse admitted with any number of bands was 1.9 times higher compared to horses admitted with no bands (Table 5). The toxic grade was similarly associated with a poor outcome in this population with horses admitted with a toxic grade of 4–9 being 2.3 times more likely to die compared to horses that presented with a toxic grade of 0–3. On the other hand, there was no association between an abnormal total WBC or neutrophil count and outcome in this population (Table 5). Survival analysis confirmed that horses admitted with band

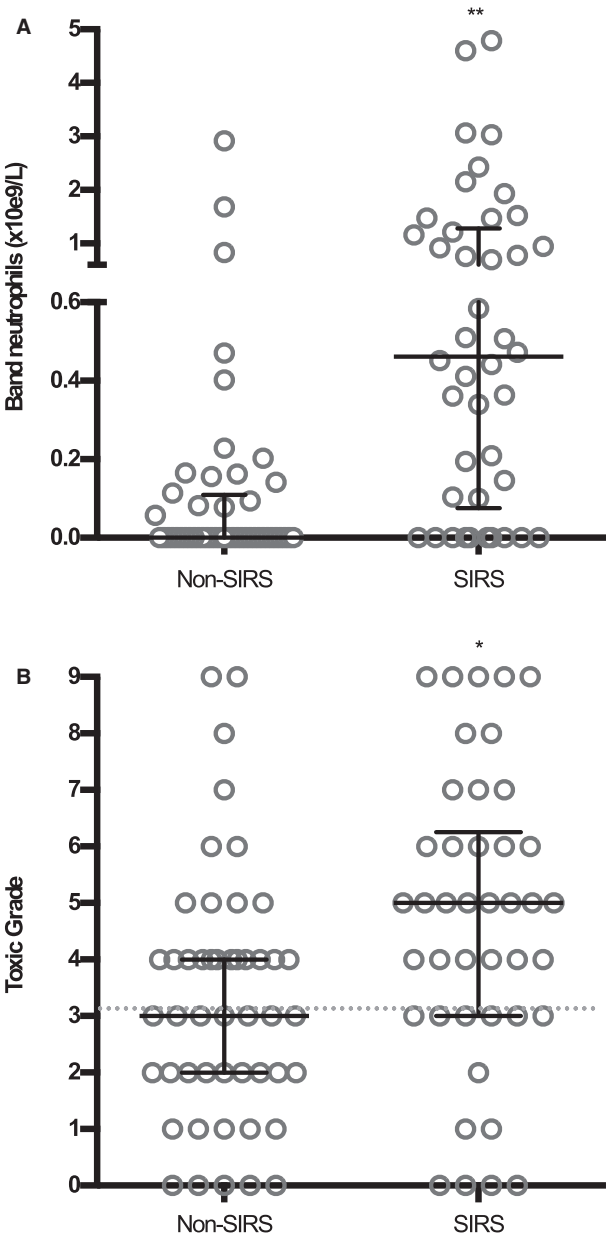

Fig 2. Band cells (**A**) and neutrophil toxic grade (**B**) in non-systemic inflammatory response syndrome (SIRS) (n = 48) and SIRS (n = 42) cases. SIRS cases had higher numbers of band neutrophils and higher toxic grade compared to non-SIRS cases. A toxic grade of 0–3 was considered mild and clinically irrelevant while a toxic grade of 4–9 was considered clinically relevant. Each individual horse value is shown, with median and interquartile range. Mann-Whitney test. $^{**}P < .001$; $^{*}P = .004$.

neutrophils or a toxic grade higher than 3 were more likely to die (Fig 4).

Finally, we detected a significant increase in case fatality rates according to the number of abnormalities detected on the blood smear (Fig 5A). Horses with no band neutrophils and a toxic grade of 0–3 on admission had a lower case fatality rate (17%) compared to horses that had one (22%) or both (48%) of these abnormalities detected. A similar, significant increase in case fatality rates was also found when combining the results of WBC and neutrophil count with the finding of the blood smear evaluation (Fig 5B). The lowest case fatality rate was found in horses presenting with normal WBC count, normal neutrophil count, and normal blood smear findings (8%). The case fatality rate then increased for horses with abnormal WBC or neutrophil count (23%), abnormal blood smear findings (31%), and abnormalities in both the cell counts and blood smear (48%).

Table 4. Association between admission band cells or toxic neutrophils and the presence of systemic inflammatory response syndrome (SIRS).

Admission Variable	Total (n)	SIRS (%)	RR	95% CI	*P* Value
Band cells > 0	48	67	2.80	1.57–4.99	<.001
Band cells = 0	42	24			
Toxic grade 4–9	51	57	1.71	1.03–2.82	.034
Toxic grade 0–3	39	33			

Discussion

Our results demonstrate that important, clinically relevant information can be obtained from the microscopic review of blood smears and that this information cannot be captured by simply looking at the WBC or neutrophil count. More specifically, we found that band neutrophils and clinically relevant toxic change (grade 4–9) are associated with disease severity (SIRS status) and poor prognosis in a population of adult horses admitted for emergency care to a primary, private, referral practice.

This study specifically investigated and documented the significance of band neutrophils and toxic change in a population of adult equine emergency admissions. In horses, very few studies have investigated the importance of bands, WBC or neutrophil count and only as part of broader risk factors studies in specific equine populations that investigated many different variables. For instance, a study investigating factors associated with outcome in colic cases found no association between WBC count and survival.[11] Similarly, in a study looking at mortality risk factors in colitis cases, WBC count was not different between survivors and nonsurvivors, although there were more neutropenic horses among the nonsurvivors.[12] Neutropenia is a predictor of death after colic surgery[13] and in neonates admitted to a neonatal intensive care unit.[14] For band neutrophils, only a few reports have described an association between band cells and increased risk of death, again as part of broad risk factors studies in specific equine populations, for example in recumbent horses[15] or in horses with acute idiopathic enterocolitis.[16] In a different study of acute colitis cases,[12] the number of bands was not found to be different between survivors and nonsurvivors. To our knowledge, there have been no studies done looking at the association between toxic neutrophils and outcome in adult horses.

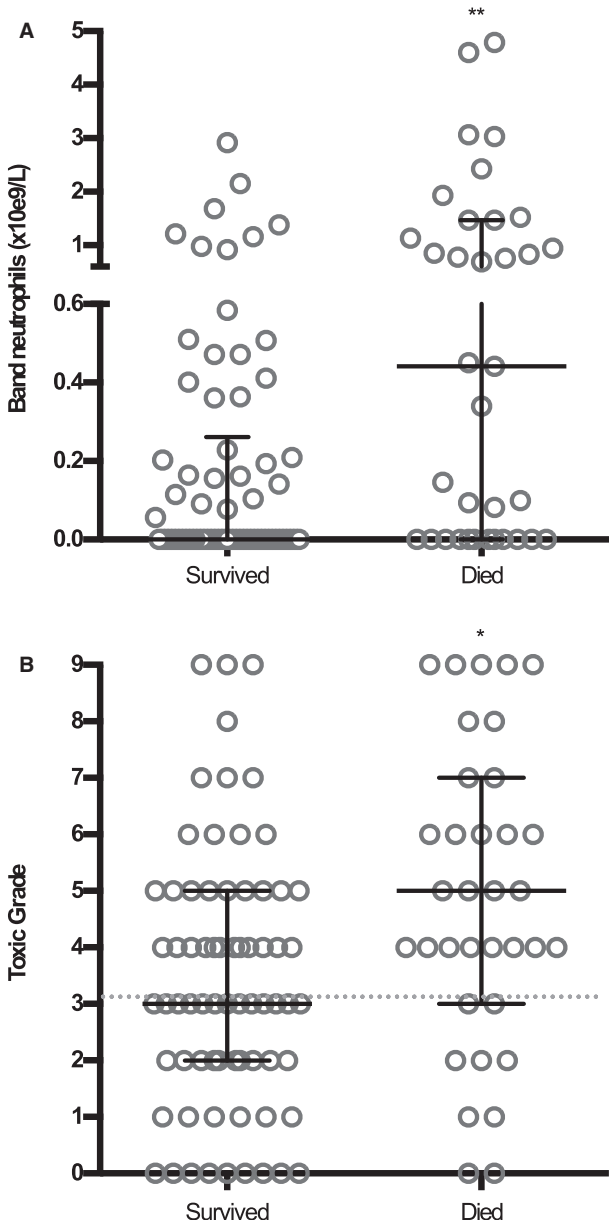

Fig 3. Band cells (**A**) and neutrophil toxic grade (**B**) in survivors (n = 70) and nonsurvivors (n = 35). Nonsurvivors had higher numbers of band neutrophils and higher toxic grade compared to survivors. A toxic grade of 0–3 was considered mild and clinically irrelevant while a toxic grade of 4–9 was considered clinically relevant. Each individual horse value is shown, with median and interquartile range. Mann-Whitney test. $^{**}P = .003$; $^{*}P = .005$.

Table 5. Relative risk of death for horses admitted with band cells, toxic neutrophils or abnormal WBC, and neutrophil count.

Admission Variable	Total (n)	Case Fatality Rate (%)	RR	95% CI	*P* Value
Band cells > 0	53	43	1.88	1.05–3.37	.038
Band cells = 0	52	23			
Toxic grade 4–9	58	45	2.34	1.22–4.50	.007
Toxic grade 0–3	47	19			
Abnormal count	66	39	1.66	0.87–3.17	.133
Normal count	38	24			

Abnormal count: abnormal WBC or neutrophil count. Normal count: normal WBC and neutrophil count.

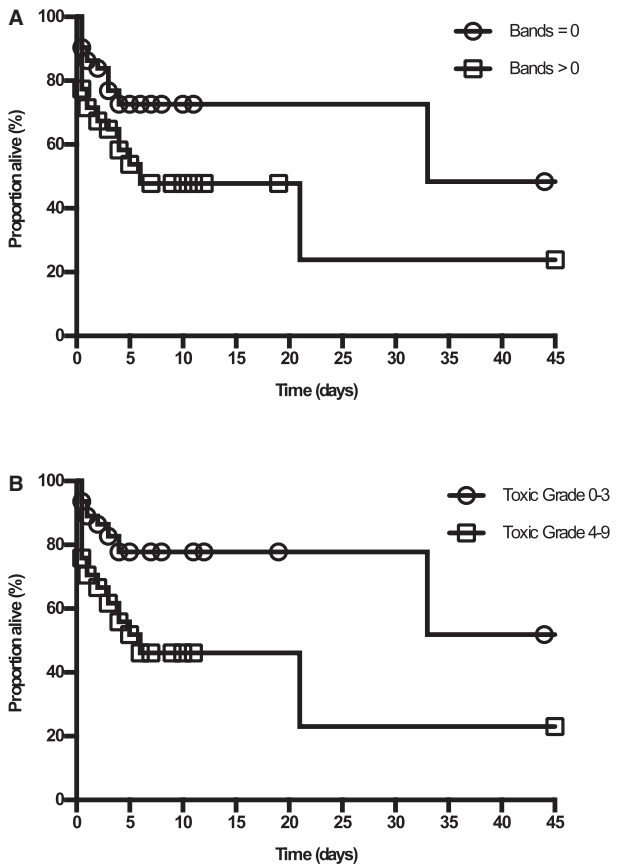

Fig 4. Kaplan-Meier survival curves, comparing (**A**) horses admitted with no band cells (n = 52) to horses with any number of band cells (n = 53) and (**B**) horses admitted with a neutrophil toxic grade of 4–9 (n = 58) to horses with a toxic grade of 0–3 (n = 47). The survival curves are significantly different with (A) $P = .036$, (B) $P = .006$ (Log-rank test). The graphs show survival up to 45 days. One case that was discharged after 108 days in the hospital is not shown on this graph but was included in the analysis.

Despite limited information in horses, the significance of band neutrophils and toxic change has been recently investigated in both cats and dogs. In ill cats admitted to a teaching hospital, the presence of a left shift was significantly associated with mortality, as was neutropenia or neutrophilia occurring with a left shift and toxic change.[17] This is similar to our results, although we found the presence of any band cells at all to be associated with increased risk of death, not just the presence of a left shift (ie, band neutrophil numbers exceeding the upper reference interval). Further, cats with DLS were more likely to die or be euthanized in hospital than cats without DLS.[1] These cats were also more likely to demonstrate marked toxic change. Only 5 of our cases presented with DLS, thus we were unable to

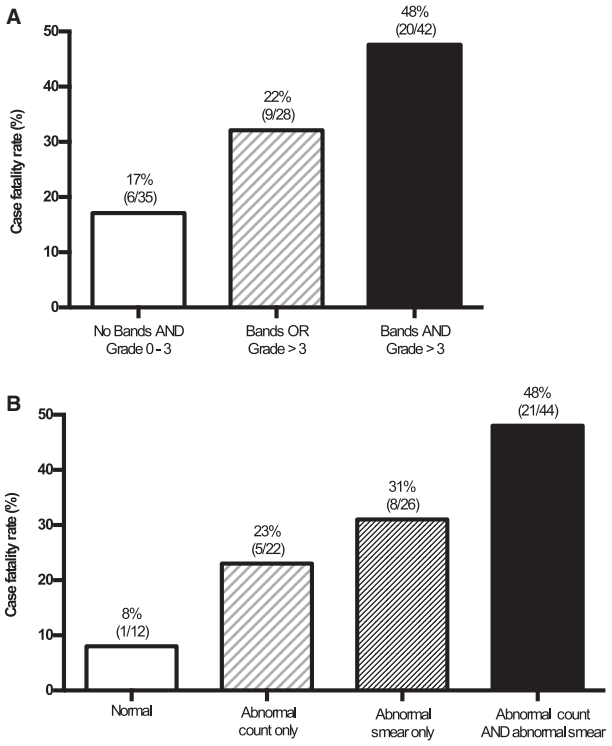

Fig 5. Increasing case fatality rates with increasing severity of (**A**) blood smear findings and (**B**) WBC and neutrophil count as well as blood smear findings. (A) There is a significant increase in case fatality rates according to the number of abnormalities found on the blood smear. Chi-square test for trend: $P = .005$ (chi-square = 8.003, $df = 1$). (B) There is a significant increase in case fatality rates according to the number of abnormalities found in both the WBC and neutrophil count and the blood smear. Chi-square test for trend: $P = .004$ ($\chi^2 = 8.461$, $df = 1$). The case fatality rate is shown above each bar with the number of death and total number of cases shown in parenthesis for each category of cases. (B) Normal: Normal WBC and neutrophil count, no band cells and toxic grade 0–3. Abnormal count only: Abnormal WBC or neutrophil count, no band cells and toxic grade 0–3. Abnormal smear only: Normal WBC and neutrophil count, band cells or toxic grade 4–9. Abnormal count AND smear: Abnormal WBC or neutrophil count and band cells or toxic grade 4–9.

assess the significance of DLS in horses because of low numbers of cases. Another study showed that hospitalized cats with toxic change had a longer duration of hospitalization and increased treatment cost compared to cats without toxic change.[8] There was no significant difference in mortality between cats with and without toxic change, and between cats with mild toxic change and cats with moderate to marked toxic change. This is in contrast to our study, however, the authors did not group toxic change by grade into "clinically irrelevant" and "clinically relevant" as we did. Findings are similar in dogs: hospitalized dogs with DLS were more likely to die or be euthanized in hospital than dogs without DLS.[6] In dogs with toxic change, case fatality, duration of hospitalization, and treatment cost were increased, and in contrast to cats, case fatality rate increased significantly with increasing toxic grade.[7] It should be noted that dogs have the largest neutrophil storage pool, thus the presence of a neutropenia or DLS is more significant than in other domestic mammals because it reflects an intense peripheral demand for neutrophils that has depleted a large storage pool. Because of the smaller size of their storage pools, neutropenia and DLS in cats and horses can develop with lesser inflammatory stimuli, and in horses in particular might not suggest the same severity of illness as in dogs.[18]

Regarding the body system involved, the greatest proportion of emergency admissions in our study population was for gastrointestinal diseases. This likely reflects the high incidence of colics in equine emergency admissions in general. Both surgical colics and colitis cases had a high prevalence of band neutrophils and clinically relevant toxic change, which probably reflects that acute gastrointestinal illnesses in horses are often associated with SIRS and are, therefore, likely to lead to the release of band cells and toxic neutrophils from the bone marrow. In cats and dogs with toxic change, a significantly higher prevalence of peritonitis was found,[7,8] however, toxic change was also significantly associated with inflammation of other body systems such as pneumonia in cats and pyometra in dogs. As well, systemic illnesses were frequently associated with toxic change, for example sepsis, shock, and metabolic disorders in cats, and septicemia, immune-mediated hemolytic anemia, and disseminated intravascular coagulation in dogs. Findings in cats and dogs with DLS are similar, with DLS significantly associated with septic peritonitis and pyothorax in cats,[1] and with septic peritonitis, parvoviral enteritis, gastroenteritis excluding parvoviral enteritis, pancreatitis, and immune-mediated neutropenia in dogs.[6]

In human medicine, an increased percentage of band neutrophils is also often viewed as a marker of acute and severe infection[19] and has been associated with sepsis[20] as well as early deterioration and poor outcome in septic patients.[21] Indeed, band neutrophils were even included in the original human SIRS definition published in 1992.[10] While we did not include band neutrophils in our definition of equine SIRS, review of our dataset revealed that including the presence of any band neutrophils in the SIRS criteria would have led to the reclassification of 7 out of 47 non-SIRS patients into the SIRS category, including 5 survivors and 2 nonsurvivors. Whether including band neutrophils in the equine SIRS definition would improve its clinical performance in defining SIRS in horses is unclear at this point, however, this addition should probably be considered.

In this study, we are proposing a grading scale for toxic change in equine neutrophils. This scale was modified from previously published tables grading toxic change in dogs and cats.[7,8] There is surprisingly little information in the veterinary literature on how to accurately and reliably grade toxic change. We modified the published grading scheme slightly by removing the category of "giant toxic neutrophils" because none were seen in our equine population, and by changing the definition for mild cytoplasmic vacuolation. Their definition of mild cytoplasmic vacuolation as "loss in cytoplasm clarity and neutral-stained granules" was not

observed in our smears, possibly because of a different staining protocol. Previous studies[7,8] considered the presence of small cytoplasmic vacuoles as a toxic change. These vacuoles can sometimes form with prolonged storage of blood in EDTA, but rarely occur in fresh samples,[8] and our samples were processed within 30 minutes, thus making it unlikely that the vacuoles seen were artifact.

Overall, this grading scheme was found to be very sensitive in detecting toxic change in equine neutrophils. A toxic grade of 0–3 identified low numbers of neutrophils with very mild toxic changes that were considered clinically irrelevant and would be unlikely to be flagged as toxic change by a hematology technician or clinical pathologist in a reference laboratory. For this reason, only a grade of 4 or greater was considered to reflect clinically important toxic change.

There are a few potential limitations to our study. First, our population size was relatively small and the final diagnoses quite heterogenous. While our intention was to prospectively collect blood smears and CBCs upon emergency admission, because of financial, time, and personnel availability constraints within a private practice, only approximately 1 in 5 emergencies had both a CBC and blood smear collected on admission. Although we were able to find a significant association for band neutrophils and toxic change with SIRS and outcome for the whole population, a finer analysis of the various final diagnostic categories was more difficult given the lower number of cases in each. Comparison of survival rates and SIRS rates between our cases and the whole emergency population admitted over the same period shows a slight bias toward collecting blood smears and CBCs on more severe cases, where possibly the clinicians could justify the cost and value of a CBC to the owner. Finally, our results might not be applicable to other adult equine emergency populations and ideally, such a study should be replicated in a different and larger population and a finer analysis within diagnostic categories performed to see if band neutrophils and toxic change have the same prognostic values in different equine populations.

Despite these limitations, the results of our study are highly relevant to equine clinicians. Many private practices are now equipped with in-house hematology analyzers, however, direct blood smear examination is not routinely performed. Our study demonstrates that valuable and clinically important information is missed when the blood smear is not reviewed for the presence of band neutrophils or toxic change. The grading system we are reporting in the present study should help in standardizing the assessment of neutrophil toxic change. While one may argue that the WBC and neutrophil count can be used in lieu of the microscopic review of the blood smear, our results show that there is no association between an abnormal WBC or neutrophil count and the presence of bands or toxic change, and that many horses with a normal WBC or neutrophil count have circulating band neutrophils and moderate to marked neutrophil toxic change. Moreover, an abnormal WBC or neutrophil count was not associated with outcome in this population, whereas the presence of band neutrophils or clinically relevant toxic change was associated.

In conclusion, our study demonstrates the potential diagnostic and prognostic value of reviewing blood smears for the presence of band cells and toxic neutrophils and reinforces the fact that this simple procedure should be done, especially when the disease severity status of the horse is unclear.

Footnotes

[a] Monoject EDTA (K3) 0.04 mL 7.5% Solution, Covidien, Saint-Laurent, QC, Canada

[b] IDEXX ProCyte Dx Hematology Analyzer, IDEXX Laboratories, Markham, ONT, Canada

[c] Hema-Tek 2000 automated stainer, Bayer, Toronto, ONT, Canada.

[d] Prism 6 for Mac OS X, GraphPad software Inc., La Jolla, CA

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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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