Association Between Necropsy Evidence of Disseminated Intravascular Coagulation and Hemostatic Variables Before Death in Horses With Colic

C. Cesarini, M. Cотовйо, J. Ríos, L. Armengou, and E. Jose-Cunilleras

Background: Disseminated intravascular coagulation (DIC) is frequent in horses with severe gastrointestinal disorders. Postmortem studies have found fibrin microthrombi in tissues of these horses, but studies relating these histopathological findings with antemortem hemostatic data are lacking.

Hypothesis: Antemortem classification of coagulopathy is related to the presence and severity of fibrin deposits observed postmortem in horses with severe gastrointestinal disorders.

Animals: Antemortem hemostatic profile data and postmortem tissue samples (kidney, lung, liver) from 48 horses with colic.

Methods: Tissue samples were stained with phosphotungstic acid hematoxylin and immunohistochemical methods for histological examination. A fibrin score (grades 0–4) was assigned for each technique, tissue and horse, as well as the presence or absence of DIC at postmortem examination. D-dimer concentration, prothrombin time (PT), activated partial thromboplastin time (aPTT), and antithrombin (AT) activity, as well as the clinicopathological evidence of coagulopathy, were determined from plasma samples collected 0–24 hours before death or euthanasia. Histologic and clinicopathologic data from the same horses were compared retrospectively.

Results: No association was found between antemortem classification of coagulopathy and postmortem diagnosis of DIC based on tissue fibrin deposition. None of the hemostatic parameters was significantly different between horses with or without postmortem diagnosis of DIC. There was no association between horses with fibrin in tissues or different cut-offs for D-dimer concentration and postmortem evidence of DIC.

Conclusions and Clinical Importance: Abnormalities of the routine clotting profile, including D-dimer concentration, were not useful in predicting histologic evidence of DIC at necropsy in horses with severe gastrointestinal disorders.

Key words: Coagulopathy; D-dimer; Equine; Thrombi.
ing horses with colic, a high percentage of affected horses had fibrin microthrombi in different tissues, and the lung was most commonly affected. This marked fibrin deposition in horses was consistent with capillary microthrombosis and DIC, which has been associated with multiorgan failure syndrome. Results from these studies suggested that both phosphotungstic acid hematoxylin (PTAH) and immunohistochemical (IHC) staining methods were reliable in demonstrating fibrin deposits in the capillary meshwork of horses at risk of DIC, with IHC being considered more sensitive. Clarifying the possible relationship between the ante-mortem diagnosis of DIC with its definitive postmortem confirmation could help identify horses with increased risk of organ failure, allowing early treatment and hopefully improving prognosis in patients at risk of uncompensated coagulopathy. In human medicine, abnormalities in the routine coagulation profile were not found to be useful in predicting cases with postmortem evidence of DIC. Additional tests that specifically detect lytic products of deposited cross-linked fibrin, such as D-dimer determination, may be more accurate in identifying patients that have evidence of DIC at necropsy. To the authors’ knowledge, no published studies in horses have analyzed coagulation data before death to determine whether any differences exist between patients with or without evidence of DIC at necropsy. Thus, the main objectives of this study were: (1) to determine the association between the ante-mortem hemostatic profile, including D-dimer concentration, and the presence of fibrin deposition in different tissues of horses with gastrointestinal disorders at postmortem examination; and (2) to evaluate the association between the ante-mortem diagnosis of DIC and the presence of postmortem evidence of DIC. Secondarily, the possible influence of diagnosis (ie, type of colic) on the previously mentioned variables was studied.

Materials and Methods

Animals

Antemortem hemostatic data and postmortem tissue samples from 48 horses with GI problems were retrospectively compared. Tissues and blood samples had been obtained in the context of 2 larger prospective studies on coagulopathies in horses with GI problems. Horses included in this study had died or had been euthanized because of poor prognosis or financial constraints a few hours after admission to the Unitat Equina, Fundació Hospital Clinic Veterinari, Universitat Autònoma de Barcelona, Spain. Owners had given their consent of sample collection for research purposes. To ensure that results of hemostatic variables were as representative as possible of the situation found at necropsy, only horses in which the last blood sample available had been obtained within 24 hours before death were included in the study.

Horses included in this study were classified into 4 groups according to their final diagnoses, based on clinical and postmortem examination findings: peritonitis group, ischemic group, enterocolitis group, and obstructive group. The peritonitis group included horses with severe inflammatory lesions in the abdominal cavity secondary to gastric or intestinal ruptures, as well as septic peritonitis (presence of intra- and extracellular bacteria in peritoneal fluid) caused by bowel devitalization, intra-abdominal abscesses and other causes. The ischemic group included horses with strangulating GI lesions, such as large colon torsion, small intestinal volvulus, epiploic foramen entrapment, inguinal hernia, and intussusception. Horses in the ischemic group were euthanized because of poor prognosis or economical constraints shortly after admission or during laparotomy. The enterocolitis group included horses with acute inflammation of the small intestine (duodenojejunitis), large intestine (colitis, typhlitis) or both, diagnosed by compatible clinical signs (eg, gastric reflux, diarrhea), clinicopathological data (eg, hypoalbuminemia, acid-base, and electrolyte imbalances) as well as results of complementary examinations (abdominal ultrasound examination, peritoneal fluid analysis). Finally, the obstructive group included all horses with nonstrangulating, noninflammatory disorders of the GI tract, without signs of intestinal devitalization, such as impactions and displacements. Horses with enterocolitis or obstructive problems included in this study did not respond to medical treatment, showed increasing abdominal pain, distension or both, and were euthanized because of poor prognosis or economic limitations. Because of the small number of horses in each of the last 2 groups (enterocolitis and obstructive), these were grouped together as “Other diagnoses” for statistical purposes.

Sampling and Processing of Tissues

Tissue samples from kidneys, lungs, and liver were taken during laparotomy (after euthanasia) or at necropsy (<24 hours after death) for further histopathologic study. Pulmonary samples were obtained from the caudal lung lobe, and the renal and hepatic samples were taken randomly from the kidney and liver. Samples were fixed in 10% neutral formalin, processed in an automated tissue processor, and embedded in paraffin. Three-millimeter paraffin sections were cut with a microtome and routinely stained with PTAH by standard procedures. For IHC, 3-mm sections were mounted on silane-coated slides and dried. Immunolabeling with polyclonal rabbit antihuman fibrinogen/fibrin antibody was performed with a streptavidin biotin complex method as described elsewhere.

The presence of microvascular fibrin deposits in glomerular and alveolar capillaries and in hepatic sinusoids was recorded separately for each sample stained with PTAH and IHC, and extravascular fibrin deposits were not considered to avoid possible misclassification of fibrin of inflammatory origin.

Regarding the PTAH-stained slides, the amount of fibrin present in kidney, lung, and liver sections was graded from 0 to 4 as described previously. Briefly, 0 meant the absence of fibrin, 1 meant the presence of fibrin deposits (occupying <50% of the capillary vessel) in some glomeruli (<50%), 2 meant the presence of partial deposits in all glomeruli, 3 meant the presence of large quantities of fibrin (fibrin deposits occupying 50–100% of the whole diameter of the capillary vessel) in all glomeruli, and 4 meant the presence of fibrin thrombi in glomerular capillaries and in noncapillary vessels. For each renal section, 10 fields were examined, and the average value from all of them was calculated. A 0 to 4 scoring system also was used for lung and liver sections, following previous descriptions. For lung and liver sections, 20 fields were examined, and the number of fields positive for the presence of intravascular fibrin in alveolar capillaries and hepatic sinusoids was counted, respectively. Sections in which none of the fields were positive received a 0 score. Sections in which 1–6 of 20 fields were positive received a score of 1; sections in which 7–12 of 20 fields were positive received a score of 2; and sections in which 13–19 of 20 fields were positive received a score of 3. Finally, sections in which 20 of 20 fields were positive received a score of 4.

For grading the amount of fibrin deposits in the 3 tissues when using the IHC method, slides were assigned a fibrin score (from 0
Hemostatic Variables and Postmortem Fibrin Deposits

Blood Sampling and Measured Parameters

Blood samples had been obtained by jugular venipuncture between 0 and 24 hours before death or euthanasia and placed into 3.8% sodium citrated tubes (1 : 9). They were centrifuged at 1000 x g for 15 minutes and plasma was frozen at −70°C until analysis. Plasma D-dimer concentration, PT, aPTT, and AT were determined in duplicate for all samples. Plasma D-dimer concentration was determined using an immunoturbidimeter (Miniquant-Biopool) with commercial reagents and controls (Miniquant Biopool). Prothrombin time and aPTT were determined with a semiautomated coagulometer with commercial reagents and controls. Antithrombin activity was determined in a semiautomatic analyzer with a chromogenic kit that measures residual thrombin activity after adding the patient sample to a known quantity of thrombin. Antithrombin activity was expressed as % of that of normal human pooled plasma.

For ante-mortem DIC classification and based on previous studies and reference values in our laboratory, the cut-off values established were as follows: D-dimer concentration >1000 ng/mL, PT >15 seconds, aPTT >65 seconds, AT <140%. If all 4 parameters determined were within the defined reference range, horses were classified as grade 0; grade 1 horses were those that had at least 1 tissue sample scored 2 and no tissue samples with higher scores; grade 2 horses were those that had at least 1 tissue sample scored 2 and no tissue samples with higher scores; and grade 1 horses were those that had at least 2 tissue samples scored 1 and no tissue samples of higher score. The remainder of the horses were classified as grade 0.

To summarize all of the information about fibrin deposition in tissues, each horse was assigned a score (0–4), depending on the amount of fibrin thrombi observed in kidney, lung, and liver. Briefly, a horse was classified as grade 4 when it had at least 1 tissue sample with a 4 score; grade 3 horses were those with at least 1 tissue sample scored 3 and no samples with higher scores; horses classified as grade 2 were those that had at least 1 tissue sample scored 2 and no tissue samples with higher scores; and grade 1 horses were those that had at least 2 tissue samples scored 1 and no tissue samples of higher score. The remainder of the horses was not included because of its low sensitivity in assessment of coagulopathies in horses with colic.

Statistical Analysis

Results were expressed as frequencies and percentages for categorical variables. For continuous variables, median (25th [P25] and 75th [P75] percentiles) was used as measurement of precision of results. The relationship between analytical results and evaluation of histopathology was assessed by estimating odds ratios (OR) and 95% confidence intervals (CI) from logistic regression models. Cut-off values used in the diagnosis of DIC were used for each of the measured parameters also were tested in logistic regression models. An additional cut-off value of >4000 ng/mL, previously shown to be associated with outcome, was used for plasma D-dimer concentrations. Based on previous studies and considering that a mild fibrin deposition (grade 1) may not be sufficient to cause organ dysfunction, a minimum cut-off score of 2 for fibrin deposits was used to classify the study population in order to evaluate the risk of organ failure. Adjusted ORs were calculated to assess the influence of diagnosis in the evaluation of analytical results. All analyses were performed using a statistical software package and a type I error of 5% was considered in all statistical analyses.

Results

Animals

Sex distribution of the 48 horses was 16 females (33%), 14 stallions (29%), and 15 geldings (31%). No sex information was available for 3 horses. The median age [P25, P75] of the population was 9 years [5, 14]. Twenty (42%) horses were Andalusians, 7 (15%) cross-bred, 4 (8%) Westfalians, 3 (6%) Friesians, and the other 8 (17%) were a mix of other breeds. No breed information was available for 6 horses. There were 19 horses in the peritonitis group, 16 in the ischemic group, 4 in the enterocolitis group, and 9 in the obstructive group. Four horses (8%) died due to severity of the primary GI disease or its complications. The other 44 horses (92%) were euthanized, 38 (86%) because of poor prognosis and 6 (14%) because the owners’ financial limitations did not allow adequate treatment (eg, surgery).

The median [P25, P75] time of blood sampling before death or euthanasia was 0 hours [4, 13] in the peritonitis group (19/48 horses), 0 hours [0, 0.6] in the ischemic group (16/48 horses), 22 hours [7, 39] in the enterocolitis group (4/48 horses), and 6 hours [4, 8] in the obstructive group (9/48 horses). The median [P25, P75] time of blood sampling before death or euthanasia was 4 hours [0, 6]. Blood samples were taken immediately before death or euthanasia in 19 of the 48 horses, within 12 hours before death or euthanasia in 44 of the 48 horses, and only in 2 horses was this time 24 hours. The median [P25, P75] time of death or euthanasia to postmortem examination was 11 hours [1, 18].

Association Between Hemostatic Profile and Fibrin Deposition in Different Tissues

None of the 4 hemostatic parameters measured (D-dimer, PT, aPTT, AT) was significantly different...
between horses with or without fibrin deposits in kidney, lung, or liver detected by either of both methods (PTAH and IHC) used in this study. No significant differences were detected when chosen cut-off values for each parameter were used to classify the study population.

Results of the association between the hemostatic profile and histopathological evidence of DIC are shown in Table 1. None of the 4 hemostatic parameters measured was significantly different between horses that were classified postmortem as having DIC and those that were not. Similarly, no significant differences were detected when horses with tissue scores $\geq 2$ were compared to those with lower scores or when cut-off values for each parameter were used to classify the population.

Association Between Antemortem and Postmortem Diagnosis of Coagulopathy

Regarding antemortem classification of coagulopathy, of the 48 horses, 5 were classified as having no coagulopathy, 25 as having activation of coagulation (1-2 hemostatic alterations), and 11 were considered to be in subclinical DIC. Seven horses could not be classified because of a lack of hemostatic data for at least 1 parameter. The effect of ventral laparotomy on plasma D-dimer concentrations could not be controlled in all cases, but, only 15/48 horses in this study underwent ventral laparotomy and in only 5/15 was antemortem DIC identified after surgery.

Of the 48 horses studied and following classification based on results of the PTAH staining for fibrin deposition, 9 horses were in DIC postmortem (microthrombi in $\geq 2$ organs), and 39 horses were not in DIC postmortem. Based on results of the IHC method for detecting fibrin deposition, 7 horses were in DIC postmortem and 38 horses were classified as not in postmortem DIC. Three horses could not be classified because of a lack of histopathologic data for at least 1 tissue.

Results of the relationship between antemortem and postmortem DIC classification are shown in Table 2. No association was found between antemortem classification of DIC and postmortem classification based on tissue fibrin deposition. Furthermore, no significant differences were detected when horses with tissue scores $\geq 2$ were compared to those with lower scores or when the proposed cut-off values (those used to diagnose DIC for each parameter and a D-dimer concentration of $\geq 4000$ ng/mL) were used to classify the population.

Influence of Diagnosis (Type of Colic)

No differences in any of the previous associations were detected when the logistic regression models were adjusted for diagnosis (type of colic).

Discussion

We did not find significant differences in the results of any of the individual coagulation variables measured

| Table 1. Results of logistic regression models including hemostatic parameters and postmortem histopathological data for each of the two methods used to detect fibrin deposition in tissues. |
|--------------------------------------------------|
| Hemostatic data YES NO | Postmortem DIC | Tissue score | Hemostatic technique Tissue PTAH staining Tissue IHC technique |
| PT (sec) | 13.8 [13.1; 14.8] | 14.9 [13.1; 19.8] | 0.384 | 14.3 [13.4; 15.8] | 15.1 [13.6; 19.8] | 0.331 |
| aPTT (sec) | 59 [51; 63] | 55 [52; 68] | 0.235 | 52 [43; 62] | 60 [54; 68] | 0.099 |
| AT (%) | 179 [149; 197] | 163 [140; 194] | 0.655 | 137 [116; 190] | 174 [134; 198] | 0.742 |
| D-dimer (ng/ml) | 3,378 [742; 5,000] | 2,603 [1,054; 4,418] | 0.829 | 2,777 [1,235; 4,418] | 2,729 [742; 4,418] | 0.942 |
| PT, prothrombin time; PTTa, partial activated thromboplastin time; AT, antithrombin; DIC, disseminated intravascular coagulation; PTAH, phosphotungstic acid hematoxylin stain; IHC, immunohistochemistry.
Table 2. Results of logistic regression models including different cut-offs tested for plasmatic D-dimer concentration, antemortem classification of coagulopathy, and its association with postmortem classification of DIC, for each of the two methods to detect fibrin deposition in tissues.

| Hemostatic Variables and Postmortem Fibrin Deposits | Tissue PT/HA staining | Tissue score | Postmortem DIC | Tissue score | OR (95% CI) | p-value |
|-----------------------------------------------------|-----------------------|-------------|----------------|-------------|-------------|----------|
| **Hemostatic Variables**                            |                       |             |                |             |             |          |
| PTAH, phosphotungstic acid hematoxylin stain, IHC, immunohistochemistry |             |             |                |             |             |          |
| Horses considered to be in subclinical DIC           |                       |             |                |             |             |          |
| Antemortem classification of DIC                    |                       |             |                |             |             |          |
| Normal                                              |                       |             |                |             |             |          |
| Activated coagulation                               |                       |             |                |             |             |          |
| Subclinical DIC                                     |                       |             |                |             |             |          |
| DIC dissection with intravascular coagulation       |                       |             |                |             |             |          |
| D-dimer, PT, aPTT, and AT results and the severity of antemortem coagulopathy were not related to the presence and severity of fibrin deposits observed postmortem, neither by PTAH stain nor by IHC methods. In another study, no differences in either the prevalence or magnitude of abnormality of any individual coagulation variable (PT, aPTT, thrombin time, fibrinogen, FDPs, and platelet count) were found between intensive care unit patients with and without evidence of DIC at necropsy, using the same criteria for histopathologic diagnosis of DIC used in the our study. Furthermore, another study that tried to find useful variables to predict DIC in patients with hemostatic abnormalities concluded that the presence of pulmonary microthromboemboi was not significantly associated with a clinical diagnosis of DIC.

Specific markers of degradation of cross-linked fibrin, such as D-dimer concentration, have been suggested to improve antemortem detection of those patients with postmortem evidence of DIC. Nevertheless, based on results of the present study, D-dimer concentration was not found to be more useful than other hemostatic parameters to detect patients with fibrin microthrombi in ≥2 organs at necropsy. D-dimer concentrations were increased not only in human patients with pulmonary microthromboemboli but also in those without embolism.

Horses considered to be in subclinical DIC antemortem were not classified more often as having postmortem evidence of DIC, based on tissue fibrin deposition scoring. This finding could have been caused by misclassification of horses based on inaccurate antemortem or postmortem criteria. The antemortem criteria used in this study were the classical laboratory criteria for diagnosing DIC in veterinary medicine, considering DIC when ≥3 abnormalities were detected in a selection of hemostatic parameters. In our study, of 11 horses considered to have subclinical DIC based on antemortem criteria, 5 did not have microthrombi in any of the tissues collected when examined by IHC, and 3 had no microthrombi when examined by H&E. These findings suggest that abnormalities of the coagulation profile usually taken to indicate the presence of DIC may not be specific for this condition.

Regarding postmortem DIC classification, general agreement does not exist about the number of thrombi and the number of affected organs necessary to diagnose DIC, but criteria used in our study already have been used in similar studies in human medicine. Furthermore, variables such as antemortem treatment, time from the occurrence of DIC to death and time from death to autopsy are known to affect thrombus lysis in human medicine.
admission, before receiving any treatment other than IV fluids and sedatives. Other factors (eg, postmortem fibrinolysis, amount of histologic material sampled, staining techniques used, missing microthrombi despite accurate microscopic examination) have created problems interpreting necropsy material in human medicine and may have contributed to failure of diagnosing postmortem DIC in some horses of our study.25 Because thrombi may no longer be detectable if tissue fixation is not performed rapidly, postmortem examination in humans is not considered a definitive diagnostic tool for this syndrome.9

The results of our study should be interpreted taking into account some limitations. The size of the studied population was small and mainly determined by the necessity of matching laboratory and histopathologic data retrospectively from the same horses. Selecting animals to minimize the time between blood collection and death (<24 hours) and necropsy decreased the number of suitable patients even more. Time of sampling relative to death or euthanasia may have affected the ability to detect horses that fulfilled criteria for the diagnosis of subclinical DIC on a time point closer to death or euthanasia. In addition, the effect of ventral laparotomy on plasma D-dimer concentrations could not be controlled in all cases. Furthermore, the delay between death and euthanasia may have contributed to missing fibrin thrombi because of postmortem lysis. Nevertheless, the human medical literature suggests that large microthrombi are more common in patients who had died within <24 hours25, which was the case for most of the horses in our study.

In conclusion, in horses with severe GI disorders, abnormalities of routinely measured coagulation variables (D-dimer, PT, aPTT, AT) do not seem to be useful in predicting cases in which histologic evidence of DIC will be found at necropsy. Based on the results of our study, antemortem plasma D-dimer concentrations in horses with colic are not associated with the presence of postmortem fibrin thrombi in lung, kidney, or liver.

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

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

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Footnotes

a Trinity Biotech, Wicklow, Ireland
b Stago ST4, Stago Diagnostics, Asnières-Sur-Seine, France
c Boehringer Mannheim, Mannheim, Germany
d Cobas-Bio, Roche, Basel, Switzerland
e STA Antithrombin III, Stago Diagnostics, Asnières-Sur-Seine, France
f Armengou L, Monreal L, Segura D, et al. Plasma D-dimers in horses with colic. Proceedings of the 8th International Equine Colic Research Symposium; 2005 pp. 2–5; Quebec, Canada

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In memoriam of Dr. Lluis Monreal Bosch.
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