Equine

Evaluation of coagulation and fibrinolysis in horses with atrial fibrillation

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OBJECTIVE
To evaluate horses with atrial fibrillation for hypercoagulability; plasma D-dimer concentrations, as a marker of a procoagulant state; and a relationship between coagulation profile results and duration of atrial fibrillation or presence of structural heart disease.

DESIGN
Case-control study.

ANIMALS
Plasma samples from 42 horses (25 with atrial fibrillation and 17 without cardiovascular or systemic disease [control group]).

PROCEDURES
Results of hematologic tests (ie, plasma fibrinogen and D-dimer concentrations, prothrombin and activated partial thromboplastin times, and antithrombin activity) in horses were recorded to assess coagulation and fibrinolysis. Historical and clinical variables, as associated with a hypercoagulable state in other species, were also recorded.

RESULTS
Horses with atrial fibrillation and control horses lacked clinical signs of hypocoagulation or thromboembolism. Compared with control horses, horses with atrial fibrillation had significantly lower antithrombin activity. No significant differences in plasma fibrinogen and D-dimer concentrations and prothrombin and activated partial thromboplastin times existed between horse groups. In horses with atrial fibrillation versus control horses, a significantly larger proportion had an abnormal plasma D-dimer concentration (10/25 vs 2/17), test results indicative of subclinical activated coagulation (18/25 vs 6/17), or abnormal coagulation test results (25/121 vs 7/85), respectively.

CONCLUSIONS AND CLINICAL RELEVANCE
Horses with atrial fibrillation did not have clinical evidence of a hypercoagulable state, but a higher proportion of horses with atrial fibrillation, compared with control horses, did have subclinical activated coagulation on the basis of standard coagulation test results. (J Am Vet Med Assoc 2016;248:201–206)

Atrial fibrillation is the most common clinically relevant and performance-limiting arrhythmia in horses.1 Atrial fibrillation is also the most common sustained arrhythmia in human beings.2 Humans in atrial fibrillation are in a hypercoagulable state3 that makes stroke and thromboembolism the major cause of morbidity and death in patients with this rhythm disturbance.4,5 The pathogenesis of the procoagulant state is multifactorial. The 3 proposed components (changes in vessel wall, blood flow, and blood constituents) of the Virchow triad for thrombogenesis change as a result of atrial tissue changes, endothelial damage and dysfunction, increased atrial size, and decreased atrial motion and inflammation, among other factors.6,7

ABBREVIATIONS
aPTT Activated partial thromboplastin time
IQR Interquartile range (25th to 75th percentile)
PT Prothrombin time

Collection of historical, clinical, and hematologic data is useful in determining the risk of thromboembolism.8 Influential historical and clinical factors include female sex, congestive cardiac failure, recent embolism, hypertension, diabetes, underlying heart disease (left ventricular dysfunction or hypertrophy), permanent atrial fibrillation (vs persistent or paroxysmal), duration of atrial fibrillation, spontaneous echocardiographic contrast observed in transesophageal echocardiogram,5,7,9 and recent cardioversion.10,11 The importance of various factors, predictive ability of scoring systems, and criteria for anticoagulation therapy has been thoroughly studied for humans. The CHA2DS2-VASc (ie, congestive heart failure, hypertension, age ≥ 75 years, diabetes mellitus, stroke or transient ischemic attack, vascular disease, age 65 to 74 years, and sex category) score is useful in human medicine; however, conflicting results regarding the accuracy of specific variables exist in the literature, possibly resulting from the differences between studies or methodologies.5 Atrial fibrillation without underlying cardiac disease (lone atrial

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fibrillation) in the young and fit is perhaps more common in horses than in humans. This and the less frequent comorbidities such as heart failure, diabetes, and hypertension in horses may account for the differences in thromboembolism development between these species.

Several clinicopathologic findings support a hypercoagulable state during and after atrial fibrillation. Some of the studied markers of a hypercoagulable state are fibrinogen, D-dimer, D-dimer von Willebrand factor, tissue plasminogen activator antigen, tissue plasminogen activator inhibitor, platelet activation, thrombin-antithrombin III complex, antithrombin activity, and P-selectin. Measurement of plasma D-dimer concentration is the single most useful clinicopathologic test to assess the risk of thrombogenesis in patients with atrial fibrillation. Thromboembolic events are described for horses with severe systemic disease, but not for horses with atrial fibrillation. Plasma D-dimer concentration is a sensitive marker of hypercoagulation in horses and is associated with various diagnoses and prognoses. The purposes of the study reported here were to determine whether a hypercoagulable state, assessed by means of clinicopathologic coagulation abnormalities, is present in horses with atrial fibrillation and whether plasma D-dimer concentration is the best marker of a procoagulant state in horses with atrial fibrillation. A second goal was to describe the coagulation profiles of horses with atrial fibrillation and their relationship to the duration of the arrhythmia and the presence of structural heart disease.

Materials and Methods

Study design

A prospective controlled study was designed to evaluate coagulation and fibrinolysis in horses with atrial fibrillation, compared with horses without cardiovascular or systemic disease. Data from standard coagulation tests to assess coagulation and fibrinolysis as well as historical and clinical variables reported to be associated with a hypercoagulable state in patients with atrial fibrillation in other species were recorded. The study was performed in compliance with institutional guidelines for research on animals, and owner consent was obtained for the use of the data.

Horses ≥ 2 years of age evaluated because of atrial fibrillation and horses evaluated because of another problem in which atrial fibrillation was found coincidentally were included in the study. Horses ≥ 2 years of age evaluated because of a problem that did not affect systemic health (eg, musculoskeletal, upper respiratory, or standardized treadmill evaluation) and in which signs suggestive of systemic disease, cardiovascular disease, systemic inflammation, or coagulopathy were not found were included in a control group.

Procedures

All horses with atrial fibrillation had a cardiovascular evaluation, following hospital standards that consisted of a physical examination, 12-lead ECG, and echocardiographic examination, including B-mode, M-mode, color flow, and continuous-wave Doppler evaluation of regurgitant jets or shunts when present. Horses that were cardioverted had echocardiography repeated following hospital protocols, with pulsed-wave tissue Doppler measurements of the left atrial wall velocity also performed. The control horses each had a general physical examination, and findings on cardiac auscultation were normal.

Historical, signalment, and clinical variables recorded for each horse included age, breed, sex, suspected duration of the arrhythmia, presence of underlying cardiac disease (vs lone atrial fibrillation), pharmacological versus electrical cardioversion (if treated), or signs of thromboembolism (eg, peripheral thrombosis, dyspnea, cough, epistaxis, signs of abdominal pain, neurologic signs, or postmortem findings when available). Underlying cardiac disease was defined as a cardiac abnormality reported to predispose horses to the development of atrial fibrillation, such as valvular or congenital heart disease causing atrial enlargement or areas of abnormal myocardial echogenicity. The suspected duration of the arrhythmia was defined as the time from a sudden decrease in performance or time from the last successful performance to initial evaluation. If a decrease in performance did not occur, the last cardiac auscultation for which results were normal was used to determine the suspected duration of the arrhythmia. The arrhythmia duration was classified as short if suspected to be ≤ 2 weeks and long if suspected to be > 2 weeks.

Blood samples were obtained by direct jugular venipuncture and placed in blood collection tubes containing sodium citrate. The plasma was separated shortly after collection and stored at -80°C until the time of analysis. Plasma fibrinogen concentration, plasma D-dimer concentration, PT, aPTT, and antithrombin activity were measured with compact hemostasis testing equipment and commercial reagents and controls. Fibrinogen concentration and PT were determined by use of a nephelometry method. D-dimer concentration was measured with an automated latex-enhanced immunoassay. Activated partial thromboplastin time was measured by adding a contact activator and calcium to citrated plasma and with automated hemostasis equipment. Antithrombin was measured with an automated chromogenic assay. Reference ranges used were those established by the laboratory for healthy horses. Activated coagulation was defined as the presence of ≥ 1 abnormal result for fibrinogen or D-dimer concentration; antithrombin, PT, or aPTT measurements; and subclinical disseminated intravascular coagulopathy as the presence of ≥ 3 abnormal results in the coagulation profiles as previously described.

Statistical analysis

Descriptive statistics were calculated for all continuous variables. Results were reported as median and IQR. Hematologic variables were treated as nonparametric data in consideration of the sample size and variability. Standard coagulation test results in horses with and without atrial fibrillation were compared by means of a Wilcoxon (Mann-Whitney) rank sum test. The proportion
of horses with atrial fibrillation and control horses with abnormal D-dimer concentrations, abnormal coagulation profiles, and abnormal coagulation parameters were compared by means of a χ² test. Results of standard coagulation tests in horses with underlying cardiac disease versus lone atrial fibrillation and in horses with short versus long duration of the arrhythmia were described, but statistical tests were not performed because of the small sample size. Statistical software was used for analysis. Values of P ≤ 0.05 were considered significant.

Results

Blood samples from 42 horses (25 horses with atrial fibrillation and 17 horses without cardiovascular or systemic disease [control group]) were obtained. Horses with atrial fibrillation had a median age of 10 years (IQR, 5 to 14 years). Horses with atrial fibrillation included 9 Standardbreds, 5 Thoroughbreds, 4 warmbloods, 3 draft horses, 1 Arabian, 1 Tennessee Walking Horse, 1 Quarter Horse, and 1 warmblood-Standardbred cross. Three stallions or colts, 17 geldings, and 5 mares or fillies had atrial fibrillation. None of the horses with atrial fibrillation had clinical signs of thromboembolism in their histories or on evaluation or during hospitalization. Seven horses with atrial fibrillation had underlying heart disease, and 18 horses did not. The underlying heart disease was described as moderate or severe valvular disease in 4 horses, focal abnormal myocardial echogenicity in 1 horse, congenital heart disease (tetralogy of Fallot) in 1 horse; and myocardial hypertrophy and pulmonary hypertension in 1 horse. Cardioversion was attempted in 16 horses and was successful in 15. Two horses were converted with transvenous electrical cardioversion and 13 with administration of quinidine sulfate via a nasogastric tube. One horse failed to respond to both electrical and chemical cardioversion. In the 9 horses for which cardioversion was not attempted, this was because of the presence of underlying heart disease, owner concerns about cost of the procedure, risks associated with cardioversion, or concerns about likelihood of recurrence. Twelve horses were classified as having a short arrhythmia duration, and 13 horses were classified as having a suspected long arrhythmia duration.

Control horses had a median age of 6 years (IQR, 3 to 11 years). The control group comprised 5 Standardbreds, 5 Thoroughbreds, and 7 warmbloods, which included 3 stallions or colts, 12 geldings, and 2 mares or fillies. The reason for evaluation of control horses was elective musculoskeletal procedures (n = 13 horses), upper airway evaluation (3), and standardized treadmill evaluation because of poor performance not attributable to cardiac disease (1).

Descriptive statistics for standard coagulation test results were summarized (Table 1). In the horses with atrial fibrillation, data for 4 horses were missing because of problems with samples or reagents. Antithrombin activity was significantly (P = 0.008) different between horses with atrial fibrillation and control horses. No significant differences were found between groups in fibrinogen concentration, D-dimer concentration, PT, and aPTT.

The distribution of horses with abnormal standard coagulation test results, activated coagulation, and D-dimer plasma concentration was summarized (Table 2). The proportion of horses with abnormal

| Table 1—Coagulation tests results in horses with atrial fibrillation and horses without cardiovascular or systemic disease (control group). |
|---------------------------------|-----------------|-----------------|-----------------|
| Variable                        | Horses with atrial fibrillation (n = 25) | Control group (n = 17) | Reference range |
| Fibrinogen (mg/dL)              | 250 (212–311.5) | 256 (219–279) | 150–375         |
| D-dimer (ng/mL)                 | 254 (206–601)  | 233 (208–293) | 39–409          |
| PT (s)                          | 11.3 (10.9–12.2)| 11.2 (10.7–11.7)| 9.0–12.1        |
| aPTT (s)                        | 45.5 (43.1–49.7)| 47.7 (45.6–49.7)| 34.3–55.3       |
| Antithrombin activity (%)       | 198 (184–222)* | 232 (202–256)* | 157–261         |

Values are median (IQR). *Values are significantly (P < 0.05) different between groups. In the horses with atrial fibrillation, 4 individual results were not obtained because of problems with samples or reagents.

| Table 2—Number of horses with abnormal D-dimer concentrations and test results indicative of subclinical activated coagulation as well as total number of all abnormal coagulation test results in horses with atrial fibrillation (subdivided as horses with underlying cardiac disease vs lone atrial fibrillation and short-term arrhythmia vs long-term arrhythmia) and control horses without cardiovascular or systemic disease. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Variable                        | Control group (n = 17) | AF group (n = 25) | AF and underlying cardiac disease | Duration of AF |
|                                | Yes (n = 7) | No (n = 18) | Yes (n = 7) | No (n = 12) | Short term (n = 13) | Long term (n = 13) |
| Abnormal D-dimer                | 2/17 (11.8) | 10/25 (40)* | 2/7 (28.6) | 8/18 (44) | 3/12 (25) | 7/13 (53.8) |
| Activated coagulation           | 6/17 (35) | 18/25 (72)* | 6/7 (85.7) | 12/18 (66.7) | 8/12 (66.7) | 10/13 (76.9) |
| Abnormal tests                  | 7/85 (8.2) | 25/121(20.7)* | 10/35 (28.6) | 15/86 (17.4) | 9/56 (16.1) | 16/65 (24.6) |

Results are given as total number (%). *Denotes significant differences (P < 0.05) in proportions, compared with the control group. AF = Atrial fibrillation.
D-dimer concentrations (2/17 in the control horses and 10/25 in horses with atrial fibrillation; \( P = 0.047 \)) and with activated coagulation (6/17 in the control horses and 18/25 in horses with atrial fibrillation; \( P = 0.018 \)) and the proportion of abnormal coagulation tests (7/85 in the control horses and 25/121 in horses with atrial fibrillation; \( P = 0.015 \)) were significantly larger in horses with atrial fibrillation than in the control horses. Two horses with atrial fibrillation and no control horses were classified as having subclinical disseminated intravascular coagulopathy.

**Discussion**

Results of the present study suggested that horses in atrial fibrillation are not in a clinical hypercoagulable state. No clinical signs of hypercoagulation or thromboembolism were detected in the horses of the present report and, to our knowledge, have not been found in horses with atrial fibrillation previously. However, our results suggested that subclinical activated coagulation exists in horses with atrial fibrillation. Antithrombin activity was significantly lower; and the proportion of horses with abnormal standard coagulation test results, subclinical activated coagulation, and high plasma D-dimer concentrations was significantly larger in horses with atrial fibrillation than in control horses.

The proportion of horses with abnormal D-dimer concentrations (10/25) was significantly higher in horses with atrial fibrillation than in control horses (2/17). However, a significant difference was not found in the plasma concentration of this marker of fibrinolysis between horses with atrial fibrillation and control horses. Therefore, the hypothesis that plasma D-dimer concentration would be the best marker of a procoagulant state in horses with atrial fibrillation was not proven. The conclusions regarding differences in plasma D-dimer concentrations should be interpreted with caution because the size of the study group coupled with the high variability in plasma D-dimer concentrations may have resulted in the study being underpowered. Taking into account the variability, a sample size of 376 horses in each group would have been needed to make calculations with a power of 80% by means of a Wilcoxon (Mann-Whitney) rank sum test with \( \alpha = 0.05 \) (2-sided test).

Reasons as to why a subclinical activated coagulation state in horses with atrial fibrillation does not result in clinically relevant thrombogenesis are uncertain. The magnitude of changes in coagulation test results was smaller in the study reported here for horses with atrial fibrillation, compared with that reported for humans with cardiac disease (in which hypercoagulation has been demonstrated) \(^{16,35-37}\) and in horses with colic or ill neonatal foals (in which a hypercoagulable state was considered clinically relevant). \(^{35,38}\) Perhaps the magnitude of the changes is not sufficient to cause thrombogenesis, suggesting that hypercoagulability is less likely to be a relevant problem in horses with atrial fibrillation in the absence of concomitant systemic disease. Studies of a larger group of horses with atrial fibrillation, including horses with comorbidities that would predispose to a hypercoagulable state such as systemic inflammation or sepsis, gastrointestinal disease, postoperative complications, neonatal sepsis, or equine herpesvirus-1 infection, \(^{39,33,35,38-43}\) are indicated to further investigate the importance of the subclinical activated coagulation state found in horses with atrial fibrillation. The effect of exercise on coagulation and fibrinolysis would also be interesting to investigate in horses with atrial fibrillation. \(^{16,44-46}\)

We studied the standard coagulation tests most often included in the clinical evaluation of horses. Prothrombin time measures the extrinsic and common pathways of the traditionally described coagulation cascade; whereas aPTT measures the intrinsic and common pathways. \(^{47}\) No significant differences existed between groups in terms of results of these classically used coagulation tests. D-dimer concentration is a marker of activated coagulation, fibrinolysis, and turnover of cross-linked fibrin associated with a procoagulant state in horses. \(^{32-47}\) D-dimer concentration is also the most commonly cited test as being useful in monitoring the risk of thromboembolism in humans with atrial fibrillation. \(^{18-22,27,28,48}\) Plasma D-dimer concentrations were more often outside the reference range in horses with atrial fibrillation, compared with control horses, but the magnitude of the change was small overall and was less than the threshold that has been suggested as clinically relevant in horses. \(^{33,47}\) Antithrombin activity was significantly lower in horses with atrial fibrillation, compared with control horses, although it was only low in 2 of 25 horses with atrial fibrillation. A decrease in antithrombin activity can be associated with inherited disorders, consumption, endotoxin inhibition, liver dysfunction, or protein loss \(^{49}\) and may occur because of consumption in humans with atrial fibrillation. \(^{25,28}\) The specific cause of the lower antithrombin activity was not evaluated in the study reported here, but it is plausible that the difference between groups was the result of consumption, similar to that reported in humans. It is a limitation of the study that results of other sensitive coagulation tests, such as thromboelastography, were not explored.

Atrial fibrillation is in and of itself a source of a hypercoagulable state in humans, \(^{35}\) and structural heart disease \(^{40}\) or signs of congestive heart failure \(^{2}\) have been shown to increase the risk of stroke in patients with atrial fibrillation. Other studies \(^{35}\) have been unable to show that organic heart disease was associated with abnormalities in D-dimer concentration; for some authors, the effect of structural heart disease on the hypercoagulable state remains controversial. \(^{4}\) In the present study, a higher proportion of horses with atrial fibrillation and underlying heart disease had standard coagulation test results and plasma D-dimer concentrations outside the reference range, compared with horses with only atrial fibrillation. Only 7 of 25 horses with atrial fibrillation also had underlying heart disease; therefore, differences in results between horses with and without underlying heart disease were not statistically tested.

The more common presence of lone atrial
fibrillation in horses, compared with humans, and the less frequent occurrence of comorbidities such as heart failure, diabetes, and hypertension may account for the differences in thrombogenesis between species.

The risk for thromboembolism is increased in human patients with nonparoxysmal atrial fibrillation and is associated with arrhythmia duration. Furthermore, some markers of a hypercoagulable state are affected by atrial fibrillation duration. Interestingly, the duration of atrial fibrillation is not a factor in the recommendations to implement anti-thrombotic therapy in patients with atrial fibrillation, and paroxysmal atrial fibrillation is seen more frequently in patients with stroke. In the present study, a higher proportion of horses with atrial fibrillation of long duration had standard coagulation test results and plasma D-dimer concentrations outside the reference range, compared with horses with atrial fibrillation of short duration. Differences in results between horses with atrial fibrillation of long duration versus horses with atrial fibrillation of short duration could not be tested statistically because of the small sample size.

The hypercoagulable state and risk of thromboembolism remain and can increase in humans in the immediate period after cardioversion (6 to 72 hours) as a result of atrial stunning, mobilization of thrombi in the left atrium, or comorbidities still affecting the hypercoagulable state. Various coagulation parameters may be altered for varying periods. Insufficient data existed to meaningfully report changes before and after cardioversion. This would be an interesting future study.

The present study had several limitations. The main limitation was the small sample size coupled with the intrinsic high variability of plasma D-dimer concentrations, which could make differences difficult to prove. The heterogeneity of the group regarding the presence of underlying heart disease, estimated duration of the arrhythmia in many cases, and lack of long-term follow-up are other limitations of this study.

In conclusion, horses in atrial fibrillation do not appear to have a clinically evident hypercoagulable state, but subclinical activated coagulation should be borne in mind when assessing these patients.

Acknowledgments

This study was performed at the New Bolton Center, School of Veterinary Medicine, University of Pennsylvania.

This manuscript represents a portion of a thesis submitted by Dr. Navas de Solis to the Universitat Autònoma de Barcelona as partial fulfillment of the requirements for a Doctor of Philosophy degree.

Supported by the Departament de Medicina i Cirurgia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona.

The authors declare that there were no conflicts of interest.

Presented as a poster at the American College of Veterinary Medicine Forum, Nashville, Tenn, June 2013.

The authors thank Judit Viu for technical assistance with data analysis.

Footnotes

1. ACL 7000, Instrumentation Laboratory, Bedford, Mass.
2. PT fibrinogen, 0009756710, HemosIL Instrumentation Laboratory Co, Bedford, Mass.
4. APPT-SP (liquid), 0020006500, HemosIL Instrumentation Laboratory Co, Bedford, Mass.
6. SPSS, version 15, IBM-SPSS Inc, Chicago, Ill.

References


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**New Veterinary Biologic Products**

<table>
<thead>
<tr>
<th>Product name</th>
<th>Species and indications for use</th>
<th>Route of administration</th>
<th>Remarks</th>
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<td>Canine Atopic Dermatitis Immunotherapeutic (Zoetis Inc, Lincoln, Neb, US Vet Lic No. 190)</td>
<td>For use in the treatment of dogs as an aid in the reduction of clinical signs associated with canine atopic dermatitis. Efficacy was demon-strated in dogs that were diagnosed with atopic dermatitis and that received a single weight-dependent dose of vaccine. A reduction in clinical signs was determined according to established PIVAS and CADESI-03 scoring guidelines.</td>
<td>SC</td>
<td>USDA conditionally licensed 7/31/15</td>
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