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Platelet Dysfunction (Glanzmann's Thrombasthenia) in Horses

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Glanzmann's thrombasthenia is a rare, inherited intrinsic platelet defect involving the platelet fibrinogen receptor (glycoprotein complex IIb-IIIa or integrin $\alpha_{IIb}\beta_3$) that is described in both human beings and dogs.[1–3] Horses with clinical features consistent with Glanzmann's thrombasthenia have been described,[4,5] but not definitively diagnosed. Clinical signs most commonly observed in humans and dogs with this disease include purpura, epistaxis, and gingival bleeding. Epistaxis was a prominent clinical feature in the 2 horses reported here. This report illustrates a possible differential diagnosis for horses with unexplained bleeding, particularly epistaxis.

A 4-year-old quarter horse mare (horse 1) was examined because of a history of chronic bilateral epistaxis that was more copious from the left nostril. The horse was in good physical condition (451 kg), and vital signs were normal. Mucous membrane color and capillary refill time were unremarkable. There was mild anemia (hematocrit 30%; reference range 32–48%) and a normal platelet count (137,000 platelets/μL; reference range 119,000–247,000 platelets/μL). No abnormalities were detected in radiographs of the head, but large petechial and ecchymotic hemorrhages in the nasopharynx were seen on endoscopy and were exacerbated by contact with the endoscope. Routine coagulation screening tests that included activated coagulation time, partial thromboplastin time, prothrombin time, thrombin time, and fibrin degradation products were normal, as were plasma concentrations of von Willebrand factor (vWF) antigen (166%; reference range 70–180%). The bleeding time, performed with a spring-loaded cassette on the gingival mucosa, was >60 minutes in the affected horse and <2 minutes in a control horse.

A clot retraction test[6] was performed in duplicate on the affected horse and on a normal horse. The degree of clot retraction was recorded after 1 and 2 hours on a scale of 1+ (minimal) to 4+ (maximal). Clot retraction was markedly reduced in the affected horse and was recorded as 1+ after 1 and 2 hours of observation compared with 2+ after 1 hour and 3+ after 2 hours in the control horse (Fig 1).

Platelet aggregation in response to various agonists was performed. Blood from the subject and a normal control horse was collected into 3.8% trisodium citrate at a ratio of 9:1. Platelet-rich plasma (PRP) was prepared by differential centrifugation of the blood as described.[7] Platelet aggregation was measured in a dual-channel aggregometer equipped with a strip chart recorder. The final concentrations of agonists evaluated included 2 μM platelet activating factor; 10, 25, and 100 μM adenosine diphosphate; and 6, 12, and 24 μg/mL collagen. Platelet aggregation responses were markedly impaired in response to all agonists tested (Fig 2).

Electron microscopy was performed on platelets isolated from the affected horse and a normal horse. Morphology of the platelets from the affected horse was comparable to that of the normal horse at the electron microscopic level.

Flow cytometry was performed to determine the number of fibrinogen receptors present on the platelets. PRP was isolated as before, and 10-μL aliquots were added to 100-μL aliquots of buffer.[8] Monoclonal antibody[a] specific to the fibrinogen receptor glycoprotein complex IIb-IIIa was added to samples of diluted PRP from the affected horse and a control horse and incubated for 20 minutes. Secondary antibody labeled with fluorescein isothiocyanate (Goat $F[ab]_2$ fragment anti-mouse immunoglobulin G) was incubated with the samples for 20 minutes in the dark on ice. Secondary antibody was added alone to PRP from both subject and control horse to assess nonspecific binding. The binding of CD41/CD61 monoclonal antibody to platelets of the affected horse was markedly reduced compared with normal equine platelets (Fig 3).

A 7-year-old 592-kg thoroughbred cross gelding (horse 2) was examined for intermittent bilateral epistaxis of 1½ years duration. The owner, who had owned the horse for 3 years, reported that the episodes of epistaxis were unrelated to exercise. Results of hematologic evaluation of several blood samples taken over the previous 18 months had been unremarkable. Previous investigations by the referring veterinarian included endoscopic examination of the upper respiratory tract and trachea with concurrent transendoscopic tracheal wash, thoracic radiography, and thoracic ultrasonography. A focal source of hemorrhage had been located in the mucosa of the left ventral meatus. Pinch biopsies from this area of mucosa had been submitted for histologic evaluation but were normal. The site had been reported to bleed copiously for 2 days after the biopsy was taken.

On physical examination, the horse was bright and in good bodily condition. Vital signs were normal. Clinical findings were normal apart from a small amount of dried blood around the left nostril.

Endoscopic appearance of the upper respiratory tract, including the guttural pouches and trachea, and radiography of the head did not show abnormalities. Routine biochemistry and CBC showed no abnormalities; in particular, the platelet count was within the normal reference range (119,000 platelets/μL; reference range 100,000–600,000

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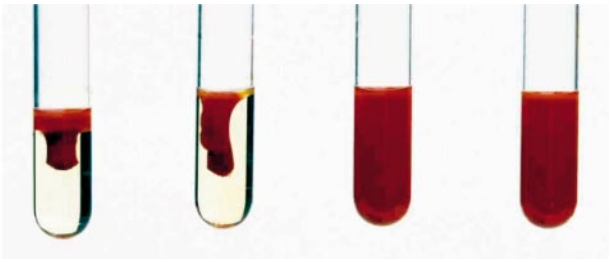

Fig 1. The 2 tubes on the left are from the control horse and demonstrate normal (+3) clot retraction after 3 hours. The 2 tubes on the right contain blood taken from horse 1 and show markedly reduced clot retraction. A similar reduced clot retraction response was observed in horse 2.

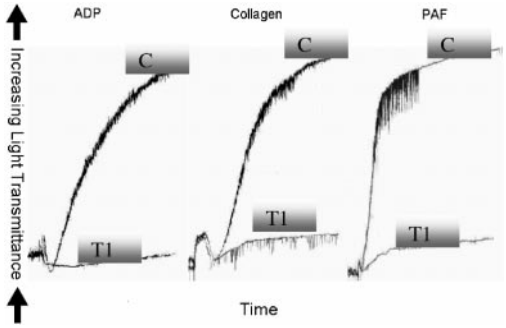

Fig 2. Aggregometry tracings demonstrating markedly reduced platelet aggregation responses to adenosine diphosphate (ADP), collagen, and platelet activating factor (PAF) in horse 1 (reduced light transmittance) compared with a normal control horse. Similar responses were observed to all concentrations of agonists. Traces from the control horse are marked C; traces from the affected horse are labeled T1. Aggregometry responses in horse 2 were similar to those seen in horse 1.

platelets/μL). Activated partial thromboplastin time, prothrombin time, thrombin time, and fibrin degradation products were normal, as was the concentration of vWF antigen (91%; reference range 80–180%).

The gingival bleeding time in horse 2 was greater than 24 hours, and bleeding had to be stopped with a cyanoacrylate glue–soaked swab.

Clot retraction and platelet aggregation responses in horse 2 were markedly reduced and comparable to those described in horse 1 (Figs 1, 2). Flow cytometric evaluation of the fibrinogen receptor also indicated markedly reduced binding of anti–CD41/CD61 antibody compared with a normal control horse (Fig 3).

The apparently late development of the condition in horse 2 raised the possibility of an acquired thrombasthenia. The subject was treated with dexamethasone, reducing from 0.1 mg/kg to 0.02 mg/kg per os in the morning over 2 months. The owners reported continued intermittent mild epistaxis. A repeat gingival bleeding time remained dramatically prolonged. Clot retraction and platelet aggregation studies were repeated, and results were unchanged from previous values.

Both owners were advised to monitor and record periods of hemorrhage. Blood sampling every 6 months, or after marked bleeding episodes, was advised in order to evaluate possible anemia.

The petechiation and ecchymoses noted in the nasal passages of horse 1 and the dramatically prolonged bleeding time in horses 1 and 2 were suggestive of a primary bleeding disorder[5] or vasculitis. Vasculitis is often characterized by demarcated areas of skin edema and is usually associated with other clinical signs of malaise not apparent in these horses.[9] There was no evidence of systemic inflammation, which is normally associated with vasculitis, in the results of blood samples taken from both subjects. Inherited clotting factor deficiencies, the most common being factor VIII,[10] or acquired factor deficiencies (caused by warfarin

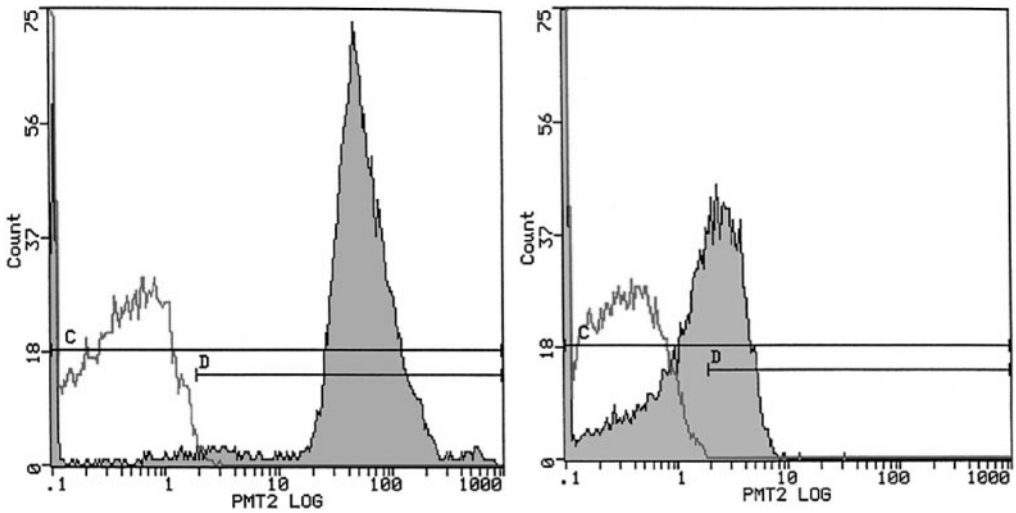

Fig 3. Flow cytometric analysis of the binding of anti–CD41/CD61 antibody to platelets from a control horse and from horse 1. The unshaded peaks (to the left of gate D) represent nonspecific binding of secondary FITC-labeled antibody only. The shaded peaks (within gate D) represent binding of antibody to control horse platelets (left panel) and affected horse platelets (right panel) incubated with primary and secondary antibody. Binding of antibody to platelets of horse 1 was markedly reduced when compared with binding of antibody to normal horse platelets. Flow cytometric results obtained with the use of platelets from horse 2 were comparable to those seen with horse 1.

toxicity, end-stage liver disease, or disseminated intravascular coagulation)[10] are characterized by abnormal coagulation tests, subcutaneous hematomas, and body cavity hemorrhage, including hemarthrosis. Petechial hemorrhages and epistaxis are unusual unless there is concurrent thrombocytopenia, platelet dysfunction or vasculitis.[10]

A platelet disorder was considered the most likely cause of the signs displayed by both horses. Platelet disorders can be subdivided into quantitative and qualitative disorders. Thrombocytopenia was ruled out in both cases because the platelet counts were normal. Von Willebrand's disease, an extrinsic platelet function disorder resulting from reduced concentrations of vWF was ruled out on the basis of normal circulating concentrations of vWF.

Platelets are required for primary hemostasis.[10] Disruption of the vascular endothelium exposes collagen, which in the presence of vWF, results in the adherence and activation of platelets. This activation causes a conformational change in the fibrinogen receptor or integrin $\alpha_{IIb}\beta_3$, enabling this receptor to bind fibrinogen which binds other platelets. During and after the platelet plug formation, a fibrin mesh forms as a result of the assembly of coagulation factors on the surface of activated platelets.[11] Thus, the platelet plug provides a scaffold for secondary hemostasis.

The primary presenting complaint in both horse 1 and horse 2 was epistaxis, which is consistent with the majority of human cases.[1] The prolonged bleeding times associated with diminished platelet aggregation responses, reduced clot retraction, and a reduction in the number of fibrinogen receptors was consistent with a diagnosis of Glanzmann's thrombasthenia.[1,3]

Two undefined thrombasthenias, with similar characteristics to those described, have been reported in Australia and Japan.[4,5] We suggest that thrombasthenia resulting from an inadequate population of fibrinogen receptors on the surface of platelets might be more prevalent in the equine population than presently recognized and that this condition should be considered as a differential in horses presenting with a bleeding diathesis but which have normal platelet numbers, normal coagulation screening tests, normal concentrations of vWF, and have no evidence of vasculitis.

Footnotes

[a] Mouse anti-ruminant CD41/CD61, MCA1095, Serotec, Raleigh, NC

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