Hemostatic Studies in Racing Standardbred Horses with Exercise-induced Pulmonary Hemorrhage. Hemostatic Parameters at Rest and After Moderate Exercise

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ABSTRACT

The purpose of this study was to determine whether a defect in hemostasis might be a factor in the etiology of exercise-induced pulmonary hemorrhage (EIPH). Hemostatic parameters were evaluated in 22 EIPH-positive and ten EIPH-negative racing horses while in a rested state. Nineteen EIPH-positive and ten EIPH-negative horses were further evaluated just before and immediately after a 15 min exercise period on a 260 m oval track. When EIPH-positive and EIPH-negative horses were compared at rest, there was no significant difference in any of the coagulation and fibrinolytic parameters studied. There was however, a significant difference in platelet function as assessed by aggregometry. The platelets from affected horses were significantly less responsive than those from nonaffected horses when exposed *in vitro* to the platelet agonists adenosine diphosphate, collagen and platelet activating factor. Exercise tended to increase the packed cell volume and factor VIII/von Willebrand factor and to decrease platelet aggregation responses to low concentrations of adenosine diphosphate. These effects of exercise however were quantitatively similar in both EIPH-positive and EIPH-negative horses. Reduced platelet function may therefore be a contributing factor in the bleeding characteristic of horses with EIPH.

RÉSUMÉ

L'objectif de cette étude était de vérifier si un trouble dans la cascade menant à l'hémostase pouvait jouer un rôle dans l'étiologie des hémorragies pulmonaires induites par l'exercice (HPIE). Les paramètres de la coagulation ont été évalués en période de repos chez 32 chevaux de course dont 22 étaient HPIE-positifs et dix étaient HPIE-négatifs. Dix-neuf chevaux HPIE-positifs et dix HPIE-négatifs furent aussi évalués immédiatement avant et après un exercice d'une durée de 15 minutes sur une piste ovale de 260 m. Lors de la comparaison des chevaux HPIE-positifs et négatifs, aucune différence significative n'a été constatée au niveau des paramètres impliqués dans les processus de fibrinolyse et de coagulation, alors qu'une différence significative a été observée au niveau de ceux de l'agrégation plaquettaire. Lorsque les plaquettes étaient exposées *in vitro* à des agonistes, tels l'adénosine diphosphate, le collagène, et le facteur d'activation plaquettaire, la réponse des plaquettes des chevaux souffrant d'HPIE était significativement moins forte que celle des autres chevaux. L'exercice avait tendance à faire augmenter l'hématocrite, les facteurs VIII et von Willebrand et à faire diminuer l'agrégation plaquettaire en présence de faibles concentrations d'adénosine diphosphate. Les effets de l'exercice étaient quantitativement semblables chez les chevaux HPIE-positifs et négatifs. La diminution de la fonction plaquettaire pourrait donc contribuer au saignement caractéristique observé chez des chevaux présentant le syndrome d'HPIE. (*Traduit par Dr André Vrins*)

INTRODUCTION

Exercise-induced pulmonary hemorrhage (EIPH) has been reported in almost all types of equine athletes. The incidence in these reports has generally exceeded 40% of the competitive population. Potential risk factors such as age, sex, and intensity of exercise have not been particularly useful in elucidating the pathogenesis of this condition (1-5).

Although the lung is clearly the source of the bleeding in horses with EIPH, the actual cause of the bleeding remains unknown. O'Callaghan *et al* (6) suggested that pathological lung changes were likely preceded by inflammatory disease (bronchiolitis), and that small airway disease stimulated bronchial vascular proliferation leading to increased bronchial blood flow and predisposing to hemorrhage during exercise. Using ventilation/perfusion scintigraphy, perfusion deficits were shown to be prominent in horses experiencing EIPH (7).

Although there is strong evidence to support the hypothesis that small air-

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way disease and vascular dysfunction play a role in the pathogenesis of EIPH, it remains unclear whether an actual defect in the hemostatic mechanism also contributes. Johnson *et al* (8) demonstrated significantly reduced platelet counts in rested "bleeder" Thoroughbred horses when compared to "nonbleeders", but this observation was not confirmed by Bayly *et al* (9). Bayly *et al* (9,10) did however demonstrate an exercise-induced suppression of platelet reactivity to adenosine diphosphate in "nonaffected" and "bleeder" Thoroughbred horses and suggested that this inhibition of platelet aggregation induced by exercise might result in a delayed sealing of damaged vessels and an increase in volume of hemorrhage. The beneficial effect of the drug furosemide was attributed to the prevention of this inhibitory effect (11). Coagulation activity in EIPH positive and negative horses has been evaluated in previous studies using intrinsic and extrinsic coagulation screening tests (partial thromboplastin time and prothrombin time respectively), and no differences between "bleeder" and "nonbleeder" horses have been noted (8,9). Plasma levels of specific coagulant/anticoagulant proteins have not been assessed.

The purpose of this study was to perform a detailed hemostatic evaluation on Standardbred racing horses identified as either having, or not having, EIPH. Horses were studied when at rest, and before and after moderate exercise. This evaluation included specific procoagulant and anticoagulant factors as well as fibrinolytic and platelet function parameters.

MATERIALS AND METHODS

CLASSIFICATION OF THE HORSES

The Standardbred horses studied were mature racing animals of either sex classified by one author (LV) as being EIPH-positive or EIPH-negative, based on historical, clinical and laboratory examination of each horse. An EIPH-positive horse met one or more of the following criteria: 1) epistaxis after racing or training reported by the trainer; 2) blood observed by the track veterinarian in the trachea on endoscopic examination after racing or training; 3) a history of poor performance in the last quarter of the race. These horses underwent a thorough respiratory examination with a subsequent bronchoalveolar lavage (BAL). The area lavaged was consistently the right and left diaphragmatic lung lobes. A horse was classified as EIPH-positive when the lavage fluid collected was bright red in color and cytologically had more than 50% of the alveolar macrophage population heavily laden with hemosiderin. The horse must have presented one or more of these criteria in the two weeks prior to being included in this study. Exercise-induced pulmonary hemorrhage-negative horses were healthy racing horses that had a BAL and endoscopic examination and met none of these criteria. Cytologically, these horses had less than 5% of the alveolar macrophages containing hemosiderin particles in the cytoplasm. None of the horses had received any drugs during the two weeks prior to study. The study was carried out with informed owner/trainer consent and cooperation, and in accordance with the guidelines of the Guide to the Care and Use of Experimental Animals of the Canadian Council on Animal Care.

BLOOD COLLECTION

Blood samples were collected from 22 EIPH-positive horses (average age 3.9 yr) and ten EIPH-negative horses (average age 3.1 yr) while at rest. The blood (45 mL) was drawn by clean venipuncture and immediately mixed with 5 mL of 3.8% trisodium citrate in a plastic container. A 2 mL volume of native blood was allowed to clot in a glass fibrin degradation product (FDP) vacutainer (Wellcome Reagents Ltd., Beckenham, England) to obtain serum for FDP measurements. Twenty mL of citrated blood were centrifuged at 4°C and 7000 *g* for 20 min to obtain platelet-free plasma (PFP). Aliquots of this plasma were immediately frozen to −70°C. A small volume of citrated blood was used for hematocrit and platelet count determinations. The remaining 20 mL of citrated blood were centrifuged at room temperature at 120 *g* for 15 min to obtain platelet-rich plasma (PRP) for platelet function studies. After careful removal of the PRP supernatant, the remainder of the contents was centrifuged for a further 15 min at 2500 *g* to obtain platelet-poor plasma (PPP). This PPP was used to adjust the platelet concentration of the PRP to 150 × 10^9/L for platelet aggregation studies.

HEMOSTATIC TESTING

Hematocrit determinations and platelet counts were performed using a microhematocrit technique and a manual count with phase contrast microscopy procedure respectively (12). Prothrombin times (PT), activated partial thromboplastin times (PTT), thrombin clotting times (TCT) and fibrinogen concentrations were determined as previously described (13). Prothrombin times, PTT, TCT, and fibrinogen were each expressed as a patient/control ratio; that is, the ratio of the patient's clotting time or fibrinogen concentration to that for a normal reference plasma. This reference plasma was a species-specific control plasma prepared by pooling plasmas from at least ten clinically normal mature horses.

Soluble fibrin monomer (SFM) titers and fibrin(ogen) degradation product titers were evaluated by protamine sulfate and latex agglutination procedures respectively (13).

Plasma factor VIII procoagulant activity (FVIII:C) was quantitated by a differential one-stage clotting assay while plasma von Willebrand factor antigen (vWF:Ag) was quantitated by electroimmunoassay as previously described (14). Plasma antithrombin III (ATIII) was measured by a chromogenic assay technique (15). All specific factors were expressed as a percentage of the activity/amount in the reference plasma.

Platelet aggregation profiles were generated using a single channel platelet aggregation profiler (Model PAP-2, Biodata Corp., Willow Grove, Pennsylvania). Fifty μL of platelet agonist were added to 450 μL of PRP (platelet concentration 150 x 10^9/μL) and the response recorded for at least 3 min. The lag phase (LP) was an estimate of the time (in s) from the addition of the platelet agonist to the initiation of an aggregation response (i.e. first evidence of a increase in light transmission). The velocity of aggregation (Av) was determined from the steepest slope of the aggregation profile and was expressed as chart

units/min. The degree of aggregation was determined by measuring the increase in light transmission (height of the aggregation curve) in chart units. The maximum degree of aggregation (A_m) was defined as the greatest increase in light transmission during the 3 min following addition of the platelet agonist, expressed as a percent of the maximum possible increase in light transmission (16).

EFFECTS OF EXERCISE

Blood samples were collected from 19 EIPH-positive horses and ten EIPH-negative horses immediately before exercise and immediately after a 15 min run on a 260 m oval track at an average speed of 10 m/s. Samples were processed and assayed as described above.

STATISTICAL ANALYSIS

Comparisons between EIPH-positive and EIPH-negative horses were made using an unpaired Student's *t*-test. Comparisons between preexercise and postexercise data were made using a paired *t*-test. A p value < 0.05 was considered statistically significant.

RESULTS

RESTED HORSES

The hematological and coagulation parameters in resting EIPH-positive and EIPH-negative horses are shown in Table I. There were no statistically significant differences in any of the parameters studied; however platelet counts were slightly lower and FVIII:C activity slightly higher in the EIPH-positive horses when compared to the EIPH-negative animals. Soluble fibrin monomer titers were similar in both groups (data not shown); all were negative (<1:10) except for one EIPH-negative horse and two EIPH-positive horses which had weak positive titers of 1:20. Fibrin degradation product titers (data not shown) were negative (<1:5) in all EIPH-negative horses; however one EIPH-positive horse had a weak positive titer of 1:10.

Comparisons of platelet aggregation responses in PRP from rested EIPH-positive and EIPH-negative horses are shown in Table II. In general platelets from affected horses responded more poorly to platelet agonists. The differences were most pronounced with the agonist adenosine diphosphate (ADP). Platelets from EIPH-positive horses took longer to respond to ADP, and the rate and degree of response was significantly less than for platelets from nonaffected horses. The differences in responses to the agonists collagen and platelet activating factor (PAF) were less pronounced but still statistically significant. Platelets derived from EIPH-positive horses showed a significantly slower response to these agonists than did platelets from horses deemed to be free of this condition.

TABLE I. Hematological and coagulation parameters in rested exercise-induced pulmonary hemorrhage (EIPH)-positive and EIPH-negative horses

Parameter	EIPH-positive horses (n = 22)	EIPH-negative horses (n = 10)
Packed cell volume (L/L)	0.36 ± 0.04[a]	0.34 ± 0.04
Platelet count ($\times 10^9$/L)	136.0 ± 45.8	157.0 ± 42.0
Prothrombin time[b]	0.99 ± 0.05	0.98 ± 0.05
Partial thromboplastin time[b]	1.10 ± 0.15	1.14 ± 0.21
Thrombin clotting time[b]	0.97 ± 0.14	1.04 ± 0.12
Fibrinogen[c]	1.20 ± 0.26	1.16 ± 0.20
Factor VIII coagulant activity[d]	126.6 ± 33.7	104.6 ± 27.8
Von Willebrand factor antigen[d]	127.8 ± 32.1	126.6 ± 29.2
Antithrombin III[d]	114.3 ± 18.1	108.5 ± 12.9

[a]Mean ± standard deviation
[b]p/c ratio = patient's time/control plasma time
[c]p/c ratio = patient's concentration/control plasma concentration
[d]% of activity in a species-specific normal reference plasma

TABLE II. Platelet aggregation parameters in rested exercise-induced pulmonary hemorrhage (EIPH)-positive and EIPH-negative horses

Aggregating agent		EIPH-positive horses		EIPH-negative horses
Adenosine diphosphate (10^{-5} M)[b]	LP	13.6 ± 3.7(20)[a]	**	9.6 ± 3.2(10)
	A_v	33.7 ± 14.2	***	63.6 ± 19.6
	A_m	60.6 ± 18.2	**	83.5 ± 23.9
Adenosine diphosphate (10^{-6} M)[b]	LP	13.8 ± 3.9(20)	*	10.3 ± 3.1(10)
	A_v	27.6 ± 14.5	***	53.4 ± 19.9
	A_m	37.4 ± 18.7	***	71.2 ± 27.8
Collagen (1:1000)[b]	LP	54.4 ± 18.8(20)		54.8 ± 15.3(10)
	A_v	47.0 ± 19.6	*	66.7 ± 27.3
	A_m	70.4 ± 23.8		89.0 ± 22.7
Platelet activating factor (0.5 ng/mL)[b]	LP	19.4 ± 4.3(13)	*	14.7 ± 4.3(10)
	A_v	63.9 ± 21.8	*	87.5 ± 17.5
	A_m	73.0 ± 21.0		90.3 ± 18.4

[a]Mean ± standard deviation (number of horses)
[b]Final concentration
LP = lag phase (in seconds)
A_v = initial velocity of aggregation (units/min)
A_m = maximum aggregation in three minutes (% of maximum possible)
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

EFFECTS OF EXERCISE

The effects of exercise on hematological and coagulation parameters in EIPH-positive and EIPH-negative horses are shown in Table III. Packed cell volume increased in both groups following exercise ($p < 0.001$). Thrombin clotting times, fibrinogen concentrations, and ATIII concentrations were not affected by exercise in either group. Although the blood platelet counts in both groups showed a tendency to increase following exercise, these increases were not statistically significant. The PTT ratios tended to increase and the PT ratios tended to decrease following exercise. However, these changes were only statistically significant in the EIPH-positive horses. Plasma FVIII:C activity and vWF:Ag increased following exercise, however the changes in FVIII:C activity and vWF:Ag were

only statistically significant in EIPH-negative horses and EIPH-positive horses respectively.

The effects of exercise on platelet aggregation responses are shown in Table IV. The major effect of exercise was to suppress the platelet responses to the agonist ADP in both groups of horses, but only at the low concentration of agonist (10^{-6} M final concentration). Exercise had no significant effect on platelet responses to the higher concentration of ADP, or to collagen or platelet activating factor. Soluble fibrin monomer titers and FDP titers were not altered by exercise in either EIPH-positive or EIPH-negative horses (data not shown).

To quantitatively compare the hemostatic responses of EIPH-positive and EIPH-negative horses to exercise, change in each parameter was assessed as a percentage of the preexercise value. For all parameters studied there was no statistically significant difference in the degree of change induced by the exercise.

TABLE III. Comparison of hematological and coagulation parameters in exercise-induced pulmonary hemorrhage (EIPH)-positive and EIPH-negative horses before and after exercise

	EIPH-positive (n = 19)		EIPH-negative (n = 10)	
Parameter	Preexercise	Postexercise	Preexercise	Postexercise
Packed cell volume (L/L)	0.36 ± 0.04[a] ***	0.45 ± 0.06	0.34 ± 0.04 ***	0.44 ± 0.05
Platelet count (×10^9/L)	134.3 ± 48.9	141.4 ± 58.5	157.0 ± 42.0	163.1 ± 56.0
Prothrombin time[b]	0.99 ± 0.05 **	0.96 ± 0.04	0.98 ± 0.05	0.96 ± 0.03
Partial thromboplastin time[b]	1.07 ± 0.12 *	1.17 ± 0.21	1.14 ± 0.21	1.17 ± 0.23
Thrombin clotting time[b]	0.95 ± 0.11	0.99 ± 0.14	1.04 ± 0.12	1.01 ± 0.08
Fibrinogen[c]	1.20 ± 0.27	1.19 ± 0.29	1.16 ± 0.20	1.09 ± 0.27
Factor VIII coagulant activity[d]	128.4 ± 35.6	137.8 ± 33.4	104.6 ± 27.8 **	127.1 ± 26.8
Von Willebrand factor antigen[d]	133.2 ± 28.0 ***	149.9 ± 30.0	126.6 ± 29.2	165.9 ± 51.6
Antithrombin III[d]	115.1 ± 18.2	122.9 ± 17.2	108.5 ± 12.9	114.4 ± 31.3

[a]Mean ± standard deviation
[b]p/c ratio = patient's time/control plasma time
[c]p/c ratio = patient's concentration/control plasma concentration
[d]% of activity in a species-specific normal reference plasma
*p < 0.05 ** p < 0.01 *** p < 0.001

TABLE IV. Comparison of platelet aggregation responses in platelets taken from exercise-induced pulmonary hemorrhage (EIPH)-positive and EIPH-negative horses before and after exercise

		EIPH-positive (n = 17)[a]		EIPH-negative (n = 10)	
Aggregating agent		Preexercise	Postexercise	Preexercise	Postexercise
Adenosine diphosphate (10^{-5} M)	LP	13.9 ± 4.1	13.9 ± 3.6	9.6 ± 3.2	9.3 ± 3.6
	A_v	34.9 ± 15.1	31.6 ± 9.6	63.6 ± 19.6	54.7 ± 14.6
	A_m	63.4 ± 19.1	59.1 ± 14.6	83.5 ± 23.9	79.5 ± 22.2
Adenosine diphosphate (10^{-6} M)	LP	13.9 ± 4.2 **	17.5 ± 5.0	10.3 ± 3.1	12.0 ± 3.6
	A_v	27.1 ± 15.3	22.8 ± 7.4	53.4 ± 19.9	38.7 ± 14.0
	A_m	37.4 ± 20.3 *	28.0 ± 10.6	71.2 ± 27.8 *	48.3 ± 24.7
Collagen (1:1000)	LP	54.9 ± 20.2	59.0 ± 16.6	54.2 ± 15.3	49.1 ± 8.5
	A_v	46.1 ± 20.8	43.0 ± 14.4	66.7 ± 27.3	54.8 ± 14.2
	A_m	68.9 ± 25.7	67.7 ± 20.9	89.0 ± 22.7	91.5 ± 17.9
Platelet activating factor — (0.5 ng/mL)	LP	19.6 ± 4.6	18.5 ± 4.5	14.9 ± 4.3	13.1 ± 4.5
	A_v	62.1 ± 23.3	66.7 ± 21.8	87.5 ± 17.5	86.7 ± 17.6
	A_m	74.6 ± 22.5	73.2 ± 20.9	90.3 ± 18.4	92.3 ± 17.0

[a]n = 10 for platelet activating factor
LP = lag phase (in seconds)
A_v = initial velocity of aggregation (units/min)
A_m = maximum aggregation in three minutes (% of maximum possible)
* p < 0.05 ** p < 0.01

DISCUSSION

The results of this study indicated that platelets derived from horses with EIPH were significantly less responsive to platelet agonists than platelets derived from horses deemed to be free of this condition. The reason for the decreased responsiveness of platelets derived from rested EIPH-positive horses is not known. Drug-induced effects were ruled out because of the thorough screening of these horses before entering the study. Although Bayly *et al* (11) showed a "tendency" for platelets from five rested EIPH-positive horses to be less responsive to ADP than control platelets, we observed a statistically significant reduction in response in platelets from affected horses, not only to ADP but to other platelet agonists. Although the effect on ADP-induced aggregation was most pronounced, responses to collagen and PAF were also significantly slower with platelets derived from EIPH-positive horses. This sluggish response of platelets from EIPH-positive horses may be a contributing factor to the increased blood loss seen in these subjects.

Platelets are the first line of defense in preventing blood loss from the circulation when vascular damage occurs; poor responsiveness of platelets to agonists would therefore predispose to increased blood loss. Vascular injury as a result of perfusion/pressure changes associated with exercise is a likely event in any horse, but particularly in a horse with concomitant lung pathology as has been described in EIPH (17).

Bayly *et al* (10) observed that platelets derived from clinically normal Thoroughbred horses failed to aggregate as readily to ADP after the animals had been exercised. They noted however that the aggregation response to a more potent agonist (arachidonic acid) was not affected by exercise. It was postulated in that study that exercise-induced release of prostacyclin from the vascular endothelium was responsible for the inhibitory effect on platelet reactivity. This inhibitory effect was detected with ADP as the agonist since it is a weak platelet activator and therefore "a more sensitive indicator of changes in the responsive capacity of platelets". Our post-

exercise studies on both EIPH-positive and EIPH-negative Standardbred horses are in agreement with these previous observations. In the present study, exercise suppressed the response of platelets to low dose ADP. Responses to more potent agonists (i.e. higher concentration of ADP, or collagen or PAF) were not affected by exercise.

Like previous researchers we were unable to demonstrate any significant differences in coagulant activity (as assessed by PT/PTT screening tests), or fibrinolytic activity as assessed by quantitation of FDP titers, in rested EIPH-positive and EIPH-negative horses (8,9,17). Although affected Standardbred horses in this study tended to have a lower platelet count than nonaffected horses, this difference was not significant. In humans, strenuous exercise has been shown to induce a significant increase in fibrinolytic activity (19). Bayly *et al* (11) found no evidence of enhanced fibrinolysis in Thoroughbred horses exercised at racing speed for 1200 m and then evaluated 5 min after exercise. Ferguson *et al* (20) found no evidence of excessive fibrinogenolysis/fibrinolysis in horses competing in a 157 km endurance race. In the present study, we found no detectable evidence of enhanced fibrinolysis in exercised clinically normal Standardbred horses. Moreover, EIPH-positive horses also showed no evidence of enhanced post-exercise fibrinolysis. Whether the apparent lack of enhanced postexercise fibrinolysis in horses represents a species difference in human/equine responses, or is related to inadequate duration and/or intensity of exercise, is not known. Our study suggests however that alterations in fibrinolytic activity associated with exercise and/or differences in fibrinolytic activity between EIPH-positive and EIPH-negative horses are not likely factors in the pathogenesis of the bleeding associated with this disease.

Antithrombin III is a major natural inhibitor of blood coagulation. The hypothesis that abnormally high ATIII activity might be a factor in EIPH was disproven as similar plasma levels were found in both EIPH-positive and EIPH-negative horses. Likewise, exercise had no effect on ATIII levels in either group.

The plasma factor VIII/von Willebrand factor (FVIII/vWF) complex was studied because vascular injury has been suggested as a basic component in the pathogenesis of EIPH. The protein vWF is a product of vascular endothelium but FVIII is not. Although the plasma levels of FVIII and vWF often tend to fluctuate in parallel, a significant increase in vWF without a concomitant increase in FVIII (i.e. increased vWF:Ag/FVIII:C ratio) has been described in humans in association with primary diseases affecting the vasculature (21,22). Disproportional increases in vWF:Ag (increased vWF:Ag/FVIII:C ratios) were reported in children with acute bronchiolitis and were attributed to pulmonary endothelial damage due to hypoxia. Ratios became normal during the recovery stage (22). Others have proposed that the vWF:Ag/FVIII:C ratio be used as a means of monitoring vascular injury (21). No similar studies however have been reported in horses. We observed normal plasma concentrations of FVIII:C and vWF:Ag in both EIPH-positive and EIPH-negative horses in this study, and similar vWF:Ag/FVIII:C ratios at rest. This may reflect a lack of "active" concurrent lung/vascular pathology and vascular damage in EIPH-positive horses at the time of testing, although tissue injury may have occurred earlier.

Increases in plasma FVIII:C activity and vWF:Ag following exercise have been extensively documented in humans (23,24). A similar effect in horses was evident in this study as both plasma FVIII:C and vWF:Ag increased following exercise. There was no difference in the degree of increase when EIPH-positive and EIPH-negative horses were compared. Since the increases in FVIII:C and vWF:Ag were somewhat in parallel, the vWF:Ag/FVIII:C ratios in both groups of horses were not significantly altered by exercise.

In conclusion this study of rested horses with EIPH points towards an underlying platelet function abnormality as a possible contributing factor in the pathogenesis of EIPH. It can be postulated that this underlying platelet defect may be exacerbated by exercise (as may the clinical expression of this defect), when the requirement for good platelet function is high. Bleeding may be prolonged and therefore blood volume loss increased by delayed sealing of injured microvessels. The present study also indicates that alterations in coagulant and fibrinolytic activities are probably not important in the pathogenesis of EIPH.

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