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Coagulation Profiles in Dogs with Congenital Portosystemic Shunts before and after Surgical Attenuation

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Background: Serious postoperative hemorrhage has been reported in dogs after closure of congenital portosystemic shunts (CPS).

Hypothesis: In dogs with portosystemic shunting, low coagulation factor activity is responsible for coagulopathy, which can cause complications after surgery.

Animals: Thirty-four dogs with CPS and 39 healthy dogs.

Methods: In a prospective study, coagulation times, platelet count, and the activity of 8 coagulation factors were measured in dogs before and after surgical shunt attenuation and in 31 healthy dogs. The effect of abdominal surgery on hemostasis was determined at ovariectomy in 8 healthy dogs.

Results: Dogs with CPS had lower platelet counts, lower activity of factors II, V, VII, and X, and increased factor VIII and activated partial thromboplastin time (APTT) compared to healthy dogs. After surgical attenuation, dogs with CPS had decreased platelet counts and activity of factors I, II, V, VII, IX, X, and XI and a prolonged prothrombin time (PT). Ovariectomy resulted in decreased activity of factors VII and X. Six weeks after surgery, portosystemic shunting persisted in 9 of 30 dogs, with no improvement of hemostatic values. CPS dogs without shunting had improved coagulation times and increased activity of factors II, V, VII, and X.

Conclusions and Clinical Importance: Dogs with CPS have lower activity of clotting factors compared to healthy dogs, resulting in a prolonged APTT. Surgical attenuation of the shunt results in increased abnormalities in coagulation times and factors immediately after surgery. Hemostasis is normalized after complete recovery of shunting after attenuation, in contrast to dogs with persistent shunting.

Key words: Coagulation factors; Hemostasis; Hepatic disease; Procoagulant proteins.

The treatment of choice for dogs with congenital portosystemic shunts (CPS) is complete or partial shunt closure at surgery. However, a wide range of perioperative complications have been reported in dogs after surgery. The outcome of surgical attenuation of portosystemic shunts depends on the type of shunt, body weight, degree of closure, and portal vein diameter.[1–7] One potential operative complication is hemorrhage because of coagulopathy, which can be fatal if not treated appropriately. Prolonged coagulation times are found in many dogs with CPS before surgery, but spontaneous bleeding disorders do not typically occur in these dogs.[8–10] However, in a previous study dramatic worsening of coagulopathy was an important cause of death after surgery.[6] The mechanisms underlying these alterations in hemostasis are not fully understood.

Liver function is closely linked to hemostasis. The liver parenchymal cells synthesize most of the clotting factors, including factors I (fibrinogen), II, V, IX, X, XI, and XIII, whereas factor VIII is thought to be produced in the liver vascular endothelium.[11] The liver is also closely involved in the regulation of coagulation by clearance of activated clotting and fibrinolytic factors. Coagulopathies are found in many patients with disturbed liver parenchymal cell function.[10,12–14] A prolonged partial thromboplastin time is observed in dogs with CPS, which might have been the result of impaired hepatic synthesis of coagulation factors.[10]

The objective of this study was to provide a more detailed understanding of the disturbances in hemostasis that occur in dogs with CPS and of the mechanisms causing further derangement of hemostasis after surgery. Hemostasis was quantified by measuring clotting times and the activity of individual plasma clotting factors (procoagulant proteins) at different points in time. We hypothesized that in dogs with CPS, plasma activities of clotting factors that are produced in the liver are lower than in dogs without CPS. As surgery can result in consumption of clotting factors, the effect of surgery on hemostasis in CPS patients was evaluated and compared to the effect of an elective celiotomy and ovariectomy in a group of healthy control dogs. Additional objectives were to see if clotting times and clotting factors were normalized by 1 month after CPS surgery and if there was a relationship between normalization of hemostasis and effective closure of the shunt.

Materials and Methods

Study Population

Thirty-four dogs that were referred for surgical ligation of a single CPS were prospectively entered into the study. Dogs were only included with fully informed consent of the owner. The diagnosis of a portosystemic shunt was made by measuring abnormal, high, 12-hour fasting plasma ammonia (NH_3) and bile acids (laboratory reference values: NH_3 24–45 μM; bile acids 0–10 μM), or an abnormal rectal ammonia tolerance test.[15] Chronic portosystemic encephalopathy was scored according to a clinical grading system.[16] The presence of a single CPS was diagnosed by demonstrating the anomalous vessel with ultrasonography. In all cases the presence of a CPS was visually confirmed during exploratory celiotomy.

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Before surgery, dogs were premedicated by intramuscular injection of methadone (0.5 mg/kg) and atropine (0.03 mg/kg), and prophylactic antibiotics were administered (amoxicillin/clavulanic acid 20 mg/kg IV). After 30 minutes, anesthesia was induced with propofol (1–5 mg/kg) intravenously and maintained using isoflurane in oxygen and nitrous oxide (1:1 ratio). During surgery, dogs received lactated Ringer's solution (10 mL/kg/h IV), and sufentanil was administered to achieve sufficient analgesia (1 μg/kg/h IV). Respiration was supported with intermittent positive-pressure ventilation. Patient monitoring intraoperatively consisted of electrocardiography, pulse oximetry, respirometry, capnography, and measurement of peripheral arterial pressure, central venous pressure, body temperature, and plasma glucose concentration. Surgical ligation of the shunt was performed by one surgeon (FJvS) with a nonabsorbable suture, according to the technique reported by Wolschrijn et al.[5]

After surgery, analgesia was continued with methadone and carprofen. The patient was postoperatively monitored at the intensive care unit until the dog had recovered sufficiently to be dismissed, usually at 2 days after surgery. Blood samples were collected 3 times: shortly before surgery, immediately after surgery, and approximately 30 days after surgery. If the dog weighed <2 kg, blood collection was done only twice (before surgery and 30 days after surgery) to prevent complications or significant plasma dilution. Packed cell volume (PCV); platelets; prothrombin time (PT); activated partial thromboplastin time (APTT); D-dimers; and factors I (fibrinogen), II, V, VII, VIII, IX, X, and XI were measured in all blood samples. Thirty days after surgery, clinical performance was scored according to the encephalopathy grading system,[16] and 12-hour fasting plasma ammonia and bile acid measurements were repeated. Abdominal ultrasonography was performed to examine hepatic development and the site and patency of the attenuated shunt and to identify abnormalities such as acquired portosystemic collateral vessels. If plasma ammonia ranged between 45 μM and 100 μM or if only bile acids were high (>10 μM), a rectal ammonia tolerance test was performed to assess ammonia metabolism. Marked increases in fasting plasma ammonia concentration (>100 μM) or abnormal ammonia tolerance testing were considered to be evidence of persistent portosystemic shunting, and the dog was classified as not recovered.

In addition to the dogs with CPS, blood was obtained from 31 healthy dogs without CPS to measure the activity of factors I, II, V, VII, VIII, IX, X, and XI (reference dogs). These dogs of various breeds and sexes were all clinically healthy, and blood was checked to confirm that creatinine, alkaline phosphatase, fasting bile acids, PCV, leukocytes, platelet counts, and coagulation times (PT, APTT) were within normal laboratory values. The reference group consisted of dogs of various ages to determine any age-related differences in factor activities; 11 dogs were younger than 6 months, 10 dogs were aged between 6 and 12 months, and 10 dogs were older than 12 months.

To determine the effect of a midline celiotomy with vessel ligation on coagulation factor activity in dogs without portosystemic shunting, blood was also collected in another 8 healthy adult dogs before and immediately after an elective standard ovariectomy performed through a midline approach (control dogs). Before surgery, blood was analyzed to confirm that creatinine, alkaline phosphatase, fasting bile acids, PCV, and leukocytes were within normal laboratory values. The activity of factors I, II, V, VII, VIII, IX, X, and XI, platelets, coagulation times (PT, APTT), and D-dimers were measured before and after surgery. All procedures were performed with approval of the owners.

Hemostatic Analysis

Coagulometric tests were used to determine the activity of specific coagulation factors in the collected plasma samples. In these tests the unknown diluted sample is mixed with undiluted plasma deficient in that specific factor. The specific factor is supplied by the unknown sample; all other factors are supplied by the deficient sample. The test principle is based on modified screening tests for PT (factors II, V, VII and X) or APTT (factors VIII, IX, XI). Plasma samples were collected from the jugular vein in 1.8-mL Becton Dickinson vacutainers and anticoagulated with sodium citrate (0.129 M or 3.8%), diluted 9:1. Automated PT and APTT determinations were performed with a coagulation analyzer,[a] and human factor-deficient plasma[b] was used in the tests. The activity of a specific factor is expressed as a percentage of the standard value (100% activity). For determination of laboratory standard values, 15 clinically healthy adult dogs of varying breeds and sexes were used for blood collection to prepare canine pooled plasma. The activities of several factors (V, VII, VIII, IX, and XI) in plasma from healthy dogs are greater than activities in human plasma by up to approximately eight (factor VIII:C) to nine (factor V) times.[17,18] To enable measurement of individual coagulation factor activities in dogs by using human deficient plasma, a series of dilutions of the canine pooled plasma was made to prepare accurate activity curves.

Fibrinogen was quantitatively determined with a commercially available assay.[c] For semiquantitative determination of D-dimers, a latex agglutination test[d] was used.

Because determination of D-dimers and factors II, V, VII, VIII, IX, X, and XI were not performed immediately after blood collection in most cases, citrated plasma was stored at −70°C until measurements were performed, with a median storage period of 2 months and a maximum of 6 months. Plasma storage at −70°C for more than 6 months may significantly shorten APTT, implying an increase in factor activity.[19]

Statistical Analysis

All statistical analyses were performed using commercial software (SPSS for Windows, release 12.0.1[e]). Kolmogorov-Smirnov tests were used to determine if distribution of variables was normal.

To compare CPS dogs to reference and control dogs and to compare paired samples at different sample times, Student's *t*-tests and paired sample *t*-tests were used to analyze differences in platelet counts, coagulation times, and factors I, II, V, VII, VIII, and X. A nonparametric test (a Mann-Whitney *U*-test or a Wilcoxon signed ranks test in paired samples) was chosen to analyze factors IX and XI. D-dimer concentrations (ordinal variable) were also analyzed using a Mann-Whitney *U*-test. In dogs with CPS, correlations between hemostatic changes that occurred after surgery (platelet counts, coagulation times, and factors; individually calculated) and type of shunt, duration of surgery, and PCV decrease were analyzed with multiple linear regression. Because of the large number of variables tested, a P value <.01 was considered significant.

Results

Hemostatic analysis was performed as a reference in 31 healthy dogs (Table 1), with a median age of 8 months (range 1.5–120 months). The group consisted of 25 dogs from 10 different breeds and an additional 6 mixed breed dogs. The 2 most common breeds were the Cairn Terrier (n = 8) and the Labrador Retriever (n = 4). APTT and factor I (fibrinogen) were slightly but significantly higher in the younger dogs. Mean APTT and fibrinogen were 15.5 seconds and 2.3 g/L, respectively, in dogs aged less than 6 months; 15.1 seconds and 2.1 g/L in dogs aged between 6 and 12 months; and

Table 1. Results of hemostatic analysis in healthy reference dogs and dogs with a congenital portosystemic shunt before and immediately after surgical attenuation of the shunt.

	Reference Dogs		CPS Dogs b.s.			CPS Dogs i.a.s.			
Variable[a]	No.	Mean (SD)	No.	Mean (SD)	*P* Value[1]	No.	Mean (SD)	*P* Value[2]	D (%)
Platelets ($\times 10^9$/L)	31	324 (87)	33	243 (88)	<.001[b]	22	161 (57)	<.001[b]	−27
PT (seconds)	31	7.9 (0.91)	34	8.8 (1.8)	.012	23	10 (2.0)	<.001[b]	+6.7
APTT (seconds)	31	14.7 (1.58)	34	19.0 (3.79)	<.001[b]	23	20.3 (4.46)	.022	
Fibrinogen (g/L)	31	2.0 (0.38)	34	2.1 (0.91)	.60	23	1.8 (0.70)	.003[b]	−14
Factor II (%)	31	79 (11)	33	56 (9.7)	<.001[b]	23	48 (11)	<.001[b]	−11
Factor V (%)	31	88 (21)	33	54 (16)	<.001[b]	23	42 (16)	<.001[b]	−16
Factor VII (%)	31	84 (29)	33	42 (15)	<.001[b]	23	36 (14)	.003[b]	−11
Factor VIII (%)	29	98 (30)	33	125 (39)	.004[b]	23	108 (36)	.018	
Factor IX (%)	30	86 (31)	32	85 (39)	.091	23	73 (41)	.001[b]	−11
Factor X (%)	31	76 (14)	33	44 (13)	<.001[b]	23	37 (12)	<.001[b]	−10
Factor XI (%)	30	106 (38)	32	104 (47)	.12	23	91 (46)	.002[b]	−11

CPS, congenital portosystemic shunt ; b.s., before surgery; i.a.s., immediately after surgery; SD, standard deviation; *P* value[1], *P* value of preoperative values in comparison with reference dogs; *P* value[2], *P* value of preoperative values in comparison with postoperative values; D, the mean degree of decrease (−) or increase (+) of postoperative compared to preoperative values; PT, prothrombin time; APTT, activated partial thromboplastin time.

[a] Laboratory reference ranges: platelets 144–603 $\times 10^9$/L, PT 6.7–9.5 seconds, APTT 10.0–17.2 seconds, fibrinogen 1.0–2.8 g/L.

[b] a significant difference.

13.4 seconds and 1.7 g/L in dogs aged more than 12 months (*P* values of .005 and .003). No age-related significant differences in PT, platelet counts, or other coagulation factors activity were found in these dogs.

Surgical attenuation of a single CPS was attempted in 34 dogs, including 21 extrahepatic shunts (16 portocaval and 5 portoazygos shunts) and 13 intrahepatic shunts (9 left, 2 central, and 2 right divisional shunts). The study population consisted of 17 male and 17 female dogs, representing 18 different breeds and 3 mixed-breed dogs. The most commonly represented breeds were Maltese dogs (n = 5), Yorkshire Terriers (n = 4) and Dachshunds (n = 3). Age at surgery varied from 3 months to 3.5 years (median 7.9 months), and body weight ranged from 0.85 kg to 33.9 kg (median 5.6 kg). Four dogs weighed less than 2 kg. Before surgery, clinical signs were scored as grade 0 (no signs) in 3 dogs, grade 1 (depression, personality changes, urologic signs) in 7 dogs, grade 2 (ataxia, compulsive behavioral changes) in 19 dogs, and grade 3 (stupor, seizures) in 4 dogs. In 1 dog clinical signs before surgery were unknown. Median fasting plasma bile acids were 52 μM (range 2–380 μM, n = 24) and median fasting plasma ammonia was 142 μM (range 33–305 μM, n = 31). A definitive diagnosis of a CPS was made with ultrasonography, and a single CPS was found at exploratory celiotomy in all patients. Median PCV before surgery was 0.40 (range 0.18–0.50), versus 0.45 in the reference dogs ($P = .009$). Other significant differences between dogs with CPS before attenuation and reference dogs were found with respect to platelet counts (lower numbers in CPS dogs) and APTT (a higher value in CPS dogs) (Table 1). Activities of the coagulation factors II, V, VII, and X were significantly lower in dogs with CPS as compared to the reference dogs. In contrast to other factors, a significantly higher activity of factor VIII was found in dogs with a CPS. No significant differences were detected in activities of coagulation factors I, IX, and XI.

In the dogs with CPS, the median duration of surgery was 110 minutes (range 35–165 minutes, n = 33). Shunt closure was complete in 9 extrahepatic shunts, and partial closure of the shunt was achieved in 10 extrahepatic and 13 intrahepatic shunts. In 17 partially closed shunts the degree of attenuation was calculated as the decrease in the cross-sectional area of the shunt: median closure was 92.8%, ranging from 75% to 97%. In 2 dogs with an extrahepatic portocaval shunt no attenuation was possible because of portal vein aplasia. One of these dogs died 6 hours after surgery because of persistent portal hypertension after temporary attenuation of the shunt. The other dog was treated conservatively after surgery and was excluded from the remainder of the study. Mortality after attempted shunt attenuation was 8.8% (3/34). Besides the dog that died as described above, 2 other dogs, both with a left divisional intrahepatic shunt, died 1 day after surgery because of acute portal hypertension. One of these dogs was euthanized because postoperative ultrasonography strongly indicated that portal hypertension was caused by thrombosis and congestion of hypoplastic portal veins. The other dog died after a second surgery to remove the ligature was declined.

In 23 dogs, blood was collected immediately after attenuation of the CPS. Hemostatic profiles were not measured in 6 animals immediately after surgery, because they had inadvertently received plasma or hydroxyethyl starch (HES) intravenously during surgery. Median postoperative PCV was 0.30 (range 0.21–0.40), which was a significant decrease ($P < .001$) compared to preoperative PCV. After surgery, PT was significantly increased, whereas platelets and coagulation factors I, II, V, VII, IX, X and XI had significant decreases (Table 1). APTT and factor VIII tended to be

increased ($P = .022$) and decreased ($P = .018$), respectively. Hemostatic changes were not significantly different between intrahepatic and extrahepatic shunt dogs and no significant correlations were found between the hemostatic changes and surgery time or decrease in PCV.

The 8 control dogs that underwent an elective ovariectomy varied in age from 5.2 months up to 5.3 years (median 2.4 years) and were all of different breeds. Median surgery time in these dogs was 83 minutes (range 63–95 minutes). Before and after surgery D-dimer values were normal in all 8 dogs (<250 ng/mL). Before surgery there were no significant differences in platelet counts, coagulation times, or factor activity between these control dogs and the 31 reference dogs. After surgery, coagulation times and platelet counts did not change significantly and remained within normal laboratory values (Table 2). A significant decrease in activity was found only with respect to factors VII and X.

Comparing both surgical procedures (Table 2), PT was significantly longer and platelet counts were significantly lower after CPS attenuation than after an ovariectomy and APTT tended to be longer ($P = .010$). Also, absolute activities of coagulation factors II, V, VII, and X were significantly lower after shunt ligation compared with ovariectomy. However, the relative degree of decrease or increase during surgery (expressed as a percentage) was only significantly different between shunt ligation and ovariectomy with respect to platelet counts ($P < .001$) and factor II activity ($P = .001$). The degree of factor V decrease tended to be different, but this was not statistically significant ($P = .015$).

The outcome after surgery was assessed in 30 dogs with CPS at a median of 44 days (range 22–447 days) after surgery. Twenty-one dogs had no clinical or biochemical evidence of portosystemic shunting and were considered to be fully recovered. In these 21 dogs, median plasma ammonia was 17 μM (range 8–62 μM). Bile acids were measured in 15 dogs, with a median concentration of 5 μM (range 1–58 μM). Ammonia tolerance tests were all normal (n = 13). In 15 of these 21 dogs ultrasonography was performed, and no functional shunting vessels could be detected.

Evidence of persistent shunting was found in 9 dogs. Clinical performance was scored as grade 0 in 6 dogs, grade 1 in 1 dog, and grade 2 in 2 dogs. Median plasma ammonia was 109 μM (n = 9, range 23–194 μM), and median bile acids were 82 μM (n = 8, range 33–237 μM). Ammonia tolerance tests were performed in 5 dogs and revealed abnormal ammonia metabolism in all 5 dogs. In 8 dogs, a functional portosystemic shunt was found with ultrasonography, and in the 9th dog no ultrasonography was performed. In 2 dogs multiple collaterals were found, in 5 dogs the original congenital shunt was still functional, and in 1 dog another single portosystemic shunt had become functional. This last dog recovered completely after surgical closure of the second CPS.

Results of hemostatic analysis of recovered and nonrecovered dogs are described in Table 3. In the dogs that recovered, coagulation times (PT, APTT) and coagulation factors II, V, VII, and X had improved significantly by approximately 6 weeks after surgery compared with preoperative values ($P < .001$). Compared with the 31 healthy reference dogs, PT was even significantly shorter in the recovered dogs ($P < .001$), the clotting factor XI had become higher ($P = .003$), and factors I and VIII tended to be higher ($P = .014$, $P = .042$). The mean value of PT was within laboratory reference ranges (7.1 seconds).

The dogs that had not made a complete recovery by 6 weeks after surgery or longer had no significant improvement of coagulation times or coagulation factors as compared to their preoperative values, although APTT tended to decrease ($P = .023$). However, in comparison with the reference dogs, only APTT was still significantly increased ($P = .006$).

Table 2. Results of hemostatic analysis in healthy control dogs before and immediately after elective ovariectomy.

	OVE Dogs b.s.		OVE Dogs i.a.s.				
Variable[a]	No.	Mean (SD)	No.	Mean (SD)	*P* Value[1]	D (%)	*P* Value[2]
Platelets ($\times 10^9$/L)	8	331 (90)	8	316 (86)	.35		<.001[b]
PT (seconds)	8	7.6 (0.57)	8	7.7 (1.3)	.96		.006[b]
APTT (seconds)	8	15.7 (2.1)	8	15.8 (1.7)	.89		.010
Fibrinogen (g/L)	8	1.9 (0.42)	8	1.5 (0.57)	.044		.40
Factor II (%)	8	77 (4.4)	8	75 (5.8)	.062		<.001[b]
Factor V (%)	8	90 (13)	8	86 (14)	.14		<.001[b]
Factor VII (%)	8	101 (25)	8	94 (26)	.004[b]	−7.6	<.001[b]
Factor VIII (%)	8	116 (17)	8	94 (10)	.016		.11
Factor IX (%)	8	80 (12)	8	70 (11)	.017		.25
Factor X (%)	8	74 (6.9)	8	69 (7.3)	.004[b]	−6.6	<.001[b]
Factor XI (%)	8	92 (13)	8	85 (12)	.090		.37

OVE, ovariectomy; b.s., before surgery; i.a.s., immediately after surgery; SD, standard deviation; *P* value[1], *P* value of preoperative values in comparison with postoperative values; D, the mean degree of decrease (−) of postoperative compared to preoperative values; *P* value[2], *P* value of preoperative values of OVE in comparison with CPS ligation; PT, prothrombin time; APTT, activated partial thromboplastin time.

[a] Laboratory reference ranges: platelets 144–603 $\times 10^9$/L, PT 6.7–9.5 seconds, APTT 10.0–17.2 seconds, fibrinogen 1.0–2.8 g/L.

[b] a significant difference.

Table 3. Results of hemostatic analysis in dogs with a congenital portosystemic shunt approximately 6 weeks after surgical attenuation of the shunt.

	Dogs Recovered			Dogs not Recovered			
Variable[a]	No.	Mean	SD	No.	Mean	SD	*P* Value
Platelets ($\times 10^9$/L)	11	288	82	3	213	37	.16
PT (seconds)	21	7.1	0.52	9	7.7	1.0	.034
APTT (seconds)	21	14.2	1.6	9	16.5	1.9	.0024[b]
Fibrinogen (g/L)	21	2.5	0.82	9	1.8	0.59	.035
Factor II (%)	19	76	9.6	8	68	13	.058
Factor V (%)	19	89	20	8	68	26	.035
Factor VII (%)	19	73	24	8	59	26	.17
Factor VIII (%)	19	117	31	8	125	36	.58
Factor IX (%)	19	100	40	8	100	44	.85
Factor X (%)	19	77	13	8	65	16	.066
Factor XI (%)	19	128	46	8	118	42	.52

PT, prothrombin time; APTT, activated partial thromboplastin time.
[a] Laboratory reference ranges: platelets 144–603 $\times 10^9$/L, PT 6.7–9.5 seconds, APTT 10.0–17.2 seconds, fibrinogen 1.0–2.8 g/L.
[b] a significant difference between recovered and not recovered dogs.

Platelet counts ($P = .037$), factor II ($P = .019$), factor V ($P = .034$), and factor VII ($P = .033$) tended to be reduced, and factor VIII tended to be increased ($P = .042$). PT and coagulation factors I, IX, X, and XI were not significantly different.

Preoperatively, only APTT tended to be different if recovered dogs were compared to nonrecovered dogs, including the dogs that had died ($P = .033$). The median preoperative APTT was 17.8 seconds in recovered dogs, whereas in nonrecovered dogs the median preoperative APTT was 20.7 seconds. Approximately 6 weeks after surgery APTT was significantly longer in dogs that were not recovered, and trends were noted toward a longer PT and lower values of clotting factors I and V in comparison with recovered dogs (Table 3). Platelet counts, PT, and the activities of factors II, V, VII, and X are shown in box- and whiskerplots, to compare differences among CPS dogs, control dogs, and reference dogs (Fig 1).

No significant differences of D-dimer concentrations were noted between dogs with CPS and the 8 control dogs before surgery ($P = .12$), dogs with CPS before and immediately after shunt ligation ($P = .054$), or between recovered and nonrecovered dogs ($P = .98$) (Table 4). However, dogs with shunt ligation revealed significantly higher concentrations of D-dimers immediately after surgery than ovariectomized dogs ($P = .008$).

Discussion

Coagulation defects in liver disease are often caused by reduced synthesis of coagulation factors or qualitative abnormalities in factor production.[14,20–22] Because a generalized bleeding tendency can also occur in dogs with a portosystemic shunt, a coagulation screening in these animals has been recommended before surgery.[8–10] The most commonly used screening tests for coagulation abnormalities are PT and APTT, although it was suggested that abnormalities in these tests reveal a poor correlation with clinical bleeding.[8,10,11,23] In single coagulation factor deficiencies, PT and APTT are predictably prolonged at factor concentrations below 35% of normal activity. Coagulopathies in liver disease are generally characterized by multiple coagulation factor deficiencies. Prolongation of coagulation times in disorders affecting multiple factors usually represents less reduction in individual factor concentrations, and abnormalities in coagulation times may therefore be detected before spontaneous clinical bleeding occurs.[11,23] Generally, acute canine hepatopathies prolong both PT and APTT, whereas chronic canine hepatic disease is often associated with prolonged APTT and normal PT.[11] Also, in dogs with chronic hepatic dysfunction caused by CPS, only APTT was prolonged, and no associated bleeding tendency during or after surgery was reported.[10] However, the effect of surgery on hemostasis could not be evaluated because hemostatic profiles were only determined before surgery. In the present study, as in earlier studies,[10,11] a prolonged APTT (19.0 seconds) and a normal PT were also found in dogs with CPS, together with lower concentrations of several coagulation factors that are produced by hepatocytes. Although APTT was prolonged and PT was normal, the specific clotting factors that were reduced represent the common pathway (factors II, V, and X) and the extrinsic pathway (factor VII). The reason for this remains unclear. A possible explanation could be found in a deficiency of factor XII (Hageman factor). Factor XII–deficient individuals do not exhibit a symptomatic bleeding tendency.[24,25] However, a deficiency of factor XII results in a dramatic prolongation of APTT and is, in fact, a laboratory abnormality, which was revealed in a family of factor XII–deficient cats.[24] Because factor XII is synthesized by the liver,[25] its activity might be decreased in dogs with portosystemic shunts and attributed to an increase in APTT, whereas the PT was normal. Another reason might be the different methodology of the coagulation tests, which might account for a difference in sensitivity. Lastly, the classical intrinsic and extrinsic pathways represent a major oversimplification of the

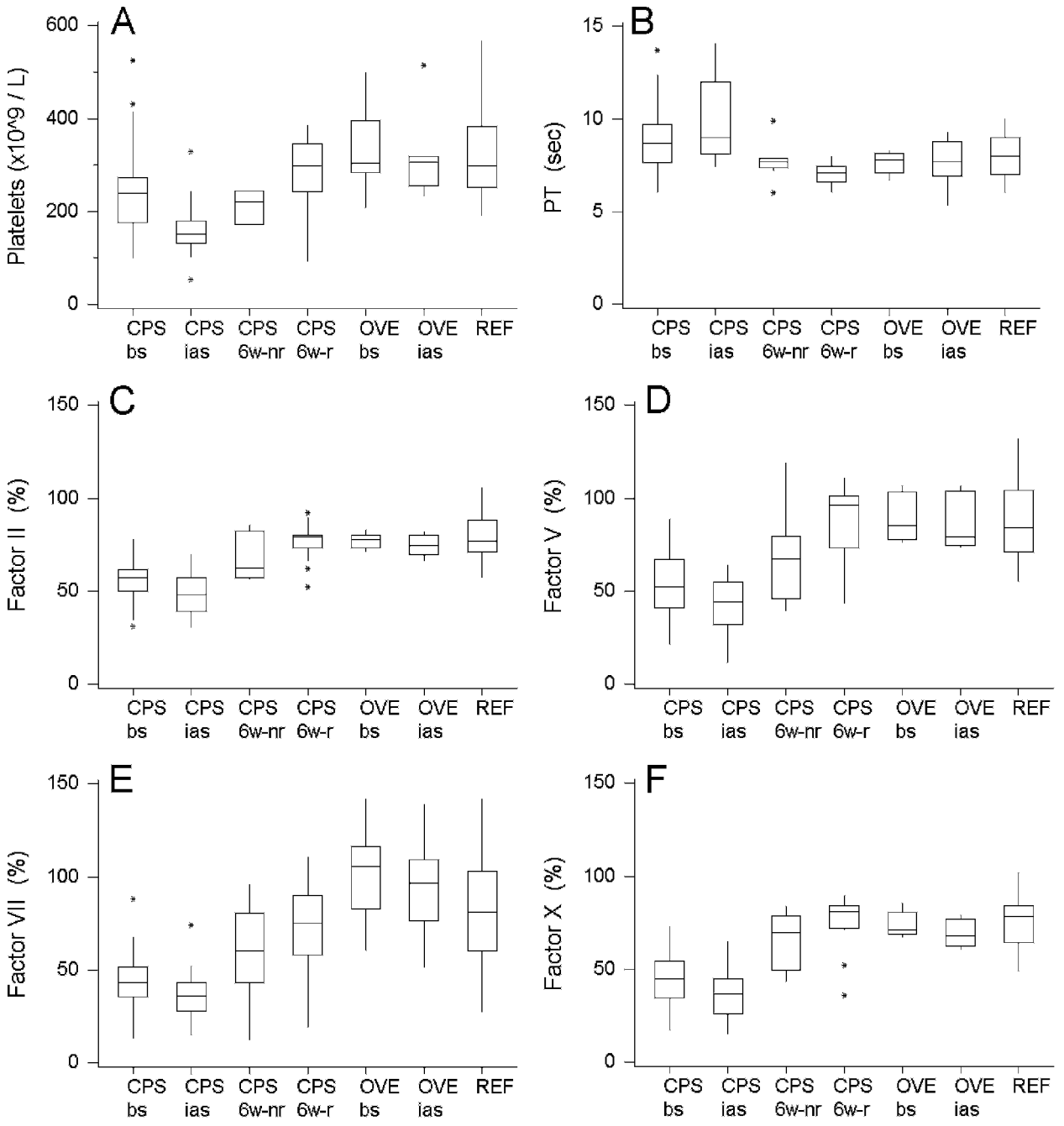

Fig. 1. Box- and whiskerplots of plasma platelet counts **(A)**, prothrombine time **(B)**, and the activity of 4 coagulation factors **(C–F)**. Hemostatic analyses were performed before surgical attenuation of a congenital portosystemic shunt (CPS bs, 33 dogs), immediately after surgical attenuation of a CPS (CPS ias, 23 dogs), approximately 6 weeks after surgical attenuation of a CPS in nonrecovered dogs (CPS 6w-nr, 8 dogs) and in recovered dogs (CPS 6w-r, 19 dogs), in 8 healthy dogs before and immediately after surgical ovariectomy (OVE bs and OVE ias, respectively), and in 31 healthy reference dogs (REF). The boxes indicate the 1st, 2nd, and 3rd quartile. The whiskers extend to the lowest and highest observations within 1.5× the interquartile range from the 1st and 3rd quartile. Outliers are plotted as *.

importance of alternate pathways, secondary amplification, and feedback mechanisms in the coagulation cascades.

Assays of individual clotting factors might help to further characterize the abnormalities present in dogs with CPS and identify possible diagnostic or prognostic indicators.[10] Because of zonal distribution of injury and specialization of hepatocytes based on their localization, hepatic diseases can result in specific coagulation factor abnormalities.[11] In the present study, abnormalities in multiple factors that are synthesized in the liver were found before surgery, with no clear diagnostic specificity or prognostic value. Factor VII is reported to show the greatest reduction in activity, both in acute and chronic liver disease, probably because it is the factor with the shortest half-life (4 to 6 hours). As the disease pro-

Table 4. Semiquantitative D-dimer plasma concentration in dogs with congenital portosystemic shunts before, immediately after, and approximately 6 weeks after surgical attenuation of the shunt.

D-dimers (ng/mL)[a]	CPS Dogs b.s. (n = 32)	CPS Dogs i.a.s. (n = 23)	CPS Dogs 6 wks, Recovered (n = 19)	CPS Dogs 6 wks, Not Recovered (n = 8)
0–250	24	10	10	5
250–500	5	8	8	1
500–1,000	1	3	1	1
1,000–2,000	2	2	0	0
>2,000	0	0	0	1

CPS, congenital portosystemic shunts; b.s., before surgery; i.a.s., immediately after surgery; 6 wks, 6 weeks after surgery.
[a] Laboratory reference value D-dimers: 0–250 ng/mL.

gresses, other coagulation factors are decreased, especially factors II, X, and V.[11,21,22,26] These factors (II, V, VII, and X) that were also decreased in dogs with a CPS are activated directly after the probably cytokine-driven increased generation of tissue factor in hepatic injury. [21] Factor IX and XI concentrations are often better preserved, possibly because of inhibition of the thrombin-induced amplification phase of coagulation.[21,22] In this study, factor VII, together with factor X, had the lowest activity of all measured factors in CPS dogs, both before and immediately after surgery. Additionally, both factors significant decreased in healthy dogs after an uncomplicated standard surgical procedure (elective ovariectomy).

There was a significantly higher concentration of factor VIII in dogs with CPS as compared to reference dogs detected in the present study. The VIII:Ag protein, which contains von Willebrand factor (VWF), is produced by megakaryocytes (VWF) and endothelial cells. Factor VIII is therefore not dependent on hepatocyte function, unlike most other clotting factors that are produced in the liver. In other studies of plasma coagulation factor abnormalities in dogs and humans with hepatic diseases, factor VIII was increased in different types of acute and chronic hepatic disease.[14,21,22] These previous studies did not include dogs with CPS, but concluded that pathologic effects of hepatic injury upon endothelial cells might cause the increased production of factor VIII. Furthermore, increases in factor VIII concentration could be due to reduced clearance via low-density receptor-related lipoprotein, which is synthesized in the liver. This effect could also be responsible for increased factor VIII concentrations in CPS dogs.

Mean concentrations of factor I (fibrinogen) remained within normal laboratory values in the dogs in this study, although a decrease was seen during CPS surgery. Factor I concentrations are rarely below normal in human hepatic disorders unless severe hepatic failure, disseminated intravascular coagulation, or cirrhosis develops. In dogs, hypofibrinogenemia is observed frequently in association with liver cell necrosis or apoptosis, seen with active acute or chronic hepatitis.[22,26,27]

Coagulation times did not increase and remained within normal limits after an uncomplicated ovariectomy in healthy dogs, although during surgery there was some apparent consumption of coagulation proteins VII and X. The activity of these factors revealed a mean decrease of 7.6 and 6.6%, respectively. The decrease in clotting factors after surgical attenuation of a CPS was significant in a larger number of factors (I, II, V, VII, IX, X, and XI), and mean decreases ranged from 10% to 16%. Compared to dogs undergoing ovariectomy, shunt attenuation also provided significant higher D-dimer concentrations after surgery. As a result of lower preoperative values and more severe apparent consumption of clotting factors, both APTT and PT were prolonged as compared to reference values after shunt attenuation (20.3 seconds and 10 seconds, respectively). Platelet counts in dogs with CPS also had lower preoperative values with a pronounced decrease after surgery (27%) as compared to healthy control dogs, although no decreased counts were found that were severe enough to cause clinical bleeding. Although clinically significant coagulation abnormalities are reported infrequently before surgery in dogs with portosystemic shunting, postoperative hemorrhage from coagulopathy was an important complication in a former study.[6] In this experience, clinical hemorrhage because of abnormalities in coagulation in dogs with CPS is mainly seen in the early postoperative period and rarely during surgery. Clinical bleeding in these animals also rarely occurs spontaneously, but is usually seen at the celiotomy wound, the site of an intraoperative liver biopsy, and the location where a peripheral arterial catheter is placed for monitoring arterial blood pressure (femoral artery) during surgery. This suggests that CPS dogs should be monitored more intensively after surgery than healthy animals at potential bleeding sites, especially when coagulation times are abnormal. Furthermore, abdominal hemorrhage must be distinguished from portal hypertension in dogs with CPS, which may also cause shock and abdominal distension. During the present study, no postoperative complications due to hemorrhage were observed. Measurement of hematologic variables, coagulation times, and plasma albumin concentration were routinely performed before and after surgery. Therefore, abnormalities or deterioration of these parameters were probably diagnosed more often and treated in an early stage. Abnormalities in hemostasis immediately after surgery might have been even more pronounced if no samples had to be excluded because of inadvertent early treatment with plasma or HES infusions.

Approximately 6 weeks after surgery recovered dogs had a significantly improved hemostasis, and all parameters had normalized. As expected, this normalization was not found in dogs with persistent portosystemic shunting at 6 weeks after surgery.

Footnotes

[a] Thrombolizer compact X, bioMérieux, Inc, France
[b] Human factor deficient plasma, bioMérieux, Inc, France
[c] Fibriquik, bioMérieux, Inc, France
[d] Minutex D-dimer, Biopool, Trinity Biotech Plc., Ireland
[e] SPSS for Windows, release 12.0.1, SPSS, Inc, Chicago, IL

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