Assessment of plasma protein C activity in dogs with portosystemic shunt

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Abstract

Portosystemic shunt (PSS) is a vascular disease that can be treated by various surgical techniques. Current veterinary studies are evaluating new prognostic markers in dogs with PSS. This study aims at evaluating the prognostic value of plasma protein C activity in dogs (n = 34) with PSS treated surgically using an ameroid constrictor. Plasma protein C activity was measured in the following time periods: preoperatively (T1), 2 days after surgery (T2), 8 weeks after surgery (T3) and > 6 months after surgery (T4). There was a significant increase in plasma protein C activity between T1/T2 and T3/T4 ($P < 0.001$). There was no significant relationship between the plasma protein C activity and sex ($P = 0.676$), age ($P = 0.172$), breed ($P = 0.573$), type of clinical signs (neurological $P = 0.993$; gastrointestinal $P = 0.924$; urological $P = 0.385$) and type of portosystemic shunt ($P = 0.516$), except for dogs with a caval type of extrahepatic PSS termination that had significant lower plasma protein C activity values compared to dogs with a diaphragmatic type of extrahepatic PSS ($P = 0.031$). No significant relationship was found between plasma protein C activity and the probability of the dog's death ($P = 0.334$) or the dog's clinical outcome ($P = 0.960$). Although not a prognostic factor, protein C activity is a laboratory marker that is useful for the diagnosis of PSS in dogs and can also be helpful in the postoperative monitoring.

Canine, vascular anomaly, ameroid constrictor, liver, prognostic marker

Portosystemic shunt (PSS) is a vascular disease caused by pathological communication between the portal and systemic circulation. The portal venous system supplies the liver with up to 80% of afferent blood (Berent and Tobias 2009). Not only does that portal blood transport the gastrointestinal tract metabolites from the splanchnic area to be utilized in the liver, but it also carries the hepatotrophic substances that aid proper liver development and function (Berent and Weisse 2007; Berent and Tobias 2009). These mechanisms are impaired in dogs with PSS.

Decreased liver function represented by increased concentration of bile acids and ammonia, increased liver enzyme activity and decreased total protein, albumin and urea concentration can usually be found in blood samples collected from dogs with PSS (Schlesinger and Rubin 1993; Bristow et al. 2017). Although there is a dominance of blood bile acid and ammonia concentration measurement in diagnostic and postoperative monitoring of dogs with PSS (Gerritzen-Bruning et al. 2006; Ruland et al. 2010; van Straten et al. 2015; Devriendt et al. 2020; Nečasová et al. 2020; Vallarino et al. 2020), current veterinary studies are evaluating new parameters that may be helpful in the postoperative monitoring of dogs with PSS. As an example, we can name serum hyaluronic acid (Devriendt et al. 2021a), serum insulin-like growth factor-1 (Serrano et al. 2021), plasma amino acid (Devriendt et al. 2021b) or serum lidocaine/monoethylglycylxylidide concentration (Devriendt et al. 2021c).

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Plasma protein C (PC) activity is one of the clinicopathological parameters measured in dogs with PSS (Toulza et al. 2006; Sunlight et al. 2022). Protein C is one of the coagulation system proteins synthesized in the liver with anticoagulation effect (Yan and Dhainaut 2001). Its decreased activity has been found in dogs with disseminated intravascular coagulopathy and in dogs with sepsis (Madden et al. 1989; de Laforcade et al. 2003; de Laforcade et al. 2008), its role has been also evaluated in dogs with gastric dilatation and volvulus syndrome (Verschoof et al. 2015; Nečasová et al. 2022). A study by Toulza et al. (2006) found a decreased PC activity in dogs with PSS compared to clinically ill dogs without PSS and also a substantial increase in PC activity after PSS ligation (Toulza et al. 2006). The authors also proved plasma PC activity to be beneficial in distinguishing PSS from microvascular dysplasia, which is named according to the latest nomenclature primary hypoplasia of the portal vein (PHPV) (Toulza et al. 2006; Cullen et al. 2006). Decreased PC activity in dogs with PSS indicates impaired liver function secondary to the liver parenchyma hypoperfusion. This was confirmed by data from human medicine, where Mack et al. (2003) described a significant improvement in decreased PC activity after the restoration of portal blood flow after surgical treatment of portal vein thrombosis (Mack et al. 2003).

Based on this data, we assume an improvement in plasma PC activity in dogs after surgical treatment of PSS, after which physiological blood flow through the portal vein should be restored. The aim of our study was to assess the PC activity levels in dogs before and after surgery of PSS using an ameroid constrictor and to find a prognostic value and risk factors of this parameter in the preoperative and postoperative period – specifically a relationship between PC activity before surgery and the dog's sex, breed, age, type of clinical signs, type of PSS, subtype of extrahepatic PSS (EHPSS) and long-term clinical outcome.

Materials and Methods

Study population and inclusion criteria

A total of 34 dogs presented to the Department of Surgery & Orthopaedics at the Small Animal Clinic, Faculty of Veterinary Medicine, University of Veterinary Sciences Brno, Czech Republic, that were diagnosed with PSS were enrolled in this prospective study. Criteria for inclusion in the study were surgical treatment of PSS using an ameroid constrictor and assessment of plasma PC activity at the time of diagnosis. A diagnosis of PSS was made based on the anamnesis, clinical examination, haematological and biochemical blood analysis (including assessment of plasma ammonia concentration and bile acid stimulation test) and diagnostic imaging (ultrasonography and/or computed tomography angiography [CTA]).

Surgical treatment and data evaluation

For the surgical therapy of PSS, dogs were put under general anaesthesia. For premedication, sufentanil 0.001 mg/kg i.v. (Sufentanil Torrex 5 µg/ml, Torrex Chiesi, Czech Republic) was used, followed by administration of propofol 1.00–4.00 mg/kg i.v. (Proposure, Richter Pharma AG, Wels, Austria). Appropriate endotracheal tube was inserted, and the procedure continued with inhalation anaesthesia, which was maintained with a mixture of medical air, oxygen and Isoflurane (1–1.5 vol. %, Aerrane Baxter S.A., Lessines, Belgium). Sufentanil 0.001–0.002 mg/kg/h i.v. was administered in continual rate infusion during the surgical procedure. A midline laparotomy with PSS identification was performed. After blunt preparation of the adventitia around the PSS, an ameroid constrictor of appropriate size was placed on the aberrant vessel in a manner to produce no or minimal (< 25%) initial constriction. The ameroid constrictor was placed on the PSS as close to its termination to the systemic circulation as possible (Plate XIV, Fig. 1). The site of ameroid constrictor placement for each type of PSS was performed in a standard manner (Berent and Tobias 2018). The surgical wound was then closed routinely. Dogs were hospitalised at the clinic and were receiving subsequent medication: amoxicillin/clavulanate 12.50 mg/kg p.o./i.m. (Synulox or Synulox RTU, Zoetis Czech Republic, s.r.o, Prague, Czech Republic) twice/once daily, lactulose (Duphalac 0.50 ml/kg p.o., Abbott Biologicals B.V., Olst, Netherlands) three times daily and metamizole 25 mg/kg i.v. (Vetalgin, Intervet International B.V., Boxmeer, Netherlands) three times daily. Dogs were hospitalised for at least the next 2 days (according to the clinical status and postoperative complications occurrence) after which they were discharged from the hospital with the following medication: amoxicillin/clavulanate (Synulox 12.50 mg/kg p.o., Zoetis Czech Republic, s.r.o.) twice daily for 7 days, metamizole (Novalgin 25 mg/kg p.o., Opella Healthcare Czech s.r.o., Prague, Czech Republic or Algifen Neo 25 mg/kg p.o.,

Teva Czech Industries s.r.o., Opava, Czech Republic) two to three times daily for the next 3–5 days, lactulose (Duphalac 0.50 ml/kg p.o., Abbott Biologicals B.V.) two to three times daily until the follow-up visit at 8 weeks after surgery, and the owners were also instructed to feed their dog a liver-supportive diet until the follow-up visit at 8 weeks after surgery.

A thorough history and physical examination as well as haematological and biochemical blood analyses (including assessment of plasma ammonia concentration and bile acid stimulation test) were performed at the follow-up examination at 8 weeks after surgery. In case of favourable results of the examination, the medical treatment with lactulose and a liver-supportive diet was ended. In case of unfavourable results, the dog was treated individually according to the attending veterinarian's recommendations.

The following data were recorded for each dog: sex, breed, age at the time of surgery, type of clinical signs, type of the PSS and subtype of EHPSS. For the purpose of statistical analysis, the dogs were subdivided according to their breed into large dog breeds (adult weight ≥ 20 kg) and small dog breeds (adult weight < 20 kg). Dogs were also subdivided into groups according to their age, namely < 6 months, 6–12 months and > 12 months of age.

Clinical signs were divided into 3 groups: neurological, gastrointestinal, and urological clinical signs. Neurological clinical signs included disorientation, ataxia, apathy, circling, head pressing, transient blindness, tremor, spasms and seizures and disorders of consciousness. Gastrointestinal clinical signs included chronic vomiting, diarrhoea and inappetence, pica, hypersalivation, and inability to gain weight with normal food intake. Urological clinical signs included polyuria/polydipsia, urolithiasis and signs related to it (strangury, haematuria, dysuria, pollakiuria). The type of PSS was determined based on diagnostic imaging and perioperative findings to EHPSS or IHPSS (intrahepatic PSS). In case of EHPSS, termination of the aberrant vessel was also recorded and was classified into one of the following groups as described by Kraun et al. (2014): caval EHPSS (shunts terminated into v. cava caudalis) or diaphragmatic EHPSS (shunts terminated cranial to the liver, either into v. azygos or into v. phrenica).

Long-term clinical outcome in the time period > 6 months after surgical treatment was also evaluated. This evaluation was performed based on either personal or telephone communication with the owner. The owner was asked whether the dog had any clinical signs of the disease, and, if so, which of them, whether the dog was on any medical therapy for the disease and whether the dog was fed a liver-supportive diet at that time. Based on this data the dog was then classified as a dog with (modified according to Mehl et al. [2005]):

- excellent clinical outcome – the dog is without any clinical signs of the disease and does not require further medical treatment;
- good clinical outcome – the dog is without any clinical signs of the disease but is receiving a liver supportive diet or lactulose or a combination of these;
- poor clinical outcome – the dog still has clinical signs of the disease or died because of PSS (at any time during the follow-up period).

Protein C activity assessment and blood analysis

The dynamics of plasma PC activity was evaluated in all dogs. Protein C activity assessment (STA Stachrom protein C, STA Satellite Max, Diagnostica Stago, S.A.S., France) was performed during a routine blood work analysis within the standard preoperative and postoperative examination of dogs with PSS. Blood was obtained at the following time periods: before surgery (at the time of diagnosis) (T1), 2 days after surgery (T2), 8 weeks after surgery (T3) and > 6 months after surgery (T4). The blood for PC activity analysis was collected into citrated tubes and was centrifuged within 1 h after sampling at 2,250 *g* for 15 min. Samples of citrated plasma were subsequently frozen at −80 °C and plasma analysis was performed after the collection of a sufficient number of samples for full utilisation of the analytical set, with a maximum storage time of 6 months. Protein C activity was considered decreased if values < 75% were measured (reference range 75–135%). Biochemical blood analysis was performed on Architect c4000 (Abbott Laboratories, Texas, USA). Haematological blood analysis was performed on Sysmex XT-2000iV (Sysmex Corporation, Kobe, Japan).

Statistical analysis

For statistical evaluation of the results, *t*-test, ANOVA and generalized linear models were used. ANOVA for repeated measures followed by Tuckey's *post hoc* test was used to compare PC activity values before surgery (T1), 2 days after surgery (T2), 8 weeks after surgery (T3), and > 6 months after surgery (T4). For comparison of PC activity values between the two groups (e.g. male and female dogs, large dog breeds and small dog breeds, etc.), *t*-test was used. The PC activity values were compared between multiple groups (3 age groups) using one-way ANOVA. A change in the probability of the dog's death with the PC activity measured before surgery was quantified and tested using a generalized linear model (GLM) with assumed binomial distribution and a logistic link function. The same model was also used to evaluate the probability of achieving an excellent clinical outcome versus an unfavourable clinical outcome (i.e. good or poor) depending on the value of PC activity. All statistical tests were evaluated at a significance level of 0.05. Statistica software, version 13.5 (TIBCO Software Inc.) and R project, version 3.4.3 (R Core Team) were used for statistical data processing.

Results

A total of 34 dogs with PSS treated surgically using an ameroid constrictor were enrolled in the study. Abdominal ultrasonography was performed in every dog and CTA was performed in 9 of the dogs. Intrahepatic PSS was found in 6 dogs (17.65%) and EHPSS in 28 dogs (82.35%), of which 18 dogs (64.29%) had caval EHPSS and 10 dogs (35.71%) had diaphragmatic EHPSS. The population included 21 females (61.76%) and 13 males (38.24%). Breeds represented in the population were the Yorkshire Terrier (n = 17), Bichon Frise (n = 3), Dachshund (n = 4), Papillon (n = 1), Toy Poodle (n = 1), Labrador Retriever (n = 1), Scottish Terrier (n = 1), Maltese (n = 1), Golden Retriever (n = 1), German Spitz (n = 1), Beauceron (n = 1), Belgian Malinois (n = 1) and a small dog crossbreed (n = 1). According to our subdivision, 30 dogs (88.24%) were considered to be of a small breed and 4 dogs (11.76%) were considered to be of a large breed. The mean body weight was 4.00 ± 4.45 kg and the mean age at the time of the surgery was 11.65 ± 13.83 months. Twenty-one dogs were < 6 months old, 5 dogs were between 6–12 months old and 8 dogs were > 12 months old. Twenty-four dogs were presented with neurological signs, 11 dogs with gastrointestinal signs and 9 dogs with urological signs, 10 dogs had clinical signs belonging to more categories.

Only two dogs had PC activity values within the reference range at T1 (Table 1). Normalisation of PC activity to the reference range occurred in 11/25 dogs (44.00%) at T3, and in 8/13 dogs (61.54%) at T4. Protein C activity values at T1 and T2 did not differ significantly (repeated measures ANOVA – Tuckey *post hoc* test: $P = 0.882$). Protein C activity values were significantly higher at T3 and T4. The values of PC activity measured in these time periods differed significantly from the values of PC activity at T1 and T2, respectively (repeated measures ANOVA: $F(3.33) = 35.721$, $P < 0.001$; Table 1). For comparison, mean values (± SD) of selected haematological and biochemical parameters suggestive of PSS together with PC activity values measured at T1, T2, T3 and T4 are shown in Table 1.

Protein C activity before surgery did not differ significantly between females (30.86 ± 15.87%) and males (33.22 ± 15.82%) (*t*-test: $t = 0.422$, df = 32, $P = 0.676$). When comparing the values of PC activity in small (32.32 ± 16.47%) and large dog breeds (27.53 ± 6.75%), no significant difference was found (*t*-test: $t = 0.569$, df = 32, $P = 0.573$). In younger dogs (age < 6 months and age 6–12 months), PC activity values (28.48 ± 14.70% and 31.25 ± 6.66%) were lower than in older dogs (age more than 12 months; PC activity 40.70 ± 19.71%); however, this difference was not significant (one-way ANOVA: $F(2.31) = 1.863$, $P = 0.172$). The mean values of PC activity in dogs with neurological signs (31.78 ± 16.38%) and in dogs without neurological signs (31.72 ± 14.59%) did not differ significantly (*t*-test: $t = 0.009$, df = 32, $P = 0.993$). The same was true when comparing the PC activity in dogs with (32.14 ± 12.94%) and without gastrointestinal signs (31.58 ± 17.07%) (*t*-test: $t = 0.097$, df = 32, $P = 0.924$) and with (27.81 ± 7.11%) and without urological signs (33.18 ± 17.66%) (*t*-test: $t = 0.880$, df = 32, $P = 0.385$). Dogs with EHPSS showed PC activity of 30.94 ± 14.80%, dogs with IHPSS showed PC activity of 35.60 ± 20.31%; the difference was non-significant (*t*-test: $t = 0.656$, df = 32, $P = 0.516$). Dogs with caval EHPSS had lower PC activity (26.51 ± 10.11%) than dogs with diaphragmatic EHPSS (38.92 ± 18.81%); the difference was significant (*t*-test: $t = 2.286$, df = 26, $P = 0.031$).

Two dogs (5.88%) in our study group died. One dog with EHPSS (a caval type) with preoperative PC activity of 15.11% died on the second day after surgery, another one with IHPSS with preoperative PC activity of 29.41% died on the fourth day after surgery; both died due to severe portal hypertension. The probability of the dog's death depending on the value of PC activity before surgery was quantified using GLM and was not significant ($P = 0.334$; Table 2, Fig. 2).

Table 1. Selected haematological and biochemical indicators and protein C activity measured in the selected time periods (T1 = before surgery, T2 = 2 days after surgery, T3 = 8 weeks after surgery, T4 = more than 6 months after surgery) including numbers of dogs with the indicator out of the reference range.

		T1	T2	T3	T4
PC	Mean ± SD	31.76 ± 15.65 [a]	32.80 ± 12.29 [a]	70.07 ± 15.32 [b]	77.55 ± 12.30 [b]
(%)	Out of RR	32	33	14	5
	n	34	33	25	13
BA	Mean ± SD	138.23 ± 139.21 [a]	24.24 ± 32.59 [b]	8.73 ± 15.62 [b]	12.35 ± 29.70 [b]
(µmol/l)	Out of RR	32	16	6	3
	n	33	33	25	13
BA PP	Mean ± SD	269.29 ± 143.06 [a]	98.72 ± 71.31 [b]	37.94 ± 61.63 [b]	35.54 ± 53.67 [b]
(µmol/l)	Out of RR	32	29	11	5
	n	32	33	24	13
AM	Mean ± SD	163.38 ± 74.00 [a]	58.79 ± 28.73 [b]	47.11 ± 12.71 [b]	46.85 ± 24.73 [b]
(µmol/l)	Out of RR	31	18	9	3
	n	33	32	25	13
TP	Mean ± SD	51.21 ± 9.22 [a]	41.61 ± 8.26 [b]	60.95 ± 4.69 [c]	-
(g/l)	Out of RR	23	32	1	-
	n	34	33	25	-
ALB	Mean ± SD	24.47 ± 4.49 [a]	20.19 ± 4.39 [b]	30.46 ± 2.52 [c]	-
(g/l)	Out of RR	16	26	0	-
	n	34	33	25	-
BUN	Mean ± SD	2.82 ± 1.61 [a]	1.95 ± 1.23 [b]	4.40 ± 2.00 [c]	-
(mmol/l)	Out of RR	22	29	9	-
	n	34	33	25	-
HT	Mean ± SD	0.39 ± 0.06 [a]	0.32 ± 0.06 [b]	0.45 ± 0.05 [c]	-
(l/l)	Out of RR	7	22	0	-
	n	34	33	25	-
MCV	Mean ± SD	59.42 ± 4.45 [a]	60.52 ± 3.81 [a]	63.12 ± 4.04 [b]	-
(fl)	Out of RR	15	10	6	-
	n	34	33	25	-

BA - bile acid; BA PP - postprandial bile acid; AM - ammonia; TP - total protein; ALB - albumin; BUN - blood urea nitrogen; HT - haematocrit; MCV - mean corpuscular volume, RR - reference range

[a, b, c] – values of haematological and biochemical indicators followed by the same upper-case letter in the row did not differ significantly (Tuckey *post hoc* test in repeated measures ANOVA).

Table 2. Parameters of the generalized linear model for dogs' mortality depending on protein C activity before surgery and their significance.

Coefficients	Estimate	Standard error	z value	*P* value
Intercept	−0.938	1.798	−0.522	0.602
Protein C activity before surgery	−0.069	0.071	−0.966	0.334

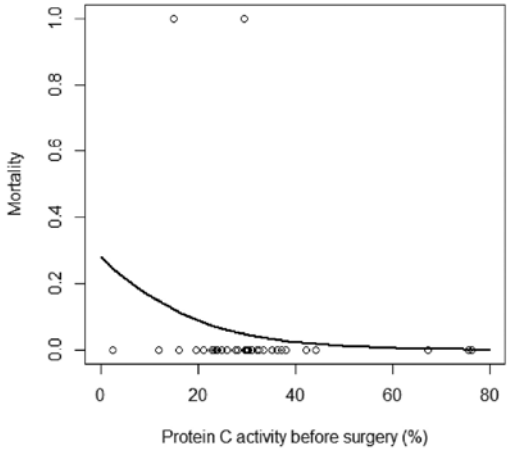

Fig. 2. Relationship between mortality and protein C activity in dogs before surgery.

Table 3. Changes in protein C activity (%) in dogs with known long-term clinical outcome > 6 months after surgery. Protein C activity (%) was measured before surgery (T1), 2 days after surgery (T2), 8 weeks after surgery (T3) and > 6 months after surgery (T4). Numbers of dogs with protein C activity values out of the reference range (RR) for each group are also listed.

		Long-term clinical outcome		
		Excellent	Good	Poor
T1	n	22	2	3
	Mean ± SD	33.63 ± 16.19	48.82 ± 26.13	22.83 ± 7.22
	Out of RR	20	2	3
T2	n	22	2	2
	Mean ± SD	33.01 ± 10.09	32.12 ± 14.65	31.59 ± 19.71
	Out of RR	22	2	2
T3	n	19	2	1
	Mean ± SD	69.72 ± 15.71	64.00 ± 6.63	97.09
	Out of RR	10	2	0
T4	n	12	1	0
	Mean ± SD	79.21 ± 11.24	57.74	
	Out of RR	4	1	

Table 4. Parameters of the generalized linear model for the long-term clinical outcome (excellent vs good-poor) depending on the protein C activity before surgery (T1), 2 days after surgery (T2), 8 weeks after surgery (T3) and > 6 months after surgery (T4).

	Coefficient	Estimate	Std. error	z value	*P* value
T1	Intercept	−1.430	1.141	−1.254	0.210
	Protein C activity	−0.002	0.031	−0.050	0.960
T2	Intercept	−1.351	1.771	−0.763	0.446
	Protein C activity	−0.011	0.053	−0.207	0.836
T3	Intercept	−3.599	3.351	−1.074	0.283
	Protein C activity	0.024	0.044	0.546	0.585
T4	Intercept	475.070	494652.735	0.001	0.999
	Protein C activity	−7.848	8088.345	−0.001	0.999

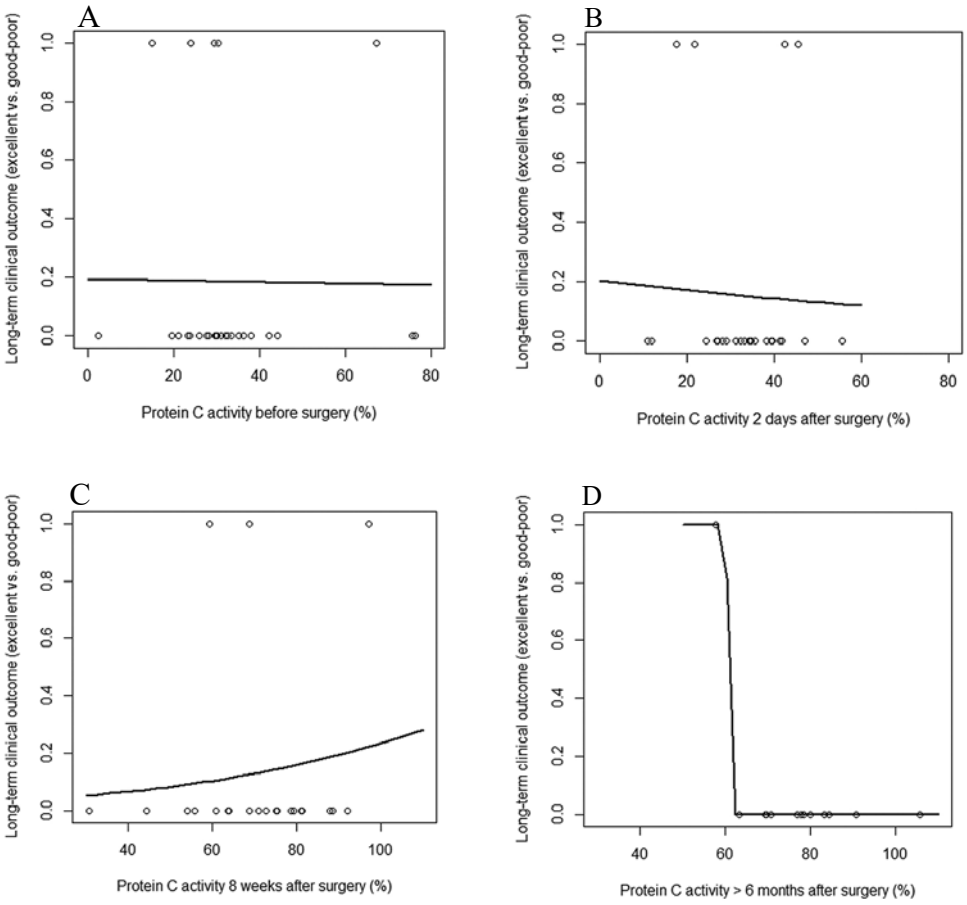

Fig. 3. Relationship between the long-term clinical outcome (excellent vs good-poor) and protein C activity in dogs before surgery (A), 2 days after surgery (B), 8 weeks after surgery (C) and > 6 months after surgery (D).

The long-term clinical outcome of > 6 months after surgery (range 6–30 months) was monitored in 27 dogs with known PC activity before surgery; the results and mean values are shown in Table 3. Data on the long-term clinical outcomes were obtained via a telephone interview with the owner in 12 cases and during a personal interview at the follow-up in 13 cases, together with the re-evaluation of plasma PC activity (at T4, range 6–30 months after surgery). Two dogs that died during the study period were automatically classified as dogs with a poor long-term clinical outcome. The probability of achieving an excellent long-term clinical outcome compared to an unfavourable clinical outcome (good or poor) depending on the PC activity value was fitted using a generalized linear model. The probability of an unfavourable long-term clinical outcome for the dog did not depend on PC activity at T1 ($P = 0.960$), T2 ($P = 0.836$), T3 ($P = 0.585$) or T4 ($P = 0.999$) (Table 4, Fig. 3A–C). The regression coefficient value (−0.002) shows that with the increasing value of PC activity at T1, the probability of an unfavourable clinical outcome decreases. However, this relationship is very weak and non-significant ($P = 0.960$) (Table 4), which is also

evident from the visualization of this relationship (Fig. 3A). Another test, based on χ^2, which compares the logistic regression model with a null model, found the statistical significance of PC activity at T4 to the clinical outcome of dogs > 6 months after surgery ($P = 0.008$) (Table 4, Fig. 3D). However, when interpreting this result, it must be considered that the model was created based on only one measurement of a dog with a poor clinical outcome.

Discussion

Protein C activity assessment seems to be a very useful parameter in dogs with PSS. Therefore, our aim was to evaluate PC activity comprehensively, with emphasis on its value in postoperative monitoring, but also to evaluate this parameter as a preoperative prognostic factor, possibly connected to important clinical variables in dogs with PSS. Protein C activity was below the reference range in the majority of dogs in our study group (32/34 dogs, 94.12%) at the time of diagnosis, similar as was observed for preprandial (32/33 dogs, 96.97%) and postprandial bile acid concentration (32/32 dogs, 100.00%) or ammonia concentration (31/33 dogs, 93.94%). Abnormalities in other blood parameters suggestive of portosystemic shunting (total protein, albumin, blood urea nitrogen, mean corpuscular volume) were not observed as often (Table 1).

Protein C seems to have a very good diagnostic sensitivity, since it was in the reference range in only 2 of 35 dogs at the time of diagnosis. We found a significant increase in plasma PC activity after surgical treatment of PSS using an ameroid constrictor between the values measured before surgery/2 days after surgery and at 8 weeks after surgery/> 6 months after surgery. Also, a significant increase/decrease in other evaluated haematological and biochemical parameters was observed compared to the preoperative values (Table 1). Our results showed that assessment of PC activity is one of the possible parameters suggestive of liver function restoration in dogs after surgical treatment of PSS, since there should be a significant increase in plasma PC activity after successful surgical treatment of PSS.

A similar trend in plasma PC activity increase after surgical treatment was observed by Toulza et al. (2006) and Sunlight et al. (2022). Toulza et al. (2006) found a substantial increase in PC activity in dogs after PSS ligation. These authors evaluated only 15 surgically treated dogs and PC activity assessment in the postoperative period or prognostic significance of the parameter was not an object of the study. Recently, PC activity was also evaluated by Sunlight et al. (2022) in their study group of 47 dogs with IHPSS treated surgically with percutaneous transvenous coil embolization. Protein C activity normalized to the reference range in 53.2% of dogs in their study group in the time period of 3–55 months after surgery (Sunlight et al. 2022). Although the time periods for postoperative monitoring of dogs were not as strictly defined as they were in our study, the results of Sunlight et al. (2022) are similar to our results since we found a normalisation in PC activity in 44.00% of dogs 8 weeks after surgery and in 61.54% > 6 months after surgery in our study group of dogs with both EHPSS and IHPSS. These results support our other finding of no significant difference between PC activity and the type of the PSS (EHPSS vs IHPSS).

The aim of our study was to evaluate the prognostic role of PC activity in dogs with PSS. Although we did not find the PC activity to be a prognostic factor for the majority of evaluated parameters in dogs with PSS, it does not mean that its assessment is of no benefit. In cases where PC activity does not normalise within the reference range after surgery, it is advisable to choose another diagnostic approach. Assessment of PC activity is less invasive and less expensive compared to other diagnostic modalities (portovenography, CT angiography, scintigraphy), hence this evaluation may be suitable as the first step in postoperative management and evaluation of dogs after PSS surgery. Based on our findings that PC activity was within the reference range in 44.00% of dogs 8 weeks after

surgery and in 61.54% of dogs > 6 months after surgery, we can conclude that a time period of 8 weeks after surgery may not be enough for hepatic function restoration and adjustment of the parameter. When using PC activity for evaluation of dogs with PSS after surgery in clinical practice, we recommend repeating the measurement after some time in cases with abnormal PC activity values at 8 weeks after surgery.

The time period of 8 weeks after surgery for follow-up postoperative monitoring was selected because the biggest expansion of the ameroid constrictor and gradual occlusion of the aberrant vessel occurs during the first 8 weeks after its implantation (Vogt et al. 1996; Hunt et al. 2014). Although a rapid occlusion of the vessel can occur after the ameroid constrictor implantation (e.g. in 8 days after implantation), changes in the expansion of the inner ring of the ameroid constrictor and changes in the vessel found at 8 weeks after the placement are very similar to those found sooner after its placement. Furthermore, it is possible to evaluate dogs with delayed shunt occlusion and dogs with re-canalisation of the aberrant vessel (Besancon et al. 2004). A decrease in PC activity in dogs with PSS can cause a dysregulation in blood coagulation and can lead to hypercoagulability (Kelley et al. 2013), which can be the cause of a rapid shunt occlusion by an ameroid constrictor. Due to the fact that the ameroid constrictor expansion causes only partial occlusion of PSS and that the complete occlusion occurs as a result of a thrombus formation within the vessel lumen (Besancon et al. 2004), we cannot exclude the hypercoagulability to be the main cause of the rapid PSS occlusion in some dogs.

In terms of biological parameters, our results show no differences in PC activity between male and female dogs and between small and large dog breeds. A trend was observed for younger dogs to have lower PC activity values compared to older dogs, but this finding was non-significant. This phenomenon may be due to more serious clinical manifestations of the disease associated with more pronounced laboratory indicators and the associated necessity for an early presentation at a veterinary clinic. Physiologically lower values of PC activity have already been found in children (Fisher and Yan 2000), but the phenomenon has not been proven in dogs yet. To the authors' knowledge, this evaluation has not been done in other studies so far. Sunlight et al. (2022) only found a correlation between the dog's age and the occlusion rate during percutaneous coil embolization of IHPSS. Dogs with complete occlusion were significantly older compared to dogs in which only partial occlusion had occurred (Sunlight et al. 2022). Our results indicate that PC activity is not affected by the dog's body weight or age.

Moreover, we did not find lower PC activity in dogs with neurological clinical signs, i.e. with a more severe clinical presentation of the disease, compared to dogs without neurological clinical signs. The same was found for any other type of clinical signs. Our hypothesis was that lower plasma PC activity would be found in dogs with neurological clinical signs (as a consequence of hepatic encephalopathy, and thus greater impairment of liver function) compared to dogs with other clinical signs. This hypothesis was not confirmed in our study. The mechanism of hepatic encephalopathy has not yet been comprehensively clarified; hyperammonaemia is believed to be the main cause, but there are probably other blood metabolites that contribute to the condition (Aronson et al. 1997; Berent and Tobias 2009; Tivers et al. 2014). The reason for the development of a specific type of clinical signs is unknown. Our results indicate that there is no relationship between impaired liver function and perfusion and the type of clinical signs. Toulza et al. (2006) found that dogs that had normal PC activity were not encephalopathic or clinically ill (Toulza et al. 2006). Our study population was smaller than the study population of Toulza et al. (2006); moreover, all dogs in our study were presented with some clinical signs in contrast to their study dogs. Therefore, we chose to compare dogs with neurological clinical signs and dogs with other types of clinical signs. These variables could have affected the results of the statistical evaluation.

In terms of the PSS type, we did not find a significant difference in plasma PC activity between dogs with IHPSS and dogs with EHPSS. Comparing types of EHPSS according to its termination, we found that dogs with caval EHPSS had significantly lower values of plasma PC activity compared to dogs with diaphragmatic EHPSS. Dogs with diaphragmatic EHPSS usually present later in their life with less pronounced clinical signs and they also tend to weigh more compared to dogs with caval EHPSS (Mehl et al. 2005; Van den Bossche et al. 2012; Kraun et al. 2014; El-Sebaey et al. 2020). It is believed that anatomical localisation is the reason for the lesser severity of the disease. Partial or complete occlusion of the shunt can be achieved during respiration and postprandial gastric distention. There might also be a higher pressure and resistance to blood flow in the phrenic vein and azygos vein compared to caudal vena cava, due to their narrower diameter and a greater distance between its origin and its termination (Van den Bossche et al. 2012; Kraun et al. 2014; Amaha et al. 2019; El-Sebaey et al. 2020). Therefore, there is probably lower blood flow through the PSS and higher blood flow in the portal vein, resulting in better liver perfusion, in diaphragmatic EHPSS compared to caval EHPSS, which is in agreement with our study results. To our knowledge, the relationship between plasma PC activity and the type of EHPSS has not been evaluated yet. These new conclusions are in accordance with studies by Kraun et al. (2014) and El-Sebaey et al. (2020). They found significantly less pronounced abnormalities in blood total protein, albumin, globulins, urea, cholesterol and triacylglycerol concentrations and liver enzyme activity in dogs with diaphragmatic EHPSS compared to dogs with EHPSS that terminated caudally to the liver (Kraun et al. 2014; El-Sebaey et al. 2020). These authors' as well as our results indicate that there is less hepatic function impairment in dogs with diaphragmatic EHPSS (because of better liver perfusion) and thus less pronounced abnormalities in laboratory parameters, including PC activity.

The disadvantage of diaphragmatic EHPSS is their worse visualisation during ultrasonographic examination (Szatmári et al. 2004a). Therefore, it is often necessary to use other imaging techniques that require general anaesthesia of the dog, e.g. CTA, for its accurate localisation. We demonstrated in our study that dogs with diaphragmatic EHPSS have significantly higher plasma PC activity compared to dogs with caval EHPSS. Assessment of plasma PC activity in dogs with PSS might help us with the prediction of PSS termination localisation during paraclinical examination (especially during ultrasonography).

To our knowledge, no other study has yet evaluated the relationship between PC activity and mortality after surgery for PSS in dogs. We presumed worse liver perfusion and function in dogs with low plasma PC activity. The liver can be so impaired by long-lasting hypoperfusion that the surgery restoring normal blood flow in the portal vein may not result in complete liver function recovery. Consequently, portal hypertension may develop in such dogs, which can be a serious postoperative complication (Berent and Tobias 2009). Protein C activity values were very low in the two deceased dogs, although they were not the lowest ones. In spite of the fact that the study was conducted on a small group of dogs, the results of the relationships are very beneficial. We do acknowledge that the results and their interpretation could be partly influenced by the small number of dogs, especially the dogs that died. For this reason, further research into this relationship with a larger cohort is recommended.

We did not find a significant relationship between the long-term clinical outcome after surgical treatment of PSS using an ameroid constrictor and plasma PC activity before surgery and 2 days after surgery; however, we did find the risk of unfavourable clinical outcome to be declining with higher PC activity values. The small sample of dogs in each group is unfortunate as PC activity was not measured in each dog in each selected time period. The reason for that is either the dog did not come back for the planned check-

up, or the dog died during the follow-up period. Our study results are in agreement with the results of Sunlight et al. (2022) who did not find a significant association between preoperative PC activity and the ultimate clinical status in dogs after surgical treatment of IHPSS with percutaneous transvenous coil embolization. Dogs with excellent clinical status after surgery had higher final PC activity values compared to dogs with fair or poor clinical status (Sunlight et al. 2022). These authors used a scale similar to the one we used in our study to evaluate the dogs' clinical status after surgery, with the exception that they did not classify the dogs that died during the study period due to PSS as dogs with a poor outcome. Despite the fact there were 16 dogs (37.2%) with an excellent clinical outcome, 19 dogs (44.2%) with a good clinical outcome and 8 of 43 dogs (18.6%) with a poor clinical outcome in their study. We achieved a better long-term clinical outcome after surgical treatment of PSS (both EHPSS and IHPSS) using an ameroid constrictor, since we found an excellent outcome in 22 dogs (81.48%), a good clinical outcome in 2 dogs (7.41%) and a poor clinical outcome in 3 dogs (11.11%). Another study achieved favourable outcomes in dogs with EHPSS after surgical treatment using an ameroid constrictor, finding an excellent outcome in 86 of 108 dogs (80%), a good outcome in 15 of 108 dogs (14%), and a poor outcome in 7 of 108 dogs (6%) (Mehl et al. 2005). According to the meta-analysis by Serrano et al. (2019), using an ameroid constrictor and complete ligation appeared to be the surgical techniques with the strongest evidence of giving a good quality of life, followed by thin film banding and coil embolization (Serrano et al. 2019). Although our study did not find a relationship between PC activity and long-term clinical outcome in dogs with PSS, we found an excellent long-term clinical outcome in 81.48% dogs, which supports the evidence of favourable outcomes after surgical treatment of PSS using an ameroid constrictor.

The residual flow through PSS after surgical attenuation was not evaluated in this study. The PSS residual flow when using an ameroid constrictor was found to be very low in previous studies (0% reported in the study by Or et al. [2016] and Traverson et al. [2018]). According to recent studies, small residual blood flow is questionable in terms of clinical significance in dogs after surgical treatment of PSS and no consensus in the management of such cases has been established yet. Some residual shunting through PSS is clinically unimportant if a major portion of blood from the portal system reaches the liver; if the blood flow through the portal vein cranial to the PSS is hepatopetal, the amount of shunting blood is insignificant and is associated with an excellent or good clinical outcome (Szatmári et al. 2004b). Therefore, we decided to evaluate plasma PC activity in relation to the clinical outcome. The residual flow and the direction of the flow in the portal vein could have possibly affected PC activity. It would be good to evaluate the possible effect of the residual blood flow on this indicator in the future.

One limitation of our study is the relatively small sample size. There was only a small number of dogs in some of the selected groups (e.g. 1–2 dogs with good or poor clinical outcome in the time period of 8 weeks and > 6 months after surgery), which was too small for statistical comparison. Nevertheless, the obtained data are valuable for the subject and may be a basis for other studies in this field of veterinary medicine.

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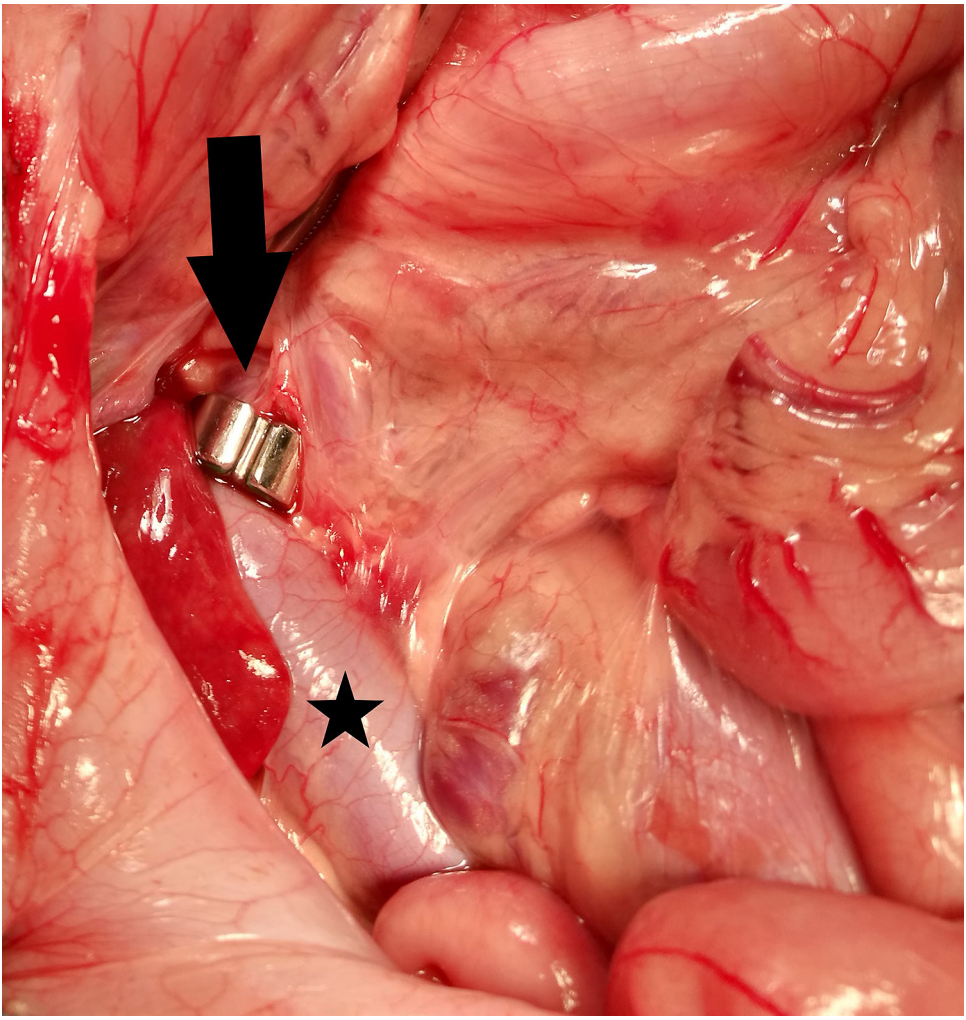

Fig. 1. Placement of the ameroid constrictor (arrow) on the portocaval portosystemic shunt as close to its termination to the caudal vena cava (asterisk) as possible.